

# Applications of Nanomaterials in Human Health

Firdos Alam Khan  
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Firdos Alam Khan

Department of Stem Cell Biology  
Imam Abdulrahman Bin Faisal University  
Dammam, Saudi Arabia

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## Preface

Over the past few years, there has been tremendous interest in the nanomaterials field because of their nanosize, better biocompatibility, better targeting of the disease and most important is the effective treatments of many diseases. The nanomaterials have revolutionized almost all the fields, especially their applications in the biomedical sciences are the most promising ones. The nanomaterials are synthesized by using different methods and they can be obtained from both natural and synthetic sources. One of the remarkable characteristics of these nanomaterials is that they can be conjugated, linked, and encapsulated with other materials, chemicals, drugs, and molecules, which make them most attractive for various biological applications. Over the past few years, several nano-based products have been created for many clinical conditions. There are many more under different phases of the trials (preclinical or clinical) for a variety of biological applications such as drug delivery, diagnosis, and treatment. As the field of nanotechnology is expanding, we thought it is time to overview the impact of the nanomaterials on human health and to write an exclusive book dedicated towards the application of nanomaterials in human health. This book provides comprehensive and updated information on all aspects of the nanomaterials related to human health. There are many books written on the application of nanomaterials on different topics, but there is no single book available that discusses all topics of nanomaterials related to human health.

In this book, we have tried to include all the topics which are directly or indirectly related to fields of nanomaterials. The primary objective of this book is to provide the students, researchers, and professionals a single source of information about different nanomaterials and their biological and medical applications. There are 15 chapters in this book and each chapter contains the updated information with beautiful illustrations. We have discussed the various topics like nanomaterials and their types and classifications, synthesis of nanomaterials, and characterization of nanomaterials. The topics such as application of nanomaterials in stem cells, tissue engineering and regenerative medicine, neuronal differentiation, neuronal protection, neurotoxicity, neurological diseases, diagnosis and treatment of genetic disorders, cancer diagnosis, drug delivery, treatment of microbial and viral infections, treatment of endocrine diseases have also been discussed. We have also discussed

major nano-based products related to nanomedicine, nanosensors, nanorobots, nanodiagnostics, commercialization of nanomaterials, IPR, and marketing of nano-products. In addition, we have also explained the negative effects of nanomaterials on human health along with ethical issues. The last chapter deals with the next-generation nanomaterials—smart nanomaterials.

Dammam, Saudi Arabia

Firdos Alam Khan

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Enjoy reading!

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Suresh Thangudu

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## About the Editor

**Firdos Alam Khan** is a Professor and Chairman of the Department of Stem Cell Biology, Institute for Research and Medical Consultations (IRMC), Imam Abdulrahman Bin Faisal University, Dammam, Saudi Arabia. He completed his Ph.D. in Zoology with a specialization in Neuroscience at Nagpur University, India, and has been involved in teaching various courses including Cell Biology, Pharmacology, Business of Biotechnology, Biomedicine, Cell and Tissue Engineering, and Bioethics and IPR to undergraduate and postgraduate students. He was a Professor and Chairman of the School of Life Sciences and served as Chairman of the Research and Development program at Manipal University (now Manipal Academy of Higher Education), Dubai Campus, United Arab Emirates. He holds three US patents in the field of stem cell biology, has authored two books: Biotechnology Fundamentals (textbook) and Biotechnology in Medical Science, and has published numerous research articles on stem cell biology, neuroscience, and nanomedicine. He is an editor and reviewer for various high-impact journals, including *Scientific Reports*, *Nanomedicine*, *IET Nanobiotechnology*, *Drug Design, Development and Therapy*, *3 Biotech*, *Journal of Biomolecular Structure and Dynamics*, and the *International Journal of Nanomedicine*.



# Nanomaterials: Types, Classifications, and Sources

1

Firdos Alam Khan

## Abstract

Over the past few years, nanomaterials (NMs) have attracted the researchers because of their nanosize, physical, biological, and chemical properties compared to their bulk materials. These NMs are classified based on their size, chemical composition, shape, and sources. Different types of NMs have been synthesized from different sources and they are being classified accordingly. Many NMs have been produced in large quantities based on the requirements for many industrial applications. The two main sources through which NMs are being produced are synthetic source and naturally occurring nanoparticles (NPs). In this chapter, we discuss the types and classifications of NMs and broadly discuss the different types of nanomaterials isolated from natural and synthetic sources.

## Keywords

Nanomaterials · Types · Classifications · Sources

## 1.1 Introduction

Nanoparticles (NPs) and nanomaterials (NMs) have attracted many researchers due to their unique properties such as nanosize, better biocompatibility and bioavailability, and most importantly their effective drug delivery capabilities. There are many factors which are responsible to make these NPs and NMs the most desirable

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F. A. Khan (✉)

Department of Stem Cell Biology, Institute for Research & Medical Consultations, Imam Abdulrahman Bin Faisal University, Dammam, Saudi Arabia  
e-mail: [fakhan@iau.edu.sa](mailto:fakhan@iau.edu.sa)

candidates for many biological and medical applications. Some of them are better thermal and electrical conductivity, better catalytic activity, and better light absorption capabilities. The definition of nanomaterials is basically related to their sizes and the materials which have diameter from 1 to 100 nm are normally known as the nanomaterials and nanoparticles. There are several rules and regulation to measure the diameter of NPs and NMs are being used by the researchers in European Union (EU) and the USA, however, there is no specific accepted definition for nanomaterials does not exist. In addition, different establishments have different definitions of nanomaterials (Boverhof et al. 2015).

As per Environmental Protection Agency (EPA) nanomaterials display exceptional properties different than the equivalent chemical compounds (United Nations 2014). In addition, the US FDA also denotes to nanomaterials as materials that have at least one dimension from 1 to 100 nm and exhibit dimension dependent phenomena (US-FDA 2016). Correspondingly, International Organization for Standardization (ISO) has named nanomaterials as a material having internal nanoscale surface structure (ISO/TS 2010). The nanomaterials such as nanospheres, nanoplates, quantum dots, nanowires, nanofibers, and other NMs have been defined based on this ISO definition (Bleeker et al. 2012). Likewise, the word “nanomaterial” is called as a produced or natural material that gets unbound, agglomerated materials where external dimensions are between 1 and 100 nm ranges according to the EU Commission (Potocnik and Off 2011). The British Standards Institution has suggested the definitions (Table 1.1) for the nanomaterials:

The field of nanotechnology is growing rapidly, and tremendous amount of NMs have been produced over the past few years, and it caused many challenges in many countries’ as the laws for the NMs are not well established and accepted materials for the biological applications. This is the major hurdle for many nanomaterial producing companies which produce materials for biological and biomedical

**Table 1.1** Definitions as per the British Standards Institution

Nomenclature	Definition
Nanoscale	1–1000 nm range
Nanoscience	The study of matter that deals with size and structure-dependent properties
Nanotechnology	Modification of materials on the nanoscale dimension for the industrial and biomedical applications
Nanomaterials	Materials with internal or external structures on the nanoscale dimension
Nanoparticles	Materials with three external nanoscale dimensions
Nanofibers	Two similar exterior nanoscale dimensions and a third larger dimension are present in a nanomaterial, it is referred to as nanofiber
Nanocomposites	The combination of two or more nanomaterials is called as nanocomposites
Nanostructured materials	Materials containing internal or surface nanostructure

applications. It is a big challenge to nanomaterials producing companies to get easy approval for the biological applications.

## 1.2 Types and Classifications

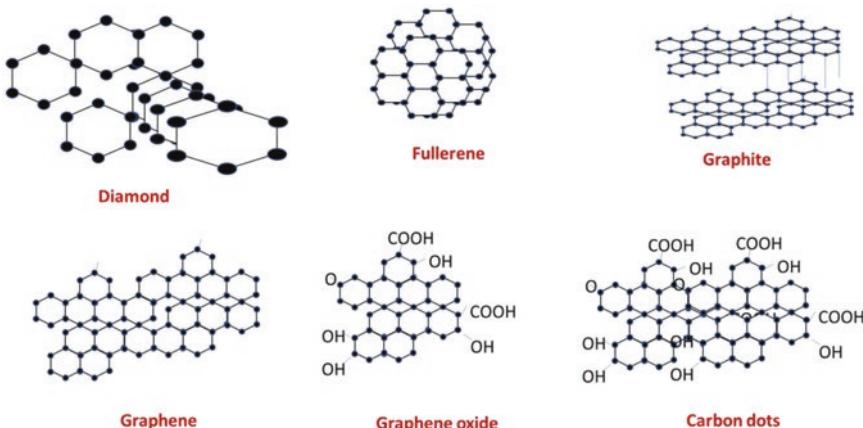
As the field of nanotechnology is growing rapidly, tremendous amount of NMs have been produced and all these NMs must be identified based on the structure, shapes, size, and chemical synthesis in order to differentiate from each other. Interestingly, NMs can be broadly classified into four categories which are described below.

### 1.2.1 Carbon Nanomaterials

The NMs which contain carbon are called carbon nanomaterials, and these carbon nanomaterials can be synthesized in different shapes such as (1) hollow tubes or (2) spheres (Kumar and Kumbhat 2016). In addition, carbon nanofibers, graphene, fullerenes, carbon black, carbon nanotubes, and carbon onions are also classified as carbon nanomaterials (Fig. 1.1).

### 1.2.2 Metal and Metal Oxide Nanomaterials

The metal and metal oxide can also be used to produce NMs which are called as metal and metal oxide nanomaterials or inorganic nanomaterials. Some of these NMs are gold (Au), silver (Ag) nanomaterials and metal oxides-based nanomaterials are titanium dioxide ( $TiO_2$ ) and zinc oxide ( $ZnO$ ) nanomaterials.



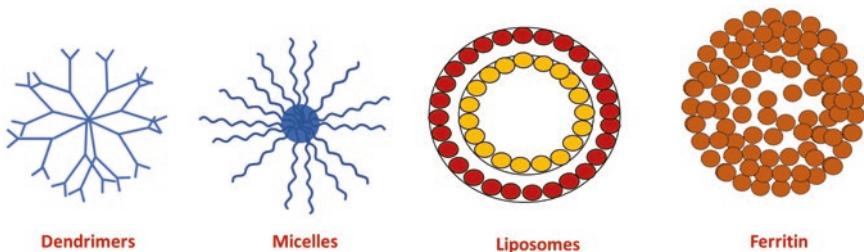
**Fig. 1.1** Different carbon-based nanomaterials

### 1.2.3 Organic Nanomaterials

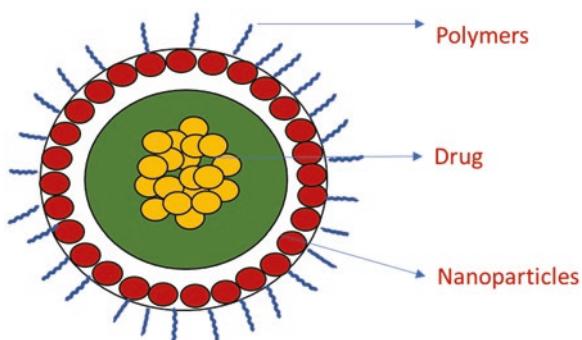
This type of NMs mostly contains organic matter, without carbon or inorganic based nanomaterials. One of the characteristics of these organic nanomaterials is that they possess noncovalent bonds (weak in nature, which can be easily broken). These organic materials can be easily modified to produce different shapes of nanomaterials like liposomes, dendrimers, micelles, and polymers (Fig. 1.2).

### 1.2.4 Nanocomposites

The combination of one type of nanomaterials with another type of nanomaterials is called as nanocomposites. The nanomaterials either combine with other types of nanowires, nanofibers, or can be combined with larger size materials. These nanocomposites may be any combinations of metal-based, carbon-based, or organic-based nanowires, nanofibers, with any form of ceramic, metal, polymer bulk materials (Fig. 1.3).



**Fig. 1.2** Different types of organic nanomaterials



**Fig. 1.3** Structure of nanocomposites

## 1.3 Classification of Nanomaterials

### 1.3.1 Dimensions and Sizes

As different types of nanomaterials are produced for a variety of applications, it becomes necessary to categorize these nanomaterials for proper applications. The nanomaterials are mostly solid particles, and their size and dimensions can be easily measured by using different methods. The idea for classification of nanomaterials was proposed by a scientist in the year 2000 (Gleiter 2000), he classified the nanomaterials based on their crystalline forms and chemical compositions. Still, this method of measuring was not fully complete as it did not measure dimensions of the nanomaterials (Tiwari et al. 2012). In another study, different groups of researchers have made a new classification which was primarily based on 0 Dimension, 1 Dimension, 2 Dimension, and 3 Dimension nanomaterial (Pokropivny and Skorokhod 2007).

The classification of nanomaterial is basically dependent on the movement of electrons in the nanomaterial. The presence of electrons is generally fixed in “0” dimension nanomaterials, whereas for “1” dimension nanomaterials, electrons can move freely along the x-axis, which is commonly less than 100 nm. Similarly, “2” dimension and “3” dimension nanomaterials have better electron movements along the x- to y-axis or x-, y-, z-axis, respectively. It has been found that the ability to predict the properties of nanomaterials determines the classification of the nanomaterials. Moreover, the characteristics of nanomaterials are basically dependent on the grain boundaries as per the Gleiter’s classification, whereas the classification by Pokropivny and Skorokhod suggested that the characteristics of nanomaterials are ascribed to the nanoparticle shapes and dimensionalities (Pokropivny and Skorokhod 2007).

### 1.3.2 Origin of Nanomaterials

The nanomaterials can be classified based on their source of origin, which means, the source materials to produce nanomaterials. They can be classified as naturally origin nanomaterials or synthetically produced nanomaterials.

#### 1.3.2.1 Natural Nanomaterials

Natural nanomaterials can be formed in biological species such as microbes, or plants and also through anthropogenic actions. The creation of natural nanomaterials is an accessible process as they are present in the hydrosphere, atmosphere, lithosphere, and biosphere. Interestingly, our planet is comprised of nanomaterials that are naturally formed and are present in the rivers, groundwater oceans, lakes, rocks, soils, magma, or lava as well as in the microbial organisms and also in humans (Hochella Jr et al. 2015; Sharma et al. 2015).

### 1.3.2.2 Synthetic Nanomaterials

The most widely used method to make nanomaterials is the synthetic method, which allows the production of nanomaterials by biological, physical, chemical, or hybrid methods. One of the advantages of the synthetically produced nanomaterials is that it is possible to produce large quantity of nanomaterials with different shapes and sizes. Another important aspect of the synthetic method is that different chemicals or reagents can be linked or conjugated with nanomaterials accurately and precisely. The major concern among synthetically designed nanomaterials is whether present knowledge is sufficient to envisage their performance. In addition, they display a different environment behavior, which is different from natural nanomaterials. Presently, diverse sources of nanomaterials are produced for various biological applications (Wagner et al. 2014).

### Byproduct Nanomaterials

The nanomaterials produced incidentally as a byproduct of industrial processes (motor vehicle engine and combustion processes) are called as subsidiary nanomaterials. These nanomaterials can also be produced by forest fires and trees burning. However, naturally produced nanomaterials can be found in the bodies of animals, insects, plants, and humans. Nonetheless there is a difference between naturally occurring or incidental and synthetically designed nanomaterials. In a few cases, incidental nanomaterials can be considered as a subcategory of natural nanomaterials.

The molecules are made up of atoms and these molecules and atoms are the basic components of all living and non-living organisms. The atoms and molecules are naturally manipulated several times to be able to make nanomaterials. The incidental and naturally occurring nanomaterials are constantly being made and dispersed in underground, water, and atmosphere. One of the main differences between incidental and synthetic nanomaterials is that the structure and morphology of synthetically produced nanomaterials can be better controlled as compared to incidental nanomaterials. Additionally, it is also possible to modify the shape and size of the synthetic nanomaterials which is however not possible in the case incidental nanomaterials. The metallic-based nanomaterials can also be produced from synthetic methods.

### Natural Nanomaterials

There are many ways to produce nanomaterials like eruption of volcanos, forest fires, and tree burning. In addition, some of the natural nanomaterials can be obtained from skin and hair shedding of plants and animals. In addition, volcanic eruptions, dust storms, and forest fires are the main sources from where high quantities of nanomaterials are produced that drastically affect the air quality of the planet. Likewise, industrial operations, and transportation are some of the human activities that also caused the production of nanomaterials. It has been reported that 10% of overall aerosols in the environment are produced by humans, while the remaining 90% of atmospheric aerosols were produced by natural method (Taylor 2002).

Dusts and cosmic dusts for (Eagle Nebula stars which are 6500 light years away from the earth) are also good sources of nanomaterials (Barnard and Guo 2012). The analysis by “astronomical infrared spectroscopy” has revealed that dust of clouds contained nitride, silicate, carbide, oxide, carbon, and organic-based nanomaterials (Barnard and Guo 2012). Interestingly, the diamond with nanometers size was discovered in the Murchison meteorite (Dai et al. 2002). Different types of nanomaterials are present throughout the world which are present in either mixed form or sorted forms. The impact of spectacular temperature variations, electromagnetic radiation, pressure gradients, physical collisions, and shock waves is generally responsible for formation of nanomaterials in the space (Barnard and Guo 2012). This precedes to the largest range of nanoscale materials with distinct isomerization and chemical spectrum (Hochella Jr et al. 2015). The satellite images showed that dust storms can move from one region to another region which can also be responsible for migration of nanominerals. It has been found that about more than fifty percentage of the atmospheric aerosol particles are in the size between 100 and 200 nm (d’Almeida and Schütz 1983; Shi et al. 2005).

### **1.3.2.3 Engineered Nanomaterials**

The engineered nanoparticles are those which are being produced due to combustion from cooking, petrol, and diesel transportation and coal for power generation (Linak et al. 2000), chemical and industrial manufacturing, oil or re-refining (Rogers et al. 2005). These nanomaterials mostly contain carbon (De Volder et al. 2013), TiO<sub>2</sub> (Weir et al. 2012), and hydroxyapatites (Sadat-Shojaei et al. 2010) which are used in the sports, cosmetics, and toothpaste production. Consequently, these synthetic nanomaterials are a new category of nanomaterials that may cause adverse environmental and human health effects (Kagawa 2002). The automobile transportation, diesel engines release 20–130 nm sized nanoparticles, whereas gasoline engines release 20–60 nm sized nanoparticles (Westerdahl et al. 2005; Sioutas et al. 2005). More than 90% of carbon nanomaterials present in the atmosphere are diesel-based nanoparticles (Kittelson 2001).

There are many applications of nanomaterials in the healthcare sectors, and they are being used in the production of cosmetics, personal, and healthcare applications. Generally, nanomaterials used in commercial applications are basically engineered nanomaterials and they are produced by using chemical, biological, and physical methods. Besides cosmetics, nanomaterials have been widely used in commercial product development ranging from personal care products to industrial products. For example, titanium oxide nanomaterials with 100 nm and above sizes are generally used in cosmetics and sunscreen production. Likewise, silver nanomaterials are used in different applications including food storage containers, shampoos, air sanitizer sprays, wet wipes, and toothpastes.

### **1.3.2.4 Naturally Produced Nanomaterials**

There are different types of nanomaterials which are present in living creatures such as plants, insects, birds, bacteria, algae, viruses, animals, and humans. Latest advancement in the field of anatomical and analytical techniques helps the

researchers to visualize nanomaterial's morphology with minute details, which in fact will eventually lead to a better understanding of these creatures. The knowledge about the presence of nanomaterials in these creatures helps the researchers to use them for the derivation and isolation of nanomaterials for various biomedical applications. For example, insects have nanomaterials which help them to stay alive in severe living or environmental conditions. In addition, plants use the nutrients available in the soil and water for their growth, which leads to the accumulation of these minerals in nanoform in their bodies. Moreover, animals and small insects utilize nanomaterials for their protection from predatory animals. Likewise, humans also have body organs that are primarily made up of nanomaterials such as bones, antibodies, and enzymes. The nanomaterials help the body to perform normal physiological functions. Moreover, DNA or RNA which are genetic materials in the animal/human cell are also made up of nanosize materials. This obviously shows that nanomaterials are the basis for life forms on earth. These nanoscale organisms are found in nanobacteria, viruses, fungi, algae, and yeast.

Among different species, viruses are the largest communities which can be a non-living catalyst and can also be a living organism inside host cells. Generally, the viruses cause diseases in plants, animals, and humans (Duckworth and Gulig 2002; Picó et al. 1996; Berg 2000; Uchiyama 1997). With the help of molecular biology tools and techniques it is now possible to genetically alter or tailor viruses for various applications. These viruses can selectively enter into smaller molecules and cause diseases and other problems (Flenniken et al. 2003; Flenniken et al. 2006). These viruses can be used in the synthesis of specialized nanomaterials (Strable and Finn 2009). There are few studies which showed that viruses can be used to produce novel nanomaterials (Langeveld et al. 2001; Wang et al. 2002).

Bacteria can bind to toxic surface of the heavy metals which can be precipitated to produce metal nanomaterials. They are called as nanobacteria. These nanobacteria are useful in the biosynthesis of low toxicity nanomaterials (Southam and Donald 1999). One of such nanobacteria *Pseudomonas stutzeri* A259 was first used bacteria in the production of silver nanomaterials (Haefeli et al. 1984). There are many metallic nanomaterials like gold nanomaterials, alloy nanomaterials, nonmagnetic nanomaterials, and metal sulfide quantum dots and ZnS (Slocik et al. 2004; Nair and Pradeep 2002; Senapati et al. 2005; Zheng et al. 2010; Jha et al. 2009a, b; Bansal et al. 2006; Narayanan and Sakthivel 2010; Sweeney et al. 2004; Mandal et al. 2006) can be synthesized by using different strains of bacteria. Moreover actinomycetes were also used to produce nanomaterials. The bacteria-based nanomaterial formation method is highly useful in a nanomedicine as they cause less cellular toxicity (Li et al. 2011). Nevertheless, the major disadvantages of these bacterial-based nanomaterials are that process is time-consuming and they are difficult to filter and produce a low yield as compared to chemically synthesized method (Jeevanandam et al. 2016).

In addition, nano-organisms are also called as nanobes and they are gaining interest as they were discovered during off-shore petroleum exploration in Australia (Uwins et al. 1998). These nanobes encompasses carbon, nitrogen, oxygen,

membrane-bound structure with dense cytoplasm. The uniqueness of these nanobes is their size and that they were found in Martian meteorite ALH84001 (Urwins 2000).

The nanomaterials can also be created by magnetotactic bacteria and they are highly effective to produce magnetic oxide nanomaterials that possess unique properties such as super-paramagnetism, high coercive force, and micro-configuration. These magnetic oxide nanomaterials can be used in various biological and biomedical applications (Li et al. 2012). Normally, biocompatible magnetite iron oxide, iron sulfides, and maghemite can be synthesized by using magnetotactic bacteria (Bazylinski et al. 1994; Bazylinski et al. 1995). They can be used in the cancer treatment through magnetic hyperthermia and magnetic resonance imaging (MRI) (Fan et al. 2009). Additionally, surface distributed magnetic iron-sulfide particles, modified iron nanomaterials, magnetic octahedral nanomaterials, and superparamagnetic nanomaterials can be produced by using magnetotactic bacteria (Watson et al. 1999; Zhang et al. 1998; Roh et al. 2001; Philipse and Maas 2002; Lee et al. 2004; Arakaki et al. 2008).

The shapes of different bacteria also play a critical role in the nanomaterial's formation, for example, ovoid, vibrio, cocci, spirilla, rod-shape, and multicellular bacteria possess unique characteristics in producing nanomaterials (Arakaki et al. 2008; Blakemore 1982; Thornhill et al. 1995; Spring and Schleifer 1995).

Nanomaterials can also be produced from algae, fungi, yeast, and bacterial spores. It has been reported that algae like Chlorella vulgaris supports the formation of silver nanomaterials (Hosea et al. 1986). Similarly, phytochelatin-coated CdS and nanocomposite and nanoporous structures can be produced by *Phaeodactylum tricornutum* and by coccoliths and diatoms (Scarano and Morelli 2003). It has been reported that fungi are excellent candidates for the synthesis of metal and metal sulfide nanoparticle. The molecular mechanism through which they help to produce nanomaterials is not well understood (Krumov et al. 2009).

It has been found that fungi which comprise a range of enzymes are responsible for providing the shapes of the nanomaterials. Furthermore, another fungus, *Fusarium oxysporum* and *Verticillium* species were used for the synthesis of gold, silver, and gold–silver alloy nanomaterials (Mukherjee et al. 2002; Bansal et al. 2004). Furthermore, enzymes present in the *Fusarium oxysporum* also helped the synthesis of Cadmium quantum dots (Ahmad et al. 2002; Dameron et al. 1989). Additionally, *Candida glabrata*, *Torulopsis* species, and *Schizosaccharomyces pombe* were used in the synthesis of Cadmium sulfur quantum dots (Reese and Winge 1988), lead sulfur nanocrystals, and silver nanomaterials, respectively.

### 1.3.2.5 Plant Based Nanomaterials

Nanomaterials can be derived from the plants as they are considered as excellent source of cellular bio-composites. It has been found that natural fibers are composites of cellulosic fibrils with 100–1000 nm long containing both crystalline and amorphous segments. The excellent strength and performance properties of natural fibers are due to their elementary structure with nano-fibrillar components (Lucia and Rojas 2009). The isolation of nanocellulose can be done by mechanical,

chemical methods (Mohammadinejad et al. 2016). The plant has many structural features such as plant leaves contain nanostructures that are used for several purposes such as insects sliding, mechanical stability, increased visible light, and harmful UV reflection and radiation absorption (Gorb et al. 2005; Bargel et al. 2006; Barnes and Cardoso Velhena 1996; Pfündel et al. 2008). The most famous nanomaterial property in plants is the super-hydrophobicity in lotus leaves that helps in self-cleaning and super-wettability of the leaves (Barthlott and Neinhuis 1997). Several studies have suggested that piles of nanomaterials are responsible for the circular layer in plants and insects which allows them to float on water without sinking (Nguyen et al. 2014; Xu et al. 2014). Taking this information, many artificial super-hydrophobic materials with self-cleaning ability have been produced [196] using electrodeposition, photolithography, and colloidal methods (Madou 2002; Ming et al. 2005; Chow 2007).

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# Synthesis of Nanomaterials: Methods & Technology

2

Firdos Alam Khan

## Abstract

Nanomaterials are synthetized by different methods based on the types and nature of the nanomaterials. In a broad sense “top-down” and “bottom-up” are the two foremost methods to synthesize nanomaterials. In top-down method bulk materials have been reduced to nanomaterials, and in case of bottom-up method, the nanomaterials are synthesized from elementary level. The different methods which are being used to synthesize nanomaterials are chemical vapor deposition method, thermal decomposition, hydrothermal synthesis, solvothermal method, pulsed laser ablation, templating method, combustion method, microwave synthesis, gas phase method, and conventional Sol-Gel method.

## Keywords

Nanomaterial synthesis · Methods · Techniques

## 2.1 Introduction

Over the past couple of decades, various methods of preparation and synthesis of nanomaterials have been developed. The main objectives of the synthesis of nanomaterials is to ensure that for what purpose these nanomaterials are being synthesized. The researchers should know the applications of the nanomaterials so that they can synthesize them accordingly. The method of production of nanomaterials to be used in the industrial application for the development of various products will

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F. A. Khan (✉)

Department of Stem Cell Biology, Institute for Research & Medical Consultations, Imam Abdulrahman Bin Faisal University, Dammam, Saudi Arabia  
e-mail: [fakhan@iau.edu.sa](mailto:fakhan@iau.edu.sa)

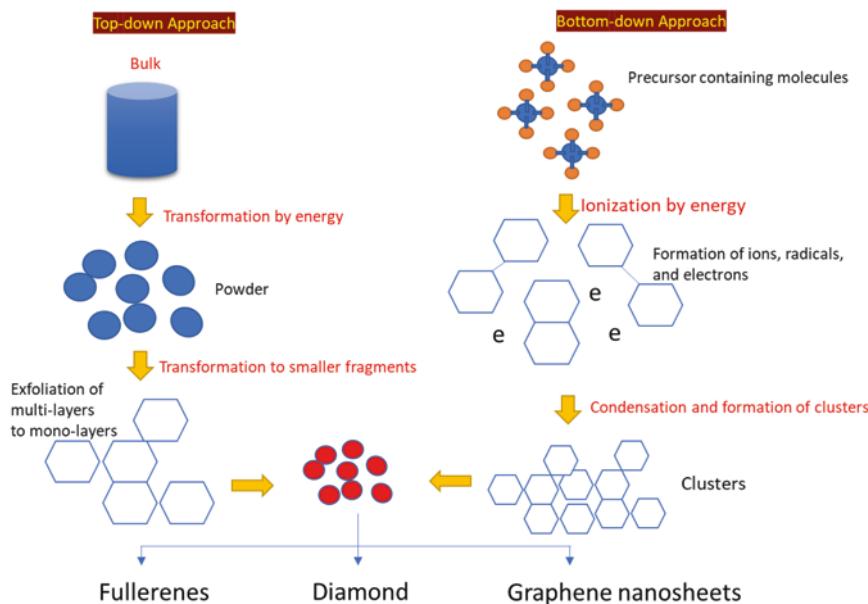
be different than the method of production to be used in biological or medical applications. Other objectives of the researchers to synthesize nanomaterials are better functionality and lower cost. Over the past few years, several physical and chemical methods have been used to improve the performance of nanomaterials demonstrating enhanced properties (Shibata et al. 1998).

## 2.2 Methods to Synthesize Nanomaterials

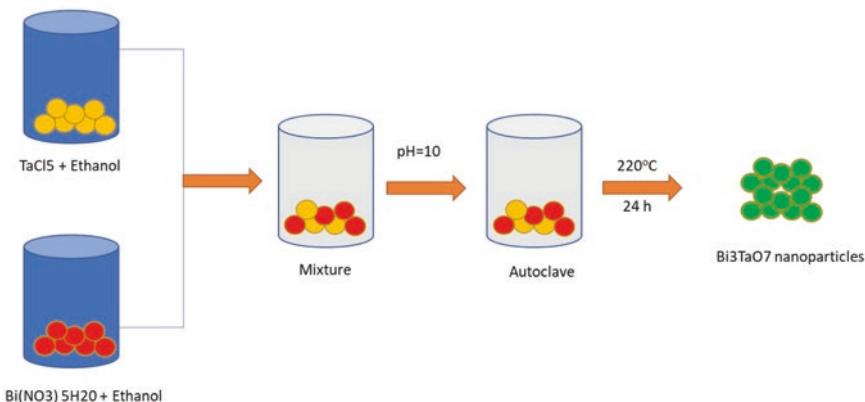
The “top-down” and “bottom-up” are the two major methods which have been used to successfully synthesize nanomaterials. We have described these methods in further detail (Fig. 2.1).

### 2.2.1 Production of Nanomaterials by Top-Down Method

In this method, solid state processing of the materials are mostly used and this method involves breaking of the bulk material into smaller particles using physical processes such as crushing, milling, and grinding methods. Generally, this method is not appropriate for formulating evenly shaped nanomaterials, and it is very difficult to get very small size nanoparticles even with high energy usages. The major difficulty of this method is the shortage of the surface structure as it has significant



**Fig. 2.1** Diagrammatic representation of top-down approach and bottom-down approach of making of nanomaterials



**Fig. 2.2** Diagrammatic representation of hydrothermal process of nanoparticle production

impact on physical properties and surface chemistry of nanomaterials. In addition, this method also causes substantial crystallographic loss to the processed shapes.

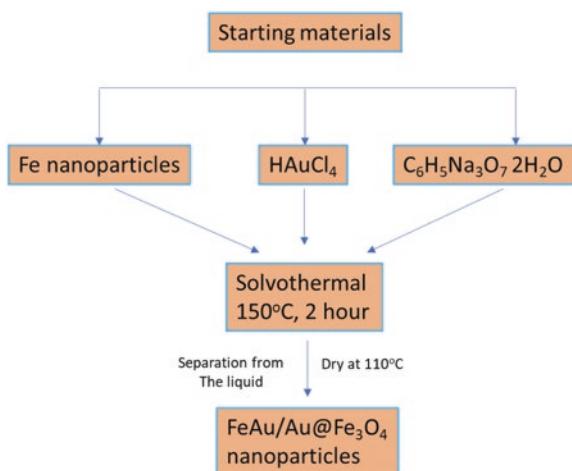
### 2.2.2 Production of Nanomaterials by Bottom-Up Method

In this method, materials are prepared by atom-by-atom or molecule-by-molecule to make large amount of materials. This method is more frequently used for producing most of the nanomaterials. This method has an ability to produce a uniform size, shape, and well-distributed nanomaterials. It basically control the chemical synthesis process in a precisely manner to prevent undesirable particle growth. This method plays an important role in the production and processing of nanomaterials with better particle size distribution and better morphology. Another important feature is that its an environment friendly and economical processes for the nanoparticle production (Hahn 1997). There are many approaches for synthesizing nanomaterials like hydrothermal (Cheng et al. 1995; Yin et al. 2001), combustion synthesis (Nagaveni et al. 2004a), gas-phase methods (Jones et al. 2003; Wang et al. 2005), microwave synthesis, and sol-gel processing (Watson et al. 2004), which we have described below.

#### 2.2.2.1 Hydrothermal Method

The hydrothermal method is normally done in a pressurized container which is called as an “Autoclave” where temperature and pressure can be controlled and regulated. During nanomaterial synthesis, the temperature can be increased at the boiling point of water, which allows the vapor to get saturated. This method (Fig. 2.2) has been extensively used in the production of different nanoparticles (Yang et al. 2001). The advantage of this method is that this can be useful to control material size, particle morphology, crystalline phase, and surface chemistry through regulation of the reaction temperature, pressure, solvent properties, solution composition, and additives (Carp et al. 2004).

**Fig. 2.3** Diagrammatic representation of solvothermal process of nanoparticle production



### 2.2.2.2 Solvothermal Method

The solvothermal method (Fig. 2.3) is like hydrothermal method, the only difference is that it uses different solvents other than water. Interestingly, this method is more effective in synthesis of nanomaterials with good distribution, especially when organic solvents or chemicals with high boiling points are selected. Moreover, this method provides better controlling method to produce better size and shapes of the materials than the hydrothermal method. This method synthesizes nanomaterials or nanorods with or without the addition of surfactants.

### 2.2.2.3 Chemical Vapor Deposition Method

The chemical vapor deposition (CVD) method is used to manufacture high performance thin nano-films. In this method, substrate is basically treated with volatile precursors which act on the substrate surface to produce the desirable films. Usually, volatile by-products are eliminated by gas flow through the reaction chamber. The quality of the deposited materials on the surface is greatly dependent on several factors like temperature, rate of reaction, and the amount of the precursors (Kim et al. 2004). It has been reported that Sn<sup>4+</sup>-doped TiO<sub>2</sub> nanoparticle films were produced by CVD method (Cao et al. 2004). Another doped TiO<sub>2</sub> nanoparticle was synthesized by CVD method where TiO<sub>2</sub> is crystallized into the rutile structures depending on the type and number of cations present in the chemical reactions (Gracia et al. 2004). The advantage of this method is getting consistent glaze of the nano film, but this method has many limitations including higher temperatures required for chemical reactions, and secondly it is difficult to scale up (Sudarshan 2003).

### 2.2.2.4 Method of Thermal Decomposition and Pulsed Laser Ablation

The doped metals can be produced by using decomposing metal alkoxides, salts, heat or electricity. Moreover, the properties of nanomaterials strongly depend on the

flow rate of the precursor's concentrations in the reactions and its environment. It has been reported that  $\text{TiO}_2$  nanoparticles with a diameter  $< 30 \text{ nm}$  can be synthesized by using the thermal decomposition of titanium alkoxide at  $1200^\circ\text{Celsius}$  temperature (Kim et al. 2005). In another study,  $\text{TiO}_2$  nanoparticles with a diameter (3–8 nm) were produced by pulsed laser ablation technique (Liang et al. 2004). In addition, doped anatase  $\text{TiO}_2$  nanoparticles were produced by the solution combustion method (Nagaveni et al. 2004b). Nevertheless, the disadvantages of this method are the high cost, low yield, and difficulty in managing the structure and morphology of the nanomaterials.

### 2.2.2.5 Templating Method

The process to construct materials with a similar morphology is known as templating method. The production of nanomaterials which uses the templating method has become exceptionally popular recently. This method uses the morphological characteristics with reactive deposition, so it is possible to prepare numerous new materials with a regular and controlled morphology by simply changing the morphology of the template materials. Over the past few years, a variety of templates have been developed to synthesize different nanomaterials (Jinsoo et al. 2005; Iwasaki et al. 2004). This method has some shortcomings, like complicated synthetic procedures where templates must be removed, normally by calcination technique, which causes an increase in the manufacturing costs and also chances of contamination (Bavykin et al. 2006).

### 2.2.2.6 Combustion Method

The combustion method includes a quick heating of a solution comprising redox groups. This method leads to production of highly crystalline nanoparticles with large surface areas (Nagaveni et al. 2004a, c). During production, the temperature reaches to roughly  $650^\circ\text{Celsius}$  for 1–2 min to make the crystalline materials.

### 2.2.2.7 Gas Phase Method

This method is good to produce thin film because it can be performed chemically or physically. Nanomaterials are formed because of chemical reaction or decomposition of a precursor in the gas phase (Jones et al. 2003; Lee et al. 2017). Moreover, physical vapor deposition (PVD) is another technique which can be used to produce thin film deposition. Interestingly, films are formed from the gas phase method without using chemical transition. To produce  $\text{TiO}_2$  thin films, a beam of electrons heats the  $\text{TiO}_2$  material and the electrons are produced and this process is recognized as Electron beam (E-beam) evaporation. There are many benefits of making of  $\text{TiO}_2$  deposited with E-beam evaporation than CVD method such as smoothness and better conductivity (Van de Krol et al. 1997).

### 2.2.2.8 Microwave Radiation Method

Nanomaterials can also be produced by using microwave radiation and there are several benefits of this method, like this method does not use high temperature

calcination for extended period of time and also is a quick method of making crystalline nanomaterials (Corradi et al. 2005). Moreover, high quality of rutile rods can be created by joining hydrothermal and microwave methods, while  $\text{TiO}_2$  hollow open-ended nanotubes can be manufactured through reacting anatase and rutile crystals in the NaOH solution (Wu et al. 2005).

### 2.2.2.9 Conventional Sol-Gel Method

This method has numerous advantages, for example, this method allows impregnation or co-precipitation of nanomaterials, which can be used to introduce dopants. To synthesize various oxide materials, sol-gel method has been used (Fernandez-Garcia et al. 2004), and this method allows better control for the texture formation, the chemical reaction, and the morphological properties of the solid materials. The major benefit of the Sol-Gel technique is the ability to scale up with a high purity of nanomaterials (Kolen'ko et al. 2005). In Sol-Gel technique process, a colloidal suspension is formed from the hydrolysis and polymerization reactions of the precursors. These precursors are usually inorganic metal salts or metal organic compounds (Pierre 1998). In addition, any factor that effects either or both reactions are likely to impact the properties of the gel formation and these factors are generally described as Sol-Gel technique factors. These factors include type of solvent, water content, acid or base content, and different type of precursor, precursor concentration, and temperature. These factors affect the structure of the initial gel formation. After this step, the wet gel can be mature in another solvent. The time between the formation of a gel and its drying is known as aging (Chen and Mao 2007).

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# Characterization of Nanomaterials: Techniques and Tools

3

Sultan Akhtar and Sadaqat Ali

## Abstract

Nanomaterials have shown excellent physical, electrical, and chemical properties compared to when they are in the bulk phase. Nanotechnology/biotechnology is dealing with synthesis, characterization, and applications of nanomaterials. The nanoscale materials contained tiny particles, often known as nanoparticles and they require special instrumentations and tools for their successful characterization and analysis. In this chapter, we will describe briefly the tools and techniques which are widely used for the characterization of nanomaterials. These techniques include but not limited to scanning electron microscopy (SEM), transmission electron microscopy (TEM), Fourier transform infrared spectroscopy (FTIR), X-ray diffraction (XRD), energy-dispersive X-ray (EDX) spectroscopy, thermal gravimetric analysis (TGA), dynamic light scattering (DLS) analysis, density functional theory (DFT), zeta sizer, etc. The illustration of each technique and some cases with graphics is provided in a separate section.

## Keywords

Techniques · Tools · Scanning electron microscopy · Transmission electron microscopy · Fourier transform infrared spectroscopy · X-ray diffraction · Energy-dispersive X-ray spectroscopy · Thermal gravimetric analysis · Dynamic light scattering analysis · Density functional theory · Zeta sizer

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S. Akhtar (✉)

Department of Biophysics, Institute for Research and Medical Consultations (IRMC), Imam Abdulrahman Bin Faisal University (IAU), Dammam, Saudi Arabia

e-mail: [suakhtar@iau.edu.sa](mailto:suakhtar@iau.edu.sa)

S. Ali

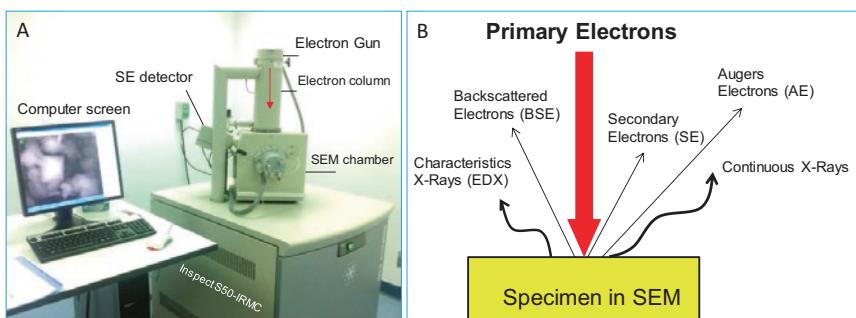
Mechanical and Energy Engineering Department, College of Engineering, Imam Abdulrahman Bin Faisal University, Dammam, Saudi Arabia

### 3.1 Introduction

A physical substance that occupies weight, space, and has volume is known as matter or material. The materials that have dimensions comparable to 100 nm or less are referred to as nanomaterials. One nanometer is equal to  $10^{-9}$  m or 0.000 000 001 m (millionth part of millimeter) which is about 100,000 times smaller than the thickness of a human hair (human hair diameter  $\approx$ 100  $\mu\text{m}$ ). Nanomaterials could be in any shape, e.g. particles, rods, tubes, fibers, or molecules, and exhibit enhanced physical and chemical properties compared to their bulk counterpart. Nano- and biotechnologies are both dealt with synthesis, characterization, and applications of nanomaterials. Most nanoscale materials are too small to be seen with the naked eye and even with conventional optical microscopes. Thus, they require special instrumentations and tools for their characterization and analysis. In this chapter, we will describe briefly the tools and techniques that are used widely for the characterization of nanomaterials. These techniques include scanning electron microscopy (SEM), transmission electron microscopy (TEM), Fourier transform infrared spectroscopy (FTIR), nuclear magnetic resonance (NMR) spectroscopy, X-ray diffraction (XRD), energy-dispersive X-ray (EDX) spectroscopy, thermal gravimetric analysis (TGA), dynamic light scattering (DLS) analysis, density functional theory (DFT), zeta sizer, etc.

### 3.2 Scanning Electron Microscopy (SEM)

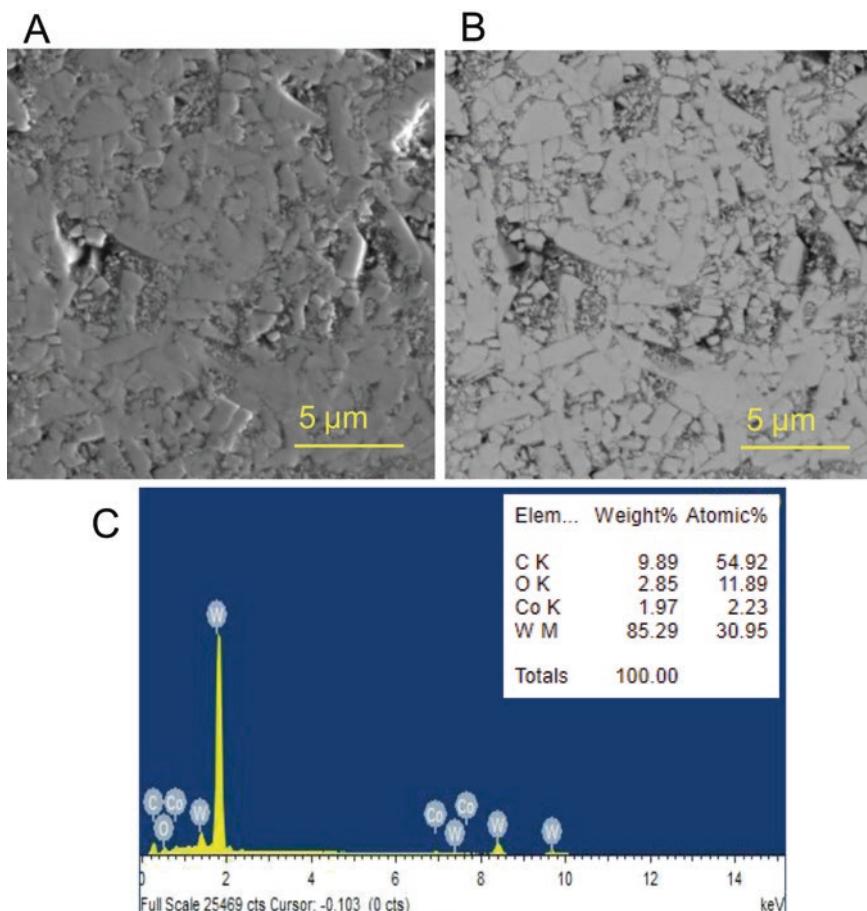
From the name, it is clear that the electron microscopes use electrons for the formation of images instead of light as the case for optical microscopes. The electronic image has often better image quality in terms of resolution than optical microscopy, thanks to their small wavelengths. In general, electron microscopes are of two types, scanning electron microscope (SEM) and transmission electron microscope (TEM). We will discuss SEM in this section and TEM in the next section. SEM is a very useful technique to obtain the surface topography and chemical composition of the specimens with a wide view (Akhtar et al. 2018, 2019; Carr and Toner 1981). SEM instrument along with a schematic of basic signals produced in SEM are shown in Fig. 3.1. SEM



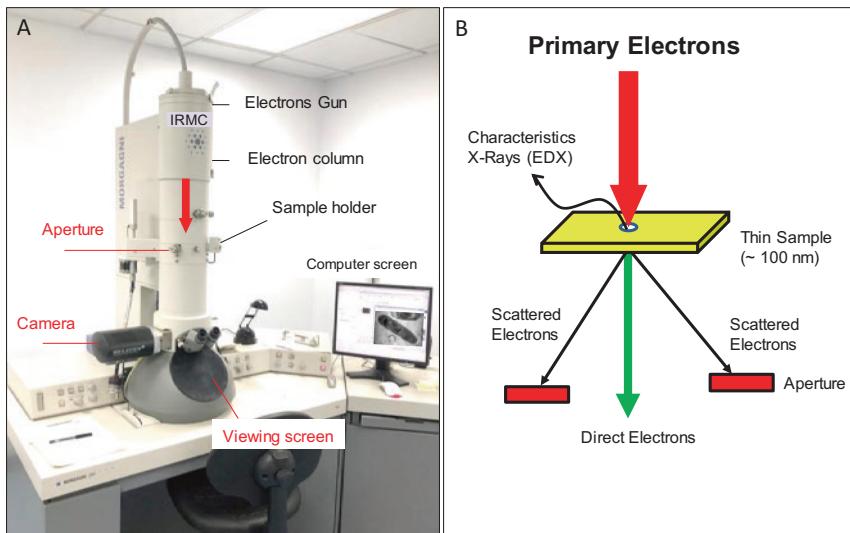
**Fig. 3.1** Photograph of SEM instrument, showing its different parts (a) and basic signals produced in the SEM after interaction of primary electrons with the specimen (b)

instrument has the following basic parts: electron gun (top), electron column (where electromagnetic lenses and coils are attached), chamber (for specimen), detectors, and vacuum pumps (not shown in this figure).

In SEM, electrons are generated by an electron gun situated at the top of the column and accelerated towards the specimen by applying a high voltage (typically 20–30 kV). The gun electrons (called primary electrons) are then focused by electromagnetic lenses while traveling through the column and scan the specimen using electrical coils. Different kinds of signals such as secondary electrons (SE), back-scattered electrons (BSE), Auger electrons (AE), X-rays, etc. are generated as energetic beam of electrons is subjected to the specimen. These signals are then detected by the dedicated detectors and produce electronic images or spectra as shown on the computer screen. The SE signals are utilized to generate surface morphological images while BSE and EDX signals are used to obtain chemical and structural information of the specimen (see Fig. 3.2). The features with lower height are



**Fig. 3.2** (a) SE image, (b) BSE image, and (c) EDX spectra of tungsten carbide doped cobalt specimen (SEM working voltage: 15 kV). EDX spectrum shows the contents of the specimen, e.g. C, O, Co, and W. W is found to be a major constituent (~85 wt.%) of this compound. The scale bars are 5 μm



**Fig. 3.3** Photograph of main TEM instrument, showing its different parts (a) and basic signals produced by primary electrons after interaction with specimen (b). Direct or scattered electrons are utilized, respectively, to perform BF or DF imaging

appeared darker in SE image compared to smooth areas while bright contrast of certain areas in BSE image means the presence of element with higher atomic number compared to other elements of the specimen and vice versa. In this way, both types of images give us different kind of information, e.g. first about the surface morphology and second type of the about the chemical nature (atomic number) of the elements present in the specimen. In the EDX spectrum, each element appeared as a peak in the spectrum with specific energy (x-axis) and intensity (y-axis). The nature of the element is identified by the analysis of the spectrum. Higher the intensity of the EDX peak means higher the content of this particular element in the compound, for instance, W has a higher content (~85 wt.%) in the current example (see table in Fig. 3.3c).

SEM samples can be prepared by mounting a small amount of powder onto the metallic stub using double-sided carbon glue and transferred into the SEM chamber for examination. In case of nanoparticles (or any biological entity, e.g. bacteria or spores) in liquid media, a droplet of the powder/biological dispersion is deposited directly onto the pre-cleaned SEM stub (no need of gluing tape) and let it dry under a clean environment prior to use it for SEM. If the samples are non-conductive such as polymers, plastics, ceramics, etc. and/or biological specimens, a thin layer of gold is sputtered onto the sample before their examination to make them appropriate (conducting) for imaging (Gad et al. 2019; Baig et al. 2019; AbuMousa et al. 2018).

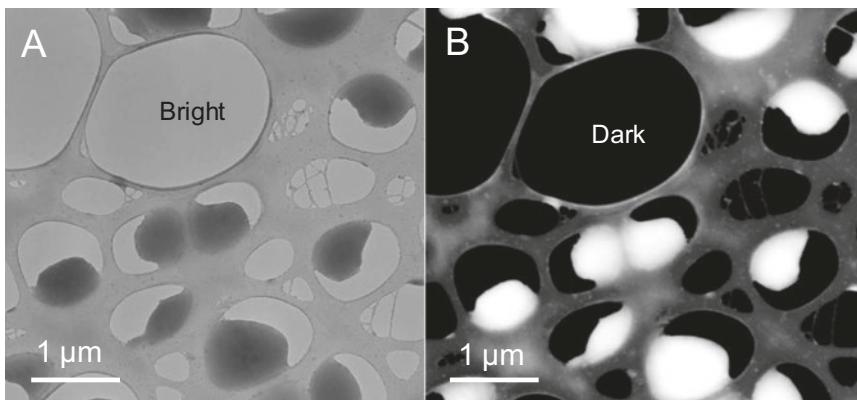
### 3.3 Transmission Electron Microscopy (TEM)

Transmission electron microscopy (TEM) is a powerful tool to attain a high-quality data of the nanomaterials. The microscope that performs this job is known as TEM instrument. In general, TEM is performed to obtain a detailed morphology and structure of the specimen which is beyond the limit of SEM. The main difference in SEM and TEM is that in TEM, the electron beam is transmitted through a thin specimen, while in SEM, the beam of electrons scans the surface of the sample instead of passing through. Another important difference is the power of the microscope (accelerating voltage; V). Typically, TEM is operated at 80–300 kV, this power is much higher than SEM (maximum: 30 kV). Thus, TEM can produce better images than SEM in terms of resolution. It is a worthy to note that the wavelength ( $\lambda$ ) of the electrons is related to V that can be varied according to the following relation ( $\lambda = h / ((2m_0eV)^{1/2})$ ); h is a Planck's constant,  $m_0$  is the rest mass, and eV is the energy of the electrons provided by V. The reciprocal relationship between  $\lambda$  and V introduces a very important concept: by increasing V, we can shorten the  $\lambda$  of the electrons and hence improve the resolution. The  $\lambda$  of electrons in TEM is comparable or smaller ( $\lambda = 0.00197$  nm at 300 kV) than the size of the atom (~ 0.1 nm) that helps TEM to reveal the finest details of the internal structure of the specimen as small as individual atoms and molecules (Akhtar 2012; Williams and Crater 2009).

The main unit of TEM along with the basic interaction of electrons and specimen is shown in Fig. 3.3. TEM has the following basic parts: electron gun (at the top), electron column, electromagnetic lenses, apertures, sample holder, fluorescence screen (camera), vacuum pumps, and high-tension tank (not shown in this figure). TEM has several modes to collect the data but important imaging modes are bright-field (BF), dark-field (DF), and high-resolution (HR) imaging, known as BF-TEM, DF-TEM, and HR-TEM, respectively (Akhtar 2012). Direct electrons are used to form the BF imaging after blocking the scattered electrons by objective aperture and vice versa for DF-TEM. HR-TEM is performed using the phase of the electrons and acquired a detailed structure of the specimens down to the atomic level.

The working principle of TEM is based on the interaction between incident electrons and specimen. In TEM, high energy electrons are accelerated towards the specimen by applying a high voltage (80–300 kV). These electrons are focused by a system of condenser lenses. A fine focused beam of electrons is then transmitted through the thin sample. Typically, the suitable thickness of the specimen is about 100 nm for material science samples and 200 nm for biological specimens. The transmitted electrons are generally of two types: direct electrons (do not change their direction) and scattered electrons that changed their trajectory after passing through the sample. Either type of electrons can be utilized to make the image. If the image is made by direct electrons, then it is known as BF image (see Fig. 3.4). On the other hand, if the scattered electrons are allowed to pass through the objective aperture for the purpose of the image and blocked the direct electrons, then it is called DF image (Akhtar 2012).

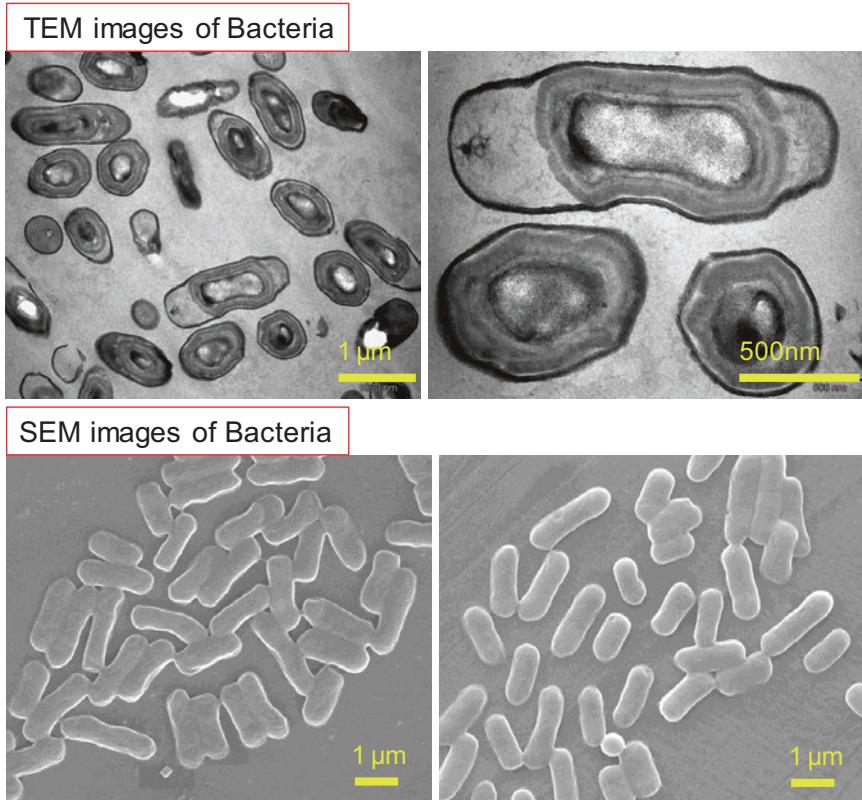
The important difference between the two images is the contrast that is opposite; for example, the bright features of BF image (such as holes of carbon support film)



**Fig. 3.4** Two imaging modes of TEM: (a) BF and (b) DF imaging. The carbon hole that has bright contrast in BF turned to dark (inverse) in DF (working voltage: 200 kV). The scale bars are 1  $\mu\text{m}$

will appear dark in DF image and vice versa. TEM image (either made by direct or scattered electrons) can be viewed on the fluorescence screen or recorded by a computer using a digital camera. Notably, images formed by TEM have proven better resolution than SEM due to the basic principle of TEM (transmission of electrons) and because of the high energy of electrons. To highlight this difference, one example is taken where bio-objects (bacteria) are examined by both TEM and SEM (see Fig. 3.5). Furthermore, EDX can also be performed in TEM like SEM for chemical analysis (see Fig. 3.2). In addition, electron diffraction can be carried out in TEM to obtain the crystalline information of the specimen and validate the X-ray diffraction (XRD) data.

Sample preparation is a very important part of TEM characterization. All kinds of samples required some preparation before introducing them into the TEM. Samples with a certain dimension and thickness can only be transferred into the TEM. In general, TEM samples can be prepared in three different ways. (1) Powder samples: powder of nanoparticles (nanomaterials) is dispersed into the ethanol/water upon applying sonication for 5–10 minutes. The sample dispersion is then deposited onto the carbon-coated TEM grids and dried prior to introduce into the TEM. (2) Bulk specimens: the preparation of bulk samples is a tedious way of preparation. Bulk specimens are prepared either by following the traditional way of preparation or prepared with the aid of a focused ion beam (FIB), mounted in the SEM. In FIB/SEM procedure, the top surface of the sample is first protected by a metallic layer (platinum layer) and then extracted a rectangular portion called lamella ( $25 \times 5 \times 0.1 \mu\text{m}^3$ ) from the bulk (Rubino et al. 2012). The prepared lamella is then finally transferred into the TEM for examination. Moreover, FIB/SEM procedure can also be applied for biological specimens with some modifications. The biological specimens required rapid freezing in liquid nitrogen before transfer into the cryo-FIB/SEM, where cryo-FIB/SEM means FIB/SEM operation under liquid nitrogen (Hsieh et al. 2006; Marko et al. 2006, 2007). (3) Biological specimens: the biological specimens can also be prepared as an alternative method using the

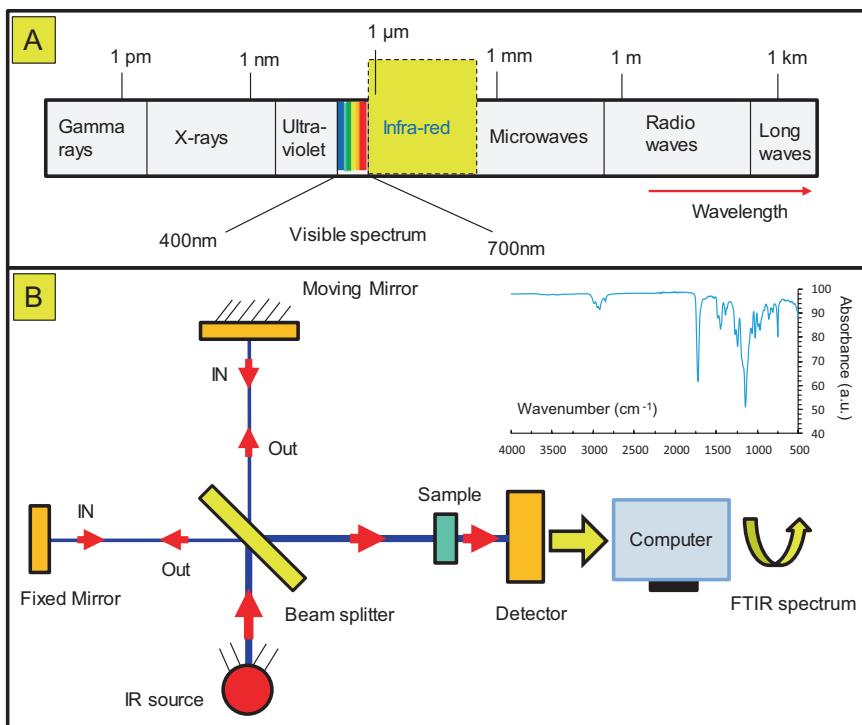


**Fig. 3.5** TEM images of the ultramicrotomy prepared bacteria specimen (method-3) (top row), and the same bacteria are imaged by SEM at two magnifications for comparison (bottom row). Herein, TEM provides the cross-sectional view and SEM surface morphology (working voltage: 15 kV (SEM) and 80 kV (TEM))

traditional way of preparation, i.e. with the use of chemicals and ultramicrotome (Fig. 3.5 (top row)). The main steps of this procedure are as follows: chemical fixation, embedding, trimming, ultra-thin sectioning, transferring the sections to TEM grids and staining with contrast enhancing agents (Akhtar et al. 2019; Fera et al. 2020; Alomari et al. 2019; Jermy et al. 2019).

### 3.4 Fourier Transform Infrared (FTIR) Spectroscopy

Fourier transform infrared (FTIR) is an infrared spectroscopy technique to study the molecular bonding of the organic/inorganic materials. IR is a region of electromagnetic radiations between the red edge of the visible spectrum at a wavelength of 700 nm to 1 mm, i.e. IR waves are longer than visible light while shorter than radio waves (see Fig. 3.6a). During FTIR, a specimen is exposed to IR radiations in order to create a spectrum of the specimen (Winter et al. 1999). A portion of the radiations



**Fig. 3.6** (a) Electromagnetic radiation spectrum where wavelengths range for IR region is from about 700 nm to 1 mm. (b) Schematic of working of FTIR instrument along with FTIR spectrum

is transmitted, while the rest of the radiations are absorbed by the specimen depending on the nature of bonding in the material. A molecular fingerprint of the specimen is created as a result of transmission and absorption at a molecular level. A wide range of information, such as identifying the material properties, quality of the specimen, and number/ratio of the individual ingredients in a mixture can be extracted by FTIR spectra.

### 3.4.1 Main Components of FTIR Instrument

The following are the main parts of the FTIR instrument:

1. *Source*: The source to emit the IR energy in the form of a beam.
2. *Interferometer*: A distinctive signal (interferogram) containing all the IR frequencies is produced by the interferometer. The interferogram signal is created as a result of the interference of two beams and contains the information of each IR frequency. Measuring the interferogram means a simultaneous measurement of all IR frequencies.

3. *Sample Chamber:* The sample is mounted into the specimen chamber. The IR beam goes into the chamber for specimen interaction. Only specific frequencies of the IR signal are absorbed/transmitted depending on the nature of the specimen.
4. *The Detector:* A special-purpose detector is designed to gauge the interferogram signal.
5. *The Computer:* To analyze the signal for identification purposes, a plot of each IR frequency is required. Therefore, the interferogram is decoded into the individual frequencies in a computer using a mathematical formulation, known as Fourier transform.

### 3.4.2 Working of FTIR Instrument

In FTIR instrument, radiations are produced by an IR light source in the form of a beam. The beam of energy then travels through the interferometer for spectral encoding. The interferogram is created as a result of spectral encoding. The working of interferometer and creation of FTIR plot is described as follows (see Fig. 3.6b). Firstly, the beam splitter (a partially reflecting mirror) of the interferometer divides the incoming radiations into two beams. These two beams are then reflected from two separate mirrors, one of the mirrors is fixed and the other is moveable. After reflecting from respective mirrors, both beams are merged back at the beam splitter.

Now, the IR beam goes into the specimen chamber. The signal from the interferometer is the result of interference of these two beams and is known as the “interferogram.” Interferogram contains the information of each IR frequency that is falling on a specimen from the source. The beam is either passed through or bounces off the specimen surface, depending on the analysis requirement. A desired range of IR frequencies representing a distinctive property of the specimen is absorbed. The interferogram signal is detected by a special purpose detector and decoded in the computer to transform into a meaningful data using a mathematical tool known as Fourier transformation. After the signal transformation, FTIR signal in the form of spectrum is available for manipulation and expositions. FTIR spectroscopic characterization can be used to analyze several kinds of materials (Vizintin et al. 2020; Haris and Severcan 1999).

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## 3.5 X-Ray Diffraction (XRD)

X-ray diffraction (XRD) is a non-destructive method to study the material's structure at the molecular and atomic level. XRD is the best method to investigate the crystalline, polycrystalline, and non-crystalline (amorphous) materials. The wavelengths ( $\lambda$ ) of the X-rays (used in the XRD) are in the range of nanometers. XRD is the elastic scattering (no-loss of energy during a collision) of X-rays by atoms of the materials. The principle of XRD is based on the interference of scattered waves. The resultant amplitude (intensity) of the scattered waves depends on the difference in

the distance traveled by the waves, i.e. path difference (phase or angle). If the two waves superimpose in such a way that they are in phase (same crests and troughs), then the resultant intensity is the sum of the two intensities, the phenomenon is known as constructive interference. On the other hand, in the destructive interference, two out of phase waves merged and the resultant intensity is the difference of two intensities. XRD analyzer plotted the  $x-y$  plot (XRD pattern) between intensity (in arbitrary units) and scattering angle,  $2\theta$  (degrees). The XRD pattern is analyzed by a mathematical formulation, known as Bragg's law and the following information can be obtained such as nature of material, atomic arrangement, crystallite size, chemical composition, etc. The XRD line patterns of more than 60,000 different crystallographic phases are available in the electronic database: JCPDS (Joint Committee on Powder Diffraction Standards). The PDF file (example: JCPDS No. 19-0628) consists of three most strong characteristics lines of the existing phase. The XRD technique applies to specimens of powders, thin films and solids.

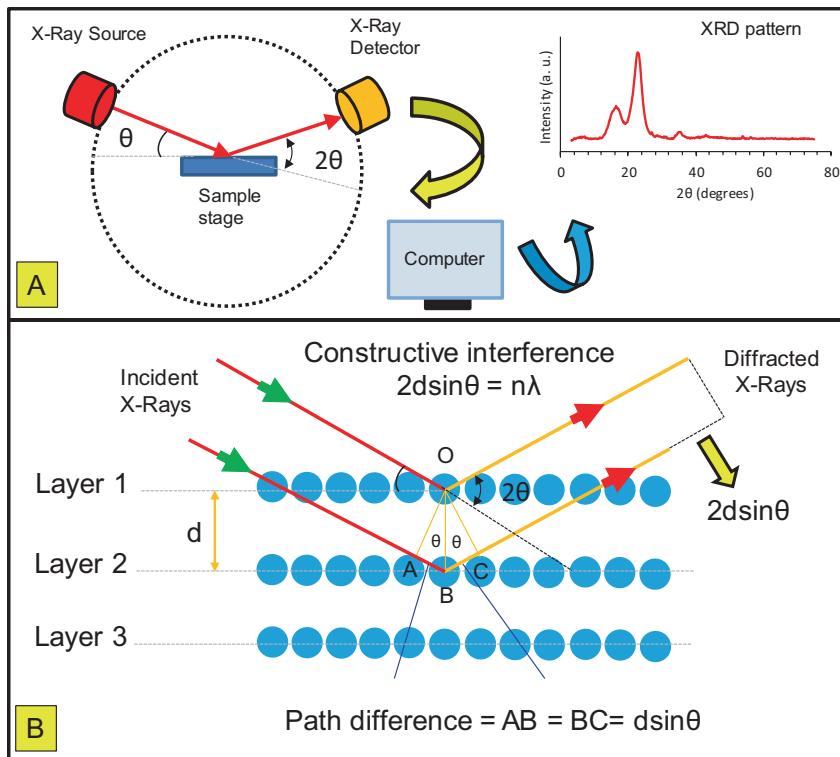
### 3.5.1 Main Components of XRD Instrument

1. *X-Rays Tube*: A beam of fast-moving electrons is collided with atoms of a metallic target (copper) to produce X-rays in the tube.
2. *Collimator*: A collimator is used to make the rays parallel before they hit the specimen.
3. *Sample Stage*: Specimens are placed in the sample stage.
4. *Detector*: The diffracted beam of X-rays is detected by a counter.
5. *Computer*: The collected data is later plotted in the form of XRD pattern ( $2\theta$  versus reflection intensity) on a computer.

### 3.5.2 Working of XRD Instrument

In XRD, a collimated beam (thin and parallel beam) of X-rays is directed towards a sample. The incoming beam of rays interacts with atoms of the specimen and scattered outward direction. Angle made by an incoming beam with the surface of the specimen is  $\theta$ , then the angle of the diffracted beam is  $2\theta$ ;  $2\theta$  is dependent on arrangement of the atoms and type of atoms, etc. The diffracted beam is then detected by a detector and sent to a computer for making an XRD pattern (see Fig. 3.7a). Now, for a given specimen consider several periodic lattice planes (atomic layers) where each layer is separated by  $d$  (inter-plane spacing). According to Bragg's Law, constructive interference occurs when the path difference is an integral number of wavelengths. We can describe this law as follows:  $AB = d \sin \theta$ , and  $BC = d \sin \theta$ , then path difference is  $(AB + BC) = 2d \sin \theta$ . According to the Bragg's laws, a constructive interface occurs when  $2d \sin \theta = n \lambda$  is satisfied (Fig. 3.7b), where  $n$  is an integer,  $\lambda$  is the wavelength of the X-ray beam, and  $\theta$  is the angle between the incident beam and the normal to the sample planes.

In general, for wider interparticle distance  $d$ , the scattering angle  $2\theta$  is lower. This means that the planes with wider  $d$  appeared earlier in the XRD patterns where



**Fig. 3.7** (a) Schematic representation of working of XRD instrument along with XRD pattern. (b) A 2-D crystal lattice and a set of imaginary planes. Constructive interference occurs when the path difference ( $AB + BC = 2d \sin \theta$ ) is equal to integral multiple of wavelength ( $n\lambda$ ) (Bragg's law of diffraction)

scattering angle is low. In XRD, the angle of the incident beam is varied to obtain all the possible reflections for a given sample. By measuring  $\theta$ ,  $d$  of every single crystallographic phase can be determined by comparing the data with the standard line patterns available in the *Powder Diffraction File* (PDF) database (Misture and Snyder 2001).

### 3.6 Energy-Dispersive X-Rays Spectroscopy (EDX)

Energy-dispersive X-ray spectroscopy (EDS or EDX) is a standard technique for identifying and quantifying the elemental composition of the material. Generally, EDX method is carried out in the SEM. In EDX, the surface atoms of the specimen are excited by a beam of high energy electrons (Guler et al. 2019; Marotta et al. 2013). Each constituent element of the specimen has a unique atomic structure and generates characteristic set of peaks in its emission spectrum. This spectrum is then analyzed by a solid-state detector often known as an energy-dispersive detector; the

detector can differentiate among the energies (Kinoshita et al. 2013). Depending on the energies, appropriate elements are assigned, yielding the composition of the atoms on the surface of the specimen. This procedure is thus called the energy-dispersive X-ray spectroscopy (EDS) and allows identification of elements and their relative proportions, e.g. weight and atomic % (see Fig. 3.2c). In EDS spectra, the energies (in electron volts) are plotted on x-axis against the peak intensity in arbitrary units (y-axis) (Scimeca et al. 2018). The EDX can be coupled with SEM, TEM, STEM, and FTIR for several applications (Utsunomiya et al. 2003; Weng et al. 2015).

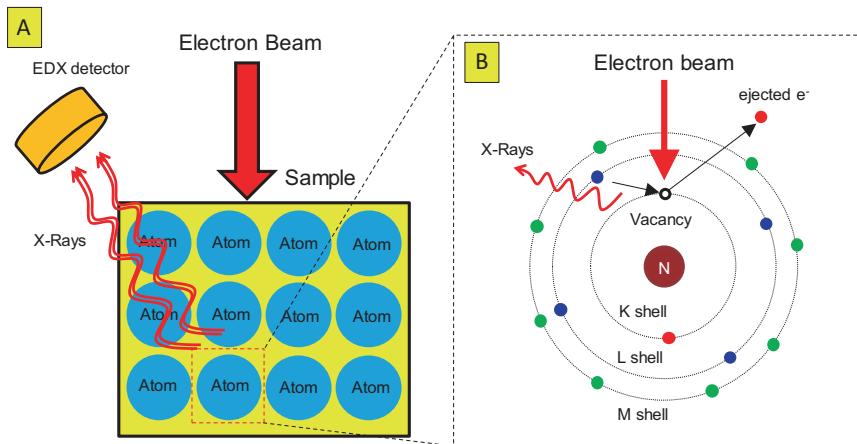
### 3.6.1 Main Components of EDX System

The main components of the EDS setup are given below:

- Electron source
- Sample holder
- X-ray detector
- Pulse processor
- Analyzer
- computer

### 3.6.2 Working of EDX System

In order to produce characteristic X-rays from the specimen, a beam of high energy electrons (produced by electron source) is focused onto the sample (see Fig. 3.8). In a normal state, an atom within the sample contains ground state (unexcited)

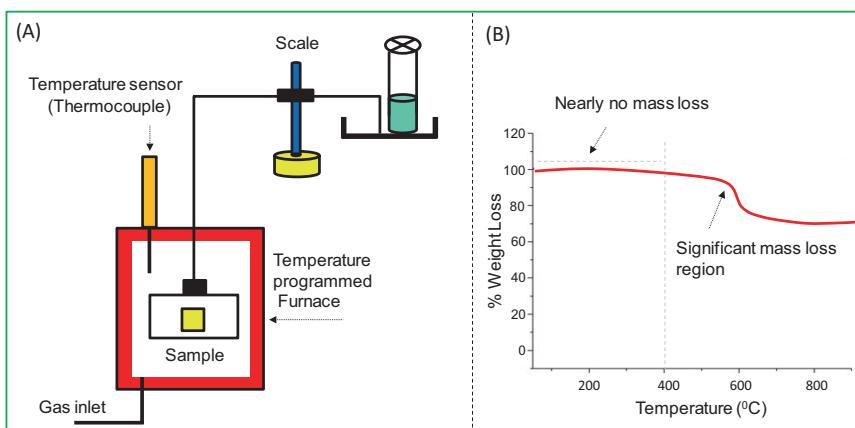


**Fig. 3.8** (a) Schematic representation of working of EDX and (b) emission of X-rays in the magnified view of one atom of the material. The emitted X-rays are the characteristics of the material and analyzed by EDX detector

electrons in discrete energy levels or electron shells. The incident beam may excite the electron in an inner shell, ejecting it from the shell while creating an electron-hole (vacancy). An electron from the upper shell then comes down to fill the vacancy. The difference in energy between the higher-energy shell and the lower energy shell is released in the form of an X-ray. The number and energy of the X-rays emitted from a specimen can be measured by an energy-dispersive spectrometer (detector) (Abd Mutualib et al. 2017). X-ray energy is converted into a voltage signal by a detector and measured by a pulse processor. Finally, the data is sent to the analyzer for computer display and analysis. Hence, EDS system measures the elemental composition of the specimen as the X-ray energies are the characteristics of difference in energy of two shells and of the atomic structure of the emitting element.

### 3.7 Thermal Gravimetric Analysis (TGA)

Thermogravimetric analysis (TGA) is often carried out to find out the chemical stability of the material against temperature (El-Sayed and Mostafa 2014). A TGA is used to measure the mass of a specimen, while its temperature varies over time. TGA is suitable for those substances, which changes their mass on heating, particularly the organic materials as they start losing their mass during a thermal reaction. TGA is performed on a device known as a thermogravimetric analyzer, see the block diagram of the TGA instrument along with a typical TGA graph as shown in Fig. 3.9. To obtain TGA plots, the temperature of the specimen is gradually raised from room temperature (i.e., 25°C) to higher temperatures (usually up to 800°C) and measured the mass of the specimen continuously over time. In TGA, temperature, time, and mass are the basic parameters, which can be further used to derive more measurements. A typical structure of a TGA system consists of a precision balance



**Fig. 3.9** (a) Schematic representation of thermogravimetric analyzer (TGA) setup along with (b) a TGA plot as an example between 25 and 800°C

placed in a furnace along with programmable temperature control. Generally, the rate of temperature increase is kept constant to incur a thermal reaction (Soliman et al. 1997). There are several favorable conditions for a thermal reaction such as inert gas, ambient air, oxidizing gases, vacuum, carburizing gases, corrosive gases, vapors of liquids, high vacuum, high pressure, constant pressure, or a controlled pressure.

In TGA analysis, the thermogravimetric data obtained from a thermal reaction is compiled by plotting mass on the vertical-axis and time or temperature on the horizontal axis. This plot is known as the TGA curve or TGA plot. Differentiating the TGA curve is used for differential thermal analysis. TGA is also useful for the analysis of polymeric materials such as thermosets, plastic films, thermoplastics, paints, elastomers, composites, coatings, fibers, and fuels (Akita and Kase 1967; Alkan et al. 2009).

### 3.7.1 Modes of TGA

Thermogravimetry has the following three types:

1. *Static Thermogravimetry or Isothermal*: In this method, the weight of the specimen is measured at a constant temperature as a function of time.
2. *Quasi-Static Thermogravimetry*: In this method, the specimen is heated to a constant weight each of the series of increasing temperatures.
3. *Dynamic Thermogravimetry*: In this method, the specimen is heated in an environment having a linearly varying temperature.

Loss or gain in weight of the specimen is recorded as a function of time or temperature. The plotted TGA curve appears to be consists of curved portions and horizontal steps. The maximum allowable temperature is around 1000°C. Calculating the loss of mass from the TGA curve is used in the quantitative analysis. The measured data of TGA is very helpful in investigating the composition and purity of the materials, identifying the stability temperatures of the compounds, and identifying the drying and ignition temperatures of materials (Hung and Autian 1972).

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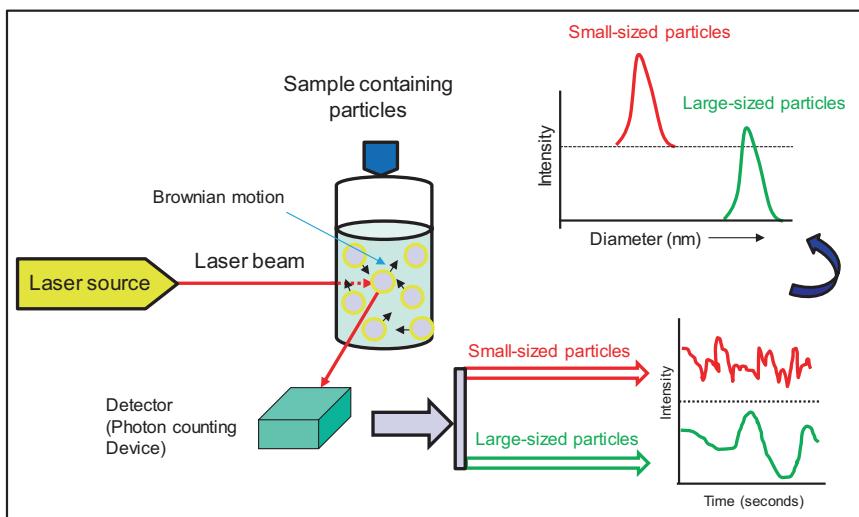
## 3.8 Dynamic Light Scattering (DLS) Analysis

A technique used to determine the size distribution of small-sized particles in solution is known as dynamic light scattering (DLS). DLS is equally be employed to investigate complex fluids like concentrated polymer solutions. In DLS method, time-domain variations are examined through the photon auto-correlation function or photon intensity. It has been demonstrated that the photon auto-correlation function is the Fourier transform of the power spectrum, and so DLS measurements can be conducted in the spectral domain (Chu 1970; Pecora 1964; Stetefeld et al. 2016).

### 3.8.1 Working of DLS System

In DLS technique, a monochromatic light (usually laser) is shined through polarizer onto the particles in suspension. The dispersed light then enters another polarizer and collected by a detector (photomultiplier) and projects the resulting image onto a screen. The image formed is called a speckle pattern (see Fig. 3.10). (Goodman 1976). The particles or molecules present in the solution (exhibiting Brownian motion) are being hit by laser beam causing the diffraction (scattering) of light in all directions. The diffracted light can have either constructive interfere (to produce a bright region of the pattern) or destructive interference to create a dark region (Schatzel et al. 1990; Urban and Schurtenberger 1998; Block and Scheffold 2010). The process is repeated, and the resulting patterns are analyzed by comparing the light intensity of each region (bright or dark) over a period. Two possible geometrical configurations of the polarizers are set up: (1) vertical/vertical (VV) geometry and (2) vertical/horizontal (VH) geometry. In VV geometry, the direction of the light in both primary and second polarizers is the same. Whereas in VH geometry, the direction of light in the second polarizer is different from the direction of the incident light.

In case of tiny particles (small-sized particles), when light hits them, as long as the size of the target particles is smaller than the wavelength of the used light (below 250 nm), the scattering of light known as Rayleigh scattering will occur. Even for a monochromatic and coherent light source, e.g. laser, there is fluctuation in intensity of scattered light over a period. The main reason behind this fluctuation is the presence of small-sized particles or molecules experiencing Brownian motion in solutions, and thus causing variable distance between the scatters. The dispersed light then experiences either destructive or constructive interference by the particles



**Fig. 3.10** Hypothetical illustration of DLS setup of two types of samples: the sample if containing small-sized particles (red) and if it has large-sized particles (green)

and formed a DLS pattern. DLS measures the variations in the dispersed light intensity due to particles diffusion, the coefficient of diffusion can be calculated. For commercial devices, DLS software shows the population of particles at various diameters (Schaetzel 1991; Urban and Schurtenberger 1998; Block and Scheffold 2010; Pusey 1999; Sidhartha et al. 2006; Sabareesh et al. 2006).

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### 3.9 Density Functional Theory (DFT)

Density functional theory (DFT) is a computational quantum modeling technique typically applied in physics, materials science, and chemistry to study electronic structures of multi-body systems (atoms, molecules, and the condensed phases). Since 1970, the DFT is very popular in solid state physics to determine material behavior by calculations. In DFT, the function is electron density that is a function of space and time. The electron density is the fundamental property of the material. In any material, the nuclei of the atoms are surrounded by a sea of electrons, referred to as many-electron system and DFT can be used to predict the properties of many-electron system by applying the electron density function.

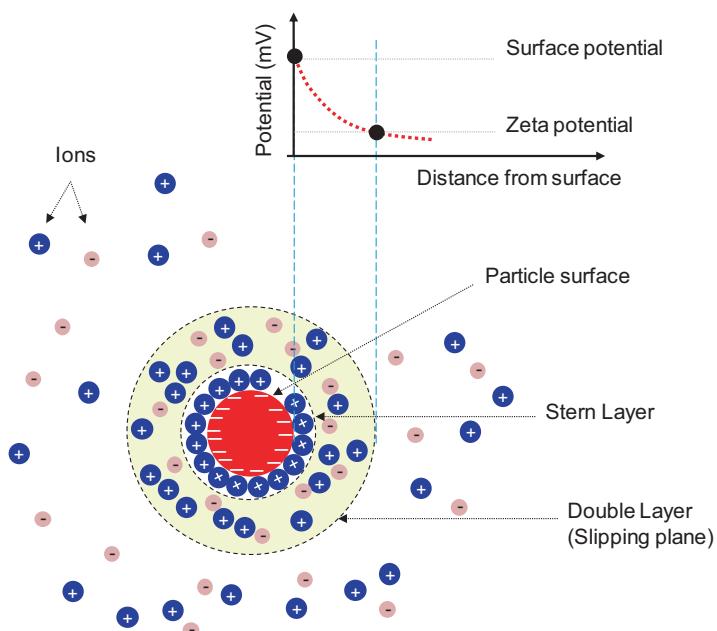
The quantum mechanical wavefunction contains the information about a given system and the Schrödinger wave equation can be applied to determine this information. For a simple two-dimensional (2-D) square potential or a hydrogen atom, the Schrödinger equation is solved to get the wavefunction of the system. By doing this, we can determine the allowed energy states of the system and hence the properties of the material. However, it is not possible to solve the Schrödinger equation for a many-body system and the solution requires some approximations to solve the problem. For example, in the case of a regular crystal in a many-body system, the atoms are not only affected by the nuclei, but they are also influenced by the electrons of surrounding atoms. Hence, in simple words we can say that the DFT is a method to obtain an approximate solution of the Schrödinger equation for a many-body system. DFT methods are employed to study the magnetic, structural, and electronic characteristics of materials, molecules, and defects. The ab initio (first principles) DFT calculations have become an important tool in material science to predict and calculate the material properties based on quantum mechanical considerations (Assadi et al. 2013; Van Mourik and Gdanitz 2002; Vondrášek et al. 2005; Tkatchenko and Scheffler 2009; Hanaor et al. 2012; Hohenberg and Walter 1964; Levy 1979).

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### 3.10 Zeta sizer

Zeta sizer or particle size analyzer is a technique in colloidal chemistry to determine the surface charges of colloidal nanoparticles in a liquid media by measuring the zeta potential (Li 2004). Zeta potential is useful to understand the physical stability of the nanoparticles while they are in the dispersion. The nanoparticle usually has a charge (either positive or negative) on the surface that attracts

oppositely charged ions to the surface of the nanoparticle and produces an interfacial double layer around the particle (see Fig. 3.11). The first layer is known as the Stern layer. The particle often diffuses through the solution along with the ionic double layer. The electrical potential at the boundary (slipping plane) of the double layer is referred to as a zeta potential that is simply the potential difference between the dispersion medium and the double layer of fluid. It is denoted with Greek letter zeta ( $\zeta$ ) and its units are volts (V) or millivolts (mV). A typical range of  $\zeta$  lies between 100 and  $-100$  mV for the colloidal nanoparticles. The magnitude of “ $\zeta$ ” is very important as it highlights the stability of the particles in the solution (see Table 3.1). A large value of “ $\zeta$ ” either positive or negative gives good



**Fig. 3.11** Schematic illustration of ionic concentration and zeta potential of the charged particle suspended in a liquid medium (Mjones1984)

**Table 3.1** Zeta potential ( $\zeta$ ) versus stability behavior of the charged particles (5-types of behaviors)

S. No.	Zeta potential (mV)	Stability behavior
1	0 to $\pm 5$	Rapid coagulation (aggregation)
2	$\pm 10$ to $\pm 30$	Incipient instability
3	$\pm 30$ to $\pm 40$	Moderate stability
4	$\pm 40$ to $\pm 60$	Good stability
5	$> \pm 61$	Excellent stability

Source: Kumar and Dixit (2017)

stability to the particles via electrostatic repulsion forces of the individual particle (Russel et al. 1992; Dukhin and Goetz 2017). In general, a value of “ $\zeta$ ” other than  $-30\text{ mV}$  to  $+30\text{ mV}$  (i.e. any magnitude value greater than  $30\text{ mV}$ ) provides enough repulsive forces among the particles to maintain their individual identity. In the case of smaller “ $\zeta$ ,” the particles can aggregate and flocculate due to the presence of van der Walls attractive forces and hence caused physical instability. There are some factors other than  $\zeta$ , such as the nature of particles, presence of surfactants, solution chemistry also contribute to the physical stability of the nanoparticles (Kirby 2010; Honary & Zahir 2013; Freitas & Müller 1998; Shah et al. 2014).

Zeta sizer or particle size analyzer, a nano range of instrument provides the ability to measure three characteristics of the colloidal nanoparticles in a liquid media, i.e. particle size, zeta potential (surface charge), and molecular weight.

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# Application of Nanomaterials: Overview and Historical Perspectives

4

Samuel Sami-Howard

## Abstract

Nanotechnology in the last few years has gained important and critical recognition in different fields and applications. Nano-fluids are fluids containing suspension of nanometer-sized particles. Their importance arose due to the need to enhance thermal performance of energy and thermal systems with noninvasive techniques. Nano-fluids have superior thermal properties over the base fluids such as water, mineral oil, vegetable oil, thermal oil, and synthetic oil. This feature makes them very attractive as heat transfer fluids in many applications and manufacturing processes. Nano-fluids have been recognized as nanoparticle of suspensions (1–100 nm) in a base fluid. Nano-fluids have other applications in biotechnology applications for drug delivery and advanced sensors technology. Nanoparticles are prepared from metal oxides, metals, or carbon in different forms. This chapter is intended to focus on the nanoparticle's characteristics, behavior in applications such as momentum and mass, enhanced energy, and heat transfer, and, enhancement of solar energy applications. Other applications involving nanomaterials were also presented and discussed.

## Keywords

Nanotechnology · Status · Types · Applications of nanobiotechnology

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S. Sami-Howard (✉)  
TransPacific Energy, Inc, Las Vegas, NV, USA

Research Center for Renewable Energy, Catholic University of Cuenca, Cuenca, Ecuador  
e-mail: [dr.ssami@transpacenergy.com](mailto:dr.ssami@transpacenergy.com)

## 4.1 Introduction

Nano-fluid is a base fluid with solid nanoparticles at size less than 100 nm are characterized by suspending nanoparticles of metals and metal oxides (Li et al. 2009; Xiang and Arun 2008; Das et al. 2003, 2007; Jama et al. 2016; Puliti et al. 2011a, b; Yang and Liu 2011; Jamshidi et al. 2012; Timofeeva et al. 2010, 2011; Nikkam et al. 2014; Abdul Hamid et al. 2015; Das 2015; Guptha et al. 2012; Xuan and Li 2000a, b; Maxwell 1873; Choi 1995; Lazarus et al. 2010; Patel et al. 2006; Das and Choi 2006; Jang and Choi 2006; Pak and Cho 1998; Zhu et al. 2004, 2006, 2007a, b; Lee et al. 1999; Singh and Raykar 2008; Li and Kaner 2005; Ding et al. 2006; Wang et al. 1999; Tseng and Lin 2003; Wang and Mujumdar 2007; Chen et al. 2007; Kole and Dey 2010; Saidur et al. 2011; Prasher et al. 2005; Abu-Nada et al. 2010; Escher et al. 2011; Namburu et al. 2009; Vajjha and Das 2009; Nelson et al. 2009; Shin and Banerjee 2011a, b; Zhou and Ni 2008; Eastman et al. 2001; Wang and Fan 2010; Shokouhmand et al. 2008; Xie and Chen 2009; Yu et al. 2007, 2008; Tzeng et al. 2005; Demirbas 2006; Kulkarni et al. 2009; Vassallo et al. 2004; Kim et al. 1999, 2007; Ma et al. 2007; Wu et al. 2010; Otanicar et al. 2010; Tyagi et al. 2009; Zhou et al. 2000; Shen et al. 2008; Vékás et al. 2007; Rosensweig 1987; Sami 2019a, b; Sami and Marin 2019; Zhang et al. 2007; Jalal et al. 2010; Jones et al. 2008; Liu et al. 2009; Pastorin et al. 2006; Singh and Lillard 2009; Fan et al. 2008; Lee and Choi 1996; Lv et al. 2014; Akoh et al. 1978; Swati et al. 2017). Nanoparticles mainly consist of nanocrystalline materials, nanocomposites, carbon nanotubes, and quantum dots. Nano-fluid particles display greater thermal, mechanical, physical, chemical properties than conventional materials. Four types of nanomaterials are presented: (1) carbon-based nanomaterials, (2) metal-based nanomaterials, (3) metal oxides, and (4) dendrimer and composites (nanosized clays) (Li et al. 2009; Xiang and Arun 2008; Das et al. 2003, 2007; Jama et al. 2016; Puliti et al. 2011a, b; Yang and Liu 2011; Jamshidi et al. 2012; Timofeeva et al. 2010, 2011; Nikkam et al. 2014; Abdul Hamid et al. 2015; Das 2015; Guptha et al. 2012; Xuan and Li 2000a, b; Maxwell 1873; Choi 1995; Lazarus et al. 2010; Patel et al. 2006; Das and Choi 2006; Jang and Choi 2006; Pak and Cho 1998; Zhu et al. 2004, 2006, 2007a, b; Lee et al. 1999; Singh and Raykar 2008; Li and Kaner 2005; Ding et al. 2006; Wang et al. 1999; Tseng and Lin 2003; Wang and Mujumdar 2007; Chen et al. 2007; Kole and Dey 2010; Saidur et al. 2011; Prasher et al. 2005; Abu-Nada et al. 2010; Escher et al. 2011; Namburu et al. 2009; Vajjha and Das 2009; Nelson et al. 2009; Shin and Banerjee 2011a, b; Zhou and Ni 2008; Eastman et al. 2001; Wang and Fan 2010; Shokouhmand et al. 2008; Xie and Chen 2009; Yu et al. 2007, 2008; Tzeng et al. 2005; Demirbas 2006; Kulkarni et al. 2009; Vassallo et al. 2004; Kim et al. 1999, 2007; Ma et al. 2007; Wu et al. 2010; Otanicar et al. 2010; Tyagi et al. 2009; Zhou et al. 2000; Shen et al. 2008; Vékás et al. 2007; Rosensweig 1987; Sami 2019a, b; Sami and Marin 2019; Zhang et al. 2007; Jalal et al. 2010; Jones et al. 2008; Liu et al. 2009; Pastorin et al. 2006; Singh and Lillard 2009; Fan et al. 2008; Lee and Choi 1996; Lv et al. 2014; Akoh et al. 1978; Swati et al. 2017). Nanoparticles can improve thermal conductivity and heat transfer of liquids when injected along with base fluids (Li et al. 2009; Xiang and Arun 2008; Das et al. 2003, 2007; Jama et al.

2016; Puliti et al. 2011a, b; Yang and Liu 2011; Jamshidi et al. 2012; Timofeeva et al. 2010, 2011; Nikkam et al. 2014; Abdul Hamid et al. 2015; Das 2015; Guptha et al. 2012; Xuan and Li 2000a, b; Maxwell 1873; Choi 1995; Lazarus et al. 2010; Patel et al. 2006; Das and Choi 2006; Jang and Choi 2006; Pak and Cho 1998; Zhu et al. 2004, 2006, 2007a, b; Lee et al. 1999; Singh and Raykar 2008; Li and Kaner 2005; Ding et al. 2006; Wang et al. 1999; Tseng and Lin 2003; Wang and Mujumdar 2007; Chen et al. 2007; Kole and Dey 2010; Saidur et al. 2011; Prasher et al. 2005; Abu-Nada et al. 2010; Escher et al. 2011; Namburu et al. 2009; Vajjha and Das 2009; Nelson et al. 2009; Shin and Banerjee 2011a, b; Zhou and Ni 2008; Eastman et al. 2001; Wang and Fan 2010; Shokouhmand et al. 2008; Xie and Chen 2009; Yu et al. 2007, 2008; Tzeng et al. 2005; Demirbas 2006; Kulkarni et al. 2009; Vassallo et al. 2004; Kim et al. 1999, 2007; Ma et al. 2007; Wu et al. 2010; Otanicar et al. 2010; Tyagi et al. 2009; Zhou et al. 2000; Shen et al. 2008; Vékás et al. 2007; Rosensweig 1987; Sami 2019a, b; Sami and Marin 2019; Zhang et al. 2007; Jalal et al. 2010; Jones et al. 2008; Liu et al. 2009; Pastorin et al. 2006; Singh and Lillard 2009; Fan et al. 2008; Lee and Choi 1996; Lv et al. 2014; Akoh et al. 1978; Swati et al. 2017). However, this can be associated with high pressure drop that occurred owing to excessive wear, sedimentation. Numerous articles have been published in the literature on nano-fluids (Li et al. 2009; Xiang and Arun 2008; Das et al. 2003, 2007; Jama et al. 2016; Puliti et al. 2011a; Yang and Liu 2011; Jamshidi et al. 2012; Timofeeva et al. 2010, 2011; Nikkam et al. 2014; Abdul Hamid et al. 2015; Das 2015; Guptha et al. 2012; Xuan and Li 2000a, b; Maxwell 1873; Choi 1995; Lazarus et al. 2010; Patel et al. 2006; Das and Choi 2006; Jang and Choi 2006; Pak and Cho 1998; Zhu et al. 2007a; Lee et al. 1999; Singh and Raykar 2008; Li and Kaner 2005) and in the following we will discuss several issues related to nano-fluids such as thermodynamic and thermophysical properties, stabilities, and applications.

Nano-fluids can be used as coolants in micro-electronic devices where the sizes have been diminishing resulting in high heat generation which needs to be removed efficiently for the optimal functioning of these devices (Timofeeva et al. 2010) and (Timofeeva et al. 2011). In the automobile industries nano-fluids can play a very important role in the removal of excess energy that is generated due to the combustion of the fuel. When flowing through the tubes of the radiator nano-fluids can lose its heat to the surrounding air through its walls. In all manufacturing processes which require heat transfers, the conventional fluids can be replaced by nano-fluids. Understanding the underlying mechanisms which cause the improvements is critical to investigate the thermal and thermophysical properties and flow characteristics of nano-fluids (Das et al. 2007).

The fluid can be significantly enhanced in heating or cooling applications and due to these reasons, the surface area with suspended nanoparticles is increased. Nano-fluids possess several benefits such as (1) absorption of solar and thermal energy can be maximized their shape, (2) material size, and volume fraction of the nanoparticles improve the thermal and thermophysical properties of the base fluid. It has been learned the nanoparticles can increase the heat transfer surface area and also the heat capacity of the base fluid. Additionally, nanoparticles enhanced the thermal conductivity which in turn advances the efficiency of heat transfer

equipment; they can transfer heat to a small area of base fluid. In addition, they can induce mixing fluctuation and turbulence of the base fluid, dispersion of nanoparticles reduces transverse temperature gradient of the base fluid, changing the volume fraction can change the fluid base properties, and finally nano-fluids enhance the temperature of solar thermal applications. This chapter is intended to address the nano-fluids thermophysical properties, nano-fluids potential for heat transfer intensification and mass transfer enhancement, and nano-fluids application in the energy and mechanical fields. A large section is devoted to the current investigations on the biological applications of the nano-fluids, which involve their antibacterial activity or drug-delivery properties.

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## 4.2 Thermophysical Properties

The different thermophysical properties of nano-fluids are (1) viscosity, (2) specific heat, (3) thermal conductivity, and (4) stability. These properties play an important role in thermal behavior of nano-fluids.

### 4.2.1 Viscosity

To better understand the issues of convection heat transfer which is connected to the enhancement of the viscosity of the base fluid and nano-fluids. It is important to understand the nature of the nano-fluids such as Newtonian or shear flow (Li et al. 2009). It has been reported that the nano-fluids acted as Newtonian when nanoparticles with 13–27 nm size when suspended in water. Shear thinning behavior was observed with the increase of particle volume fraction. It was also reported and observed that with large increase of viscosity, the behavior of Newtonian or shear nano-fluids, cannot be predicted by standard empirical methods.

It was shown (Li et al. 2009) that thermal conductivity and viscosity of nano-fluids with carbon nanotubes (CNTs) behave reasonably different than other nano-fluids. It has been found that viscosity is equally important as thermal conductivity in determining pumping power and the heat transfer coefficient in thermal engineering process and system. Whereas higher nanoparticle volume fractions result in more viscous, attenuating the velocity and reduces convection. In addition, lower reduction of convection and velocity can increase the thickness of the thermal boundary layer thickness and consequently, lower the Nusselt number.

### 4.2.2 Specific Heat

The measurements of specific heats of nano-fluids are very limited as reported by Xiang Q W and Arun S M (Xiang and Arun 2008). It has been found that a water-alumina nano-fluid appeared to have improved thermal conductivity and lowered

specific heat as compared to the base fluid. It has been reported (Das et al. 2007; Jama et al. 2016) nano-fluids with 2–10% by volume of  $\text{SiO}_2$ ,  $\text{Al}_2\text{O}_3$ , and  $\text{ZnO}$  nanoparticles in the ratio 60:40 ethylene glycol and water mixture also gave better results. In addition, there are several reports which suggested that the agglomerated nanoparticles tend to precipitate out of the water solution (Puliti et al. 2011a; Yang and Liu 2011; Jamshidi et al. 2012; Timofeeva et al. 2010). It was found that the interfacial effect is caused by the extremely high specific surface area of the nanoparticles (Puliti et al. 2011a; Timofeeva et al. 2010). Furthermore, solid like liquid layers attaching to the nanoparticles were due to a shorter intermolecular mean free path compared to the bulk fluid.

#### 4.2.3 Thermal Conductivity

It has been reported that nano-fluids display greater heat transfer features to conventional method. One of the explanations is that the suspended nanoparticles strangely increase thermal conductivity of nano-fluids over the base fluid. Additionally, formulas have been developed and used for the 2-phase mixtures that contain powder with nanoparticle from micrometers to millimeters. Nonetheless, the formulas can be applied to obtain a rough estimation of the thermal conductivity of nano-fluids. In addition, it must be noted that the effective thermal conductivity of using suspensions increased by decreasing the sphericity of the particles under the condition of the same volume fraction as per Table 4.1. Apparently, these results clearly show that increase of the thermal conductivity and the conventional heat transfer relays upon the nanoparticles material (Xiang and Arun 2008; Puliti et al. 2011a; Guptha et al. 2012; Xuan and Li 2000a, b; Maxwell 1873; Choi 1995; Lazarus et al. 2010; Patel et al. 2006; Das and Choi 2006).

**Table 4.1** Thermal conductivity of materials (Puliti et al. 2011a; Guptha et al. 2012; Xuan and Li 2000a)

Materials thermal conductivity [W/mK]	
Metallic materials; copper	401
Silver	429
Nanometallic materials silicon	148
Alúmina ( $\text{Al}_2\text{O}_3$ )	40
Carbon nanotubes	2000
Ethylene glycol	0.253
Engine oil	0.145
Nano-fluids (nanoparticle concentration %)	
Water/(1.50)	0.629
EG/ $\text{Al}_2\text{O}_3$ (3.00)	0.278
EG-water/ $\text{Al}_2\text{O}_3$ (3.00)	0.382
Water/ $\text{TiO}_2$ (0.75)	0.682
Water/CuO (1.00)	0.619
Base fluids water	0.613

### 4.3 Stability of the Nano-fluids

Several methods have been established to assess the stability of nano-fluids (Das et al. 2007; Jang and Choi 2006). In the sedimentation method, nanoparticles nano-fluid were used under an external force field which is a sign of the stability. The difference of particle size with sediment time can be achieved by special device (Das et al. 2007). In addition, nano-fluids are stable when the concentration or size of nanoparticle of supernatant is constant. The sedimentation method has been used to examine the stability of the graphite suspension (Singh and Raykar 2008) and centrifugation method is also developed to evaluate the stability of nano-fluids. Thereafter, the centrifugation method has been applied to detect the stability of silver nano-fluids (Li and Kaner 2005).

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### 4.4 Applications of Nano-fluids

Applications of nano-fluids include broad range of engineering applications in particular in the areas of thermal system and in the following paragraph we discuss some of these applications in fields related to thermal systems (Li et al. 2009; Xiang and Arun 2008; Das et al. 2003, 2007; Jama et al. 2016; Puliti et al. 2011a; Yang and Liu 2011; Jamshidi et al. 2012; Timofeeva et al. 2010, 2011; Nikkam et al. 2014; Abdul Hamid et al. 2015; Das 2015; Guptha et al. 2012; Xuan and Li 2000a, b; Maxwell 1873; Choi 1995; Lazarus et al. 2010; Patel et al. 2006; Das and Choi 2006; Jang and Choi 2006; Pak and Cho 1998; Zhu et al. 2007a; Lee et al. 1999; Singh and Raykar 2008; Li and Kaner 2005; Ding et al. 2006; Wang et al. 1999; Tseng and Lin 2003; Wang and Mujumdar 2007; Chen et al. 2007).

#### 4.4.1 Electronic Applications

The tight and compact space of the design of electronic components makes heat dissipation a significant problem in electronic applications. Therefore, reliable thermal management system is critical to efficient operation of the advanced electronic devices. Moreover, nano-fluids are characterized with higher thermal conductivities and higher convective heat transfer coefficients compared to those of base fluids. Therefore, they can contribute significantly in improving of the performance in electronic applications. Recent studies have demonstrated that nano-fluids increase the heat transfer coefficient by increasing the thermal conductivity of coolants (Jang and Choi 2006; Li and Kaner 2005; Wang and Mujumdar 2007; Kole and Dey 2010; Saidur et al. 2011; Prasher et al. 2005; Abu-Nada et al. 2010; Escher et al. 2011; Namburu et al. 2009; Vajjha and Das 2009; Puliti et al. 2011b; Nelson et al. 2009; Shin and Banerjee 2011a, b; Zhou and Ni 2008; Eastman et al. 2001; Zhu et al. 2004, 2007b; Wang and Fan 2010; Shokouhmand et al. 2008; Xie and Chen 2009) with compared to apparatuses which use pure water as working heat transfer fluid. Moreover, nano-fluids can increase the thermal resistance and the temperature

difference between the heated microchannel wall and the coolant (Jang and Choi 2006; Shokouhmand et al. 2008; Xie and Chen 2009). The above-mentioned references found that microchannel heat sink combined with nano-fluids has noteworthy potential as the next generation cooling devices for removing ultra-high heat flux. Apparently, this improvement is triggered by the increase in the thermal conductivity of coolant. The nanoparticle thermal dispersion effect and small pressure was found to be diminished with the use of the nanoparticles and low volume fraction (Xie and Chen 2009).

#### 4.4.2 Transportation

Automotive industry can be upgraded by dispersing more heat by using the cooling system and nano-fluids can play a significant role in dissipating that thermal waste heat and enhancing the efficiency and subsequently decreasing the weight and reducing the complexity of thermal management system. The energy and fuel consumption are very beneficial to high performance and high fuel economy of car and truck (Yu et al. 2007; Tzeng et al. 2005; Demirbas 2006). It has been reported that ethylene glycol-based nano-fluids have been used in various applications such as engine coolant. Researchers have used (Demirbas 2006) nano-fluids for the cooling of automatic transmissions in a 4-wheel drive vehicle. The nano-fluids and copper oxide ( $\text{CuO}$ ) and  $\text{Al}_2\text{O}_3$  nanoparticles were injected into engine transmission oil and the results showed that  $\text{CuO}$  nano-fluids lowered the transmission temperature.

#### 4.4.3 Industrial Cooling Applications

The United States auto industry has been using nano-fluids for cooling and heating water and it is estimated to save 1 trillion British thermal unit (BTU) of energy (Demirbas 2006; Kulkarni et al. 2009; Vassallo et al. 2004; Kim et al. 2007; Ma et al. 2007; Wu et al. 2010). Additionally, the US electric power industry could save about 10 to 30 trillion BTU/year which was basically equal to the yearly energy intake of 50,000–150,000 homes (Kulkarni et al. 2009; Vassallo et al. 2004). Moreover, there was also reduction in the emission of approximately 5.6 million metric tons of  $\text{CO}_2$ , 8,600 metric tons of  $\text{NO}_2$ , and 21,000 metric tons of sulfur dioxide ( $\text{SO}_2$ ) (Vassallo et al. 2004). It was reported that (Kim et al. 2007) the performance of polyalphaolefin nano-fluids containing exfoliated graphite nanoparticle fibers in cooling of specific heat of nano-fluids was found to be 50% higher for nano-fluids compared with polyalphaolefin as base fluid.

#### 4.4.4 Heating Building and Reducing Pollution

It has been reported that nano-fluids can be used effectively in heating the buildings in cold environments compared to base fluid ethylene or propylene glycol mixed

with water in diverse proportions (Vassallo et al. 2004). It was shown that using nano-fluids in heat exchanger reduces volumetric, mass flow rates and reduces overall pumping power. Moreover, nano-fluids when used in heating systems can deliver equal amount of thermal energy but with economical rates. This method also reduces the environmental pollution.

#### **4.4.5 Space and Defense**

It has been reported that the increases in the critical heat flux in pool boiling with nano-fluids was associated with the use of nanofluids compared to the base fluid (Kim et al. 2007). This study was significant as large number of military procedures and systems are required high heat flux cooling to high levels. There are other nano-fluids which have great potential to supply required cooling to armed systems. Consequently, nano-fluids can make a significant improvement in space and security fields.

#### **4.4.6 Mass Transfer Enhancement Process**

Several studies have been reported where mass transfer enhancement of nano-fluids was used in  $\text{NH}_3/\text{H}_2\text{O}$  absorption system (Demirbas 2006; Otanicar et al. 2010; Tyagi et al. 2009) and the addition of nanoparticles enhanced the absorption performance. In addition, the impact of nanoparticles and surfactants on the absorption properties showed that surfactants and nanoparticles improved the absorption performance during the ammonia-bubble absorption process and also numerical investigations of thermo-diffusion and diffusion-thermo were also reported.

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### **4.5 Energy Applications**

As discussed previously, more nano-fluids are utilized in the energy field as presented below.

#### **4.5.1 Energy Storage**

There is a significant emphasis on storage of thermal energy in energy management and conservation of the wasteful heat and solar energy (Demirbas 2006). Latent heat storage is one of the most effective means of storing thermal energy (Wu et al. 2010; Otanicar et al. 2010). The materials ( $\text{Al}_2\text{O}_3\text{-H}_2\text{O}$  nano-fluids) can be used as a new phase change material for the thermal energy storage of cooling systems. It has been found that the addition of  $\text{Al}_2\text{O}_3$  nanoparticles decreased the supercooling degree of water and also increased the freezing time and decreased the total freezing time.

#### 4.5.2 Solar Absorption

Solar absorption technology can be combined with the emerging technologies of nano-fluids and liquid nanoparticle suspensions that can create a new class of nano-fluid which is based on solar collectors (Tyagi et al. 2009; Zhou et al. 2000). The efficiency was reported to increase by 5% in solar thermal collectors by utilizing nano-fluids as the absorption media. Moreover, when compared to the experimental data results that showed an increase in efficiency with volume fraction, it was shown that direct absorption solar collector with the presence of nanoparticles increased the absorption of incident radiation.

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### 4.6 Mechanical Applications

It is well known that nanoparticles in nano-fluids form a protective film with slight hardness and elasticity which make the nano-fluids an excellent lubricant. Magnetic fluids are considered as special nano-fluids as it operates with no maintenance and with low leakage (Yu et al. 2008; Shen et al. 2008; Vékás et al. 2007; Rosensweig 1987; Kim et al. 1999; Zhou et al. 2000).

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### 4.7 Friction Reduction

Nanoparticles possess better load carrying capacity, can further improve lubricants integrated nano-fluids (Yu et al. 2008; Shen et al. 2008; Vékás et al. 2007; Rosensweig 1987; Kim et al. 1999; Zhou et al. 2000). It has been studied that the tribological behavior of Copper nanoparticles in oil on a 4-ball machine and results showed that Copper nanoparticles were better friction reduction and possessed anti-wear properties than zinc dithiophosphate especially at high applied load. Likewise, nano-fluids can strikingly improve the load carrying capacity of the base oil (Yu et al. 2008). Moreover, nano-fluids decrease the grinding forces and improved surface roughness (Shen et al. 2008).

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### 4.8 Magnetic Sealing

Ferromagnetic fluids are special nano-fluids and they are extremely stable colloidal suspensions of small magnetic particles such as magnetite. Their physical properties can change their size and adjust their surface coating to meet the requirements of colloidal firmness of magnetic nano-fluids (Shen et al. 2008; Vékás et al. 2007; Rosensweig 1987; Kim et al. 1999; Yu et al. 2008; Sami 2019a; Sami and Marin 2019).

## 4.9 Thermal Solar Collectors

Nano-fluids can also be used to improve solar photovoltaics (PV) efficiency (Sami 2019b). Researchers have developed a new concept of combined photovoltaic-thermal solar panel hybrid system where the PV cells were cooled by water and due to this, the excess thermal energy was generated/degenerated. The degenerated and excessed thermal energy can increase the cell temperature and in turn reduce the conversion efficiency of the cell. Consequently, the net result was an enhancement of the combined photovoltaic-thermal efficiency of the hybrid system. A simulation model was established to forecast the behavior of a hybrid system composed of PV-Thermal panel and thermoelectric generator using nano-fluids (Zhang et al. 2007). Recently, the performance of nano-fluids was studied in a power generation and thermal energy storage system (Jones et al. 2008). This study was done to investigate the improvement effect of nano-fluids. In this study, the refrigerant mixture was used to enhance the efficiency. The results showed that power absorbed and power collected are enhanced with the increase of the solar radiation.

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## 4.10 Biomedical Application

Nano-fluids can be used in various biomedical applications such as antibacterial activities, drug-delivery properties meanwhile they have applicable properties and extensively studied (Jones et al. 2008; Liu et al. 2009; Pastorin et al. 2006; Singh and Lillard 2009; Zhu et al. 2006; Fan et al. 2008; Lee and Choi 1996; Lv et al. 2014; Akoh et al. 1978; Swati et al. 2017; Storm et al. 1995; Torchilin and Trubetskoy 1995; Hwang et al. 2008; Jalal et al. 2010; Mahapatra et al. 2008). Nano-fluid is useful for cell biology to separate and purify different types of cell populations, tissue repair, and in cancer treatment. Some pharmaceutical applications include targeted nanodrug-delivery system such as antibacterial activity and future possibility is to develop high performance nano-fluids for nontoxic or biodegradable nanoparticles. There are commonly used materials such as lipids, ceramic, polymers, and metals (Torchilin and Trubetskoy 1995) and natural and synthetic polymers and lipids are typically used as drug-delivery vectors (Hwang et al. 2008; Jalal et al. 2010; Mahapatra et al. 2008). The nanomaterials which comprise chemotherapeutic agents are consumed by phagocytes and speedily cleared by the reticuloendothelial system. Additionally, different technologies have been developed to withstand the nanoparticles in bloodstream one of which includes an alteration of the polymeric composition of the carrier. On the other hand, nanoparticles anchored with hydrophilic polymers were used to keep the nanoparticles in the bloodstream for a longer period of time which also helps in successfully targeting the cancerous cells (Hwang et al. 2008; Jalal et al. 2010). The hydrophilic polymers coated on nanoparticle's surface prevent the plasma proteins from being opsonized and also the hydrophilic polymers are poly-ethylene-glycol (PEG), polysaccharides, and poloxamines. The poloxamers are the most commonly used polymers (Hwang et al. 2008; Jalal et al. 2010; Mahapatra et al. 2008).

## 4.11 Antibacterial Activity

Zinc oxide (ZnO) nano-fluids can be used for antibacterial agents as ZnO nano-fluids have bacteriostatic activity against bacteria (Jalal et al. 2010; Jones et al. 2008; Liu et al. 2009; Pastorin et al. 2006; Singh and Lillard 2009). Moreover, electrochemical measurements suggest some direct interaction between ZnO nanoparticles and the bacterial cell membrane. The antibacterial activity of ZnO nanoparticles against *Escherichia coli* has been assessed by estimating the decrease ratio of the bacteria treated with ZnO and it has been found that bacterial survivability was decreased with increasing the concentrations of ZnO nano-fluids (Liu et al. 2009; Pastorin et al. 2006; Singh and Lillard 2009; Zhu et al. 2006). Furthermore, it has been reported (Liu et al. 2009; Pastorin et al. 2006; Singh and Lillard 2009; Zhu et al. 2006) that ZnO nanoparticles have a wide range of antibacterial impacts on a number of different microorganisms. The antibacterial activity of zinc oxide (ZnO) nano-fluids shows that ZnO nano-fluids have better bacteriostatic activity against *Escherichia coli*. It has been reported (Pastorin et al. 2006) that *Escherichia coli* growth was decreased with increasing concentrations of ZnO nano-fluids. It has been suggested that ZnO nanoparticles have a wide range of antibacterial on different microorganisms (Mahapatra et al. 2008; Pastorin et al. 2006; Bianco et al. 2005). Metal oxide nanomaterials such as ZnO and CuO also exhibit anti-microbial behavior against pathogenic bacteria. It was also reported that ZnO, CuO, and iron oxide nanoparticles have excellent anti-microbial activity against Gram-positive and Gram-negative bacteria. Nevertheless, it was shown that ZnO has the greatest anti-microbial activity against both Gram-positive and Gram-negative bacteria (Bianco et al. 2005).

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## 4.12 Nanodrug Delivery

The drug molecules can be successfully delivered to target site by using nanoparticles. The nanoparticles with small size, customized surface improve solubility, and multi-functional capabilities open new prospects of biomedical applications. It was found that gold nanoparticles provide nontoxic carriers for drug and gene delivery applications and another feature of gold nanoparticle is their interaction with thiol groups, providing an effective and selective means of controlled intracellular release of drug (Jones et al. 2008). Moreover, the attachment of molecules that target specific receptors on tumor cells helps improve response to anti-cancer agents. It has been reported that gold nanoparticles can provide nontoxic carriers for drug and gene delivery applications (Bica et al. 2007; Chiang et al. 2007). The gold core imparts stability and allows tuning of surface properties such as charge and hydrophobicity.

Carbon nanotubes (CNTs) have been reported as a new another and efficient tool for transportation drug molecules (Bica et al. 2007; Chiang et al. 2007). It was also found that CNTs can work with bioactive nucleic acids, peptides, proteins, drugs and used to deliver at the targeted sites. Moreover, it was reported that CNTs show

lower toxicity in the nanomedicine. A novel technique strategy was developed for working of CNTs with two different molecules using the 1, 3-dipolar cycloaddition of azomethine ylides. The molecules will target the specific receptors on cancer tumor cells and will help in accelerating cancer treatment. Clinical hyperthermia can treat organs for tumor/cancer treatment. It is also named as hyperthermia and can be generated by radio frequency, microwave and laser wavelengths. Blood vessels are not developed in the cancerous cells/tissues and possess lower thermal resistance than healthy tissue (Bica et al. 2007; Chiang et al. 2007). Moreover, magnetic nano-fluids can be used to target the tumor with magnets. This allows to deliver local doses of drugs or radiation without damaging nearby healthy tissue, which is a significant side effect of traditional cancer treatment methods. Additionally, adhesion to tumor cells than non-malignant cells makes magnetic nanoparticles as an excellent candidate for cancer therapy (Jeevanandam et al. 2018).

Magnetic nanoparticles possess excellent characteristic for manipulation of the nano-fluids by magnetic force as compared to other metal type nanoparticles (Kumar and Kumbhat 2016). The nano-fluid magnetic nanoparticles also act as a super-paramagnetic fluid which absorbs energy and this enhances the chemotherapeutic treatment. The hyperthermia is intentional to produce a preferential radiation effect on the malignant cells. Additionally, iron based nanoparticles can be used as drug-delivery vehicles without damaging healthy cells or tissues. It has been suggested that particles can be attached to tumor using the magnets. Moreover, nano-fluids can also be used for safer surgery by producing effective cooling effect near the surgical region. It is well acknowledged that magnetic nanoparticles absorb much more electrical power than micro-particles and this is vital as nanoparticles are more adhesive to cancer tumor cells than normal cells. Consequently, magnetic nanoparticles excited by a magnetic field are considered as favorable for cancer therapy.

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## **4.13 Other Applications**

### **4.13.1 Intensify Microreactors**

The improvement of heat transfer by using nano-fluids has potential in the area of process intensification of chemical reactors. It has been studied that nano-fluid  $TiO_2$  material can be dispersed in ethylene glycol in reactor heat exchanger. It was found that the overall heat transfer coefficient has been increased up to 35% and this resulted in a closer temperature control in the reaction of selective reduction of an aromatic aldehyde by molecular hydrogen.

### **4.13.2 Nano-fluids as Vehicle Brake Fluids**

It was studied that the nano-fluids can be used in the process of braking and the brake fluid in the hydraulic braking system and as breaking oil (Nelson et al. 2009).

Nano-fluids are produced by using the arc-submerged nanoparticle synthesis system and the plasma charging arc system (Lv et al. 2014). It was found that nano-fluids have improved properties than that of traditional brake fluid.

### **4.13.3 Nano-fluids-based Microbial Fuel Cell**

It has been found that microbial fuel cells (MFC) utilized the energy which is found in the proteins, carbohydrates, and other natural products to generate electrical power (Wang and Mujumdar 2007) and their performance of MFC was mostly dependent on the electrodes and electron mediator. The performance of the MFC using CNT-based nano-fluids and CNT-based electrodes has been studied against plain graphite electrode based MFC which showed as high as six fold increase in the power density compared to graphite electrodes.

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## **4.14 Limitations of Nano-fluids**

The applications of nano-fluid constrained by many factors which we discuss below:

### **4.14.1 Poor Long-term Stability of Suspension**

It has been reported (Lv et al. 2014) that physical or chemical methods have been applied to get stable nano-fluids like an addition of surfactant, surface modification of the suspended particles, and applying strong force on the clusters of the suspended particles. It was found that  $\text{Al}_2\text{O}_3$  nano-fluids kept after one month exhibit some settlement compared to fresh nano-fluids (Akoh et al. 1978).

### **4.14.2 Increased Pressure Drops and Pumping Power**

The efficacy of nano-fluid application is determined by pressure drop developed and required pumping power during the flow of coolant (Swati et al. 2017). It is recognized that higher density and viscosity lead to higher pressure drop and pumping power. However, different studies have showed significant increase of nano-fluids pressure drop compared to base fluid (Akoh et al. 1978).

### **4.14.3 Lower Specific Heat**

Several studies have shown that nano-fluids exhibit lower specific heat than base fluid.

#### 4.14.4 High Cost of Nano-fluids

Preparing nano-fluids requires advanced and sophisticated equipment and normally associated with higher production cost of nano-fluids.

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#### 4.15 Application of Different Types of Nanomaterials

Nanoparticles (NPs) and nanostructured materials (NSMs) can be organized into four material-based categories (Khan et al. 2019) as described in Table 4.2.

It has been reported that nanoparticles can be also obtained from the animals (bird and Mollusk shells). It was reported that the human body consists of nanoparticles that help in the formation of many body tissues, cells, and genetic. Additionally, genetic materials which are present in the organisms can interact with other nanoparticles and play a major role in the nanodrug development. The nanoparticles which are present in the bone inorganic nano-hydroxyapatite and organic collagen. Furthermore, microorganisms like viruses and bacteria are classified as nanostructures that can cause diseases in humans (Khan et al. 2019; Abd Ellah and Abouelmagd 2016). Nanoparticles can be further classified into different classes based on their properties, shapes, and sizes. There are different types of nanomaterials like ceramic NPs, fullerenes, metal NPs, and polymeric NPs (Gong et al. 2015). Moreover,

**Table 4.2** Application of nanomaterials

Carbon-based nanomaterials	Inorganic-based nanomaterials	Organic-based nanomaterials	Composite-based nanomaterials
NMs contain carbon, hollow tubes, ellipsoids, or spheres. Fullerenes (C60), carbon nanotubes (CNTs), carbon nanofibers, carbon black, graphene (Gr). Carbon onions are included under the carbon-based NMs category. Laser ablation, arc discharge, and chemical vapor deposition (CVD) are important production methods for these carbon-based materials fabrication	NMs include metal and metal oxide NPs and NSMs. These NMs can be synthesized into metals such as Au or Ag NPs, metal oxides such as TiO <sub>2</sub> and ZnO NPs, and semiconductors such as silicon and ceramics	NMs made mostly from organic matter-excluding carbon-based or inorganic-based NMs. The utilization of noncovalent (weak) interactions for the self-assembly and design of molecules helps to transform the organic NMs into desired structures such as dendrimers, micelles, liposomes, and polymer NPs	NMs are multiphase NPs and NSMs with one phase on the nanoscale dimension that can either combine NPs with larger or with bulk-type materials complicated structures, such as a metalorganic framework. The composites may be any combinations of carbon-based, metal-based, or organic-based NMs with any form of metal, ceramics

semiconductor materials are present in between metals and non-metals and are found to be efficient in water splitting applications. In addition, polymeric nanoparticles are used in many biomedical applications such as drug carriers and delivery and RNA release in cancer therapy (Abd Ellah and Abouelmagd 2016)

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## 4.16 Future Research in Nano-fluids

Research must be conducted in the area of nano-fluids with low viscosity. Studies in this area reported are very limited and also on nano-fluids under high temperature applications. This is important and critical for nano-fluids since it may change their thermophysical properties. It is also recommended to study the enhanced thermal conductivity of nano-fluids for specific applications like biomedical applications. More research are required in the production of nano-fluid and its cost since it has become a barrier for its applications and commercialization.

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# Application of Nanomaterials in Stem Cells, Tissue Engineering and Regenerative Medicine

5

Peter Hollands

## Abstract

This chapter reviews the current state of the art in the use of nanomaterials in stem cell technology, tissue engineering and regenerative medicine. This new approach to therapy brings together physicians, clinical scientists, bioengineers and physicists in novel collaborations to produce innovative products and delivery systems which will revolutionise the practice of clinical regenerative medicine.

## Keywords

Nanomaterials · Stem cells · Tissue engineering · Regenerative medicine

## 5.1 Introduction

*“Though she be but little, she is fierce!”*  
(Shakespeare, A Midsummer Night’s Dream)

Stem cell technology has developed across the past 50 years to a point today where we know of stem cells present in bone marrow (Edwards 2004), umbilical cord blood (Gluckman and Rocha 2005), umbilical cord tissue and amniotic fluid (Joerger-Messerli et al. 2015), placenta (Macholdova et al. 2019), adipose tissue (Si et al. 2019), skin (Gonzales and Fuchs 2017), teeth (Hollands et al. 2018), human embryos (Ilic and Ogilvie 2017) and many more are discovered almost every day. There has also been the development of induced pluripotent stem cells (iPSC) which

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P. Hollands (✉)  
Fertility and Gynaecology Academy, Lipocube Ltd, London, UK

are “man made” stem cells created from somatic cells and they have potential in both therapeutic and research applications (Hamazaki et al. 2017). A natural progression from iPSC technology has been the trans-differentiation of somatic cells directly to the target cells of interest without going through the pluripotent stem cell or progenitor cell stage (Rackham et al. 2016) using key transcription factors. In addition there has been considerable development in the isolation of acellular exosomes (cellular vesicles 1–4 µm in diameter) recently which have many potential uses in regenerative medicine (Bjorge et al. 2017) and similarly for very small embryonic like (VSEL) stem cells found in the peripheral circulation and many tissue which are dormant naturally but may have considerable potential in regenerative medicine and longevity (Ratajczak et al. 2008) and even in the better understanding of psychiatric disorders (Kucharska-Mazur et al. 2018).

In parallel to these ground-breaking discoveries in stem cell science there has been considerable developments in nanomaterials and other products which can enable or enhance the use of stem cells in various applications. These include alginate hydrogels to promote osteogenic differentiation (Pullisaar et al. 2015), mesoporous silica nanoparticles to enhance the development of cardiomyocytes from embryonic stem cells (Ren et al. 2015) and the use of graphene to enhance stem cell growth and differentiation (Lee et al. 2011a). Further recent research in nanomaterials includes the discovery and development of quantum dots (Li et al. 2014; Qui et al. 2015; Yukawa and Baba 2017), carbon nanotubes (Ruenraroengsak et al. 2016) and DNA nanostructures (Li et al. 2013).

These parallel advances in stem cell technology and nanomaterials bioengineering have already shown great promise in areas such as the differentiation of a range of stem cells into target cells such as the use of gold nanoparticles to induce osteogenic differentiation using human adipose tissue mesenchymal stem cells (Choi et al. 2015) and the production of cardiomyocytes from human embryonic stem cells (Tung et al. 2014). This chapter will explore the current use of nanomaterials in stem cell technology, tissue engineering and regenerative medicine and where possible look into the possible future of this critical symbiosis of technologies.

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## 5.2 Mesenchymal Stem Cells (MSC)

Perhaps one of the most important cells in future regenerative medicine technology is the mesenchymal stem cell (MSC) (Wolmarans et al. 2019). It is therefore extremely important to assess the interactions of nanomaterials and MSC as part of the translational process from the laboratory to the clinic and the possible safety issues which may arise during this process. These clinical applications will have to be preceded by a clear understanding of the fate of stem cells on transplantation which can be accomplished using nanoparticles in technology which has become known as stem cell imaging (Solanki et al. 2008). Several workers have used nanoparticles (NP) including iron oxide NP in the non-invasive tracking and

homing of MSC (Huang et al. 2014) and iron oxide NP have also been used to track stem cells *in vivo* using magnetic resonance imaging (MRI). This is a significant step towards the development of regenerative medicine-based therapy based on clear knowledge and understanding of the fate of the cells used (Mahmoudi et al. 2011). A similar approach using gold NP has been taken using computerised tomography for stem cell tracking in the brain in patients suffering from neuropsychiatric disorders (Betzer et al. 2014). The utility of quantum dots (QD) has also been shown to be useful as contrast agents as another example of nanomaterials being used in clinical applications (Taylor et al. 2012; Dupont et al. 2010).

The nanoparticles which are being used in this type of research are defined as being in the range of 1–100 nm in diameter and a surface area > 60 m<sup>2</sup>/cm<sup>3</sup> as determined by atomic force microscopy (Napierska et al. 2010; Baalousha et al. 2014). Initially nanoparticles were spherical but various nanoparticle products have now been developed including rods and tubes (Xiao et al. 2007), prisms (Hu et al. 2005; Jitianu and Goia 2006), cubes (Long et al. 2010) and hexagons (Ahmed et al. 2009). Nanoparticles have been developed for clinical use using their magnetic and optical properties to enable real-time tracking of intracellular processes to enable stem cell imaging in regenerative medicine (Deb et al. 2012; Villa et al. 2010; Solanki et al. 2008). In addition to these important applications in regenerative medicine nanoparticles have also been used to enable drug delivery using microgel carriers (Lehmann et al. 2014), dendrimer complexes have been used for gene delivery in gene therapy (Shcharbin et al. 2013) and as nanocarriers in chemotherapy (Perez-Herrero and Fernandez-Medarde 2015). A combination of drug delivery and imaging, referred to as nanotheragnosis is one of the many advanced methodologies being based on nanoparticles (Arias 2011; Jung et al. 2013) along with the novel applications of nanoparticles in therapeutic approaches to cancer (Da Rocha et al. 2014; Shenoi et al. 2011). It should however be remembered that this is relatively new technology and, despite initial data showing it to be safe, long-term studies are needed to ensure safety (Accomasso et al. 2016).

Endocytosis of nanomaterials by cells, and the formation of lysosomes or endosomes containing nanomaterials, could result in a toxic effect to the cell even in very small doses (Wang et al. 2012). Particular attention needs to be paid to the possible immunotoxicity, genotoxicity and epigenetic toxicity of nanomaterials (Dusinska et al. 2017). Another route to possible toxicity through the clinical use of nanomaterials is the possibility that nanomaterials could damage the cytoskeleton within the cell, thus potentially disrupting the natural physiological functions of the cell such as adhesion, motility and cellular interactions with the surrounding microenvironment (Gupta et al. 2004; Laffon et al. 2018). Once internalised by the cell nanoparticles could also contribute to membrane damage (Nel et al. 2006; Karlsson et al. 2013), DNA damage (Singh et al. 2009; Mortezaee et al. 2019) and disruption of cellular transcription and translation (Pisanic et al. 2007; Chun et al. 2018).

### 5.3 Graphene and Mesenchymal Stem Cells

Graphene oxide has been shown to be a useful growth factor delivery carrier to promote chondrogenic differentiation of human mesenchymal stem cells in 3D hydrogels (Zhou et al. 2019). Such technology could provide a route to therapy for diseases such as osteoarthritis which not only represents high morbidity on a global scale but also a considerable socioeconomic burden (Hunter et al. 2014; Lewis et al. 2019). Traditional therapy of osteoarthritis relies partly on the innate regenerative potential of cartilage (Vinatier et al. 2009) and most significantly symptomatic therapy (Zhang et al. 2016) using anti-inflammatory medications such as steroids and non-steroidal anti-inflammatory drugs (NSAID).

The standard approach to the use of mesenchymal stem cells in tissue engineering is to have the stem cell of choice (in this case a mesenchymal stem cell), a 3D scaffold on which the stem cells differentiate into the target tissue and selected growth factors to induce the differentiation of the target tissue (O'Brien 2011; Lee et al. 2011b; Link et al. 2019). One of the key challenges in this approach is to provide the correct dose of growth factors and to ensure that these growth factors are present and active on the target cells for the correct amount of time (Ikada 2006). More recent developments in growth factor delivery technology have shown a controlled growth factor release when using 3D-printed hydrogels (Wang et al. 2019) and the use of nanomedicine loaded hydrogel for the delivery of angiogenic growth factors to ischaemic myocardium (O'Dwyer et al. 2019).

Graphene and graphene oxide have also shown promise in the construction of cellular substrates for the adherence of human osteoblasts to mesenchymal stromal cells (Kalbacova et al. 2010) and as an in vitro biosensing material in neuroscience as a neuron culture substrate (Fischer et al. 2018). Graphene oxide coating on a 2-D substrate has been shown to be bioactive in supporting of adipose derived mesenchymal stem cells in the differentiation towards bone, adipose tissue and epithelium (Kim et al. 2013). The safety and efficacy of graphene oxide as an ex vivo cell expansion substrate for umbilical cord (Wharton's jelly) mesenchymal stem cells has demonstrated that graphene oxide is novel, safe and biocompatible to these mesenchymal stem cells which for clinical regenerative medicine procedures will probably always require expansion before use (Jagiello et al. 2019). Such technology illustrates the safety, efficacy and promise of graphene nanomaterials in regenerative medicine which with the correct level of funding will lead in the future to better, more accessible, regenerative medicine protocols with the resultant reduction in morbidity and mortality.

### 5.4 Quantum Dots

Quantum dots are best described as nanocrystal semi-conductors with a typical diameter of 2–10 nm (Lei et al. 2008). Quantum dots have narrow, symmetrical and varying emission spectra resulting from the quantum-confinement effect (Norris and Bawendi 1996) resulting in fluorescence which can be used to track and identify

cell types with increased brightness and efficiency compared to traditional fluorophores. Following on from thus initial observations there was a rapid growth in the use of quantum dots in bio-imaging applications (Chan and Nie 1998) which has led on to more recently to the investigation of the potential of graphene quantum dots for theranostics and bio-imaging applications (Schroeder et al. 2016). Similar work has proposed the use of graphene quantum dots in the bio-imaging of cancer cells and real-time molecular imaging (Li et al. 2018a) which could enhance future diagnosis and treatment of cancer. The most recent work on quantum dots seeks not only to use their photoluminescence properties but also to investigate the use of spectrally tunable carbon dots to enable high resolution bio-imaging (Zhi et al. 2019). Equally recent work supports the concept that quantum dots are safe to use in diagnostics and biosensor studies (Fatahi et al. 2019) although there is still much work to do on quantum dot safety and efficacy because some animal studies have already shown possible cardio-toxicity of quantum dots which raises the need for further cautious clinical trial (Li et al. 2019). Perhaps the most exciting application of quantum dots to date is to use them for *in vivo* tracking of cells in regenerative medicine procedures (Fath-Bayati et al. 2019). Such applications will enable a clearer understanding of the mechanisms behind the complex cellular interactions which occur between cells during cell transplantation and cell regeneration.

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## 5.5 Nanofibers

Polymeric nanofibers, created by phase separation, self-assembly or electrospinning, are becoming the nanomaterial of choice when needing to replicate the morphology and structure of the natural extracellular matrix (Lu et al. 2013). By using self-assembly and phase separation it has been possible to create short nanofibers, of 10 nm diameter, such as RADA16 peptide nanofiber. The RADA16 peptide nanofiber has been used in the support of the growth of hippocampal neurons which in turn produced synaptic neurites (Zhang et al. 1993; Holmes et al. 2000). Peptide nanofiber scaffolds have been shown to be able to contribute to the re-growth of brain tissue (Leung et al. 2012) and axon regeneration resulting in the return of vision (Ellis-Behnke et al. 2006). These studies have led to further innovations in the use of nanofibers in nerve generation such as using mussel protein-glue based nanofiber nerve regeneration conduits (Cheong et al. 2019). There is clearly considerable promise in the utilisation of nanofibers in regenerative neurology.

Nanofibers can also be created using electrospinning technology to produce long continuous strands of nanofiber with diameters from 50 to 1000 nm (Lu et al. 2013). Electrospinning allows the formation of mesh structures with pore sizes which facilitate cell proliferation and migration (Lao et al. 2011). The development of electrospinning technology to produce nanofiber structures has introduced the possibility of using them to enhance and promote wound repair (Ghosala et al. 2019) and to the production of cellulose nano-whiskers, in combination with silver nanoparticles, which has produced an antibacterial polymer membrane with extremely promising applications in areas such as medical packaging, membrane filtration, wound

dressings and even in the manufacture of clothing for medical professionals (Spagnol et al. 2018).

Further developments in electrospinning technology of nanofibers have shown that it is possible to incorporate various biological proteins, peptides and growth factors into the nanofiber scaffold to enhance the biological activity of nanofiber scaffolds especially in bone regeneration (Sun et al. 2011). The use of mesenchymal stem cells, in combination with electrospun nanofibers, has also been shown to have enormous potential in bone and cartilage tissue engineering (Li et al. 2005; Li et al. 2006; Shih et al. 2006; McCullen et al. 2007; Shafiee et al. 2011). More recently human mesenchymal stem cells have been co-cultured with endothelial cells, on an electrospun scaffold, and such cultures have been able to generate bone tissue with significant osteogenesis when compared to mono-culture technology (Yao et al. 2019). It is of particular note that adipose derived stem cells (mesenchymal stem cells) can differentiate to osteogenic cells without any induction media if the adipose stem cells are cultured with electrospun poly (L-acetic acid)/collagen nanofibers which can retain calcium phosphate (Ravichandran et al. 2012). These studies open up significant opportunities to introduce such technology into repair of traumatic bone damage in orthopaedic procedures and also in the regeneration of bone tissue in chronic bone disease. Such an approach in the future could significantly reduce the morbidity and mortality of many orthopaedic patients.

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## 5.6 Nanomaterials in Neural Regenerative Medicine

Nanomaterials and nanodevices have been shown to have considerable potential in the field of neural cell regeneration (both in the peripheral and central nervous system) when they are incorporated with the relevant growth factors (Faustino et al. 2017; Sarker et al. 2018). It has also been demonstrated that synthetic, negatively charged, nanoparticles can modulate the immune system. Such immune-modifying nanoparticles could have a big part to play in pharmacotherapy based on nanoparticles (Getts et al. 2015) and have been shown to interact with and modulate antigen presenting, T, B and NK cells (Ben-Akiva et al. 2018) opening up opportunities to treat a wide range of immunological based neurological pathologies.

One of the major challenges in neuronal regenerative medicine is to develop technologies and clinical translation to repair traumatic damage to the spinal cord (Hutson and DiGiovanni 2019). Nanomaterials have a significant role to play in this context acting as neuronal stem cell scaffolds, microenvironment provision and growth factor release in order to facilitate repair (Song et al. 2019). An interesting nanomaterial approach to traumatic spinal cord damage is the use of Paclitaxel (chemotherapy) encapsulated liposomes held in a collagen microchannel scaffold (Li et al. 2018b). This approach using nanomaterials provides a sustained release of Paclitaxel which enhances the differentiation of both grafted and endogenous neural stem cells and in this animal study there were signs of locomotion recovery for up to 8 weeks. This concept of growth factor and/or drug delivery using nanomaterials has been shown to increase neuronal cell survival, cell retention and cell recruitment

(Andreas et al. 2014). Hyaluronic acid in cell regulation has been shown to be a good candidate especially in the context of stem cell niche regulation (Tiwari and Bahadur 2019).

There has also been interest in the use of polysialic acid incorporated into an electrospun nanofiber scaffold as a potential treatment for traumatic spinal cord injury (Zhang et al. 2018). Nanomaterials based on reduced graphene oxide foam have been shown to support the infiltration and migration of endogenous cells and resultant myelination and angiogenesis in an animal model (Domínguez-Bajo et al. 2019). A similar concept has been used to assess the use of electrospun graphene oxide-PLGA hybrid nanofibers containing insulin-like growth factor 1 (IGF1) and brain derived neurotrophic factor (BDNF). This approach showed that the graphene oxide/growth factor combination provided protection for neuronal stem cells against oxidative stress and enhanced the proliferation and differentiation of neuronal stem cells (Pan et al. 2019). A recent publication has described the use of carbon nanotube micropillars to support the growth and development of complex human neuronal stem cell networks. This observation that neural stem cell networks can be developed using nanotechnology opens up the possibility of regenerative medicine technology being used to repair and restore clinically significant damage or trauma to the central nervous system (Lorite et al. 2019). These experimental studies provide a solid base for further experimental verification and clinical trials of the nano-material technology in central nervous system neuronal regeneration in both trauma and disease.

The peripheral nervous system is also an important clinical target for regenerative medicine technologies using nanomaterials. The delivery of growth factors and their related genes, such as brain derived neurotrophic factor, to the area of peripheral nerve damage has been shown to be promising (Lopes et al. 2017). In addition, the use of nanomaterial chitosan conduits to provide a bridging structure in peripheral nerve regeneration is showing equal promise (Li et al. 2018c). A similar approach has been used using nanoengineered chitosan/CaTiO<sub>3</sub> hybrid scaffolds to promote Schwann cell growth and subsequent peripheral nerve regeneration (Li et al. 2017) and more recently a similar result has been achieved using electrospun peptide scaffolds (Nune et al. 2019).

Arguably one of the most promising areas of nanotechnology in neuroscience has developed around the use of gold nanoparticles (Wang and Guo 2016). Particular interest has developed in the modulation and stimulation of neural activity including the modulation of intracellular calcium using laser exposure of gold nanorods (Paviolo et al. 2014) and thermosensitive ion channel activation using plasmonic nanoparticles (Nakatsuji et al. 2015). Gold nanorods, when exposed to laser light, can stimulate neuronal cell outgrowth (Paviolo et al. 2013) and intraspinal administration of polyethylene glycol coated gold nanoparticles has been shown to produce functional recovery following traumatic damage to the spinal cord (Papastefanaki et al. 2015). It has also been shown that gold nanorods aid the stimulation of auditory neurons (Yong et al. 2014) and a similar result has been found when using plasmon resonance of gold nanorods which resulted in neural stimulation (Eom et al. 2014). It is also interesting to note that infrared sensitive nano-transducers can

inhibit neural activity by photothermal inhibition which could be a model for the regulation of neural activity in diseases such as epilepsy (Yoo et al. 2014).

The use of gold nanoparticles in the manipulation and regeneration of peripheral nerves in experimental models has shown considerable promise in terms of the elongation of axons and the establishment of complex neuronal networks (Baranes et al. 2016). Gold nanoparticles, when combined with silk fibers, have been shown to be capable of promoting the adhesion and proliferation of Schwann cells in an experimental model *in vitro* and most importantly there were no signs of either toxicity or immunogenic responses *in vivo* (Das et al. 2015). Similar work has shown that chitosan/gold micro-grooved nerve conduits can enable neuronal stem cells to support the regeneration of the sciatic nerve (Lin et al. 2008). This work has been confirmed more recently and represents a significant step forward in the future clinical use of gold-based nanomaterials in peripheral nerve regeneration (Saderi et al. 2018). It is also possible that the electrical properties of gold nanoparticles may be used to promote peripheral neurite elongation (Park et al. 2009).

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## 5.7 Nanomaterials in Bone Regenerative Medicine

The repair of bone following traumatic injury or the regeneration of bone in disease has been the focus of attention of stem cell biologists for many years (Lopes et al. 2018) because of the potential positive impact it could have on the morbidity and mortality of a considerable number of patients on a global scale. There are many factors which can limit or inhibit bone regeneration and one key factor is infection resulting in osteomyelitis (Garcia del Pozo et al. 2018). Osteomyelitis results when there is a deep set, intractable infection in bone and getting antibiotics into the site of infection, often very deep inside the bone, proves difficult if not impossible. An innovative, nanomaterial based, approach to this problem has recently been proposed involving the use of silver nanowires (De Mori et al. 2019). These workers developed a hydroxyapatite, chitosan and silver nanowire scaffold which was shown to be both antibacterial and bioactive *in vitro*. Such technology can now proceed to clinical trial as a potential therapy for chronic and acute osteomyelitis by utilising the antibiotic properties of silver in the silver nanowire.

An equally important use of nanomaterials in bone regeneration is the regeneration of bone in dental applications which is a complex and challenging field (Langer and Vacanti 1993; Guazzo et al. 2018). The incorporation of nanomaterials into the many scaffolds also under investigation in dental bone regeneration is already showing great promise (Nishida et al. 2016). Perhaps one of the most promising nanomaterials in the context of dental bone regeneration and countless other applications is graphene and its derivatives (Shin et al. 2016). There are an ever increasing number of graphene nanomaterials being developed including graphene oxide, graphene nanosheets and few-layered graphene which can provide differing size, surface properties and number of layers (Jastrzebska et al. 2012) and the biosafety of graphene oxide on cells *in vitro* seems to be acceptable (Chang et al. 2011).

In order to use graphene-based nanomaterials clinically, and indeed all other nanomaterials, there must first be a clear demonstration of biocompatibility to ensure that the nanomaterial does not result in any harm to the recipient (Lee et al. 2018). Many studies have been carried out on the biosafety of nanomaterials and the consensus at present is that more work is needed to be sure that the graphene nanomaterials are safe for human use (Singh and Nalwa 2007; Zhao and Nalwa 2007).

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## 5.8 Discussion & Conclusion

This chapter has introduced the potential use of nanomaterials, in conjunction with stem cell technology, in regenerative medicine. There is clearly an enormous clinical potential in this area of biotechnology which brings together clinical scientists, physicists, biotechnologists, physicians, biotechnology companies and all other related professions to create successful research and development of this technology. Much of the data at present are experimental but encouraging. In the future we need to develop and carry out clinical trials to demonstrate the safety and efficacy on nanotechnology in regenerative medicine. Once these trials are safely and effectively completed this will lead to the introduction of nanotechnology into routine clinical practice which will also enable the long-term epidemiology of the technology to be assessed.

An important factor in the use of nanomaterials in regenerative medicine will be the source and type of stem cells used in conjunction with nanomaterials. This could from an autologous source such as very small embryonic like stem cells (VSEL) which are pluripotent and easily collected in platelet rich plasma. Laser activation (Zamani et al. 2019) of VSEL, to make VSEL into active cells, could be an excellent partner for nanomaterials. Another autologous approach could be the use of adipose tissue stem cells which are easily collected from the patient and, with non-enzymatic processing technology, can be collected and processed as and when required by the patient. Potential allogeneic sources of stem cells could be any type of mesenchymal stem cell as these can be transplanted without a concern for rejection by the recipient patient. Such mesenchymal stem cells could be collected from donors, processed, cryogenically banked and released for patient use when required. A more complex approach could include the use of induced pluripotent stem cells (iPSC) or even target cells derived from transduction technology.

Research is needed to evaluate whether autologous or allogeneic are the best choice of stem cells to combine with nanomaterials. It may be that each different disease or trauma calls for a different nanomaterial/stem cell combination which is why it is important to keep an open mind on which is the best way to proceed. Whatever happens any future nanomaterial/stem cell therapy must be evidence based and supported by clear beneficial data from clinical trials.

This final step, bringing the nanotechnology into routine clinical practice, will need careful regulatory input to ensure that the correct policies and procedures are utilised to protect patients and that the new nanotechnology medical devices are manufactured and tested to the highest possible standards. The production of stem

cells for nanomaterial use will need national and international regulation to ensure safety and best practice as will the nanomaterials which will be categorised as medical devices. This combination of innovation, collaboration, basic research and clinical trial using nanotechnology will open up a new era in the treatment of many diseases and injuries.

*“Nanotechnology in medicine is going to have a major impact on the survival of the human race”*

Bernard Marcus

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# Applications of Nanomaterials in Neurological Diseases, Neuronal Differentiation, Neuronal Protection, and Neurotoxicity

Amani H. Alhibshi, Widyan A. Alamoudi, and Rai K. Farooq

## Abstract

The central nervous system (CNS) is one of the most important systems in the human body, and thus, CNS disorders are causing a significant threat to human health. Researchers from around the world are making impressive efforts to come up with therapeutics and solutions to treat neurodegenerative disorders. However, the issue of brain targeting remains an unsolved challenge due to the blood-brain barrier (BBB) existence. Due to the many unique properties of engineered nanomaterials, their use could make it possible to overcome difficulties in the diagnosis and treatment of neurodegenerative disorders, provide promising neuroprotective strategies, and stimulate neuronal differentiation and nerve generation as a therapeutic approach. In contrast, despite the rapid development of the nanomaterials industry and the spread of its applications in the biomedical field, there is lacking evidence regarding their possible adverse health effects, and very little is known about their toxicity. Numerous *in vivo* and *in vitro* studies have emerged, providing evidence of neurotoxic effects of various types of nanoparticles (NPs), and therefore the advantages of nanomaterials should be weighed against their potential effects. In this chapter, we focused on the applications of nanomaterials in neurological disorders, neuronal differentiation, neuroprotection, and neurotoxicity.

## Keywords

Nanodiagnostics · Neurodegenerative disorders · Neurotoxicity · Oxidative stress · Neuroprotection · Drug delivery · Blood–brain barrier · Neural differentiation · Neurite outgrowth · Structure scaffold

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A. H. Alhibshi (✉) · W. A. Alamoudi · R. K. Farooq

Department of Neuroscience Research, Institute for Research and Medical Consultations (IRMC), Imam Abdulrahman Bin Faisal University, Dammam, Saudi Arabia  
e-mail: [a.alhibshi@iau.edu.sa](mailto:a.alhibshi@iau.edu.sa)

## 6.1 Introduction

Nanotechnology is a considerable interdisciplinary field that governs the synthesis and characterization of materials in nanoscale and their utilization in various areas ranging from cosmetics, electronics, food to biotechnology, and biomedical research (Han et al. 2017). Nanomaterials are fabricated to give different forms, for instance, spherical, rod, crystalline, and pyramidal nanoparticles (NPs) (Quadros and Marr 2010). Others give rise to more complex forms such as carbon nanotubes, nanowires, metallic, liposomes, semiconductors, polymeric, and viral NPs (Dayem et al. 2014). Comparing to viruses, cells, and proteins, NPs have a comparable size ranging from 1 to 100 nm dimensions, which enable them to interact with distinct biological processes (Khan et al. 2018a).

Unlike large scale materials, NPs have better physical properties, higher functionalities, and cellular uptake ratio and larger surface to mass ratio (Oberdörster et al. 2007). Therefore, nanomaterials demonstrate profound promising capacities in drug delivery, cancer imaging, treatment, and other clinical oriented purposes (Portney and Ozkan 2006; Park and Webster 2005). NPs have become even more favorable to be used for the central nervous system (CNS) insults in terms of diagnosis and treatment (Kumar et al. 2017). The effective intervention of CNS disorders has encountered many challenges. For instance, the complex nature of the brain in terms of structure and function with a lack of proper biomarkers adds more complications for neurological disorders diagnose and therapy. Moreover, there are treatments available that can only reverse the process of degeneration and inflammatory processes associated with neurological disease and mainly focuses on slowing the progression of the degeneration only (Shadfar et al. 2015). In addition, these treatments are insufficiently efficient due to the high selectivity of the blood–brain barrier (BBB), which makes nanotechnology extra advantageous (D’Agata et al. 2018).

NPs have been reported to be used as promoters for neural growth, protection, and regeneration, owing to their distinctive physicochemical properties (Khan et al. 2018b). Reasonable studies have been conveying the NPs usage to overcome some challenges in neuroscience, such as neuronal cell differentiation and nerve regeneration (Riggio et al. 2012; Riggio et al. 2014; Richert et al. 2008). Evermore, the combination approach of NPs and stem cell transplantation represents an important avenue to tackle previously mentioned hindrances. However, due to the highly active surfaces of the NPs, health-threatening issues such as cytotoxicity have been elicited. In this chapter, we elaborate on the applications of nanomaterial in neurological diseases, neuronal differentiation, neuroprotection, and toxicity, with supporting findings from both *in vivo* and *in vitro* studies.

## 6.2 Applications of Nanomaterials in Neurological Diseases

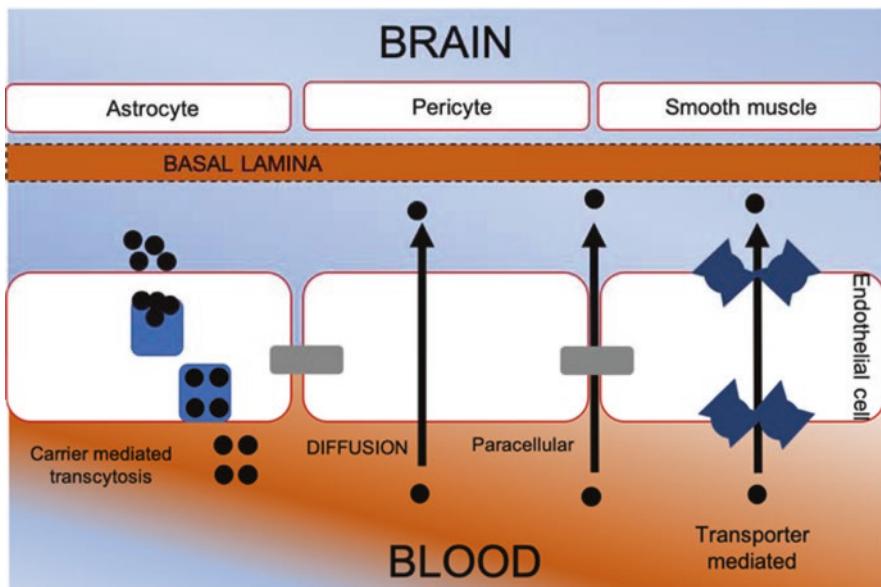
Neurological disorders are the diseases that affect the function of the brain, spinal cord, and nerves. Essentially, the whole body is under the control of the nervous system. The nervous system disorders can, thus, result in dysfunction of any of the body parts. From higher mental functions to vegetative functions and autonomic nervous systems, brain disorders may result in significant deterioration of well-being. Loss of brain and spinal cord cells is usually linked to neurodegenerative diseases and results in cognitive impairment, which initially represents a loss of memory and dementia. Important diseases in this category include Alzheimer's disease (AD), multiple sclerosis (MS), Parkinson's disease (PD), Creutzfeldt–Jakob disease, brain cancer, and ischemic stroke. Inflammation is an essential part of the degenerative process. Degeneration is defined as a process that involves inflammation, breakdown, and eventual loss of the structure and the function of the tissue involved (Skaper et al. 2018). Neurological control over bodily functions is exerted through motor and sensory nerves, neurotransmitters, and hormones. Dysregulation of neurotransmission is implicated in many neural systems malfunctioning. Another factor that is closely regulated in neuronal function is neuroplasticity. This is defined as the ability of neuronal tissue to regenerate to its original state after an injury or inflammatory event.

Despite many signs of progress, the currently available early diagnostics and strategies for treatments are limited. The brain has proved to be a difficult organ as a therapeutic target. The attempts to apply therapeutic approaches for brain disorders have had many obstacles due to its fragile and complex nature, barriers, limited regeneration capacity, and localization inside a bony cavity. Thus, the diagnosis and treatment of CNS disorders represent a considerable challenge.

With the help of a network of endothelial cells of different types, brain and spinal cord tissue are separated away from the blood by the BBB. The BBB is composed of brain endothelial cells making tight junctions that form the cerebral microvascular endothelium (Fig. 6.1).

This barrier stops the entry of molecules and cells from the blood to the brain tissue. The primary purpose of these junctions is to stop harmful compounds or molecules from entering the brain. However, small lipophilic molecules that are eventually needed for essential nutrients such as precursors and cofactors may enter. In addition to protection, BBB has proven to be a barrier against therapeutic molecules, as well. In addition, the BBB is also equipped with mechanisms that decrease the concentration of therapeutic molecules inside the brain. Crossing the BBB is a challenge that only a multidisciplinary approach can solve. It involves the creation of non-toxic, biocompatible carriers of small size that can penetrate the BBB and stay in the circulation long enough to have a therapeutic effect. Although many such candidates are racing for being the best to deliver drugs, vaccines, nucleic acids, and peptides, the clinical application remains somewhat elusive.

Nanomaterials can help overcome some of the problems associated with the availability of therapeutic molecules inside the brain. Due to their versatility, physical properties, and chemical functionalization, nanomaterials could cross the BBB



**Fig. 6.1** Blood–brain barrier: Structure and means of transport across the brain

alone or be modified to help increase drugs crossing over the BBB and increase the time and concentration of presence inside the brain (Fernandes et al. 2010). Currently, strategies are depending on the use of nanomaterials that can interact on a molecular level with BBB cells without interfering with the normal functions of the BBB. The methods being used to advance NPs include adsorptive-mediated transcytosis, which depends on an electrostatic interaction on the luminal surface of the endothelial cells. And receptor-mediated transcytosis, which is the more investigated physiological method which depends on the existence of receptors on the BBB that lead modified NPs through the BBB endothelium. In addition, lipoprotein receptors and transferrin receptors are also targeted to facilitate the transport of NPs across the BBB. Other possible mechanisms include retrograde transport, transport exploiting monocyte/macrophage infiltration in the CNS, and NPs mimicking activated monocytes (Bhaskar et al. 2010).

Nanotechnology has made advances in diagnosis, as well. The use of nanotechnology for this purpose is now officially called nanodiagnosis. It refers to diagnosing the events that occur at the nanoscale using a purposefully designed device in at least one dimension on that scale. Nanodiagnosis can help achieve early diagnosis and better sensitivity. It can also be used in conjunction with other conventional diagnostic and imaging technologies. This technology is helping us develop platforms for the detection of single nucleotide polymorphisms and other biomarkers of the diseases that are not detectable conventionally.

Brain tumors are among the most common CNS disorders with glioma representing 80% of all brain tumors. The sensitivity and complexity of the brain make it

difficult to surgically resect the tumors, especially after infiltration into normal tissues. Besides the BBB, the blood–tumor barrier (BTB), and drug efflux pumps on brain tumor cells are other obstacles that further restrict drug uptake into brain tumors. Therefore, nano-therapeutics can be utilized to encapsulate drugs and increase their crossing possibility through both the BTB and BBB and then prevent them from getting noticed by the drug efflux pumps. After that, the drugs are directly and effectively reach the tumor and display treatment (Gaoe et al. 2012). To overcome both the BTB and the BBB, several strategies have been developed.

One of the strategies aimed to deliver drugs into the brain is through adsorption-mediated endocytosis. Cationized albumin (CBSA) conjugated to NPs were developed to deliver the tumor necrosis factor-related apoptosis-inducing ligand (TRAIL) gene and cancer drug aclarubicin to tumors in mice brain. The concentration of aclarubicin in the tumors treated with CBSA-NPs was higher than its concentration in tumors treated with unmodified NPs. Additionally, mice repeatedly injected with TRAIL gene-loaded CBSA-NPs showed better anti-tumor effect compared to the unmodified NPs as additional treatment cycles significantly increased the mice survival time (Lu et al. 2007; Lu et al. 2006).

In addition, NPs encapsulated with the aptamer AS1411 (AsNPs) exhibited a better binding ability compared to control NPs and displayed higher localization and uptake by tumor cells. *In vivo*, it slowed the growth of the tumor and extended the mice median survival time compared to control particles (Guo et al. 2011).

Another strategy aimed to deliver therapeutics through the BBB's glutathione (GSH) transporter by using GSH conjugated liposomes to deliver drugs through the BBB which is called G-Technology. Another carrier used GSH pegylated liposomal doxorubicin, which may become the first nanomedicine due to its favorable attributes (Gaillard et al. 2012). In another report, an angiopep-2 modified cationic liposome (ANG-CLP) conjugated with (PEGFP-hTRAIL) plus paclitaxel (PTX) was used as therapeutic gene against glioma (Sun et al. 2012). This study utilized a dual co-delivery targeting strategy that enhances both the liposome uptake and gene expression in U87MG cell line, brain capillary endothelial cells (BCECs), and glioma bed. Compared to the unmodified co-delivery system and single therapeutic platform, the selective induced glioma cells apoptosis by this system was extensively noticeable (Sun et al. 2012).

Another targeting delivery study used RNA NPs (RNPs) as a vector for siRNA to silence genes within glioblastoma cells. The conjugated folate (FA) ligand within siRNA loaded RNPs mediated successful targeting and delivery. It also destroyed glioblastoma stem cells without causing non-specific accumulation of the NPs (Lee et al. 2015). Moreover, detecting brain glioma *in vitro* and *in vivo* models was achieved by using superparamagnetic iron oxide (SPIO) NPs conjugated with epidermal growth factor receptor monoclonal antibody (EGFRmAb) as an MRI contrasting agent (Mu et al. 2015).

Among neurological disorders, the neuronal damage in AD is associated with an abnormal amyloid-beta (A $\beta$ ) protein processing and accumulation extracellularly and the intraneuronal filaments of hyperphosphorylated tau protein that results in gradual cognitive and motor deterioration (Ittner and Götz 2011). Some strategies

tried to target A $\beta$  using NPs to reduce its levels in the brain and treat AD (Matsuoka et al. 2003). Nanoliposomes (NL) have shown a high affinity toward A $\beta$  (Bereczki et al. 2011) and the ability to reduce its toxicity in vitro and in vivo in postmortem AD brain samples when coated with an anti-A $\beta$ -antibody (Canovi et al. 2011). Additionally, curcumin coated NL showed inhibiting ability against A $\beta$  aggregates (Taylor et al. 2011). Using another type of nanomaterials, fullerenes C60 showed the ability to rescue rats against A $\beta$ -induced cognitive impairment (Podolski et al. 2007). Another study used H102 encapsulated liposomes for AD treatment. H102 is a novel breaker peptide that can interfere with the  $\beta$ -sheet in A $\beta$ , which results in inhibiting its formation and resolving its backbones, thus reducing neuronal inflammation and increasing neuronal survival (Zheng et al. 2015). H102 encapsulated liposomes were administered intranasally and were able to ameliorate the impaired memory of the AD rat model with both low and high doses (Zheng et al. 2015).

Considerable efforts are being focused in the research laboratory on using nanoneuromedicine for disease treatment. In AD, nanodiagnostics are useful tools for the early diagnosis of the disease by measuring concentrations of specific markers in human cerebrospinal fluid (CSF) that are not detectable by traditional assays. Using gold (Au) NPs, an ultra-sensitive method was developed to detect the approximate concentration of AD biomarkers (Georganopoulou et al. 2005; Neely et al. 2009). Moreover, an *in vitro* study showed that quantum dots (QDs) conjugated with streptavidin can effectively detect the presence of the amyloid precursor protein (APP) with high sensitivity compared with traditional method Fluro immunoassay (Feng et al. 2010).

*In vivo* studies have also been carried out to detect AD. A study developed ultra-small superparamagnetic iron oxide (IO) NPs, chemically coupled with A $\beta_{1-42}$  peptide, to detect amyloid deposition using magnetic resonance microimaging ( $\mu$ MRI) in AD transgenic mice (Yang et al. 2011). A developed online high-performance liquid chromatography-mass spectrometry (HPLC-MS) method based on nanoseparation of HPLC was able to monitor major phospholipids implicated in AD using CSF from AD patients (Kosicek et al. 2010). In a mouse model of AD, encapsulated Polymeric n-butyl-2-cyanoacrylate (PBCA) NPs were designed to detect amyloid plaques *in vivo* for early AD diagnosis providing a noninvasive technique (Roney et al. 2009).

PD is another neurologic disease seen in elderly individuals above the age of 65 that is characterized by the abnormal accumulation of cytoplasmic aggregates of the protein  $\alpha$ -synuclein forming Lewy's bodies that subsequently alter the substantia nigra area of the brain by causing loss of dopaminergic neurons. Difficulties in movement control characterize the disease, and on the cellular level, the dysfunction of the mitochondria, proteasome, and endoplasmic reticulum stress are thought to be the mechanisms (Cole and Murphy 2002).

Different studies focused on applying nanomaterials to detect  $\alpha$ -synuclein for PD diagnostic purposes. A photoelectrochemical immunosensor was designed using Au-doped titanium oxide (TiO<sub>2</sub>) nanotube array to detect  $\alpha$ -synuclein. The used nanotubes played an important role in the generation of the photocurrent during the assay and the stabilizing of the protein molecules. The sensor additionally provides

enhanced sensitivity and excellent stability, and a high ability that could be used to target and bind to a wide range of neurologically involved proteins (An et al. 2010). Researchers also developed a nanotechnology tool using the AFM force spectroscopy approach to detect early the protein misfolding and self-assembly of  $\alpha$ -synuclein, which are the initial steps that lead to its aggregation, a phenomenon seen in neurodegenerative diseases which will help in understanding disease development (Yu and Lyubchenko 2009). Another assay was developed using AuNPs to quantify neurotransmitters related to PD (Baron et al. 2005).

Regarding PD treatment, severe side effects are associated with long term usage of dopamine replacement therapy (DRT), which has been reported, such as sleep attacks and variation in disability (Group, P.S. 2004; Hobson et al. 2002). Gene therapy is currently adopted to treat PD; SNCA gene was considered as a therapeutic targeted gene (Khodr et al. 2011) and therefore, nanotechnology is applied to overcome some limitations with the non-viral delivery system. Nanocarrier strategy used magnetic iron (II, III) oxide ( $\text{Fe}_3\text{O}_4$ ) NPs coated with oleic acid molecules and loaded with  $\alpha$ -synuclein shRNA plasmids an effective inhibitor for  $\alpha$ -synuclein synthesis in PD in both *in vitro* and *in vivo* models (Niu et al. 2017). These super magnetic particles reduced toxicity effect of  $\alpha$ -synuclein by decreasing its expression and further suppressing the programmed cell death process (Niu et al. 2017). Also, gene delivery for PD therapy has been investigated using lactoferrin (Lf)-modified NPs as a non-viral gene vector with the ability to cross the BBB (Huang et al. 2010). The results showed an improved significant reduction in dopaminergic neuronal loss, locomotor activity, and enhanced dopamine levels in the PD rat brain.

Prion disease is a family of infectious neurodegenerative disorders such as Creutzfeldt–Jakob disease that occurs as a result of the abnormal misfolding of prion protein accumulation.

A group of researchers made efforts to remove the pathogenic isoform of the prion protein from infected cells using oppositely charged polyelectrolytes coated AuNPs to inhibit the prion protein aggregation and convert the isoform in neuronal GT1 and N2 cells, and prion treated mice (Tran et al. 2010). In another experiment, the coated AuNPs were able to cross the BBB and translocate into different areas of the brain, including the hippocampus, thalamus, hypothalamus, and the cerebral cortex close to locations of prion aggregations in mice, which shows the potential of AuNPs to serve as a diagnostic tool (Sousa et al. 2010). In prion disease diagnosis, a method was developed by using aptamers, micromagnetic particles, and QDs in which prion protein isoform was sandwiched and discriminated from cellular prion protein. The method additionally showed the ability to detect the protein in 0.01% brain homogenate compared to 10% in traditional methods (Xiao et al. 2010). In another study, PEG-interspersed nitrilotriacetic acid (NTA)-QDs were functionalized to specifically label prion protein expressed on cell surfaces (Xie et al. 2010).

More studies on neurodegenerative diseases have been conducted. Among other diseases amyotrophic lateral sclerosis (ALS) is defined by the loss of neurons responsible for voluntary muscle control in the brain and the spinal cord (Ambesh and Angeli 2015). Mutations in the gene of the antioxidant enzyme superoxide dismutase (SOD1) can cause the generation of free radicals and form deposits

intracellularly, which are implicated in the pathologies of ALS. Conjugated AuNPs and SOD1 monomers were used to detect aggregates of SOD1 with high sensitivity and colorimetric detection abilities compared to former methods (Hong et al. 2009).

Additionally, solid lipid NPs were prepared using the warm oil-in-water microemulsion technique and used as a drug carrier for riluzole, the drug used in ALS treatment. The NPs containing riluzole were administrated to rats, and rats were sacrificed after different time intervals and the riluzole concentration in the blood and organs such as the brain, liver, spleen, heart, and kidney was determined. The results demonstrated that the nanocarriers' solid lipid NPs successfully carried riluzole into the CNS in addition to other the organs, which shows a high drug loading and lower nonselective biodistribution abilities, and a greater efficacy compared to free riluzole in rats (Bondì et al. 2010). Immune disbalance is also one of the characteristics seen in ALS. MRI was utilized to monitor immune T-cells labeled with ultra-small paramagnetic iron oxide (USPIO) NPs in familial ALS rats with multiple copies of mutated human SOD G93A gene. The MRI revealed that CD4+ lymphocyte infiltrated the mid- and inter-areas of the brain, whereas the brainstem region only was infiltrated by CD8+ cells.

Moreover, the BBB was compromised in areas that were congruent with the MRI foci of T-cell infiltration. The activation of microglia was also observed using immunocytochemistry, indicating the exciting of inflammatory in rats' brains and the multifocality and complexity of ALS that causes inflammation in the CNS (Bataveljić et al. 2011). In a similar study, in addition to using MRI technology, nonlinear optical microscopy was also used to monitor USPIONPs cross-linked with antibodies (CLUSPIO) in ALS *in vivo* or *ex vivo*, respectively. Abnormal accumulation of lipids in motor neurons may be critically involved in the pathology of ALS. T-cell CD4+ was labeled in the transgenic rat model expressing the human SOD G93A gene using CLUSPIO antibodies. MRI showed an enhancement in signal intensity of pathological ALS rat brain regions specifically compared to controls and the nonlinear optical microscopy indicated cellular interactions based on lipids association to anti-CD4 CLUSPIO (Machtoub et al. 2011).

Other applications of NPs in CNS disorder investigation, intervention, and treatment have been reported. For instance, 2D carbon nanotubes (CNTs) derive synaptogenesis by altering neuronal membrane lipids homeostasis and shaping neurotransmission in hippocampal neurons culture throughout synapses development (Pampaloni et al. 2018). Carbon dots (C-dots) have a modulatory effect on synapses and neurotransmitters as well (Borisova et al. 2015). C-dots decreased synapses acidification, attenuated glutamate, and GABA uptake in nerve terminal (synaptosomes) in Wistar rat brain. Additionally, C-dots possess a great bioanalytical potential with fluorescent emitting properties that make them suitable for labeling and visualization purposes (Borisova et al. 2015). Moreover, silica NPs (Orlando et al. 2017) and curcumin–docosahexaenoic acid loaded carriers (Guerzoni et al. 2017) have been reported to enhance neuron viability and increase cell survival, respectively. The synergic effect of nanomaterial and tissue engineering combined approaches are potentially promising to employ as a strategy for neurodegenerative disorders by providing support for neurite and axon growth (Gilmore et al. 2008).

Another strategy was aimed to reduce the oxidative stress in neurodegenerative disorder by antioxidant NPs. Owning to their interactive ability with biological molecules, using antioxidant NPs will be much effective in treating reactive oxygen species (ROS) in damaged areas, which will be indicated in detail in the applications of nanomaterials in neuroprotection part of the chapter (Sandhir et al. 2015).

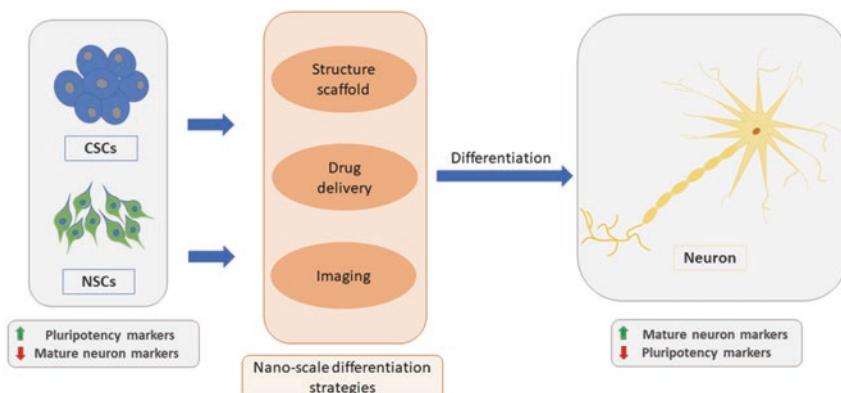
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### 6.3 Applications of Nanomaterials in Neuronal Differentiation

Cell differentiation covers a wide range of implications such as cancer cell differentiation, stem cells, teratocarcinoma stem cells, and neuronal-like cell differentiation. Each implication triggered by different factors, whether its changes in genetical, nutritional, or chemical elements (Murakami and Tashiro 2015). In neural cell differentiation, the neuronal body goes through various mechanisms such as growth, elongation, and bifurcation (Polak and Shefi 2015). Typically, several cellular modifications characterize this process involving alteration of cellular morphological, the elevation of the expression of cytoskeletal marker of neuron, which are the genes responsible for neural differentiation such as  $\beta$ -tubulin III (class III beta-tubulin or TuJ1). These markers are essential to promote neurite growth and maturation (Polak and Shefi 2015). Differentiation as therapy is considered as a promising approach and an alternative treatment of brain tumors owing to its ability to transform cancer stem cells (CSCs) to differentiated cells by the abolished self-renewable ability and thus limiting tumor growth (Massard et al. 2006). Additionally, the discovery of neural stem cells (NSCs) existence in the mammalian brain throughout lifetime boosts the potential of using this renewable ability in response to injury and neurodegenerative disorders (Santos et al. 2016).

Many researchers have implied stem cells (SCs) capability to grow, proliferate, differentiate into a lineage, recruit endogenous cells to the injury site, and stimulate these cells to enhance tissue regeneration and repairing (Alghazali et al. 2019). Thus, the differentiation process holds a huge prospect in cancer and neurodegeneration treatments, even gains more importance in case of injured neurons recovery. However, the low survival rate of NSCs progeny within the damaged environment and inefficient cells regenerative by endogenous neurogenic cells deliberated a hindrance for CNS insults treatment (Santos et al. 2016). Additionally, the availability of microenvironments, such as extracellular matrix (ECM) or scaffolds, is crucial to enhance and regulate cells' self-regeneration and differentiation by forming a supporting structure. In vitro studies deprived of such biophysical and biochemical supported niches make it challenging to maintain cell growth and differentiation (Jhala and Vasita 2015). Moreover, having control and guided tool for growth factors and chemicals to trigger their growth and differentiation in a controlled manner is also required.

The unique properties of NPs were employed as a guided tool for controlled growth and differentiation (Berns et al. 2016; Haddad et al. 2016; Saraiva et al. 2016). Moreover, micro- and nano-scaffolds have been synthesized to provide an artificial



**Fig. 6.2** Schematic outline of neuronal cell differentiation approaches via nanoparticles

ECM to address the previous issue (Alghazali et al. 2019). Numerous mechanisms have been reported in which NPs effectively stimulate neural cell differentiation and neurite growth with a wide range of NPs, such as metallic, metallic oxide, silica, QDs, lipid-based, polymeric, and hydrogel NPs. Through diverse strategies, NPs overcame conventional limitations to promote neural differentiation via drug delivery, scaffolds, and real-time imaging systems (Fig. 6.2) (Carradori et al. 2017).

In the nanoscaffolding strategy, NPs act as mimicry extracellular environments to support cell differentiation upon NSC transplantation to the injured target (Carradori et al. 2017). As a result, it might increase the viability of the cells by providing a favorable niche and boost neurogenesis in damaged or non-neurogenic regions (Carradori et al. 2017). The gold optical (plasmonic) properties have been integrated with synthetic ECM or different dimension structures and used as plasmonic surfaces for neuronal cell activation purposes (Alghazali et al. 2019). In another study, a 2D nanocomposite system was created by incorporating an active layer of gold nano-rods (Au NRs) on a plastic coverslip (Thermanox) to investigate their effect in stimulating neurogenesis potential of human mesenchymal stem cells (hMSCs). This study showed an acceleration in hMSCs differentiation into a neural lineage upon cells seeding on the Au NRs surface. The expression of the neural progenitor cells and mesenchymal specific biomarkers such as GFAP, calcium-binding protein B (S100 $\beta$ ) was detected after 24 hrs of seeding and preserved after 6 days of differentiation. The assessed morphological changes by type III intermediate filament (IF) protein (Vimentin) antibodies reveal a neural-like characteristic morphology (Alghazali et al. 2017).

The effect of bioactive materials in determining cells faith or phenotype is influenced by several factors of the nanomaterial (Farrukh et al. 2018) including 1-concentration: high concentration of graphene oxide change neural markers toward oligodendrocyte (Shah et al. 2014), 2-modification: modified hydrogen with Fc-tagged N-cadherin protein-enhanced neural differentiation by around 80%

comparing to unmodified hydrogel (Lee et al. 2016), 3-stiffness: soft matrix with around 1 kPa raise neurogenic marker expression of MSCs and mimic neurogenic brain environment (Engler et al. 2006), 4-functionalization: RADA16-BMHP appears to be the best scaffold for NSCs proliferation, where RADA16-RGD seems like best support for differentiation (Cunha et al. 2011), 5-topology: the topology of laminin-covered polyethersulfone (PES) fiber meshes exhibits a key role in influencing cells propagation and differentiation (Christopherson et al. 2009). The 283-nm dimension of PES meshes enhanced rat NSCs (rNSCs) differentiation to oligodendrocyte by 40 %, whereas 749-nm fibers meshes show a 20% increase in the differentiation rate (Christopherson et al. 2009).

The notion of utilizing NPs as a drug carrier or transporter opens the door for transporting degradable or even non-biocompatible drug agent to the CNS. The ideal features of delivery-oriented particles should be traceable, biodegradable, able to target specific cells, capable of releasing drugs kinetically at different dose and time, escape endolysosome, and have high internalization to the cells (Santos et al. 2016). Many studies have followed the drug delivery approach for treatment CNS insults using NPs.

The therapeutic potential of a polymeric miR-124 loaded NPs to improve neural cell survival and differentiation upon exposure to ischemic insult was evaluated in vitro using subventricular zone (SVZ) derived NSCs that have been isolated from postnatal C57BL/6 J mice. miR-124 is the best plentiful and measured miRs within the brain (Saraiva et al. 2016). The results indicate no toxic impact for treated cells and exhibit enhancement of SVZ differentiation as NeuN, a mature neuronal marker, increased by 1.6-fold compared to control cells (Saraiva et al. 2018).

Self-renewal ability and differentiation of NSCs indirectly were improved as Ferritin NPs mediate the compact formation of neurospheres during the growth of human-induced pluripotent stem cells (hiPSC), adult human brain neural progenitor cells (NPCs), and human fetal NSCs (hfNSCs). The incorporated Ferritin NPs with low concentration showed unremarkable cytotoxicity, with an increase in the expression of glial and neuronal differentiation markers, glial fibrillary acidic protein (GFAP), and Tuj1, respectively (Lee et al. 2018). Another drug delivery system which controls NGF release into canine bone marrow-derived mesenchymal stem cells (cBM-MSCs) for nerve tissue regeneration purpose was achieved using NGF loaded chitosan NPs (Mili et al. 2018). The NGF-chitosan NPs provided a stable, biocompatible system with sustained NGF release and increased cBM-MSCs proliferation with 2–4 mg/ml concentration. NGF-chitosan NPs and free NGF were able to transdifferentiate cBM-MSCs into neurons and exhibited morphological change of fibroblast cell, positive immunostaining of mature neuron markers such as tubulin  $\beta$ III and microtubule-associated protein 2 (MAP2) and nestin (belong to intermediate filaments (IF)) (Mili et al. 2018). Therefore, chitosan acts as a nanodelivery tool for neurotrophic factors and further potentially enables both in vivo and in vitro differentiation of stem cells (Mili et al. 2018).

Another delivery strategy used small peptide (SpBMP-9) derived peptide from bone morphogenetic protein nine growth factor (BMP-9), which can promote cholinergic neurons differentiation aimed at treating AD (Lauzon et al. 2017). The following

study treated human neuroblastoma SH-SY5Y cells an *in vitro* model of neural cells with SpBMP-9 encapsulated alginate and chitosan (Alg/Chit NPs) following NGF encapsulated nanoscale delivery strategy. This system achieved SH-SY5Y differentiation to mature neurons via sustained release of SpBMP-9 peptide, increase expression of neuronal markers such as neuron-specific enolase (NSE) and vesicular acetylcholine transporter (VAcT) that result in neurite growth. A high load efficiency with 70 % encapsulation capacity was also reported (Lauzon et al. 2018). Reduced neurogenesis in AD can be induced *in vitro* and in SVZ by internalization of curcumin-encapsulated PLGA (Cur-PLGA-NPs) into hippocampal NSC of adult rats (Tiwari et al. 2013). Increased gene expression of neural proliferation and differentiation markers was reported, for example (Pax6, nestin, and reelin) and (neurogenin, Stat3, neuroligin, neuroD1, and neuregulin), respectively. Cur-PLGA-NPs boost neuronal differentiation through activating neurogenesis regulatory pathway, Wnt/β-catenin pathway. Moreover, they increased the activity of TCF/LEF transcriptional factor and cyclin-D1 promoter, enhanced β-catenin nuclear protein translocation, and decreased GSK-3β. Subsequently, the AD-induced rat showed reverse cognitive impairment using these NPs (Tiwari et al. 2013).

Another study aimed to increase NSCs and promote their differentiation as a helpful strategy for PD therapy (Saraiva et al. 2016). Accordingly, the researcher applied this approach to allow *in vitro* and *in vivo* delivery of microRNA (miR)-124 loaded NPs to manage NSCs' activity and stimulate their differentiation (Saraiva et al. 2016). The miR-124 NPs intracerebroventricular administration to PD mice model enhanced the neurogenesis in the olfactory bulb and ultimately ameliorated the PD motor symptoms. This seems to be achieved by enhanced neurogenesis through increasing NeuN in both study models. Likewise, boosting neuronal differentiation *in vitro* was attained by inhibiting the expression of embryonic developmental genes such as Sox9 and Jagged1, and in the SVZ derived NSPCs culture (Saraiva et al. 2016).

In the imaging approach, NPs have been used as contrasting material for different purposes such as labeling and tracking cells such as *in vivo* migration and localization of implemented stem cells. In addition, NPs are used for directed drug delivery and intervention via imaging (Kiessling et al. 2014). Spermine-modified amylose cationic superparamagnetic IONPs (ASP-SPIONPs) nanocomplex, for example, was developed as a biocompatible, biodegradable complex to label MSCs for acute ischemic stroke treatment purpose (Lin et al. 2017). This approach efficiently enabled *in vivo* MSCs tracking via MRI in ischemic stroke with no negative impact on the biological behavior of the cells (Lin et al. 2017).

Optical and plasmonic features of Au make it a perfect metal for imaging, and due to that, different researches have used it as a light-based neuro-stimulation mediator (Abdal Dayem et al. 2018). A study used AuNRs with low power laser to treat NG108-15 neuronal cells, rat glioblastoma, and mouse neuroblastoma cell line *in vitro* models (Male et al. 2016). Different approaches have been studied to overcome the limitation of nerve tissue regeneration ability and nerve growth factor (NGF) short half-life (Mili et al. 2018; Lauzon et al. 2018). A dramatic enhancement of neuronal differentiation (83%) and neurite outgrowth (51%) was observed

using an engineered nanomedicine consisting of nerve growth factor functionalized superparamagnetic iron oxide-gold core-shell nanoparticles (NGF-SPIO-Au NPs) and irradiated by a low-intensity light-emitting diode (LED) which provides a non-invasive root for neuronal regeneration (Yuan et al. 2019). PC-12 cells (neuroendocrine tumor cells), neurite outgrowth, and further differentiation were triggered upon treatment with  $1.9 \text{ mW/cm}^2$  of LED illuminated NPs with low reported toxicity. NGF-SPIO-Au NPs increased integrin  $\beta 1$ , and Class III  $\beta$ -tubulin, and the influx of  $\text{Ca}^{+2}$ , locally associates with neurite extension (Yuan et al. 2019). Another guided neuronal regeneration study investigates the involvement of functionalized  $\text{Fe}_3\text{O}_4$  with rat sarcoma (RAS), brain function modulator (Hayashi et al. 2017; Kim et al. 2003) in neurite outgrowth of induced human neurons (Schöneborn et al. 2019).

Likewise, the differentiation outcome can be attained via NPs solely physiochemical effects such as electrical stimulation, own properties, or in some cases, the synergistic effect of combined NPs with loaded agents in one platform. Silver (Ag) NPs, for example, are well known for their use as commercialized NPs and have been widely used in biomedical applications as antimicrobial, anti-fungal, anti-inflammatory, and anti-cancer agents and as cargo carriers for drug delivery purposes (Han et al. 2017). Many studies investigate the AgNPs role in neural differentiation due to its facility to penetrate BBB that makes a good model for drug delivery, despite its reported high toxicity (Carradori et al. 2017). A group of researchers in Korea investigated the interaction of spherical AgNPs with SH-SY5Y (Dayem et al. 2014). The study revealed that a low dose of AgNPs led to a morphological change of SH-SY5Y cells and increased the neurite length. Moreover, it upregulates the expression of  $\beta$ -tubulin III, microtubule-associated protein 2 (Map2), Synaptophysin, growth-associated protein 43 (Gap-43), dopamine receptors type 2 (Drd-2), and neurogenin-1, neuronal differentiation markers. On the other hand, this treatment increases the intracellular ROS during cell differentiation and activates some kinases like AKT serine/threonine kinase (AKT) and extracellular signal-regulated kinase (ERK). Additionally, it modulates the expression of dual-specificity phosphate (DUSPs) (Dayem et al. 2014). Another study examined the dual effect of AgNPs in exhibiting cell toxicity and differentiation for cancer treatment purposes using F9 teratocarcinoma stem cells as an *in vitro* model (Han et al. 2017). In a dose dependent manner, AgNPs show modulation of F9 cytotoxicity and neuronal differentiation. Low dose AgNPs increase the expression of differentiation biomarkers such as Laminin B1(LMNB1), Collagen Type IV, and Retinol-binding protein (RBP), in contrast, decreased the essential marker for retaining the pluripotency of embryonic stem cells like Sox2, Oct3/4, and NANOG transcriptional factors (TFs). Nevertheless, high concentration promotes apoptosis by increasing ROS and lactate dehydrogenase (LDH), mitochondrial membrane dysfunction, and DNA fragmentation. Furthermore, it upregulates apoptotic genes and downregulates anti-apoptotic genes. AgNPs treatment combined with a chemotherapeutic agent like cisplatin inhibits F9 cells viability, which experienced differentiation induced by AgNPs (Han et al. 2017).

Another study treats NSCs by electrical conducting NPs such as graphene oxide (GO) or reduced graphene oxide (rGO) to stimulate their differentiation upon the

help of exogenous electrical fields (EFs). The studies demonstrate the ability of rGO, to promote the differentiation of NSCs to mature neuron, based on the upregulation of both MAP2 and Tuj1(Akhavan and Ghaderi 2014; Talukdar et al. 2014). Comparing to rGO, GO has a better outcome in improving the NSCs differentiation. This might be due to its hydrophilic surface (Feng et al. 2018; Weaver and Cui 2015). Remarkably, these graphene derivatives NPs can stimulate cell differentiation with or without the support of exogenous EFs (Halim et al. 2018).

The synergistic effects of combined Ag and GO in a single NP (GO-AgNPs) provide a novel NP with unique physiochemical properties that play a role in pluripotency inhibition and promotion of neuroblastoma cancer cells differentiation. This differentiation is influenced by the dose, time, and oxidative stress level induced by Ag and GO oxidation. GO-AgNPs induced differentiation of SH-SY5Y cells by increasing the expression of neuronal differentiation markers, NEUN, MAP2, NSE (gamma neuronal), and TAU (microtubule-associated protein tau). Furthermore, it attenuated the self-renewable ability of the neuroblastoma cells by decreasing pluripotency markers, except for c-Myc (Gurunathan and Kim 2017). Additional NPs studies with similar schemes for neuronal differentiation are listed in Table. 6.1. And both types of stem cells whether pluripotent stem cells such as embryonic stem cells (ESCs) and iPSCs and multipotent stem cells such as MSCs and NSCs have been investigated for their therapeutic potentials for neurodegenerative disorders (Vissers et al. 2019).

NPs can influence cell differentiation through different roots according to NPs types (Polak and Shefi 2015). Metal NPs facilitate differentiation via cell attachment modulation (Lein et al. 2000; Yoo et al. 2004; RUSSELL et al. 2002). Although the exact mechanism of changing gene expression by metal ions is still unknown, different hypotheses were also proposed, such as ROS production (Ostrakhovitch and Semenikhin 2013), direct NGF influence (Ostrakhovitch and Semenikhin 2013), or function as coenzyme (Polak and Shefi 2015). Currently, some studies were directed to investigate the differentiation mechanism upon NPs treatment. A study was relied on a comparison of AgNPs differentiation effect with well-known neural cell differentiator, retinoic acid (RA), to unveil the mechanism of neuronal differentiation by AgNPs using SH-SY5Y cells (Abdal Dayem et al. 2018). Generally, the enhancement of neurite growth was stimulated by increasing some differentiation markers and activation of ERK and AKT kinases. Intriguingly, the neurite outgrowth by AgNPs was opposite to RA in its mechanism. Underwent differentiation, DUSPs was downregulated by AgNPs while upregulated by RA.

Similarly, AgNPs suppressed antioxidant genes expression substantially, resulting in mitochondria dysfunction and high ROS. The opposite was observed with RA treated cells. Both treatments activate the signaling pathway of ERK and AKT and illustrate minor cell viability, though. Despite the resulting free radicals in cells exposed to AgNPs, it has been shown that pretreatment of the corresponding group with free radicals scavenger such as N-acetyl-l-cysteine (NAC) eradicates the oxygen radicles, subsequently obliterate cells differentiation, unlike RA exposed cells (Abdal Dayem et al. 2018). Another study investigates the underlying mechanism of neuronal differentiation induced upon NSCs treatment with CNTs (Shao et al.

**Table 6.1** Nanoscale approaches for neuronal differentiation

Strategy	NPs name	Cell type	Study design	Study ref.
Drug delivery	PS80 PBCA NC	Mouse iPSCs	In vitro	(Chung et al. 2017)
Drug delivery	siRNA loaded nanotopography	NSCs	In vitro	(Solanki et al. 2013)
Drug delivery	Cur-PLGA-NPs	NSCs/ hippocampal NSC	In vivo	(Tiwari et al. 2013)
Drug delivery	microRNA-124 loaded NPs	NSPCs derived SVZ	In vitro/in vivo intracerebroventricular injection	(Saraiva et al. 2016)
Drug delivery	Polyamidoamine (PAMAM) dendrimers	hNSPCs	In vitro	(Zeng et al. 2016a)
Drug delivery	RA and BDNF encapsulated nanofiber	mNPCs	In vitro	(Low et al. 2015)
Delivery and structure	DNA-peptide nanotubes	NSCs	In vitro	(Stephanopoulos et al. 2014)
Structure scaffold	Patterned gold and silicon-based photonic crystals	NSCs	In vitro	(Huang et al. 2016)
Structure scaffold	Rolled graphene oxide foam (GOF)	hNSCs	In vitro	(Akhavan et al. 2016)
Structure scaffold	Graphene-nanofiber	NSCs	In vitro	(Shah et al. 2014)
Structure scaffold	Bioactive DNA-peptide nanotubes	NSCs	In vitro	(Stephanopoulos et al. 2014)
Structure scaffold	HA-laminin with salmon fibrin combined hydrogels	hNSPCs	In vivo implantation	(Arulmoli et al. 2016)
Structure scaffold	SAPs-PCL-PLGA nanofiber	Murine NSCs derived SVZ	In vivo implantation	(Raspa et al. 2016)
Structure scaffold	YIGSR and GYIGSR decorated silk fibroin films (SFFs)	hMSCs	In vitro	(Manchineella et al. 2016)
Structure scaffold	IKVAV functionalized cyclodextrin nanofibers (CDNFs)	PC-12 cells	In vitro	(Hamsici et al. 2017)

(continued)

**Table 6.1** (continued)

Strategy	NPs name	Cell type	Study design	Study ref.
Structure scaffold	Hyaluronic acid-based hydrogel	PC-12 cells	In vitro	(Xing et al. 2017)
Structure scaffold	CNTs-multilayered nanocomposites	Primary mNSCs	In vitro	(Shao et al. 2018)
Structure scaffold	3D carbon nanotubes mesh	3D organotypic cultures derived C57BL mice	In vitro/in vivo	(Usmani et al. 2016)
Imaging and delivery	Multimodal magnetic core-shell NPs	NSCs	In vitro	(Shah et al. 2013)
Real-time imaging	Bicistronic vector polymeric nanovehicle	NSCs	In vivo	(Wang et al. 2015)
Imaging	Modified deoxythymidine oligonucleotides loaded gold NPs	hNSCs	In vivo transplantation	(Nicholls et al. 2016)
Miscellaneous	SiPCL-NPs	SH-SY5Y cells	In vitro	(Wiedmer et al. 2019)

Induced pluripotent stem cells (iPSCs), neural stem cells (NSCs), human (h), neural stem/progenitor cells (hNSPCs), mesenchymal stem cells (MSCs), neural progenitor cells (NPCs), mouse (m), hyaluronic acid (HA), carbon nanotubes (CNTs), curcumin-encapsulated PLGA nanoparticles (Cur-PLGA-NPs), self-assembling peptides (SAPs), poly( $\epsilon$ -caprolactone) and poly(D,L-lactide-co-glycolide) (PCL-PLGA), subventricular zone (SVZ), neural stem/progenitors cells (NSPCs), retinoic acid (RA), brain-derived neurotrophic factor (BDNF), bioactive laminin derived epitope (IKVAV), three dimension (3D), silica- $\epsilon$ -polycaprolactone NPs (SiPCL-NPs)

2018). The study reveals that in the absence of exogenous differentiators, multilayered CNTs substrates have more significant differentiation capability than the smooth substrate. The NSCs differentiation displays a dynamic mechanism that encompassed interaction among CNT and NSCs via integrin, thereby activating focal adhesion kinase, which consequently activates signaling pathways involved in neuronal differentiation regulation and synaptic formation (Shao et al. 2018).

## 6.4 Applications of Nanomaterials in Neuronal Protection

The mechanism or strategy to slow down the progress of a neurologic disorder is called neuroprotection, and it could be achieved by preventing or reducing further neuronal loss. Among the many strategies used, alleviating oxidative stress and neuroinflammation, decreasing excitotoxicity, and proposing growth factors and anti-apoptotic peptides have been documented (Kumar et al. 2017). The brain, due to its rich content of lipids, the high rate of oxygen consumption, and the relatively scarce

level of antioxidant enzymes compared to other organs, is extremely susceptible to oxidative stress and, therefore, to its damage that can negatively impact the CNS (Salim 2017). Oxidative stress elevated in the brain has been historically considered to be involved in the pathologies of most neurodegenerative disorders such as AD, PD, MS, ischemia, traumatic brain injury (TBI), cerebrovascular disorders, and its recent recognized role in neuropsychiatric disorders such as depression and anxiety (Patel 2016). Presently, the incidence of CNS diseases increases gradually, causing a threat to human health. Therefore, oxidative stress is considered a key therapeutic target for neurological diseases (Loane and Faden 2010). Nanomaterials, due to their many advantageous traits, can be used as strategies for neuroprotection. One of the approaches is to use nanomaterials that have antioxidant properties or load nanomaterials with antioxidants to eliminate ROS—the toxic byproducts of oxidative stress—*inflammation mediated by oxidative stress and neural degeneration* (Gilmore et al. 2008).

For example, NPs composed of cerium oxide (CeONPs) possess extraordinary antioxidant attributes by mimicking the actions of two essential antioxidant enzymes, SOD, and catalase (CAT), possibly diminishing all toxic intracellular ROS *through* a self-regenerating mechanism (Celardo et al. 2011). Additionally, CeONPs provided excellent free radical scavenging abilities when compared to other antioxidants, including vitamins E and C, melatonin, and n-acetylcysteine by providing higher neuronal protection and survival rate in response to oxidative stress (Singh et al. 2007). On the cell culture level, CeONPs extended the lifespan of diverse neuronal cell cultures and the neurons within these cultures, including astrocytes, neurons, microglia, and some oligodendrocytes while maintaining normal calcium signaling during the extended lifespan due to their potent and regenerative antioxidant characteristics (Rzigalinski 2005). This unique neuronal cell culture protective abilities of CeONPs prevented cell dysfunctions mediated by oxidative stressors, decreased inflammatory functions, and increased neuroprotection associated genes, neurite outgrowth, and dopamine secretion (Das et al. 2007; Ciofani et al. 2013; Schubert et al. 2006).

In the context of treating neurological diseases associated with oxidative stress, several studies have been reported. In AD oxidative injury induced by A $\beta$  protein, CeONPs protected SH-SY5Y cells against apoptosis and maintained cell viability and morphology. It also increased brain-derived neurotrophic factor (BDNF) protein level, which performs a crucial role in memory development and storage (D'Angelo et al. 2009). In another report, CeONPs attached with an anti-A $\beta$  antibody specifically targeted A $\beta$  aggregates and rescued neuronal survival by regulating the BDNF signaling pathway (Cimini et al. 2012). Furthermore, CeONPs reduced the level of free radicals in rat primary cortical neurons and blocked mitochondrial fragmentation and cell death by A $\beta_{1-42}$  (Dowding et al. 2014).

Moreover, CeONPs effectively inhibited the formation of A $\beta$  aggregates, decreased cellular ROS, and protected cells from A $\beta$ -derived toxicity in PC12 cells (Li et al. 2013). The potent antioxidant activity of CeONPs is dependent on size, composition, and particle surface area. A single 10nM dose of CeONPs protects against multiple free-radical related damages and toxicity induced by A $\beta_{1-42}$ . It also

extended the life span of the neurons when compared to 7nm and Fe-doped CeONPs (Singh et al. 2008).

Only a few reports have been made available for the treatment of PD with CeONPs. In a designed PD biochemical pathway, CeONPs act as a potential inhibitor of  $\alpha$ -synuclein, a protein involved in PD pathogenesis, and is proposed to be employed as a nano-drug against PD (Kaushik et al. 2018). In a mouse model of PD, CeONPs could protect dopaminergic neurons and maintain striatal dopamine in the substantia nigra (Dillon et al. 2011). In another study, a PD model of Drosophila showed improved survival when pretreated with CeONPs, which could also preserve motor function (Rzagalinski et al. 2017). Also, CeONPs significantly protected PC12 cell's survival and dopamine metabolism against oxidative stress induced by manganese chloride, which is responsible for an occupational form of Parkinson-like disease (Pinna et al. 2015).

The use of CeONPs as a potential therapeutic agent for MS has also been explored. In mouse models of MS, CeONPs significantly improved recovery in a later stage of the disease by attenuating white matter pathology, and associated inflammation and suppressing oxidative stress and ROS in the brain (Eitan et al. 2015; Heckman et al. 2013), which demonstrates that CeONPs maintain their anti-oxidant properties and can be used for the treatment of oxidative stress in MS.

Furthermore, in brain slices of a rat stroke model, CeONPs dose-dependently reduced ischemic cell death and concentrations of nitric oxide (NO) and superoxide by 50% and 15%, respectively (Estevez et al. 2011). And in a rat model of ischemic stroke, CeONP can efficiently eliminate ROS and decrease neuronal loss in brain sections without penetrating the brain of normal animals targeting the damaged area by disruption of the BBB after ischemia (Kim et al. 2012).

Moreover, CeONPs increased neuronal survival and preserved intracellular free signaling in a tissue culture model of TBI, reduced oxidative stress, preserved endogenous calcium antioxidant activity, and ultimately improved cognitive function when administrated after mild TBI in rats (Bailey et al. 2016). It was established that the accumulation of CeONP in brain tissues could reduce levels of oxidative stress markers, which could be due to their capability to cross the BBB (Heckman et al. 2013). The previous findings demonstrate the potential for CeONP as a novel nanopharmaceuticals for the treatment of neurodegenerative disorders.

Among other NPs, chitosan NPs have received considerable attention for their neuroprotective abilities. Chitosan is a biodegradable and biocompatible polymer commonly used in biomaterial research and has been broadly experimented in a diversity of areas such as drug delivery, tissue engineering, and wound healing (Cho and Borgens 2012). Chitosan NPs exhibited neuroprotective effects in BV-2 rat microglial cells when exposed to hydrogen peroxide ( $H_2O_2$ )—a well-known source of oxidative stress—by rescuing the cells from induced cell death due to its physical sealing ability of cell membrane breaches (Chen et al. 2018). Additionally, chitosan NPs protected PC12 neurons against acrolein, a highly toxic endogenous aldehyde associated with CNS pathologies and neurodegenerative diseases, by reducing membrane integrity damage, lipid peroxidation, and secondary oxidative stress effectively and statistically (Cho et al. 2010).

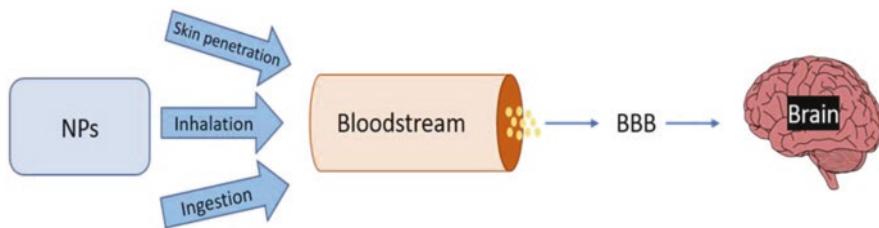
Currently, in PD treatment, the administration of the neurotransmitter dopamine is employed to reduce the disease symptoms of the pathology, although it also causes several side effects, including cell-induced oxidative stress (Ragusa et al. 2018). When administrated to SH-SY5Y cells, dopamine encapsulated in chitosan NPs induced lower H<sub>2</sub>O<sub>2</sub> production compared to the free dopamine drug, thus reducing the oxidation rate and potentially exerting neuroprotective effects (Ragusa et al. 2018). In a stroke mice model, the functionalization of chitosan NPs with both anti-apoptotic peptide and the basic fibroblast growth factor enabled the NPs to cross the BBB via receptor-mediated transcytosis and reduce the infarct volume significantly (Yemisci et al. 2015). This makes chitosan NPs a promising drug carrier to transport BBB-impermeable therapeutics for neuroprotection against stroke and other neurologic diseases.

In the past several years, owing to their unique properties, investigations about AuNPs have increased along with their applications in different fields such as in sensors, drug delivery, electronics, probes, DNA labeling, and cancer therapy. AuNPs capped with the marine brown alga *Dictyopteris divaricata* (DD) were tested against oxidative stress in an ischemic stroke model using SH-SY5Y cells. The DD-AuNPs protected the cells from loss of cell viability, induced LDH release, ROS production, and apoptosis (Park et al. 2019). Moreover, a recent study also suggested that AuNPs capped with extract of *Kalopanax Cortex*—a dried stem bark of *Kalopanax pictus*—exhibit neuroprotective properties by regulating the expression of the antioxidant protein NRF2 signaling pathway, restoring cell viability, inhibiting ROS production, mitochondrial membrane potential (MMP) disruption, and apoptosis (Park et al. 2017). In addition, AuNPs synthesized using the root extract of the traditional Chinese medicinal plant *Paeonia mountan*, reduced ROS, and inflammatory cytokines levels in rat BV-2 cells (Xue et al. 2019). It also alleviated the dopaminergic neuroinflammation and improved motor coordination in Parkinson induced mice (Xue et al. 2019). In another PD study, composites of AuNPs were able to inhibit the cellular toxicities induced by the neurotoxin MPP+ after they were transfected into PC12 cells and dopaminergic neurons via endocytosis, they could also cross the BBB successfully in PD mice models (Hu et al. 2018).

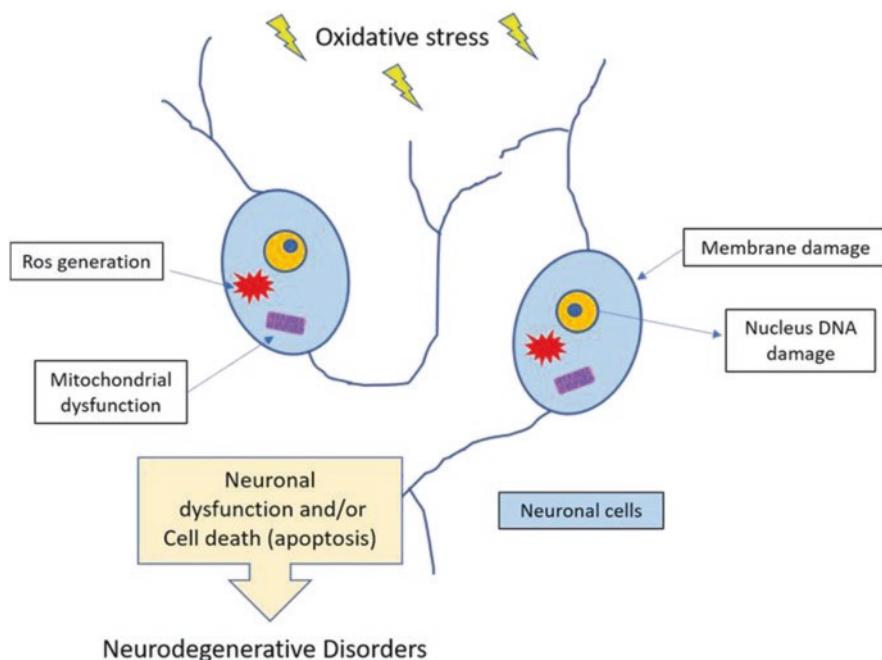
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## 6.5 Applications of Nanomaterials in Neuronal Neurotoxicity

Despite the increasing interest in applying nanomaterials in the biomedical field, only little is known about their inherent risk on human health, especially their possible toxicity on the CNS. Due to their very small size (<100 nm), NPs could easily enter the human body via several routes by inhalation, ingestion, dermal contact, or a combination of these routes (Fig. 6.3) and their penetration depends on their size and surface modifications (Liao et al. 2014). NPs can cross most biological membranes, including the BBB, and subsequently accumulate in the brain (Sarkar et al. 2017). In the brain, NPs may induce adverse neurotoxic effects such as oxidative stress, inflammation, and apoptosis, supporting the growing evidence that shows an



**Fig. 6.3** Routes of exposure to NPs. Once the NPs pass by the first barriers, the skin, lung, or the gastrointestinal tract through deposition, inhalation, and ingestion, they may reach the bloodstream, and then the brain if they cross the BBB



**Fig. 6.4** A brief illustration of nanoneurotoxicity

association between neurodegeneration and NPs (Figure 6.4) (Win-Shwe and Fujimaki 2011). Hence, along with the development of novel NPs, and the massive exposure to NPs by consumers, specialists in related scientific fields are calling for simultaneous evaluation of the toxicological and environmental impacts of NPs (Seaton and Donaldson 2005).

Yet, long term studies that detect NPs retention levels assess brain elimination management and classify NPs deterioration effects are rare and limited (Bencsik et al. 2018). The long term brain ability to eliminate the NPs via classical routes includes extracellular degradation, intracellular degradation, lymphatics vessels, and cervical nodes, and ABC transporters mediated brain-to-blood efflux have been reported

to influence the NPs clearance (Gao et al. 2017). Some features of NPs may influence the clearance, for example, their stability (Gao et al. 2017), particle size, surface properties, and NPs functional group and coated ligand (Lockman et al. 2004).

Nanomaterials have several parameters to their classification including their chemical composition (organic and inorganic), their shape and size, their formation and their industrial or biomedical applications (Saleh and Gupta 2016). Organic NPs are of three-dimensional form via self-assembly. Organic NPs are manufactured using organic molecules from natural and synthetic origin such as protein aggregates, lipid bodies, milk emulsion, and other complex structures such as Viruses. This type of NPs plays a vital role in industries that include food and cosmetics. For example, food materials including chocolate, cakes, and creams offer nanoemulsions in their characterization. Two approaches are used nowadays to fabricate organic NPs: first is the top-down method that uses techniques such as mechanical milling, microfluidics, and lithography.

The second method or bottom-up produces NPs by precipitation and condensation. Inorganic NPs are typically formed by precipitation of inorganic salts. They are more stable than organic NPs but their stability is limited and depends on the chemical or mechanical and nature of the particle (Mageswari et al. 2016).

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## 6.6 Organic Nanoparticles

### 6.6.1 Polymeric Nanoparticles

One of the most studied organic nanomaterials are the organic polymeric NPs. They are mostly used in nanomedicine because of their rapidly growing outstanding properties in drug delivery and disease diagnosis (Crucio and Barros 2017). Their most important advantages are their abilities to protect the carried drug molecules, specifically target drug release sites and the ability to release the drug in a controlled manner, in addition to their ability to simultaneously diagnose and treat various diseases (Singh et al. 2017). Although we have mentioned the various protective effects of chitosan, some studies emerged, pointing to its toxicity. Polysorbate 80-modified chitosan NPs were localized and evaluated for their neurotoxicity in rats. Chitosan NPs were systematically injected into rats for 7 days (Yuan et al. 2015). The rats had decreased weight over time, which was dose-dependent, and deposits of chitosan were found in the frontal cortex and cerebellum with slight inflammatory responses. Neurons showed signs of apoptosis, necrosis, and a decrease of glial fibrillary acidic protein (GFAP) which all contributed to the neurotoxicity of chitosan. In another study, chitosan NPs were incubated with zebrafish embryos in the presence of ZnONPs as positive control. The embryos had a decreased hatching rate and malformations and increased mortality, which was concentration-dependent. It additionally increased cellular apoptosis, ROS expression, and HPS70 protein indicating that chitosan NPs can cause physiological stress in zebrafish (Hu et al. 2011a).

### 6.6.2 Liposomes

Liposomes are nanosized artificial vesicles consisting of an aqueous core that can encapsulate hydrophilic therapeutics and a phospholipids bilayer that can carry hydrophobic therapeutics. Thus they have been used as nanocarriers for not only drugs but also vaccines, proteins, enzymes, nucleic acids, and imaging agents (Montesinos 2017). Recently they have been proposed as carriers for drugs to the CNS since they can cross the BBB and as diagnostic tools for neurologic diseases (Montesinos 2017). In this context, two liposomal formulations carrying the chemotherapy medication cisplatin were developed as potential therapeutic agents for the treatment of F98 rat glioma. The results indicated a high cytotoxic effect against glioma cells and tumor retention in glioma bearing rats compared to the cisplatin free controls. However, when neurotoxicological tests were carried in non-tumor bearing Fischer rats, one of the formulations carrying the drug had severe neurotoxic effects and resulted in death after a few days. Similarly, hollow liposomes showed similar neurotoxicity, indicating that this was due to the liposomes themselves rather than the cisplatin. In contrast, the other liposomal formula showed dose dependent neuropathologic changes from none to severe following 10 to 14 days after administration (Huo et al. 2012).

### 6.6.3 Dendrimers

Dendrimers similar to liposomes have the ability to carry therapeutics of both hydrophilic and hydrophobic nature; thus, they are used as nanocarriers for therapeutics and imaging agents (Srinageshwar et al. 2017). And because they can overcome the BBB, they have been used extensively in therapeutics to cure brain diseases (Srinageshwar et al. 2017). However, their different characteristics play a role in inducing neurotoxicological responses in the CNS (Albertazzi et al. 2012). Therefore, the safety of dendrimers needs to be assessed when used to carry drugs to treat brain disease. The safety of polyamidoamine (PAMAM) dendrimers was assessed in human NPCs cultured as a 3D neurosphere model. The dendrimers were shown to highly affect cell proliferation and neuronal migration in higher concentrations (Zeng et al. 2016b). In zebrafish embryos and larvae, the dendrimers induced a dose and concentration-dependent innate immune response and neurotoxicity (Calienni et al. 2017). PAMAM dendrimers also showed toxicity in human NPCs by affecting neuronal differentiation, mitochondrial activity, apoptosis, gene expression, which is related to DNA damage and oxidative stress, and this cytotoxicity was influenced by the particles numbers and surface density (Zeng et al. 2016a). A study established a strategy to reduce the toxicity of PAMAM dendrimers by surface modification, which prevented neurotoxicity and synaptic activity alterations in hippocampal neurons (Vidal et al. 2018).

## 6.7 Inorganic Nanoparticles

### 6.7.1 Silver Nanoparticles (AgNPs)

Nanosilver is one of the nanomaterials that are under the most inspection today due to the antimicrobial activity of silver (Wijnhoven et al. 2008), and its release and effects are studied widely (Scheringer et al. 2010). Nanoscale silver particles have been known for about 120 years and are commercially available for over 100 years and were used in diverse applications (Nowack et al. 2011). AgNPs exhibit strong antimicrobial and anti-inflammatory activities; thus, they are used in the medical field in medical devices, products, and they have been proposed in medical implants and instruments for the prevention of infection (Lee et al. 2007). In addition to the medical field, AgNPs nowadays are widely included in many applications and consumer products such as textiles, water purification systems, refrigerators, mobile phones, antimicrobial agents, plasters, and toothbrushes (Chernousova and Epple 2013). It is also increasingly used in cosmetics that are being equipped with silver to obtain an antibacterial effect (Wiechers and Musee 2010). That represents approximately 30% of the total consumer products in the nanomaterial industry. Therefore, humans are being exposed to AgNPs increasingly via various pathways, including inhalation, ingestion, dermal exposures that lead to the accumulation of AgNPs in different body organs (Lansdown 2006). In the CNS, AgNPs tend to stay for a considerably longer time compared to other organs (Panyala et al. 2008). They can enter the brain by passing through the BBB, causing its dysfunction and neuronal damages after continued exposure (Xu et al. 2015).

Moreover, AgNPs can provoke oxidative damage and inflammatory response in the CNS, which leads to the induction of many neurodegenerative disorders such as AD, MS, stroke, and brain tumors (Myhre et al. 2013). The continuous accumulation of AgNPs inside the brain affects neurological functions and induces neurotoxicity via a variety of mechanisms (Tang et al. 2009; Strużyńska and Skalska 2018; Safari et al. 2016). This AgNPs mediated neurotoxicity causes high free radicals' production, a decline in antioxidant agents, and apoptosis in different neuronal cell types (Yin et al. 2013; Liu et al. 2015; Ghooshchian et al. 2017; Khan et al. 2019). In this context, some studies have tested the neuroprotective activities of some agents against the neurotoxic effects of AgNPs.  $\alpha$ -lipoic acid and *Ginkgo biloba* ameliorated the series impact of AgNPs on BBB function and tight junctions' proteins through their antioxidant efficacies (Lebda et al. 2018). Furthermore, the bioflavonoid rutin mitigated the imbalance in brain neurotransmitters, oxidative stress, and neurotoxic effects caused by the oral administration of AgNPs in rats (Ahmed and Hussein 2017). Moreover, nasal administration of AgNPs caused bodyweight loss, activation of neuroglial cells, and destruction of the granule layer of the cerebellum in rats, which was strikingly attenuated by vitamin E (Yin et al. 2015).

### 6.7.2 Titanium Dioxide Nanoparticles ( $\text{TiO}_2$ )

Nowadays, titanium oxide ( $\text{TiO}_2$ ) NPs are consumed in many fields, such as renewable energy devices (Kapilashrami et al. 2014), sensors (Bai and Zhou 2014), food (Martirosyan and Schneider 2014), and sunscreens (Newman et al. 2009). This widespread  $\text{TiO}_2$ NPs could lead to unintentional human exposure and environmental pollution.  $\text{TiO}_2$  NPs could be absorbed through inhalation, indigestion, and skin penetration with the ability to enter the body tissues such as the lung, heart, liver, spleen, kidney, plasma, lymph nodes, and be translocated into the brain (Song et al. 2016). Several findings demonstrated that rats and mice exposed to  $\text{TiO}_2$ NPs via various routes of administration had NPs in most parts of their brain zones with an increased level of Ti in their brain (Wu et al. 2009; Disdier et al. 2015; Geraets et al. 2014). Based on current research, the nasal administration or intranasal installation is considered the most efficient route for  $\text{TiO}_2$ NPs to be translocated into the brain (Wang et al. 2008). Additionally, the injury induced by  $\text{TiO}_2$ NPs is believed to occur when the particles are transported into the blood, they then pass through the BBB and cause disruption of its integrity and a reduction in its permeability as they finally accumulate in the brain (Li et al. 2010; Brun et al. 2012). The ability of  $\text{TiO}_2$ NPs to pass through the BBB is dependent on particle size, shape, and surface modifications, which could adjust the toxic impacts of  $\text{TiO}_2$ NPs on the brain (Liu et al. 2013; Zhang et al. 2011).

The toxic effects of  $\text{TiO}_2$ NPs on the CNS have been established *in vivo* as several studies revealed that  $\text{TiO}_2$ NPs cause oxidative stress by reducing antioxidant enzymes and non-enzymatic antioxidant content, increase oxidative stress biomarkers and expression level of genes regulating oxidative stress, leading to mitochondrial dysfunction, inflammation, and apoptosis with upregulated Ti concentrations in the brain (Ma et al. 2010; Ze et al. 2013; Meena et al. 2015; Nalika and Parvez 2015). Furthermore, the hippocampus area of the brain—which is responsible for memory and learning—is easily affected after  $\text{TiO}_2$ NPs exposure (Hu et al. 2011b).  $\text{TiO}_2$ NPs concentration was upregulated in the hippocampus in Wistar rat neonates after intragastrical administration to the pregnant mother suggesting that  $\text{TiO}_2$ NPs are translocated through the maternal circulation system, which could impact the embryos brain and their development (Mohammadipour et al. 2014). The hippocampus and spatial recognition memory were impaired in mice treated with  $\text{TiO}_2$ NPs, which could lead to neurodegenerative disorders such as AD (Bird and Burgess 2008; Ze et al. 2014; Hu et al. 2010; Ashbrook et al. 2014).

Besides, several neuronal cell types have been affected by the administration of  $\text{TiO}_2$ NPs. For example, treated primary microglia had increased inflammatory biomarkers (Xue et al. 2012). In PC12 cells,  $\text{TiO}_2$ NPs had apparent cytotoxicity, activated oxidative stress, decreased MMP, and elevated apoptosis (Liu et al. 2010; Wu et al. 2010). In primary hippocampal neurons,  $\text{TiO}_2$ NPs reduced cell viabilities, enhanced LDH activity, elevated apoptosis, reduced MMP, increased  $\text{Ca}^{2+}$  concentration, and disrupted neurite outgrowth (Wu et al. 2010; Hong et al. 2015). In SH-SY5Y neuronal cells,  $\text{TiO}_2$ NPs disrupted cell microtubules, elevated apoptosis, increased genotoxicity, damaged cell membranes, and affected mitochondrial

function (Valdiglesias et al. 2013a; Mao et al. 2015; Coccini et al. 2015). Moreover, in rat and human glial cells (C6 and U373), respectively, TiO<sub>2</sub>NPs caused apoptosis, changes in morphology and cytoskeleton, depressed cellular proliferation, impaired mitochondria, and induced oxidative stress (Márquez-Ramírez et al. 2012; Huerta-García et al. 2014). In human cerebral endothelial cells, TiO<sub>2</sub>NPs induced significant cytotoxicity, ROS generation, and marked DNA damage (Kenzaoui et al. 2012).

From the studies mentioned above, oxidative stress, apoptosis, and inflammation might mainly demonstrate the molecular mechanisms underlying the neurotoxicity of TiO<sub>2</sub>NPs. It is important to re-evaluate the biosafety of TiO<sub>2</sub>NPs, and therefore more research is required to examine their parameters.

### 6.7.3 Iron Oxide Nanoparticles (IONPs)

Iron oxide NPs (IONPs) are a type of magnetic NPs because they possess unique native magnetic and highly stable colloidal properties. Therefore, they are the most frequently used nanoconstituents in biomedical applications such as drug delivery, cancer and gene therapy, MRI, and tissue repair (Iv et al. 2015). The brain highly depends on iron because it is an essential factor in the production of several neurotransmitters, myelinogenesis, and the brain's respiratory activity (Pisanic et al. 2009). It has been well documented that iron dysregulation plays a critical role in the pathogenesis of several neurodegenerative disorders, including AD and PD (Myhre et al. 2013). The usage of IONPs in the CNS has been increasing due to their ability to conjugate to peptides and antibodies for the treatment of numerous diseases, including tumor targeting, drug delivery for targeted tissues across the BBB, and NSCs tracking for therapy purposes (Iv et al. 2015). Like other NPs, IONPs can get into the body by various routes via inhalation, ingestion, skin penetration, and injection and then distributed to other body tissues (Shang et al. 2014). The ability of IONPs to pass through the BBB into the brain occurs via receptor-mediated endocytosis, which is dependent on the physicochemical characteristics of the NPs (Kreuter 2015; Hoff et al. 2013). Many investigations were carried to explore the effects of IONPs on several neuronal cell types. Dose-dependent exposure to IONPs in PC12 cells caused decreased cell viability, ROS generation, and resultant oxidative stress (Wu and Sun 2011; Deng et al. 2014). Moreover, in a rat *in vitro* BBB model and SH-SY5Y cells, the inhibition of cell proliferation, ROS generation, decreased number of active mitochondria, and compromised BBB integrity were observed after exposure to IONPs (Imam et al. 2015). The microglia is another type of neuronal cell that plays a role in the brain similar to that of the macrophages in the rest of the body, which makes it relevant to the accumulation of IONPs (Pickard and Chari 2010). The exposure to IONPs for 6h led to a strong iron content increase in microglia, followed by a major increase in ROS and cytotoxicity (Petters et al. 2016). This cell toxicity is time and concentration-dependent as short time exposures did not cause toxicity (Luther et al. 2013; Wu et al. 2013). Nonetheless, the effect of IONPs exposure to microglia is still controversial (Petters et al. 2014).

Astrocytes are another type of glial cell that has been used to test the effect of IONPs and was generally not acutely damaged by the presence of IONPs (Hohnholt et al. 2013). In different cultures of astrocytes, IONPs did not cause cell loss or cytotoxic effects (Geppert et al. 2009; Yiu et al. 2012; Lamkowsky et al. 2012). Contrarily, some studies demonstrated the toxicity of IONPs, including accumulation of cellular iron and ROS formation induction, mitochondria dysfunction, and decreased cell viability (Geppert et al. 2012; Au et al. 2007).

A few *in vivo* studies have also been carried to show the effect of IONPs on the CNS. After an intraperitoneally injected to zebrafish with IONPs, a gradual increase in iron content in the brain was observed accompanied by cell toxicities and triggered apoptosis (de Oliveira et al. 2014). Additionally, markers of inflammation and apoptosis were increased after intraneuronal injection with IONPs into an intact sciatic nerve in Sprague–Dawley rats (Kim et al. 2013). In another study, the rats were intranasally treated with IONPs for 7 days; the concentration of the NPs was found to be particularly high in the rat striatum and hippocampus. The NPs additionally caused oxidative damage in striatum and neuronal fatty degeneration in the hippocampus after 14 days (Wang et al. 2007). Thus, the previous findings and others demonstrate the adverse effect of IONPs on the CNS, yet, some factors like biocompatibility, coating type, experimental settings, and ION concentration may determine the implied neurotoxicity induced by ION exposure (Petters et al. 2014). Further investigations are needed to understand the impact of IONPs on the CNS in neurobiological applications.

#### 6.7.4 Zinc Oxide Nanoparticles (ZnONPs)

Due to their many attributes, zinc oxide (ZnO) NPs have been widely used in many consumer products and biomedical applications (Akhtar et al. 2012). This led to increased human exposure to ZnONPs as they can enter the organs, including the brain, via several routes, causing a risk to human health (Kao et al. 2012). In the brain, ZnONPs could alter the function and formation of cerebral proteins and ultimately affecting several biological processes (Shim et al. 2014). ZnONPs had a deleterious neurotoxic effect on the neuronal network responsible for respiration in mammalian neonates (Nicolosi et al. 2018). Additionally, the exposure to ZnONPs in developing zebrafish resulted in abnormalities in the development of dorsal root ganglion, blood vessels, and the axonal projections, which can continue to the next generation (Kteeba et al. 2018). Furthermore, the chronic exposure of ZnONPs given orally to rats caused momentous oxidative stress indicated by increased malonaldehyde (a lipid peroxidation marker) and the decline in cellular antioxidants, followed by DNA fragmentation, inflammation, and apoptosis (Attia et al. 2018). It was also demonstrated that in mice, old individuals exposed to ZnONPs are more susceptible to its induced neurotoxicity marked by oxidative stress, impaired learning, and memory abilities, changes in hippocampal pathological, and systemic inflammation, thus pointing to a synergistic relation between ZnONPs and aging (Tian et al. 2015). Moreover, Wistar rats treated for 30 days with ZnONPs via tongue installation resulted in particles transferring into the CNS via the taste nerve after causing glial

cell activation and neuroinflammation, and reduced viability of BV2 and PC12 cells in addition to inflammatory response and calcium influx (Liang et al. 2018).

The neurotoxic potential of ZnONPs was addressed in primary astrocytes. The particles were dose and time-dependently toxic, as evidenced by the reduced cell viability and apoptotic cell death. They also altered cell morphology and MMP and increased ROS production (Sudhakaran et al. 2019). In rat C6 glial cells, the exposure to ZnONPs induced a toxicological response that depends on dose and exposure time (Sruthi and Mohanan 2015). Similarly, in mouse neural stem cells, ZnONPs induce a dose but not size-dependent neurotoxicity by inducing cytotoxicity and apoptosis (Deng et al. 2009). In SH-SY5Y cells, ZnONPs induced different forms of toxicities, including a decrease of viability, cell cycle alterations, and genotoxicity in a dose- and time-dependent manner (Valdiglesias et al. 2013b), it also enhanced the production of ROS and decreased GSH activity, and increased apoptosis and necrosis (Kim et al. 2015). From the previous *in vitro* studies, the neurotoxicity of ZnONPs appeared to be time and dose-dependent, which emphasizes the importance of considering the time, clearance, and dose of the NPs in biomedical applications as their persistence may still induce damage to the cells even at small concentration.

### 6.7.5 Manganese Nanoparticles (MnNPs)

Manganese is a hydrophilic essential metal ion that is transported through the BBB into the brain to maintain the homeostasis of the CNS (Albrecht et al. 2007). It is also a major element used in producing different types of steel and cast iron in addition to other several uses. Manganese oxide (MnO) is used in food supplements, textile printing, ceramics, glass painting, and paints. Manganese is known to cause neurotoxicity and is involved in neurological disorders such as PD, AD, Huntington disease, and ALS (Peres et al. 2016). Inhalation of MnONPs led to their translocation through the BBB via the olfactory bulb, resulting in inflammatory changes, which indicates the neurotoxicity of air born NPs (Elder et al. 2006). Investigations additionally showed that MnNPs could enter N27 rat dopaminergic cells inducing a series of neurotoxic effects resulted in oxidative stress, activation of autophagy, and apoptotic signaling pathway, leading to dopaminergic cell death (Ngwa et al. 2011).

### 6.7.6 Carbon-based Nanoparticles

Carbon-based NPs (CNPs) mainly consist of one material, which is carbon with a size smaller than  $\geq 100\text{nm}$ . Due to their unique structure that has excellent properties, they are used in numerous applications such as environmental sensors, composite materials, antimicrobial agents, renewable energy technologies, and hydrogen storage fuel cells (Mauter and Elimelech 2008). There are three widely known and well-developed types of CNPs, which are fullerene, carbon nanotubes (CNTs), and the recently emerged graphene. Some factors could contribute to the toxicity of CNPs, such as their size, shape, physicochemical properties, surface charge, and

production method. Neurotoxicity has been becoming a concern when it comes to CNPs applications in the CNS. Among fullerenes, C<sub>60</sub> is the most studied and used type of fullerenes, and it was found that they cause oxidative damage by inducing lipid peroxidation in largemouth bass brain and glutathione (GSH) depletion in fish gills (Oberdörster 2004).

Previous research has demonstrated that CNTs can pass by the olfactory neurons and accumulate into the olfactory bulb in the brain, causing inflammation and microglial activation (Yamamoto et al. 2006). There are two well-known forms of CNTs: the single-walled CNTs (SWCNTs) and the multiwalled-CNTs (MWCNTs). The SWCNTs are found to be the most toxic form of CNTs, among other forms causing both *in vitro* and *in vivo* toxicities and oxidative stress in biological systems (Jia et al. 2005; Ren and Zhong 2010). The data from rainbow trout exposed to SWCNTs for 10 days suggested that SWCNTs are a respiratory toxicant and they caused the fishes to suffer from vascular injury in the brain that may alter the permeability of BBB, and it also increased Zn and Cu concentrations in the brain which may prevent the fishes from defending themselves in a fight due to high metal levels (Smith et al. 2007). Also, increased concentration of SWCNTs induced apoptosis in PC12 cells and inhibited proliferation specifically by causing a reduction in MMP and intracellular antioxidant enzymes, ROS generation, and lipid peroxidation in a dose-dependent manner (Wang et al. 2011a).

Furthermore, another study has indicated that the neurotoxicity of MWCNTs inhibited voltage-gated potassium channel in rat hippocampal CA1 pyramidal neurons leading to neuronal excitability by increasing both the firing frequency and spike half-width significantly and, therefore, inducing an abnormal change of neuronal action potential properties which is considered as a new demonstrative event of dysfunctional neuronal diseases (Chen et al. 2013). Additionally, another study indicated that MWCNTs with iron impurities reduced cell viability and inhibited the ability to make mature neurites by increasing cytoskeletal disruption in undifferentiated rat PC12 cells (Meng et al. 2013). Moreover, MWCNTs significantly impaired axonogenesis regeneration in dorsal root ganglia neurons without associated cell death, which indicates a damaging effect on nerve regeneration and may trigger axonal pathology (Wu et al. 2012). It was also found that after exposure to graphene, it generated ROS and induced apoptosis in a time and dose-dependent manner in human neuronal cells (Zhang et al. 2010).

Despite the cytotoxicity issues of CNTs, they still exhibit strong potentials for numerous applications. Nowadays, researchers are trying to configure ways to minimize their toxicity by various methods to benefit from their excellent features for further medical applications.

### 6.7.7 Silica Nanoparticles

Silica NPs are of special importance since they are used in various industries and applications such as the food industry, cosmetics, engineering, biomedical, bioimaging, and drug delivery applications (Teleanu et al. 2019). Neurotoxicity wise, the intranasal route for the distribution and localization of silica NPs has been

frequently investigated as it leads to the accumulation of NPs in the brain causing synaptic changes, cognitive dysfunction, and similar pathologies to neurodegeneration (You et al. 2018).

Various *in vitro* studies showed that silica NPs display toxicity on neurons and the related silica NPs neurotoxicity is mostly correlated with increased ROS levels and lipid peroxidation, reduced GSH, oxidative stress, inflammation, DNA damage, and apoptosis in microglia, PC12 cells, human SH-SY5Y, SK-N-SH, and N2a neuroblastoma cells (Choi et al. 2009; Wang et al. 2011b; Kim and Yang 2011; Yang et al. 2014). In vivo studies also underlined the effect of silica NPs on the CNS of animals such as mice and fish, including disturbance of neural behaviors, and a possible physiological rationalization for this phenomenon was given (Lockman et al. 2004). This could be a result of the BBB bypassing of the particles, which allow them direct entry into the brain, which depends on their size and chemical design (Lockman et al. 2004). Overall, it is suggested from the previous studies the possible neurotoxicity of silica NPs, and they should be considered as a possible hazard for neurodegenerative dysfunctions.

### 6.7.8 Quantum Dots (QDs)

Due to their electro and optical properties QDs have gained considerable interest in the biomedical field in the past years. Those zero-dimensional nanomaterials offer great application potentiality in biology and medicine including cell labeling and tracking, drug delivery, cancer therapy, and bioimaging (Cintenza 2010). Thus, assessing QDs neurotoxicity is an essential step for their application in biomedicine. Many factors play a determinate role in the neurotoxicity caused by QDs including their size, shape, surface charge and coating, concentration, their purity and nature, and solubility of the constituent materials. QDs are capable of crossing the BBB and causing neurotoxicity in the CNS. *In vitro*, they caused damage to neuronal cells by causing oxidative stress, increased Ca<sup>2+</sup> levels in the cytoplasm and autophagy, and *in vivo* they caused impairments in brain functions, and synaptic transmission and plasticity (Wu et al. 2016). A study assessed the neurotoxicity of QDs in RMEs motor neurons responsible for controlling foraging behavior. It was demonstrated that QDs induced developmental deficits in the neurons and abnormal foraging behavior via the combination of oxidative stress, cell identity, and bioavailability effects, which can be regulated by QDs surface modification with zinc sulfide coating (Zhao et al. 2015).

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## 6.8 Strategies to Reduce the Neurotoxicity of Nanomaterials and Nanomedicines

Cytotoxicity, aggregation, biodegradation, and biocompatibility are major concerns that need further investigation and action. For instance, cytotoxicity was reported with AgNPs in a dose depending manner (Han et al. 2017; Dayem et al. 2014), although it has a role in differentiation (Lin et al. 2017). Another study reported that

increased surface area with low size particles causes inflammation for the same NPs mass (Iswarya et al. 2016). Particle aggregation, poor stability, and biocompatibility have been reported with uncoated IONPs (Muthiah et al. 2013). Therefore, different modification measures have been studied and provided to address these problems and limits. It has been reported that maximizing the NPs permeability through BBB might be enhanced by coating NPs with ligands or antibodies for brain endothelial cell epitopes (Santos et al. 2016). Au has been used as a shell to avoid aggregation and improve NPs biocompatibility (Muthiah et al. 2013). Alginate and chitosan are polysaccharides with bioresorbable capacity, providing biocompatibility with high loading efficiency (Lauzon et al. 2018). Conducting biological synthesis approaches instead of chemical methods for synthesizing metallic NPs can reduce toxin from the used chemical material and provide much stable NPs (Abdal Dayem et al. 2018; Soltani Nejad 2015). To get an optimal NPs design, reducing NPs inheritance neurotoxicity and improving their selectivity is essential. A dual-targeted strategy in drug delivery showed a promising result (Gao and Jiang 2017) to improve brain selectivity, treatment outcomes, and reduce unselective distribution of NPs and neurotoxicity (Huile et al. 2011).

## 6.9 Conclusion

Researchers have to pay a lot of attention while designing their NPs, considering all the aspects of treatment efficacy, safe delivery, and proper clearance assessment. When using NPs, researchers should also consider their induced toxicity, BBB integrity, brain selectivity, and physiological safety. In terms of regulation and implementation of nanotechnology in clinical practices, understanding the toxicity effect upon the use of NPs and their cellular and molecular mechanism would be a preliminary step in applying safety guidelines.

## References

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# Application of Nanomaterials in the Diagnosis and Treatment of Genetic Disorders

7

Alaa A. Aljabali, Mohammad A. Obeid, Haneen A. Amawi,  
Meriem M. Rezigue, Yassmen Hamzat, Saurabh Satija,  
and Murtaza M. Tambwala

## Abstract

Genetic testing is focused on identifying chromosome, gene, or protein changes between healthy and diseased cells or person. Genetic test outcomes can either verify or rule out possible genetic conditions and help determine whether a person is likely to develop or pass a genetic disorder. There are currently more than 1000 genetic testing and many more in the development pipeline. Therefore, the need to develop a susceptible and reliable method is vital in the diagnosis of genetic disorders. Nanomaterials offer a futuristic diagnosis platform for genetic diseases as it is a non-invasive, simple, portable, inexpensive diagnostic platform. Different nanomaterials have also been developed and functionized with the target molecules to provide therapeutic selectively and for molecular imaging. For these reasons, the development of nanomaterials for the early detection of specific disease biomarkers in tiny amounts reaches to part-per-billion (ppb) levels, in real-time, with high sensitivity and selectivity and reliability is of great importance in disease diagnosis and disease progression monitoring. Such nanomaterials should have exceptionally high sensitivity and selectivity that combines

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A. A. Aljabali (✉) · M. A. Obeid · M. M. Rezigue · Y. Hamzat

Faculty of Pharmacy, Department of Pharmaceutics and Pharmaceutical Technology,  
Yarmouk University, Irbid, Jordan  
e-mail: [alaaj@yu.edu.jo](mailto:alaaj@yu.edu.jo)

H. A. Amawi

Faculty of Pharmacy, Department of Pharmacy Practice, Yarmouk University, Irbid, Jordan

S. Satija

School of Pharmaceutical Sciences, Lovely Professional University, Phagwara, Punjab, India

M. M. Tambwala

School of Pharmacy and Pharmaceutical Science, SAAD Centre for Pharmacy and Diabetes,  
Ulster University, Coleraine, UK

the optical, magnetic, and electrical properties of nanomaterials with the biological selectivity and sensitivity toward their targets.

### Keywords

Gene detection · Biosensors · Microarray · Genetic diseases · Nanomaterials

## 7.1 Introduction

Genetic testing can provide information about individuals' genes and chromosomes information. Currently, some of the available genetic screening of newborns for genetic disorders that can be treated earlier, identified or excluded by diagnostic testing for a specific genetic or chromosomal disease are widely available in clinics. Besides, predictive and presymptomatic testing to identify the defected gene associated with specific genetic disorders after birth or identifying the risk of some people to develop some genetic diseases based on their family history is vital for genetic screening. Besides, predictive and presymptomatic testing to identify the defected gene associated with specific genetic disorders after birth or the risk of some people to develop some genetic diseases based on their family history. Therefore, it is paramount essential to develop reliable techniques and very rapid and sensitive methods at the same time. The genetic testing registry at NCBI (<https://www.ncbi.nlm.nih.gov/gtr/>) lists around 60,046 tests, 12,059 conditions, 18,631 genes involved in genetic disorders.

Technological development has been pending for successful diagnosis and clinical treatments for genetic diseases. Molecular biology and recombinant DNA have provided these diseases with diagnostic and therapeutic methods during the last two decades of the last century. Because researchers have understood biological mechanisms that prioritize hereditary factors and developmental progress, great scientific achievements have been used in clinics. Recently, the advancement in the nanotechnology field almost revolutionized the drug delivery and nanomedicine field. It is now possible to deliver therapeutics at precise locations and cells using targeted nanoparticles (NPs). One of the significant impacts of nanotechnology in medicine can mainly be seen in disease diagnostics, drug delivery, and controlled release. Diagnostic approaches are essential for the early detection of disease to enable prompt treatment and preserve severe damages to the tissues or organs. Diagnostic techniques that are based on NPs have proven to have higher sensitivity and play a vital role in the early detection of disease, leading to better prognosis and give the patient higher chance of survival and treatment (Aljabali et al. 2018b; Deng et al. 2014; Gao et al. 2014). Once the disease is diagnosed, the fight against the disease begins. Current diagnostic technology sensitive is deficient in some disease and can only be detected when at a very advanced or late stage, losing the chance to treat the disease. Nanodiagnosis will be used in this chapter for the use of nanoparticles for diagnostic purposes and early prognosis.

Nanodiagnostics offers to solve such problems with selective delivery and controlled release on specific locations. The recent technological advancement led to a new generation of nanodevices that are smaller, cheaper, faster, and require small samples to give accurate readings. The smaller samples required for the analysis imply less invasive extraction techniques and can obtain more accurate and reliable multiple measurements at the same time.

This chapter will explore the use of various nanomaterials for the diagnosis of genetic diseases and disorders. Conventional genetic disease screening and detection rely primarily on fluorescence and radioactive labeling, polymerase chain reaction (PCR), Northern blotting (RNA detection), Southern blotting (DNA detection), and enzyme-linked immune sorbent assay (ELISA) (Lien and Lee 2010; Sassolas et al. 2008). These technologies have provided the golden standard for genetic-based diagnostics that require careful primer design and accurate temperature to control the PCR amplification. Herein, excluding the conventional methods for the detection of genetic disease, we describe the applications of various nanomaterials in genetic-disease detection methods, including colorimetric, fluorescence, electrochemical, microarrays, and surface plasmon resonance.

Since the release of human genome sequencing in 2003, the sequence revealed a lot of genetic details about genetic variation. Many research papers have shown the relationship between SNPs with tumor development and progressing and the diagnosis of genetic diseases (Baptista et al. 2011). SNPs detection plays a pivotal role in identifying SNPs responsible for the early diagnosis of risk patients. The identification technique relies on either the identification of the mismatch sequence between healthy and infected samples. Knowing the connection between genetic factors and disease development has helped to uncover the molecular factors of the disease from the sequencing of the genome. In the last ten years, interest over detecting unique nucleotide sequences, particularly in the areas of medicine, biology, and chemical biology, has increased tremendously. The identification of genetic disorders and diseases plays an important role and is especially useful in early-stage intervention and surveillance. The focus of this book chapter will be on a noble NP, especially AuNPs, dendrimers, nanoshells, micelles, quantum dots (QDs), magnetic NPs, and liposomes as nanotemplates for the screening or the treatment of genetic disorders.

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## 7.2 Types of Nanoparticles Applied in Diagnosis and Treatment of Genetic Disorders

The efficacy of many conventional diagnostics and therapeutic preparations can be significantly improved using nanoparticle drug delivery systems. These nanoparticles are designed to improve the pharmacokinetics and the biodistribution of the encapsulated agents and ensure the accumulation of these agents at the target organ. The loaded therapeutic or diagnostic agents are usually either encapsulated inside or conjugated to a specific drug delivery carrier. The size of the nanoparticles delivery systems used for delivering diagnostic or therapeutic agents can range from 10 to

**Table 7.1** The common types of nanoparticles applied in diagnosis and treatment of genetic disorders

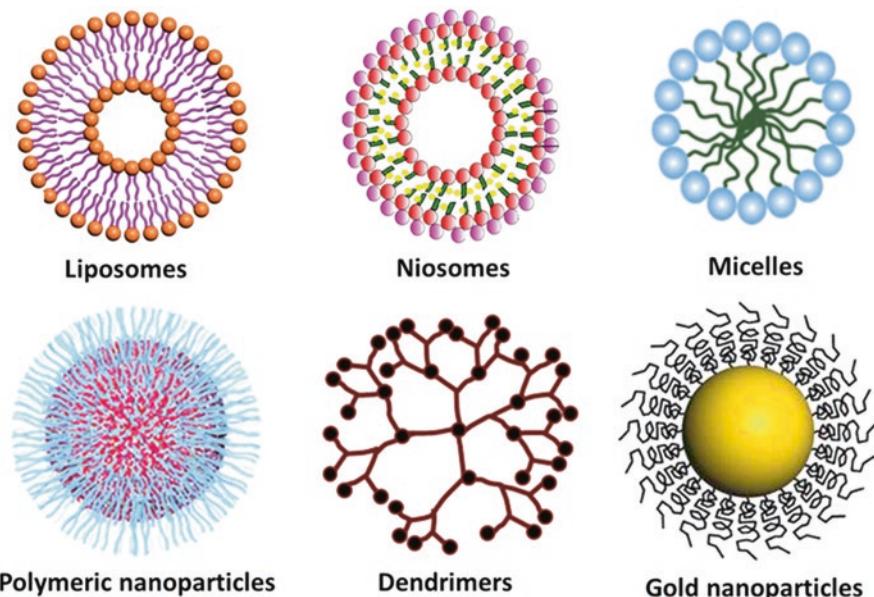
Nanocarrier	Composition	Size
Liposomes	Phospholipids, cholesterol	20–200 nm
Niosomes	Nonionic surfactants, cholesterol, charging agents	50–300 nm
Micelles	Amphiphilic molecules	2–300 nm
Polymeric nanoparticles	Different types of polymers such as polylactic acid, polysaccharides, poly(cyano)acrylates, poly(lactide- <i>co</i> -glycolide)	50–300 nm
Dendrimers	Branched polymers	5–50 nm
Gold nanoparticles	Chloroauric acid, sodium citrate	5–200 nm

300 nm. With this small size, these carriers will be able to penetrate diseased tissues such as tumors and inflamed tissues with high specificity based on the changes in the pathophysiological structure in these diseased tissues compared to healthy tissues that have normal tight junctions that prevent molecules at this size to penetrate these tissues. This is the basis of the passive targeting approach that helps the nanocarriers drug delivery system to be selective to diseased tissue and avoid the healthy tissues. Moreover, the surface of the nanocarriers used to deliver diagnostic agents can be functionalized with a specific ligand that can bind specificity to a complementary receptor at the target site, which will increase their specificity through the active targeting. Different types of nanoparticle delivery systems can be used to deliver diagnostic or therapeutic agents for the diagnosis and treatment of genetic disorders. The most used delivery systems and their compositions will be discussed here and summarized in Table 7.1 and Fig. 7.1.

### 7.2.1 Liposomes

Liposomes are spherical nanoparticles consisting of membrane bilayer structure encapsulating an aqueous moiety. The bilayer structure of the liposomes is usually composed of natural and synthetic phospholipids with cholesterol (Obeid et al. 2018). This structure of liposomes makes these nanocarriers versatile in which they can encapsulate hydrophilic agents in their aqueous core and hydrophobic agents in their membrane bilayer. Liposomes are expected to protect the loaded therapeutic or diagnostic agents until these carriers reach the target tissues and bind with the outer membrane of the target cells. Moreover, the surface of the liposomes vesicles can be functionalized with a specific ligand for specific receptor binding for active targeting (Akbarzadeh et al. 2013).

Cholesterol is also used in the preparation of liposomes, which will be embedded in the membrane bilayer of these nanoparticles. Cholesterol will decrease the fluidity and increase the rigidity of the bilayer structure of liposomes, which will help in preventing the leakage of the encapsulated drug from these vesicles (Obeid et al. 2018). Liposomes can be prepared through different methods such as hydration of a



**Fig. 7.1** Schematic representation for the common types of nanoparticles applied in the diagnosis and treatment of genetic disorders

thin film of lipids, microwave method, ethanol injection, and microfluidic mixing methods. Each method will end up in the preparation of liposomes with different characteristics in terms of size, polydispersity, and lamellarity. For example, the hydration of the thin film lipid will end up in the formation of large and multilamellar vesicles with a broad polydispersity index, which requires a size reduction step after preparation through extrusion or sonication. Microfluidic mixing, on the other hand, can produce small, unilamellar, and monodisperse particles without the need for a size reduction step for the prepared particles (Patil and Jadhav 2014). Liposomes have certain limitations in terms of production cost and stability. The phospholipids used in the preparation of these vesicles are expensive and are susceptible to oxidation, which might affect the long-term storage of these nanoparticles. However, liposomes are still the most studied type of nanoparticles as a drug delivery system.

### 7.2.2 Niosomes

Niosomes, or sometimes referred to as nonionic surfactant vesicles, are liposomes like vesicles in which they are composed of a membrane bilayer encapsulating an aqueous compartment. The replacement of the phospholipids in liposomes with a nonionic surfactant will end up in the formation of niosomes (Obeid et al. 2019). Different types of nonionic surfactants were used in the preparation of niosomes

such as Tweens, Spans, and Brij surfactants. These surfactants will have different vesicles forming abilities based on the difference of their hydrophilic–lipophilic balance (HLB) values. Cholesterol will also be used in the formation of niosomes to increase the membrane bilayer rigidity. Other components that can be used in niosomes preparations are charging lipids, which are used to induce a specific charge on the surface of niosomes. For example, diacetyl phosphate was used to produce negatively charged niosomes, while sterile amine was used in the preparation of niosomes with a positively charged surface (Marianecci et al. 2014). Niosomes can be prepared with the same methods that are used in the preparation of liposomes and, similar to liposomes, can encapsulate both hydrophilic and hydrophilic therapeutic or diagnostic agents. However, niosomes have superiority over liposomes in terms of stability and production cost as the surfactants used in niosomes are stable and very much cheaper compared to phospholipids used in liposomes (Obeid et al. 2017b, c).

### 7.2.3 Dendrimers

Dendrimers are another type of nanoparticle drug delivery system, which can be used in the delivery of therapeutic and/or diagnostic agents in genetic disorders. Dendrimers are made up of different types of repetitively branched polymers such as poly(ethylene glycol) (PEG), poly(l-glutamic acid) (PGA), polyethylenimine (PEI), and polyamidoamine (PAMAM) using different step-growth polymerization (Aulenta et al. 2005). Dendrimers consist of an inner core with different levels of branches coming out of this core. Dendrimers are monodispersed nanoparticles, and the outer terminal of these branched nanoparticles can be modified to achieve active targeting of the loaded drug or for selective imaging of different diseased tissues (Kesharwani et al. 2014).

### 7.2.4 Polymeric Nanoparticles

Polymeric nanoparticles are considered among the highly investigated solid colloidal particles for drug delivery of therapeutic and diagnostic agents. The intended therapeutic or diagnostic agents to be delivered will be conjugated or encapsulated onto or within the surface of these nanoparticles. Polymeric nanoparticles can be used to achieve sustained release of the loaded cargo at the target site (Masood 2016). Different methods can be applied in the formulation of polymeric nanoparticles for drug delivery, and these include emulsification, solvent extraction, nano-precipitation, and many other methods (Rao and Geckeler 2011). Depending on the formulation method, the final composition of the prepared polymeric nanoparticles may vary nanospheres or nanocapsules. Nanospheres are a polymeric nanoparticle's structure where the loaded drug is dispersed throughout the particles where the drug in the nanocapsules will be dispersed into an aqueous or oily cavity surrounded by a polymeric membrane (Prabhu et al. 2015).

Polymeric nanoparticles will protect the loaded therapeutic or diagnostic agents against the degradation effect of the body enzymes. The release of the loaded cargo from the polymeric nanoparticles will happen through diffusion, hydrolysis, enzymatic degradation, or through a combination of all these methods (Edlund and Albertsson 2002). Several types of polymers can be used in the preparation of polymeric nanoparticles, such as poly(lactide-co-glycolide) (PLGA), polylactide (PLA), polyglycolide, polycaprolactone (PCL), poly(d,l-lactide), and many others. Polyethylene glycol can also be incorporated for the development of polymeric nanoparticles with active targeting (Prabhu et al. 2015).

### 7.2.5 Micelles

Micelles are a drug delivery system in which amphiphilic molecules will be self-assembled into spherical particles. The type of these amphiphilic molecules in micelles preparation will determine the final micelles category. This includes lipid micelles, lipid-polymeric hybrid micelles, or polymeric micelles. The core of these monolayer vesicles will be composed of the hydrophobic part of the copolymer, while the hydrophilic part of the copolymer will be exposed to the outer aqueous environment (Obeid et al. 2018). The formation of the micellar structure will depend on the concentration of the amphiphilic molecules where the lowest concentration of these molecules that are required for the self-assembly and micelles vesicle formation is called the critical micelle concentration (CMC) (Letchford and Burt 2007). This micellar structure enables hydrophobic therapeutic or diagnostic agents to be localized into the core of the micelles and be protected from the outer degradative enzymes (Obeid et al. 2017a). However, because of their limited hydrophobic volume, micelles usually have limited loading capacity for hydrophobic therapeutic or diagnostic agents (Obeid et al. 2018).

Biosensors are defined as analytical devices composed of a biological recognition element and an optical/magnetic transducer (Fan et al. 2005). The binding of the biological moieties to the sensor generates a measurable signal (electronic or optical). Biosensors are highly sensitive resulted from the high specificity binding of the biomolecules to their target and required only a tiny amount of the analyte to be detected (i.e., antibody and antigen). Also, the selectivity of the sensor relies on the selectivity of the biological recognition (i.e., the enzyme and its substrate). Biosensors can be classified based on their recognition properties such as enzyme-based, immunological, and DNA biosensors. Another way of classifying biosensors is based on their type of inducers; electronic, optical, electrochemical, fluorescent, and piezoelectric biosensors (Park et al. 2002; Xu et al. 2005; Aljabali et al. 2018b). Over the past few decades, biosensors were developed into commercial products such as lab-on-a-chip to facilitate molecular diagnostics for various genetic disorders. Biosensors' portability and their sensitivity make them an ideal candidate for the development into easy to use clinical tools.

### 7.2.6 Gold Nanoparticles (AuNPs)

AuNPs are considered as attractive drug delivery systems for the delivery of various molecules such as nucleic acids, proteins, and anticancer agents. The core of this NPs is inert and non-toxic. Moreover, these NPs can be easily prepared with different sizes ranging from 1 to 200 nm with monodisperse size distribution (Ghosh et al. 2008). AuNPs can be prepared through the reduction of gold salts by the addition of reducing agents in the presence of a stabilizing agent to prevent the formation of agglomerates. The final particle size of these NPs will depend on the used gold salts and type of stabilizers. These NPs can deliver different therapeutic and/or diagnostic agents by binding the active agent to these nanoparticles through thiol linkage. Gold nanoparticles have been investigated by many researchers for the delivery of contrast agents for X-ray (Hainfeld et al. 2008), for immunostaining (Mallidi et al. 2015), and as a delivery vehicle for many therapeutic agents to enhance their uptake by the target cells (Jain et al. 2011; Pissuwan et al. 2011). See Table 7.1 and Fig. 7.1.

## 7.3 Sensory NPs for Genetic Disease Detection

Gene polymorphism plays a pivotal role in the rapid detection of genetic variations allowing for a very reliable early diagnosis of genetic diseases way before any symptoms of the disease appear. The basic principles of the detection of any genetic disorders rely on direct hybridization with fluorescent (Maxwell et al. 2002; Fang et al. 2000), chemiluminescent, and radioactive (Pathak et al. 2001). Additionally, the indirect detection methods for the identification of genetic diseases rely on the enzymes, which catalyze the reaction to generate colorimetric or fluorescent signals that can be quantified using spectroscopy techniques (Campbell et al. 2002; Liu and Lu 2004). Maxwell et al. (2002) developed a single base mutation detection sensor in which thiolated fluorescently labeled oligonucleotide sequence attached to the surface of 2.5 nm gold nanoparticles AuNPs. Once the target binds, allowing the fluorescent to leave the surface of the nanoparticles as after this structural change, a fluorescent signal is generated and highly selective toward its target.

Another significant development in clinical detection of cancer biomarkers is the use of “nanolock-nanopore” for the detection of a single molecule of a serine/threonine-protein kinase gene BRAF V600E mutation in thyroid cancer patients (Wang et al. 2017). The strategy was focused on sequence-specific nanolock mutation identification. The formed nanolock produced on the mutant allele duplex can be separated into two concurrent phases in the nanopore by the duplex dehybridization approach. Such unzipping kinetics generates a unique electrical signal which allows the detection of the tumor mutant allele single DNA molecule compared to the wild-type allele. This method for diagnosis not only allowed detection as a diagnostic tool but also as a quantitative method (Wang et al. 2017). The BRAF V600E mutation is well studied, and there are treatments available that specifically target it. Due to the high sensitivity and lower identification limits, there is indeed a potential

to use in non-invasive cancer diagnostic methodologies, or, if detected earlier, therapy would be very efficient.

Another remarkable example of such digitalized biosensors is the use of nanowires to differentiate between mutant and wild-type genes of cystic fibrosis of one of its transmembrane receptor proteins through the monitoring of the change in the chemical potential generated from the binding event of the target/analyte (Gao et al. 2014). The uniqueness of such a system relies on conductivity changes after the binding event without the need for target amplification. For instance, 20 nm wide silicon nanowire was created through a vapor deposition procedure where a set of biotinylated peptide nucleic acid was paired with functionalized avidin proteins necessary for elemental nanowire recognition. The complete DNA sequence of the transmembrane receptor of the cystic gene fibrosis was contained in the nucleic acid specimens of the peptide nucleic acid probes leading to an abrupt twofold increase in the conductivity of the nanowire (Hahm and Lieber 2004).

One of the methods for cancer detection relies on SNPs for the detection of mutation in the breast cancer gene (BRCA1). Mutations in the BRCA1 gene are responsible for more than 80% of inherited breast cancers. Therefore, a sensitive and reliable method was developed to detect SNPs in exons, 2, 3, 9, 10, 11, and 16 of BRCA-1 and BRCA-2 (Gharatape and Yari Khosroushahi 2019; Rebelo et al. 2019; Saylan et al. 2019). Furthermore, the detection of the mutated tumor suppressor gene TP53, which undergoes missense mutation, in which single nucleotide is substituted for another have also been reported. The identification of such mutation in the TP53 is of great value for the prediction of the prognosis of a tumor and has been identified using electrochemical nanosensors (Esteban-Fernandez de Avila et al. 2015).

More than 12 hereditary disorders in humans have been identified to be linked to extend trinucleotide repetitive sequences (TNRs) such as CTG, CGG, and GAA repeats, including Myotonic Dystrophy (CTG repeat in the first intron of *Zinc Finger 9* (ZNF9)), Fragile X Syndrome (CGG-CCG triplet), Huntington disorder (CAG-CTG), multiple spinocerebellar ataxias (CTG-CAG), and Friedreich Ataxia (GAA-TTC triplet). For example, Huntington disease is mainly caused by the expansion of CAG repeats in the huntingtin gene (HTT). Patients develop symptoms when more than 27 CAG repeats in their HTT gene are present. To confirm the clinical diagnosis, genetic screening for families with risks and the early identification of heterozygous carriers are necessary for the diagnostic of these conditions at the DNA levels. The existing techniques for the identification of triplet repeat include southern blotting and PCR amplification of DNA domains (Campuzano et al. 1996).

Unfortunately, there are no effective treatments available to treat such damaged diseases because so little is known regarding pathogenesis pathways. In 2005 researchers from Japan developed a small molecule biosensor capable of identifying CAG repeats hairpin length. The small-molecule ligand, naphthyridine-azaquinolone (NA) binds selectively to the CAG-CAG triad. The research team led by Kazuhiko Nakatani developed a surface plasmon resonance biosensor based on AuNPs that is highly selective and sensitive to CAG repeat length. The repeat

sequence bound to the immobilized NA on the sensor more efficiently than the shorter CAG sequence. This binding was converted to surface plasmon resonance signal intensity and could be used to establish the severity and the stage of the disease (He et al. 2013). Besides, new sensitive methods rely on the electrochemical recognition of the length of the triplet expansion based on the multiple hybridizations of the repetitive sequence with a short labeled reporter probe (Fojta et al. 2004).

Lymphoma cells exhibit different non-glycosylated CD20 antigens on their surface. The CD20 plays a critical role in the B-lymphocyte differentiation and activation of the cells. The overexpression of the CD20 antigens on the tumoral B-lymphocyte made it a unique target for capturing the circulating tumor cells (CTCs) in blood and other fluids. QDs or magnetic NPs functionalized with Rituximab (binds selectively with the CD20 antigen) and detected through flow cytometry with higher sensitivity and specificity (Shariatifar et al. 2019; Bao et al. 2019).

Furthermore, another approach was developed for the detection of phenylketonuria (PKU), in which defective enzyme phenylalanine hydroxylase (PAH) fails to metabolize the phenylalanine amino acid. The researchers developed an electrochemical based nanosensor for the recognition of a PAH mutation using AuNPs decorated with alkanethiol single-strand DNA deposited on the reduced graphene oxide sheet with a detection limit of 21.3 fM of the target DNA concentration (Seifati et al. 2018).

The development of polynucleotide molecules known as a molecular beacon (MB) is a fluorescent-based sensor that has been developed for gene analysis (Fang et al. 2000). MBs are intended to create a stem-loop structure with a 3' end quencher and a 5' end fluorophore. The quencher's relative proximity to that of the fluorophore will not permit any fluorescence to show up. The quencher and fluorophore were spatially separated after the hybridization of the target oligonucleotide with the MB, allowing the fluorescence signal to be restored. MBs have been utilized for the DNA sequence changes on single base mutations in various genetic diseases, measure different gene expression levels (Ferguson et al. 1996; Taton et al. 2000), forensic analysis, and drug screening (Wang 2000; Falus et al. 1998).

According to the International Federation, Diabetes mellitus (DM) is one of the top five death causes in most developed countries. DM has many etiologies such as low fat, carbohydrate and protein metabolism as a chronic hyperglycemic disease. Type 1 DM is the absolute insulin deficiency and is classed in 1A and 1B types. Autoimmune destruction of  $\beta$  cells constitutes to 1A, while idiopathic insulin deficiency belongs to 1B type (Alberti and Zimmet 1998). Oral hyperglycemic medications and insulin and glucagon-like parenteral formulations are commonly used in conventional therapy (Meier 2012). Zhang et al. established a liposome-based lipid formulation system by mixing lipid: cholesterol of 3:1 with maximum efficiency in insulin trapping (Zhang et al. 2014). Chitosan-coated liposomes facilitate insulin delivery in an alternative approach. The hypoglycemic effect increases with the increasing molecular weight of chitosan (Wu et al. 2004).

Furthermore, folic acid functionalized liposomes improved the targeted delivery of insulin and anionic poly(acrylic acid) and cationic poly(allylamine) coatings

resulting in sustained hypoglycemic conditions observed for 18 h after an advanced dose (Agrawal et al. 2014). Another study was carried out using a reverse-phase evaporation approach to load metformin hydrochloride into a Span-40-based niosome. Pharmaceutical research has confirmed that metformin-charged niosomes are more effective than mere metformin in lowering blood glucose levels (Hasan et al. 2013). A biodegradable transmucosal nano-fiber patch has also been produced by electrospinning of poly(vinyl alcohol)-alginate composite, and by dynamic loading for diabetes management, the patch has been impregnated with antidiabetic medication insulin. Besides, ~100% of drug releases took place within 12 h of patch release. The sequence of the release follows the kinetics of first order with an initial explosion of the drug. The *in vivo* experiments confirmed that drug-induced nanofibers have been effective since the level of glucose in diabetic male Wistar rats was significantly decreased (Jin et al. 2012).

Alzheimer's disease is the most common neurodegenerative disorder based on premortem and *in vivo* brain imaging diagnosis. The current screening method for markers examines the total protein content of the tau and amyloid- $\beta$  gene. Alternatively, the approach relies on the targeting of tau protein or amyloid- $\beta$ -derived diffusible ligands (ADDLs) as a diagnostic marker. Although this approach could help to detect pathogenic markers, levels are deficient and cannot be accurately identified by the ELISA test.

Nam et al. have published a colorimetric bio-barcode system that minimizes the requirements of traditional non-enzymatic cytokine detection assays. They used porous microparticle materials, which allowed the loading of large quantities of barcode DNA per AuNPs. The sensitivity of this test is consistent with the identification of 30 aM concentrations of cytokines (about three orders of magnitude more active than other non-enzyme assays). This test is intended to be very useful in the diagnosis of immunological disorders containing cytokines as a marker (Nam et al. 2005). A successful application for the bio-barcode is the identification of amyloid-B-derived diffusible ligands (ADDLs), which are potentially soluble markers for the detection of the Alzheimer (Georganopoulou et al. 2005).

Hemophilia is a hereditary bleeding disorder triggered by the absence or dysfunction of coagulation factors VIII (FVIII) or IX (FIX). The FVIII and FIX genes are positioned in the X chromosome. Conventionally hemophilia is treated with injections of the missing coagulation factor (FVIII or FIX), as these injected factors have a very short half-lives (10–12 h) and require three weekly infusions to keep the levels of FVIII (20–40 IU/kg) above the spontaneous bleeding level, multiple injections are required. Therefore, the successful development of PEGylated liposomes of a size between 80 and 100 nm with surface functionalization to coagulation factor FVIII (Yatuv et al. 2010). The generated nanomaterials were well tolerated and appropriate for long-term therapy of hemophilia. The liposome-based approach confirms that particles are not toxic in mice and dogs, as confirmed by this study.

A relevant field of research in nanomaterials fabrication is to provide exogenous enzymes to the cell/tissue to replace defective enzymes through the replacement therapy approach. One of the early examples of the use of nanomaterials approach is the diagnosis of lysosomal storage disorders, primarily due to genetic deficiencies

causing the lysosomal degradation of different substrates. The list includes neuro-pathic Phenylketonuria (PKU) as a result of a phenylalanine hydroxylase (PAH) defect (Harding and Blau 2010), or the delivery of uricase enzyme for gout treatment (Sherman et al. 2008).

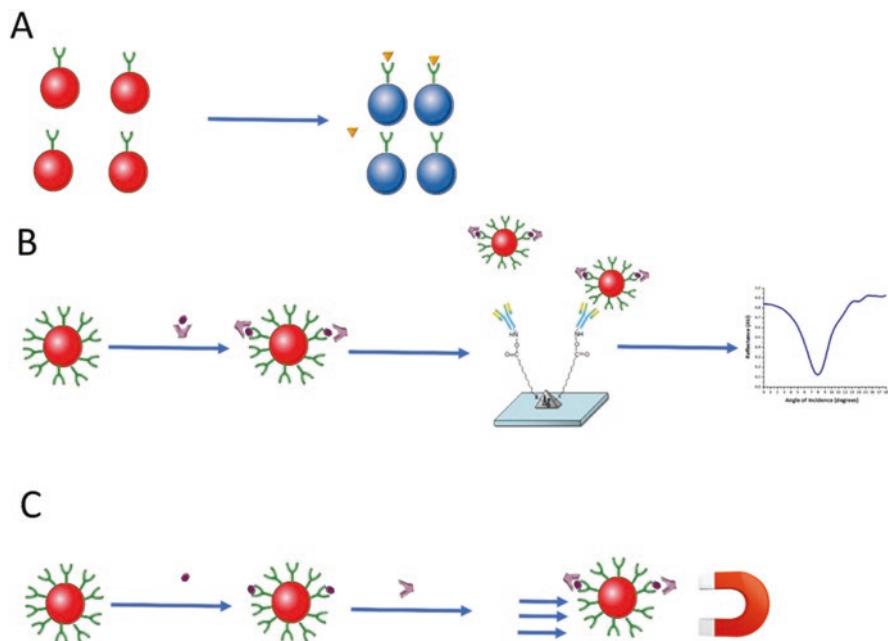
One (A-T) gene mutation causes sickle-cell disease. Beta-globin gene, a debilitating and life-shortening illness mostly infects people of African descent. Successful application of custom-designed engineered essential substances (nuclease enzyme) to treat human disease cells for the detection of possible off-target cleavage events (cleavage at locations other than the intended target site). An active area of the development of custom-design, enzyme-based critical substances in the treatment of cancer cells in human cells and for the identification of possible off-target cleavage incidents, which may contribute to single point mutations, deletion of nucleotides, and also responsible for chromosomal reordering. One of the examples of such an approach is the engineering of the FokI nucleases that are required for the dimerization of (right and left) to dimerize. A mutation that renders the enzyme inactive, resulting in single-strand breaks (nicks) as opposed to double-strand break. An example of treating single-gene disorder is the use of Zinc-finger nucleases (ZFNs) and transcription activator-like effector nucleases (TALENs) (ZFN/TALEN) based treatment of sickle-cell disease. ZFN and TALENs precisely bind to the  $\beta$ -globin gene and deliver them to hematopoietic progenitor cell nuclei via donor templates carrying stable  $\beta$ -globin. The delivered nuclease in the targeted area near the  $\beta$ -globin locus is used to activate the homologous recombination route to the correct gene sequence. The correct hematopoietic stem and progenitor cells can re-engrafted in the sickle-cell disease to produce healthy red blood cells and replace the sickle cells (Sun et al. 2012; Bertolini et al. 2007).

### 7.3.1 Types of Detection

High sensitivity nanosensors utilize electronic, mechanical, electrical, and magnetic relaxation properties to drive biomarkers identification limits below reasonable concentrations. The design of the nanosensor to detect deficient concentrations of biomarkers to provide a distinctive signal that might be optical, magnetic, or electronic nanosensors, as shown in Fig. 7.2.

### 7.3.2 Electronic Detection

One of the earliest examples of the electronic nanobiosensors was the BioDetect™ system developed by Integrated-NanoTechnologies (Henrietta, NY). The nanobiosensor was developed to detect the binding of a target DNA electronically to a microchip. The BioDetectTM system operates by digitally identifying the adhesion of DNA from a target molecule to an INT microchip sensor. The DNA forms a bridge between two wires divided by electricity. The bound target DNA is then chemically formed to form a conductive wire that, like that of an on/off key, “turns



**Fig. 7.2** Principle of the identification of hereditary mutation biomarkers by nanomaterials (A). Monodisperse NPs functionalized with (complementary oligonucleotide sequence, antibody) upon the binding between the surface moieties of NPs and the analyte, the solution color changes immediately from ruby-red to blue as a result of surface plasmon resonance (SPR) property of AuNPs (B) Sensogram signal of SPR signal reporting the binding response versus time that monitors the binding between the ligand-analyte and can be converted into digital. (C) Magnetic detection sensors that rely on the conjugation of antibodies to the surface of magnetic nanomaterials and using an external magnet to pull the nanomaterials and the target

on” the sensor (Bhalla et al. 2016; Malekzad et al. 2018). In addition, some of the latest research into this field has demonstrated how a biosensor for single nucleotide (SNP) detection from DNA has been developed. In contrast to conventional methods of SNP detection, the system combines the use of DNA-tweezers with high-quality graphene with a higher sensitivity almost 1000-fold (Hwang et al. 2018).

### 7.3.3 Optical Detection

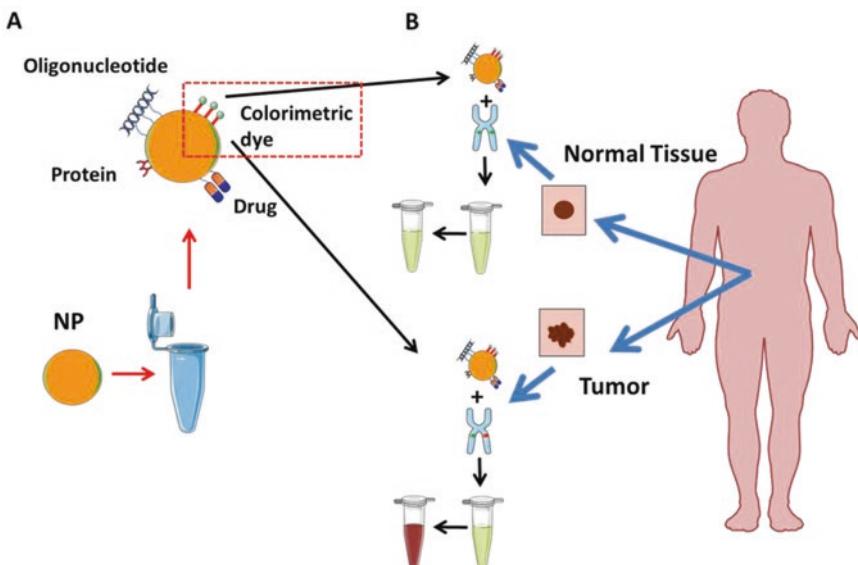
One of the essential properties of gold nanoparticles (AuNPs) is their optical properties, relative ease of synthesis, and the ability to modify the surface to induce targeting moieties for selective targeting, diagnosis, or imaging. AuNPs have a surface plasmon resonance (SPR), which describes the oscillation of the conductive electrons of the gold atoms that are triggered by electromagnetic waves (light). SPR properties are highly dependent on the AuNPs size, geometrical shape, and surface modifications (Nugroho et al. 2016; Aljabali and Evans 2014; Aljabali et al. 2018a).

Usually, AuNPs colloidal solution of a size under 100 nm has a ruby-red color. As the AuNPs size increases, particle extinction shows a red-shifts, resulting in a bluish (purple) solution (Yang et al. 2017). This color change (aggregation of AuNPs) can be achieved through a cross-linking mechanism based on compatible molecules between the analyte and what is on the surface of the AuNPs. Alternatively, the adsorption of the biomolecules such as DNA and peptide can agglomerate or adsorb on the surface of the AuNPs leading to the color change. Either way, this phenomenon allows for higher sensitivity and lower detection limits (Liu et al. 2011). This red-shift property is the essence of developing an immunoassay for the detection of prostate-specific antigen (de la Rica and Stevens 2012). This colorimetric detection approach based on the use of AuNPs can be a valuable tool in monitoring in real-time and on-site detection in a straightforward manner without the need for expansive devices and is superior to many other detection methods with higher sensitivity (Deng et al. 2014; Ma et al. 2011).

### 7.3.4 Microarrays

Microarrays are tools for the simultaneous identification in each sample of various nucleic acid-based fingerprints. Different microarrays of different target materials, including DNA, cDNA, mRNA, protein, small molecules, tissues, or other quantitatively analyzable substances have been developed. In the last ten years, DNA microarray technology has evolved rapidly and enables significant quantitative analysis of gene expression at the same time. Some of the main applications of microarrays in diagnosis fall within one of the following categories: analysis of gene expression (to determine expression patterns and allow quantifiable comparison between healthy and diseased cells), mutation and polymorphism detection (identifying SNPs and mutations in involved genes and its subsequent impact on the cells/organism). Enable genetic characteristics which could affect the response to a therapy as a follow-up treatment approach, to develop knowledge about genetic characteristics of diseases before symptoms begin to appear and to prevent them in a preventive medicine approach, and finally microarrays enabling fast diseases detection by using the correct genetic markers (Fig. 7.3).

DNA microarrays is one of the most used techniques for the detection and mapping of the changes that correlate the DNA alterations with the subsequent genetic diseases. Conventional DNA microarrays have been used for genotyping and the determination of genetic disease-relevant genes that are responsible for causing the disease, screening of single nucleotide polymorphisms (SNPs), detection of chromosome abnormalities, and determining genetic diseases related to posttranslational modification. The two primary sources of genetic disease are either chromosome abnormality or DNA mutations. Mutations can lead to the change of the amino acid sequence and change the 3-D structure. Common SNPs detection methods include polymerase chain reaction (PCR) for DNA amplification followed by gel-electrophoresis based fragments identifications, restriction fragment length polymorphism (RFLP), and single-strand conformation polymorphism (SSCP)



**Fig. 7.3** Nanoparticles are tools for diagnosis of genetic disorders (Nanodiagnosis). (A) The preparation of nanoparticle intended as nanodiagnostic tool. The nanoparticle can be loaded by different molecules, including oligonucleotides, proteins, drug, and colorimetric dyes among others. (B) Nanoparticle-based colorimetric test as an example of nanodiagnostic tools; in summary, the DNA is initially purified from a clinical sample. The nanoparticle based-colorimetric dye is then added directly to the isolated DNA for the detection of genetic alterations. Visual observation of the sample is carefully conducted to detect any changes in the color of the mix. Healthy tissue does not contain a complementary DNA and does not show any change in dye color. Tumor tissue contains a complementary DNA with mutation (ex: SNP) and produces radially detectable color change when mixed with the colorimetric dye

(Acquila et al. 2001; Korkko et al. 2002). Despite their effectiveness, these techniques are time-consuming and laborious, hence the need for the development of DNA microarray for SNPs screening.

Chromosomal microarray (also known as DNA microarray) is one of the most powerful diagnostic tools for genetic disorders by determining the extra (duplicated) missing (deleted) segments alternatively looking for the copy number of the genes. The technique is very reliable in detecting homozygosity specifically the recessive disease disorders, karyotype, mosaicism (a mixture of normal and abnormal cells). A DNA microarray is generally composed of a solid surface (gold, mica, or carbon) onto which DNA with known sequences hybridized with the clinical sample under investigation. Kang and coworkers developed a DNA microarray for the detection of Down's syndrome (associated with chromosome 21), Patau syndrome (associated with chromosome 13), Edward syndrome (associated with chromosome 18), Turner syndrome (associated with chromosome XO), Klinefelter syndrome (associated with chromosome XXY), alpha-thalassemia retardation-16, Prader-Willi syndrome, Rubinstein-Taybi syndrome, and Williams syndrome

(Fiorentino et al. 2013; Riggs et al. 2014; Roberts et al. 2014; Yatsenko et al. 2013). Furthermore, SNPs arrays designed to examine the association of specific nucleotide alteration with its corresponding disease. SNPs rely on oligonucleotides that are 25–50 base pair (bp) long with a recommended quantity of the DNA between 200 and 250 ng of the DNA, which is an achievable target for most prenatal samples for the early detection of chromosome abnormalities (Shaffer et al. 2012).

### 7.3.5 Viral Vectors for Gene Delivery

The development of various nanomaterials as a therapeutic tool to increase the therapeutic effectiveness, lowering the toxicity. Nanomaterials have enhanced bioavailability with lower doses administered in comparison to free drug molecules and thereby minimize the adverse side effects. One of the most effective methods for safe gene transfer to the cells depends on the use of viral-based vectors for gene therapy and vaccines. Viruses present the classical approach for long-term gene therapy applications. Viruses are obligate intracellular parasites with the ability to use the host cell machinery for their biosynthesis and propagation. Viruses have a minimal genome with only the necessary genes for their nucleic acid integration with the host cell and their viral-protein genes.

Viruses like the retrovirus, lentivirus, adenovirus, and adeno-associated viruses have been widely used for vaccination and neurological disorders treatment and as a viral vector in the transduction process (Gradishar et al. 2005; Nyman et al. 2005). Viral vectors play a vital role in gene delivery to the targeted cells. Virus based systems are deemed safe as the viruses can only replicate in susceptible and permissive cells. The spectrum of viral-based vectors is comprehensive and has been developed for transient (short-term) and permanent (long-expression).

Adeno-associated viruses (AAV) low pathogenicity and toxicity with its long-term transgene expression consider one of its significant advantage in gene delivery. However, AAV can only carry 4 kb insert on its single-strand ssRNA, and an immune response is triggered by repeated administration of AAV, which can be addressed by applying a different AAV serotype for each re-administration (Mingozzi and High 2013). In this context, X chromosome-linked neurodevelopmental disorder named Rett Syndrome (RTT) has shown dose-dependent side effects, and extensive survival for RTT mousses was demonstrated by the AAV vector expressing the transcription regulator methyl CpG-binding protein 2 (MeCP2) directly delivered to the cerebrospinal fluid (CSF).

Moreover, AAV-based therapy was tested in the neurodegenerative Huntington (HD) mouse model (Pfister et al. 2018). Furthermore, HD transgenic sheep expressing the full-length human huntingtin (HTT) gene was generated, the findings showed that there was a decrease in human HTT mRNA levels between 50 and 80% HTT protein upon the successful targeting of exon 48 of the HTT gene (Pfister et al. 2018). Although, cystic fibrosis AAV-based expression system of the cystic fibrosis transmembrane conductance regulator (CFTR) has been developed in several animal models showing an excellent safety profile, however, there were no significant

medical benefits reported from such approach (Guggino et al. 2017). Besides, in 17 patients with colorectal cancer, non-small cell lung cancer, urothelial cancer, and renal cancer, tumor-selective chimeric enadenotucirev adenovirus vector was intravenously delivered. Tumor-specific delivery was reported in most tumor tissue with no adverse side effects reported (Garcia-Carbonero et al. 2017).

The AAV-based delivery system, this approach has been developed for hemophilia. AAV system has provided a long-term expression of factors VIII (FVIII) and IX (FIX), which cured the patients of severe bleedings and joint damage associated with hemophilia. Currently, there are 11 clinical trials for hemophilia gene therapy and six ongoing Phase I/II tests for liver-directed AAVs delivery that produce either FVIII or FIX with some success (Spencer et al. 2016).

One of the first viral-based gene therapy was approved for the oncolytic adenovirus (AV) expressing the p53 gene known as Gendicine™ and AdH101 containing the E1b-55 K deletion sequence for the treatment of head and neck cancer (Raty et al. 2008; Zhang et al. 2018). Gendicine™ has been used in over 30,000 patients with an excellent safety record for 12 years and has received considerably better results in tandem with chemotherapy and radiotherapy than in conventional treatments. Most recently, a second-generation oncolytic herpes simplex virus (HSV) vector with the GM-CSF has been approved for melanoma treatment in Europe and the USA (Fukuhara et al. 2016; Kaufman et al. 2010). The future is auspicious in this area and many other viral-based drugs will most likely be in the market in the future.

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## 7.4 Conclusions

In summary, there has been significant progress in the field of genetic disorder testing in almost 3000 clinical trials carried out by 2017, and the estimated number would be higher by now. Not surprisingly, the rapid advancements and innovations in the area of nanomedicine have regularly changed the field of disease diagnostics. Throughout biomedical research and clinical practice, the use of nanotechnology is established in the nanomedicine area, which could directly affect human health in the next few years. In diagnosis, imaging and targeted use of drug carriers' nanomaterials are commonly used. Continuous clinical care for the patient's health can be provided through nanoscale sensors and tools. In the early stage of illnesses, including leukemia and inflammations, nanoscale detectors and software have helped improved diagnosis. Recent advances over the past two decades in nanotechnology have allowed the researchers in the area to develop new and very reliable tools for the diagnosis of genetic diseases. None of the systems we are aware of can reach the sensitivity and the selectivity of the nanomaterial-based approaches. The future of genetic disorders screening or treatment is very promising even taking a cautious approach. With the use of diverse nanomaterials, the approval of several therapies for the treatment of various genetic diseases allows tremendous flexibility and alternative approaches. However, nanomaterials have advanced significantly for gene therapy and in diagnostics and have become a central intervention in modern medicine.

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# Application of Nanomaterials in Cancer Diagnosis, Drug Delivery, and Therapy

8

Shahid S. Siddiqui, Mashael Saeed Al-Qahtani,  
Faisal Ahmed Khalil Al Allaf, Loganathan Sivakumar,  
and Zeba Kidwai Siddiqui

## Abstract

Cancer diagnostics and therapy has a lot to gain from advances in nanotechnology. Liposomes like nanoparticles can be loaded with probes and anti-cancer drugs to target cancer tissues. Drug delivery requires the specificity of targeting the cancer tissue; prolonged circulation of the nanoparticles in the blood; assessment of the tumor microenvironment (TME) and the controlled release of nanoparticles. This is particularly important from enhanced permeability and retention of nanomaterials, also known as the EPR effect. Thus, controlling the nanoparticles for different cancer types and in different formulations is critical. Efficacy and access of nanoparticles to the cancer cells may be monitored and

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S. S. Siddiqui (✉)

Department of Medicine, University of Chicago, Chicago, IL, USA

Department of Basic and Clinical Oral Sciences, Faculty of Dentistry, Umm Al Qura University, Makkah, Saudi Arabia

Department of Medical Genetics, Faculty of Medicine, Umm Al Qura University, Makkah, Saudi Arabia

M. S. Al-Qahtani

Department of Basic and Clinical Oral Sciences, Faculty of Dentistry, Umm Al Qura University, Makkah, Saudi Arabia

F. A. K. Al Allaf

Department of Medical Genetics, Faculty of Medicine, Umm Al Qura University, Makkah, Saudi Arabia

L. Sivakumar

Department of Medicine, University of Chicago, Chicago, IL, USA

Department of Environmental Science, Periyar University, Salem, India

Z. K. Siddiqui

McGenome LLC, Glenview, IL, USA

regulated for specific tumor types that could lead to patient specific precision medicine. Hence, innovative nanotechnology can supplement existing molecular, cellular, and genetic techniques to study alterations across different cancer types, enabling the sorting of normal and malignant cells and tissues. For diagnostics, nanoparticle biosensors may be used in monitoring molecular signals specific to tumorigenesis, to assess tumor specific changes occurring in the malignant tissues. Here we also review novel nanotechnologies including the use of CRISPR/Cas9, CAR-T immunotherapy, and DNA and RNA nanotechnology studies in cancer theranostics design.

### Keywords

Nanomaterials · Nanoparticles · CRISPR/Cas9 · CAR-T · Immunotherapy · Cancer theranostics · EPR · Nanotechnology · Nanomedicine · Drug delivery · Cancer therapy · Spherical nucleic acids

## 8.1 Introduction

Cancer is a complex and devastating global disease that results in morbidity and poor health in all ethnic populations and age groups. About 18 million new cases of cancer and 9.6 million cancer related deaths have been reported in 2018; an estimated 20% of men and women are predicted to develop cancer in their lifetime globally (IARC Global Cancer Observatory; <http://gco.iarc.fr>). Several factors contribute to the increasing incidence of cancer such as population growth, aging, social, economic, and environmental disparities.

Early diagnosis of cancer provides better treatment options, reduced health burden, and improved survival. A key technology in helping better diagnosis and drug delivery is the use of nanomaterials that have shown improvement in cancer targeting, diagnosis, and potential therapy developing clinical translation of nanomedicine. Nanomedicine encompasses aspects of engineering, molecular biology, cell biology, material science, and developing related technology that works at nanoscale level to translate in effective diagnosis, drug delivery and therapy. These novel approaches promise in the improved theranostic of cancer and thereby help in better survival of the cancer patients.

Here in this chapter, we focus on the application of nanomaterials in cancer diagnosis, novel approaches of drug delivery using nanomaterials, and potential use of nanomedicine in cancer therapy. For cancer diagnostics, drug delivery and possible therapy, nanomedicine has made great strides, since the established conventional cancer therapies have not been able to overcome limits for safer and improved cancer diagnosis and therapy. Despite significant progress in nanotechnologies, the main challenge remains in understating the complexity and heterogeneity of the cancer tissue, and the mechanism(s) by which the nanoparticles interact with the cancer tissue. For nanomedicine to succeed and have meaningful commercial impact, physical, biochemical, and physiological properties of the nanomedicine

carrying particles have to be understood, and how such nanoparticles can be manufactured in a controlled and scalable manner for drug delivery and therapy.

We will also review development in cancer immunotherapy and progress in gene editing technology using CRISPR/Cas9 system in cancer diagnosis and therapy. Some recent breakthroughs such as the Nobel Prize winning approach of immunotherapy ushers a new development in cancer therapy, but even this approach has some serious limitations. There is a huge excitement in basic and clinical research emanating from gene editing technologies such as CRISPR/Cas9 that may help in diagnosis and treatment of cancer, but the journey is still in early steps and little is known about the winding road of off target effects of genome editing and ethics of throwing dice.

Chapters (Chaps. 1, 2, 3, 4, 5, 6, and 7) in this book describe characteristics of nanomaterials based on their size and shapes, and their synthesis as it relates to human health. Other nanotechnology areas are described in other chapters (Chaps. 9, 10, 11, 12, 13, 14, and 15) include, historical perspective and characterization of nanomaterials; application of nanomaterials in tissue engineering and regenerative medicine, neural development and neurodegenerative disease and diagnosis and treatment of genetic disorders. Some other chapters cover fabrication of novel nanomaterials and their use in microbial and viral infection, endocrine disease, and commercialization of such as new generation nanomaterials. Chapters are also included addressing negative effect of nanotechnology on human health and associated ethical issues. Although all of the above topics are relevant to cancer research, we will not discuss these aspects of nanomaterials in this review, as other chapters in this book may adequately cover these studies.

Last two decades show increasing crescendo of research papers published (as seen on PubMed and related databases) on cancer nanomedicine doubling approximately every five years, suggesting that there is a rapid realization of the use of nanotechnology in cancer diagnosis, drug delivery and efforts to use nanoparticles for cancer therapy. This is largely because such nanoparticles carrying medicines accumulate in solid tumors through the EPR (***Enhanced Permeability and Retention***) effect. The EPR effect is obviously not uniform for different types of solid tumors and has complex dynamics due to the various constitution and heterogeneity of cancer cells types within the solid tumors. Therefore, it will tremendously benefit cancer patients if specific biomarkers of the EPR effect can be identified and harnessed for specific drug delivery to the target cancer tissue, leading to more personalized approaches to treat cancer patients.

Several issues have been recognized for nanotechnology to be effective in cancer biology, drug delivery, and cancer therapy. These include, improving the drug efficacy and reducing its toxicity; cell and tissue specific targeted delivery of the nanomedicine; improving the pharmacokinetics, such as the stability, drug sustainability, cancer cell specificity, and half-life of the nanomedicine, with the possibility of multiple drugs targeting the tumor tissue simultaneously. For better cancer diagnosis, improved and sensitive biomarkers are being tested that could be targeted and imaged in cancer tissues, and may be activated through light and other stimuli (such as gold nano-shells, iron oxide liposomes). For example, photo-thermal

cancer therapy is one of the most significant therapeutic uses of specifically designed nanoparticles.

An important attribute of nanotechnology is to harness nanoparticles as contrast agent for anatomical and functional imaging of the cancerous tissue; and such nanoparticles may also be used for *in vitro* and *in vivo* screening outside the human body. Such non-invasive *in vivo* imaging of nanoparticles in drug delivery could benefit in the evaluation of cancer treatment responses in early stages of tumorigenesis. Furthermore, development of miniature medical devices to deliver nanomedicine (*s*) in diagnosis, screening, and the ability of nanomedicine to overcome the endocytosis and transcytosis barriers across endothelium and epithelium; such potential therapeutic applications will have positive outcome for the cancer patients. Delivery of specific biological drugs such as the small interfering RNA (siRNA), microRNA (miRNA), and DNA molecules through novel nanotechnology to reach within the targeted cancer cells is an area of increasing focus.

To develop nanomedicine for solid tumors, the key tasks are to investigate mechanistically the dynamics of nanoparticles in blood circulation, extravasations (leakage of intravenously injected drugs into the extra-vascular tissue at the site of infusion), and interaction of drug carrying nanoparticles with the microenvironment of the cancer tissue. Other challenges for nanomedicine are the tissue permeability, and nanoparticles trafficking within the tumor cell. Similarly, the physical, biochemical, and physiological attributes of nanoparticles, such as their shape, size, surface properties, elasticity, hardness, and porosity may all influence the EPR effect. Furthermore, choice of the drug ligand, its chemical composition and the dynamics of drug release and its absorbance by the solid tumor will also have bearing on therapeutic success or failure.

Advances are being made in understanding the nature of microenvironment and harnessing this information for early diagnosis of cancer before metastasis sets in. Similarly, at the commercial and manufacturing level, there are efforts to produce nanoparticles in a controlled and reproducible manner that can be scaled up, so that sufficient quantities of precise nanoparticles are available for drug delivery and potential therapy.

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## 8.2 Nanotechnology & Nanomaterials

### 8.2.1 Delivery of Cancer Nanomedicine Across Biological Tissues

Nanotechnology may offer new ways of drug delivery and could have enormous therapeutic potential and translational efficacy; however, there are some caveats that face the use of nanoparticles in cancer therapy. Among many challenges is the removal of nanoparticles after the drug release and non-specific adverse effects on non-intended tissues and organs, and related toxicity. This will require examining the properties of each nanoparticles design, their intended target, and pharmacokinetics of this drug delivery (Xu et al. 2016). Drug delivery is important component for effective treatment; nanotechnology has been utilized in drug delivery to enhance

the therapeutic outcomes of different ailments, including cancer. Research has been directed on developing new kinds of nanoparticles with improved drug delivery potential and function (Goyal et al. 2016). Nanoparticles offer the advantage of increased permeability and improved retention in tumor tissues, as the drug carrying nanoparticles can release loaded drug molecules from nanoparticles in a controlled manner to allow optimum drug exposure needed for therapy, or these nanoparticles may be stimulated by a specific trigger at the specific release site. Previously tried drug candidates may now be re-examined through nanoparticles delivery for enhanced permeability, stability, and solubility of the delivered drugs. In addition, specific ligands can be added to the design of nanoparticles for better imaging and monitoring of drug delivery (Goyal et al. 2016).

There is a growing need to investigate the design and performance of these novel nanoparticles, the pharmacokinetics of packaged drugs, and the nanoparticles-based drug delivery process. For cancer treatment new approaches such as synergistic interactions of drug-drug and drug and nanoparticles formulations to overcome the multidrug resistant cancers are being explored. Similarly, exploiting the blood circulatory system to maximize the drug delivery to the tumor tissue and cancer cells (e.g., over-expression of integrin receptors for cancerous lung tissue). Furthermore, nanoparticles that harbor lipoproteins and moving them across the blood–brain barrier for brain tumors, and nanoparticles with manganese dioxide for use in radiotherapy. Similarly, specially designed nanoparticles to deliver insulin that could release insulin at physiologically appropriate glucose level and at optimum time for insulin release for the tissues (Li et al. 2016).

### **8.2.2 Nanomedicine and Theranostics**

Combining diagnostics with therapy has evolved as a novel notion in cancer treatment. It harnesses efficiency of nanoparticles as carriers to deliver drugs at the tumor target for both tracking the drug by imaging and delivery of potential therapeutic drugs. The design of such nanoparticles requires the following attributes; first, the choice of a therapeutic drug (small molecule to macromolecules such as oligonucleotides or large peptides); secondly, selection of a carrier with optimum half-life in blood circulation. The other two properties are, ability to targeting the target in a specific and controlled manner, and finally the use of such a nanoparticle as an imaging agent (Chi et al. 2017; Arranja et al. 2017; Muthuraj et al. 2016).

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## **8.3 Liposomes as Drug Delivery Carriers**

Liposomes provide the basic design of nanoparticles in nanomedicine, as liposomes resemble natural cell membrane in basic composition; these laboratory designed vesicles consists of a bilayer structure made of amphipathic lipids in aqueous medium (Bangham et al. 1965; Gregoriadis et al. 1971; Akbarzadeh et al. 2013). Since liposomes are highly compatible to biological systems and relative safety;

these are vehicle of choice for drug delivery. The basic bilayer structure of liposomes is made of cholesterol, phosphatidylcholine, and other hydrophobic moieties and they can carry a number of small drug molecules and large therapeutic drugs of hydrophilic or hydrophobic nature (Akbarzadeh et al. 2013; Allen and Cullis 2013). Liposomes can entrap a specific drug and can fuse with the cellular membrane to deliver the drug to the target cells. Since the liposomal surface can be modified by incorporating polyethylene glycol (PEG) that extends their stability in blood circulation, a number of cancer treatment drugs have been incorporated in liposomes for drug delivery. For example, two Doxorubicin liposomes, namely Doxil (also known as Caelyx, outside the USA) and Myocet got FDA approval in mid-1990s. Later, a generic version of Doxil (manufactured by Sun Pharma) was approved by the FDA (Chou et al. 2015). Several new liposome formulations are in clinical trials and use (Table 8.1).

### **8.3.1 Combining Multiple Antitumor Drugs in One Carrier: Erythrocytes and Platelets**

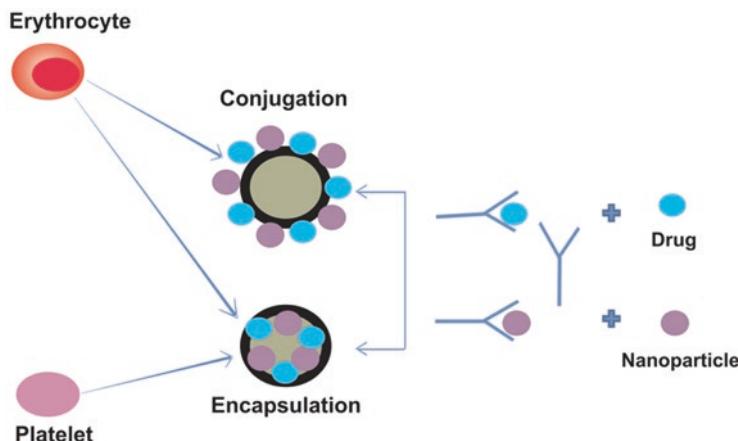
In addition to the conventional liposome-based nanoparticles, blood platelets and erythrocytes are being used as drug delivery vehicles that have certain advantages and limitations for the drug delivery (Piao et al. 2014). Drug delivery uses blood platelets as drug carriers (Hu et al. 2012; Li et al. 2016), which requires overcoming technical issues with platelets, including deformation in shape, easy activation of platelets, breakdown by thrombosis, and problems in platelet preservation. However, platelets can be used to combine multiple drugs, if the platelet vehicles can be produced and delivered in a controlled manner. Erythrocytes provide the advantages of being natural carrier of oxygen and carbon dioxide, long circulation time, low immunogenicity and the ability of erythrocytes to move across the membrane barrier and can carry small molecules, liposomes, and other engineered drug candidates. Basic idea of the use of erythrocytes and platelets in drug delivery is shown in Fig. 8.1.

Erythrocytes can be incorporated with proteins, nucleic acids (miRNA, siRNA, and small DNA fragments), and small molecule drugs, such as vincristine and methotrexate; and have been now approved for clinical trials (Bossa et al. 2008; Favretto et al. 2013; Magnani and Rossi 2014; Sun et al. 2017; Luk et al. 2016). By modification of their membrane composition, erythrocytes can be targeted to specific cancer tissue. Another advantage of using erythrocytes for drug delivery is that they are engulfed by macrophages in the spleen and liver tissues (Luk et al. 2016; Du and Chen 2019). The limitations of erythrocytes as a drug delivery system are due to changes in osmotic concentration when loaded with drugs, changes in permeability, mechanical stability, and plasticity of the modified erythrocytes (Bossa et al. 2008; Hu et al. 2012; Li et al. 2016).

In addition to erythrocytes, platelets also offer vectors for drug delivery, as these are easily accessible from blood and may be challenged with agonists to deliver drugs. The technical issues with the use of platelets are their tendency to aggregate

**Table 8.1** Liposomal composition, anti-cancer drugs and cancer targets

No.	Active component	Liposomal composition	Size nm	Targeted cancer	References
1	DNR	DSPC:cholesterol (2:1)	50	Kaposi's Sarcoma	Petre and Dittmer (2007)
2	DOX	1-Palmitoyl-2-oleoylphosphatidylcholine: cholesterol (55.8:44.2)	180	In clinical Trial for Metastasis	Allen and Cullis (2004); Safra (2003)
3	DOX	Cholesterol, DSPC, DSPE and DSPE-PEG2000	100	Prostate Cancer	Mock et al. (2013)
4	DOX	HSPC/DSPE/cholesterol (12.5:1:8.25)	130	Colorectal Cancer	Hardiansyah et al. (2014)
5	ATRA	DPPC:cholesterol:1,2-disaturated-sn-glycero-3-phosphoethanolamine-Methoxy PEG2000 (6:3:1)	200	Human Thyroid Carcinoma	Cristiano et al. (2017)
6	PCX	Egg phosphatidylcholine: cholesterol: TPGS1000-TPP (88:3:5:8.5)	80	Lung Cancer	Zhou et al. (2013)
7	MXT	HSPC: DSPE-PEG2000: anacardic acid (0.55:0.05:0.05)	112	Melanoma Cell Lines	Legut et al. (2014)
8	ATRA	DOTAP, cholesterol and ATRA (70:20:10)	263	Lung cancer in vivo	Berlin Grace and Viswanathan (2017)
9	ATI-1123	Docetaxel	60–80	NSCLC Gastric Pancreatic Cancer	Mahalingam et al. (2014)
10	DNR	DSPC: Cholesterol (molar ratio 2:1)	50	Kaposi's Sarcoma	Petre and Dittmer (2007)



**Fig. 8.1** Schematic drawing of the use of erythrocytes (red blood cells), blood platelet and nanoparticles as vectors for drug delivery for potential therapeutic uses. The liver, bone marrow and spleen like organs that comprise the reticuloendothelial system (RES) can be targeted by erythrocytes. Platelets are most commonly used to deliver drugs to cancerous tissue, and nanoparticles are used to target circulating system and tumors (Cartoon is adapted from Du and Chen 2019)

(forming a thrombus) and get activated, difficulty in platelet collection as they are sensitive to centrifugation, washing and variations in temperature, and can only be preserved for relatively short time scale (Du and Chen 2019).

### 8.3.2 Incorporation of Drugs into Cells

Two major approaches of loading cells with small drug molecules are either encapsulation or decoration of cell surface with drug molecules. However, since endocytosis in erythrocytes is poorly understood, there are no standard ways of controlled encapsulation of drugs into erythrocytes. Drugs are encapsulated using the hypotonic method, as red blood cells can be sensitive to osmotic concentrations and have the ability to expand in volume up to 25% of the original volume, making pores in the cell membrane for the entry of drugs. By careful monitoring the osmolarity of the erythrocytes these pores can be restored to previous membrane porosity. Although quite simple process hypotonic encapsulation is limited in scope as the cells are damaged by hemolytic shock and the process is not easily controlled. However, with better monitoring of osmotic concentration and handling erythrocytes may provide a useful drug delivery vector.

### 8.3.3 Electroporation

Is a method of choice to load cells with drug candidates. The method is based on electric transfection principle, and is used to transfet virus particles, nucleic acids (DNA, RNA) into bacteria or eukaryotic cells and the method has low

toxicity and high efficiency. For example, bleomycin could be transfected in cats with periocular cancer.

Another method for drug delivery is the use of membrane penetrating peptides. The two commonly used peptides are arginine rich or proline rich peptide that have been shown to penetrate cell membranes and consist of 16 amino acid residues (containing seven positively charged residues) and two hydrophobic tryptophan residues. In cancer drug delivery use of such peptides has been widely reported, due to enhanced membrane permeability, drug release kinetics, and drug accumulation in tumor cells. Thus, a variety of techniques are available to deliver cancer specific drugs into erythrocytes, allowing newer approaches for cancer treatment.

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## 8.4 Cancer Immunotherapy and Nanotechnology

Nobel Prize was accorded in 2018 to James P. Allison of the University of Texas MD Anderson Cancer Center and Tasuku Honjo of Kyoto for their discovery of cancer therapy by inhibition of negative immune regulation. Cancer immunotherapy has provided a kind of silver bullet and better alternative to the conventional cancer treatment regimen, including chemotherapy, surgery, and radiation treatment. Following the conventional therapies, immunotherapy has ushered a new treatment strategy for the follow up therapy. The basic premise of cancer immunotherapy works by activating the individual's own immune system, using effector immune cells to recognize cancer cells and destroy them. Among the cancer immunotherapy are the use of checkpoint inhibitors (CPI), adoptive cell transfer (ACT), cancer specific monoclonal antibodies, cytokines, cancer vaccines, and therapy based on viruses that can attack cancer cells (Yap et al. 2012; Oliveira Pinho et al. 2019). FDA (Food and Drug Administration, USA) has approved the use of immunotherapy in the treatment of many different types of cancers, such as lung cancer; head and neck cancer, pancreatic ductal adenocarcinoma, lymphoma and renal cell cancer.

### 8.4.1 Nanotechnology Innovations in CAR-T Immunotherapy

The newly revolutionary CAR (Chimeric antigen receptor) T-cell therapy comprises the infusion of engineered T-lymphocytes that harbor a chimeric antigen receptor on the cell membrane. The extracellular domain of this receptor molecule is designed to bind to a specific molecule on a cancer cell; as this binding occurs, the internal signaling domain of the molecule is activated; resulting in the stimulation of T-cell to attack the cancer cell. Several chimeric antigen receptors have been developed that consist of modified internal domains that can further increase the immune response against the intended cancer target cell. The basic treatment using CAR-T approach is first to isolate T-cells from the patient under treatment (also known as leukapheresis). These extracted T-cells are then genetically altered to express a chimeric antigen receptor and cell population is expanded in the lab. These engineered

T-cells are injected back into the patient to battle the cancerous growth. Hematological malignancies that do not respond to the traditional cancer therapy have been successfully treated by the emerging CAR (Chimeric antigen receptor) T-cell therapy, as this cancer tissue seeking cells move throughout the bloodstream and follow cell expansion and contraction kinetics (Grupp et al. 2014). The CAR is constituted from a tumor antigen binding domain that is the targeting component, fused to trans-membrane signaling domains (Srivastava and Riddell 2015). CAR-T cell expansion, activation, and effector role are based on their ability to reach the cognate malignant antigen target.

The success of CAR-T treatment has yielded impressive remission results for leukemia cases, and it is noteworthy since most of the subjects in the clinical trials are those who have not responded to conventional cancer therapies. Thus, CAR-T therapy is not the miracle cure for cancer, yet. Many adverse effects such as cytokine release syndrome and neurotoxicity, and eventual death has been reported to be associated with the CAR-T therapies against the CD19 antigen that is present on immune B lymphocytes. In lymphoma and leukemia CD19 is the often most used target molecule.

Commercially two major biotech companies have invested in CAR-T technology; Novartis and Gilead. The CAR-T therapy called Kymirah was launched by Novartis for B-cell acute lymphoblastic leukemia (ALL); that showed an impressive above 80% remission rate after three months of treatment in patients not responding to the conventional cancer therapy. However, about half the patients treated with Kymirah showed adverse effects such as cytokine release syndrome, and resulted in some deaths. The other company Gilead who acquired Kite Pharma used the FDA approved YesCarta CAR-T therapy, that successfully resulted in more than 70% patients showing remission from aggressive B-cell non-Hodgkin lymphoma cancer. Nevertheless, even in this therapy deaths were linked to the adverse effects of the YesCarta therapy. The clinical success of CAR-T immunotherapy directly relies on CAR-T cell exposure to the cancer target. However, in some instances CAR-T therapy leads to unwanted adverse effects leading to neurotoxicity and lethality, thus it is critical to monitor CAR-T cell dynamics and kinetics in a non-invasive manner.

In assessing the progress for hematological cancer treatment, CAR-T immunotherapy though has made some successful advances; yet, it is challenged by a number of uncertainties, such as the number of T-cells that actively engage the target cancer tissue, the half-life of such functional T-cells, and the subsequent growth and control of this cellular interaction. Another issue is whether the cell toxicity can be tolerated. To address these issues on T cellular interactions a reporter gene (with prostate specific membrane antigen, PMSA) has been studied in a mouse model by transduction of CD19 CAR-T cells since PSMA is from human origin and tissue specific expression and can be coupled with a number of therapeutic radioligands for PET (positron emission tomography) scanning (Milone and Bhoj 2018; La-Beck et al. 2019; Minn et al. 2019). It was shown that CD19-tPSMA(N9del) marked CAR-T cells can be imaged with [18F]DCFPuLPET in a Na1m6 mouse model of ALL (acute lymphoblastic leukemia), and was successfully used to quantify the number of CD19-tPSMA(N9del) CAR-T cells in the bone marrow and peripheral

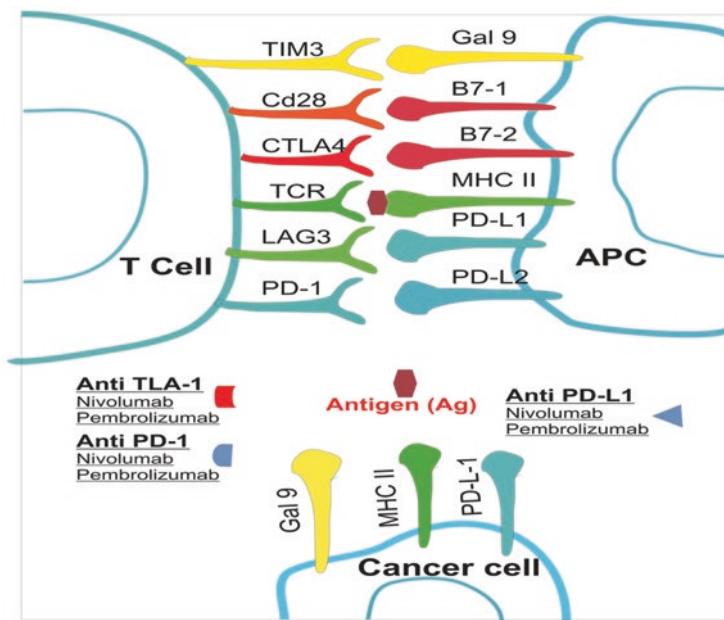
blood and these cells in the cancerous tissue (Minn et al. 2019). Such non-invasive tracking of CAR-T cells in a clinical setting improves the prospects of CAR-T immunotherapy in lymphoblastic malignancies (Milone and Bhoj 2018). To improve upon the efficacy and reducing the adverse effects of CAR-T therapy, is to combine CAR-T cells with combining the use of CHI (checkpoint inhibitor) technology that inhibits tumor's defense mechanisms against T-cells, by enhancing efficacy and by lowering the treatment doses regimen.

### 8.4.2 Checkpoint Inhibitors in Cancer Therapy

Checkpoint inhibitors (CPI) in cancer therapy have gained recognition by virtue of highly specific targeting of cancer cells, thus providing great hope in cancer treatment. These CPI include PD-1 (programmed death receptor-1), PD-L1 (programmed death ligand-1), TIM-3 (T-cell immunoglobulin and mucin domain-containing-3), LAG-3 (lymphocyte activation gene-3), and CTLA-4 (Cytotoxic T-lymphocyte associated protein-4). These molecules are expressed on cancer cells and immune cells and could be induced with specific triggers. The interaction of these checkpoint inhibitor molecules and their receptors results in the induction of cellular signaling that causes inhibition of immune responses against cancer cells that can be rescued by inhibiting the checkpoint inhibitor molecules. Highly investigated checkpoint receptor/ligand interactions are PD-1/PD-L1 and the CTLA-4/CD28 interactions. (Keir et al. 2008; Postow et al. 2015; Karyampudi et al. 2016; Jenkins et al. 2018). The checkpoint inhibitor receptor/ligand interaction results in the phosphorylation of receptor tyrosine residue that is located in the ITSMs (immunoreceptor tyrosine based switch motifs) that leads to the SHIP-2 (Src-Homology domain containing Phosphatase-2) involvement, causing dephosphorylation of adjoining signaling complexes, e.g. ZAP70, PKC-Theta, and PI3K on T-cells. These events lead to suppression of cancer cell proliferation, production of cytokines. As a result of this different T cell signaling cascade; there is also an induction of killer activity of T-cells and enhances programmed cell death of T-cells (Keir et al. 2008; Koonin and Krupovic 2015; Iwai et al. 2017; Jenkins et al. 2018; La-Beck et al. 2019). Several labs have discovered that agents targeting these receptors could induce the antitumor responses, suggesting a powerful approach for cancer therapy. Studies done on monoclonal antibodies that prevent the interaction of checkpoint molecules showed efficacy in treating cancer leading to the FDA approval of such antibodies in a number of cancers (Jenkins et al. 2018). However, this approach has met with very limited success as most patients fail to respond to these antibodies treatment and mechanistically little is understood on such interactions.

In cancer therapy, several inhibitor drugs have been developed that are agonist or antagonists to the T-cell and ligand interactions (Fig. 8.2).

For example, Pembrolizumab, Pidilizumab, Nivolumab, and MED10680 inhibit PD-1, whereas Atezolizumab, Durvalumab, Avelumab, and BMS 936559 inhibit PD-L1 resulting in activation of T-cells. Another way of T-cell gets activated is when



**Fig. 8.2** T-cell activation and proliferation response mediated by a multitude of receptor and ligand interactions. As the TCR binds to the APC MHC II receptor presenting antigen, resulting in antigen specific T-cell activation and cell multiplication (proliferation). On the other hand, LAG3 binding to the APC MHC may inhibit T-cell proliferation and activation. In addition, PD-1 present on T-cells can bind to PD-L1 on APCs, tumor cells or T regs, resulting in the inhibition of this activation. Pembrolizumab, Pidilizuma, Nivolumab and MED 10680 inhibit PD-1, whereas Atezolizumab, Durvalumab, Avelumab, and BMS 936559 inhibit PD-L1 resulting in proliferation and activation of T-cells. Another way of T-cell gets activated is when B7-1 or B7-2 binds to CD28; however this cell activation is inhibited if CTLA-4 binds to B7-1 or B7-2. The two drugs Ipilimumab and tremelimumab block CTLA-4, which induces T-cell proliferation and activation. Enoblituzumab inhibits CD3 and CD276; not depicted here. (Schematic adapted from Somasundaram and Burns 2017)

B7-1 or B7-2 binds to CD28; however, this cell activation is inhibited if CTLA-4 binds to B7-1 or B7-2. The two drugs Ipilimumab and tremelimumab block CTLA-4, which induces T-cell activation and cell proliferation. Enoblituzumab inhibits CD3 present on the T-cell and Enoblituzumab inhibits CD276 present on the Antigen presenting or tumor cell.

## 8.5 CRISPR/Cas9 and Gene Editing

The revolutionary technology of genome editing called CRISPR/Cas9 has taken media by a storm and belongs to the genome editing using nucleases that can nick DNA in a sequence specific manner. Such technology has been in the laboratory from 1980s as researchers from Japan (Yoshimi Ishino, Osaka University) first

reported existence of special nucleotide sequences in bacteria; these were short sequences with repeating pattern of palindromic DNA sequences, separated by short, non-repeating DNA sequences called “spacer” DNA sequences, (Ishino et al. 1987).

Later in 2012 these sequences were named CRISPR (clustered regularly interspaced short palindromic repeats) and have been found in many bacterial species and archaea since then and it was discovered that with a combination of class of RNA molecules (called guide RNA) and Cas (CRISPR-associated) proteins constitute a bacterial immune system to defend against viral and plasmid infections. This combination of Cas proteins, specific RNA species, and the complex of DNA repeat sequences was called the CRISPR/Cas system. The technology that could harness CRISPR/Cas system in eukaryotic cells came from the groups of Jennifer Doudna (Gilbert et al. 2013).

Others have also made pioneering studies in CRISPR technology such as from the labs of Feng Zhang and George Church, and other groups have been active since exploiting the approach in a number of organisms ranging from the baker’s yeast, nematode *Caenorhabditis elegans*, Drosophila, Zebra fish, mouse, monkeys, and humans (Mojica et al. 2005; Dickinson et al. 2013; Zetsche et al. 2015; Taylor et al. 2015; Wang et al. 2014; Wright et al. 2015; Ho et al. 2015; Oakes et al. 2016; Slaymaker et al. 2016). The convenience of using this technology has revolutionized gene manipulation ranging from basic research to the clinical and therapeutic applications. These successes have allowed development of new categories of gene editing systems based on Cas9, Cas12, and Cas13 protein orthologs (Mojica et al. 2005; Wang et al. 2014; Slaymaker et al. 2016).

The CRISPR/Cas9 based technique can be utilized for both non-homologous end-joining (NHEJ) and as single nucleotide editing to achieve gene alterations. Similar approaches are being made to manipulate RNA sequences, for diagnostic and therapeutic applications.

Both in vitro and in vivo use of CRISPR/Cas9 gene editing technology have facilitated cancer research by allowing development of lab models to help in screening cancer markers and therapeutic targets (Wang et al. 2014; Chu et al. 2015; Yin et al. 2019). Important progress using CRISPR technology has been made to edit genes and regulating sequences in the genome to characterize genetic elements mediating tumorigenesis. The CRISPR system has been used to screen high throughput screens, at a relatively modest cost to identify novel cancer markers and therapeutic targets.

### 8.5.1 Prime Editing in CRISPR/Cas9 Realm

In the conventional CRISPR/Cas9 genomic editing based on nuclease and base editing approach only a small number of gene variants can be altered. In a pioneering study David Liu’s lab at the Broad Institute in USA has provided the potential to allow more flexibility and improved precision to gene editing. One of the major obstacles to the use of CRISPR/Cas9 technology in correcting gene variant causing

disease is the inefficient manner and off target effects that are associated with gene editing with conventional CRISPR/Cas9 approaches. Prime editing is a sort of “Search and Replace” method that allows targeted deletions, insertions, and all possible types of transversions (base-to-base conversions); and has the potential to combine different editing modules with one another. In brief, by using a catalytically defective Cas9 endonuclease that has been fused to an engineered reverse transcriptase (RT) enzyme, and programming with a prime guide editing guide RNA (pegRNA) that encodes the intended edited variant and also specifies the gene target sequence, prime editing of a defective gene can be achieved without the need of a double-strand break or donor DNA template (Anzalone et al. 2019).

The Liu group performed genomic editing in 175 cases in human cells that included specific deletions and targeted insertions and all 12 different types of point mutations, without the necessity of double-strand breaks or the requirement of donor DNA template sequences, with great efficiency of prime editing to correct genetic variants, and with small number of byproducts (Anzalone et al. 2019), and were able to make a transversion in *HBB* locus (cause of sickle cell disease) and a deletion in *HEXA* locus (causing Tay-Sachs disease), and edited a protective transversion in *PRNP* gene. This approach of genetic manipulation was also successful in to insert different epitopes and gene tags precisely into the target gene. With the promise of about 89% correction of all known disease causing genetic variants and few off target effects “Prime Editing” appears to be a powerful tool to tackle correcting genetic diseases.

Coupled with great advances in immunotherapy to treat cancer, CRISPR technology has also been utilized to improve CAR-T based therapies by reducing cell toxicity, improving manufacturing capabilities, and providing new approaches for cancer treatment. Finally, CRISPR/Cas9 system may be introduced into the cancerous tissue to inhibit tumor growth and the gene editing CRISPR/Cas9 technology may help to reveal rare mutations in a malignant tissue sample, and the relatively low number of transformed cells (Doench et al. 2016).

### 8.5.2 CRISPR-Cas Technology in RNA Manipulation

With the advent of RNA-targeting Cas proteins that are inactive as nuclease a number of proteins have been developed to control gene expression, alter epigenome, and study chromatin dynamics. For example, have used RNA-targeting Cas13 RNases (dCas13s) that are nuclease deficient and can target RNA in observing RNAs in vivo in live cells to investigate RNA function. By using 20–27 base long guide RNA (gRNA) real time imaging and tracking of RNA can be achieved. This is an improved system as it can be used to track RNA in live cells. These authors used the dCas13 system to label NEAT1, SatIII, MUC4, and GCN RNAs and could track NEAT1 in a dynamic manner. Combing dCas13 with MS2-MCP permitted dual color imaging of RNA molecules in single cells. Remarkably, dCas13 and dCas9 combinations have been utilized to observe genomic DNA and RNA moieties in live cells. Collectively these approaches allowed RNA visualization of

CRISPR-dPspCas13b and dPguCas13b; tracked NEAT1 with dPspCas13b and single RNA molecules in single cells, and finally allowed tracking of NEAT1 to confirm “kiss-and-run” and “fusion” models of paraspeckle kinetics in live cells.

### 8.5.3 Delivery of CRISPR/Cas9 System into Cancerous Cells

Nanotechnology has a great potential in drug delivery to the targeting cancer tissue for treatment. Most commonly used, liposomes are the best carriers in the delivery of CRISPR/Cas9 due to their surface characteristics, such as the ability to modify the surface charge, polyethylene glylation (PEGylation), and ligand engineering; thereby providing high efficiency gene silencing. By varying surface charges, PEGylation and other modifications, longer lasting blood circulation half-life, improved access to the cancerous cells, and enhanced endocytosis. However, there are technical issues such as endosomal escape and decreased cellular uptake in the use of PEG mediated liposomal drug delivery (Lino et al. 2018; White et al. 2017; Hosseini et al. 2019; Ran et al. 2015; Rui et al. 2019; Zhen and Li 2019).

Cholesterol rich nanoparticles have been developed to increase stability in blood circulation and improving the transfection efficiency, have described 2, 3, 4-component cationic liposomes to determine the combined effect of cholesterol moiety, DOPE (dioleoyl phosphatidylethanolamine) a lipid that promotes fusion, and PEG (polyethylene glycol) on transfection efficiency and other characteristics such as cellular toxicity, serum stability, and endosomal escape capacity of the designed liposomes. In a HEK293 cell line in vitro, these authors measured the transfection efficiency using the GFP (green fluorescent protein) reporter to show that Cas9 and sgRNA plasmid delivery can be achieved by modifications of cholesterol composition in the liposome design at about 39% efficiency, and that such liposomes apparently do not have cytotoxicity. These results suggest that improved cholesterol/DOPE and other components can be used for efficient delivery of Cas9/sgRNA and other gene editing complexes.

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## 8.6 Ribonucleic Acids and Cancer

The RNA world plays critical role in gene regulation mechanisms, including translational control of messenger RNA (mRNA), the role of transfer RNA (t-RNA), and the key constituent of ribosomes, ribosomal RNA (rRNA) all are involved in cellular homeostasis. In addition there is a class of non-coding RNAs such as miRNA, siRNA, and ncLRNA (non-coding long RNA) all play critical role in gene expression during development and in the progression of disease across metazoans. For example, mRNA translation initiation factors are critical for cell growth and their deregulation has been associated with many disease conditions; resulting in abnormal translation that induces cell division, angiogenesis, cell survival, and tumorigenesis (Robichaud et al. 2019).

### 8.6.1 RNA Translational Control

In cancer cells, for instance, complex molecular perturbations result in increased translation of initiation factors and signaling cascade controlling them (Robichaud et al. 2019). In cancer cells it was shown that eukaryotic initiation factor eIF4E has increased expression and its over-expression can cause transformation of NIH3T3 cells in a cell culture (Lazaris-Karatzas et al. 1990). Initiation of translation can also be controlled by phosphorylation of eIF4F components, e.g. eIF4E phosphorylation by the two kinases MNK1 and MNK2 may induce tumor formation in prostate tissue and metastasis (Furic et al. 2010; Proud 2018).

### 8.6.2 Gene Silencing Via siRNA

Discovered originally in the nematode *C. elegans*, RNA interference (RNAi) is an important mechanism to inhibit endogenously expressed proteins (Fire et al.). There are many uses of RNAi technique in various biological investigations, and is being utilized to inhibit mRNA encoding disease causing proteins and peptides for therapeutic purposes. RNAi is useful technology to inhibit expression of endogenously synthesized peptides and proteins, and the ability to silence mRNA transcripts for disease causing proteins though RNA has met with critical clinical success offering great therapeutic potential. Delivery of RNAi reagents (RNA ribonucleotides, etc.), liposomes carrying siRNA and viral vectors that are designed to trigger RNAi have been developed for both in vitro and in vivo use. Success in RNAi technology has entered clinical trials and siRNA drugs have been approved for human use by FDA. This powerful approach holds great promise as an alternative to conventional small molecule drug development and may treat life threatening ailments and syndromes. In an early study on cancer therapy, RNAi based approach was used in the liver tissue. The cell cycle polo like kinase PLK1 was targeted using specific siRNAs, as PLK1 kinase is involved in phosphorylation of several protein factors mediating cell cycle; blocking PLK1 expression results in the arrest of cell cycle and programmed cell death of cancer cells (Steegmaier et al. 2007; Davidson and McCray 2011). A number of anti-cancer RNAi based drugs have been in various stages of clinical development and Phase I and Phase II trials, such as FANG for solid tumors targeting Furin (Gradalis Inc.); SPC2996 for Chronic myeloid leukemia, targeting BCL-2 (Santaris Pharma) and many other RNAi drugs in different phases of clinical testing (Davidson and McCray 2011). Table 8.2 summarizes some RNAi based drugs that are being developed in cancer therapy.

### 8.6.3 Major Role of microRNA (miRNAs) in Gene Regulation

Originally discovered in the nematode *C. elegans*, microRNAs (miRNAs) are non-coding RNAs of about 22 nucleotide long ribonucleic acid, mediate gene expression by inhibiting target mRNA translation by their ability to bind complementary

**Table 8.2** Lipid delivery systems to deliver siRNA for cancer treatment in clinical trials

No.	Cancer type	Drug target	Identifier	Drug/Company	Phase status
1	Advanced cancers	EPHA2	NCT 0159356	siRNA-EphA2 DOPC M.D. Anderson Cancer Center	Phase I Recruiting
2	Solid Tumors	KSP and VEGF	NCT 00882180 NCT 01158079	ALN-VSP02 Alnylam Pharmaceuticals	Phase I Completed
3	Advanced Solid Cancers	PKN3	NCT 00938574 NCT 01808638	Atu 027 Silence Therapeutics	I / Completed I/II Completed
4	Cancer	Polo Like Kinase PLK1	NCT 01437007 NCT 01262235 NCT 0219878	TKM-080301 Tekmira Pharmaceuticals	I Completed I/II Completed I/II Completed
5	Solid Tumors Multiple Myeloma Non-Hodgkin Lymphoma	MYC	NCT 02110583 NCT 02314052	DCR-MYC Dicerna Pharmaceuticals	I Terminated I/II Terminated

Adapted from Barba AA, Bochicchio S, Dalmoro A, Lamberti G. Lipid Delivery Systems for Nucleic-Acid-Based-Drugs: From Production to Clinical Applications. *Pharmaceutics*. 2019; 11(8):360

sequences in the 3'-untranslated region (3'-UTR) of mRNAs (Lee et al. 1993; Wightman et al. 1993). MicroRNAs can regulate a number of genes and are involved in normal development and also in pathological pathways, including tumorigenesis (Calin et al. 2004; Calin and Croce 2006; Mitamura et al. 2013). A number of studies have shown the role of miRNAs in developing drug resistance in cancer cells (Si et al. 2019). The miRNA action is tissue specific and one specific miRNA can target multiple transcripts and can mediate drug resistance in cancer cells by inhibiting genes involved in cell cycle, checkpoint regulation, cell proliferation, and apoptosis (Lengauer et al. 1998; Dar et al. 2011; Nishida et al. 2012; Mitamura et al. 2013; Peng and Croce 2016; Si et al. 2019). Wengong Si and his colleagues have described drug resistance in cancer therapy in a number of cancer and tissue types including breast cancer, ovarian cancer, pancreatic cancer, non-small cell lung cancer, gastric cancer, colorectal cancer, gastric cancer, lung cancer, etc. and listed different miRNAs that have been shown to play a role in drug resistance (For details on each cancer tissue specific miRNA and its predicted mRNA target see Table 8.1, in Si et al. 2019). The miRNA database (<http://mirbase.org>) reports 1872 human miRNAs that have been annotated and there are many more miRNAs whose function remains unknown. Elucidating miRNA function in normal development and in cancer resistance can allow developing novel approaches to regulate miRNAs and utilize this knowledge in theranostic pursuit in tumorigenesis.

## 8.7 The Master Molecule DNA and Its Usage in Nanomedicine

The genetic material deoxyribonucleic acid (DNA) is the bedrock of life and transmits information that is inherited in an offspring. Recent developments in nanomedicine have harnessed DNA sequence information to design structural motifs and assemble these motifs together, allowing remarkable control on self-assembly. This approach has found applications in designing nanoparticles, protein assembly, and diagnostics. These DNA assemblies can also serve as vehicles for drug delivery, structure determination, and in synthetic biology.

### 8.7.1 Packing the DNA Punch in Novel Nanoparticles: SNAs

Spherical nucleic acids (SNAs) have been developed by Chad Mirkin's group at Northwestern University that offer the advantages of specificity, mobility, and access to the target tissue that can cross blood–brain barrier and these SNAs are polyvalent nanoparticles capable of packing high density of nucleic acid “spines” into a tiny space allowing strong ligand-cell interaction with receptors on the target cell surface (Bang et al. 2014; Cutler et al. 2010, 2012; Young et al. 2012; Mirkin et al. 1996).

Due to the specificity of complementary DNA or RNA nucleotide sequences to a given DNA sequence, it is possible to design highly specific nanoparticle that can hone on the specific tumor cell, avoiding cell-cell interactions with other bystander cells. Due to the nanoparticle size in the range of billionth of a meter across, SNAs may be able to travel in the body and target cells avoiding detection by immune defense mechanisms. As the SNA are constituted with specific nucleotide sequences - these SNA's are highly oriented. These nanoparticles SNAs can be highly specific in targeting cells that are critical in the disease process. In the initial stages such SNAs were constituted from gold nanoparticles AuNP, (Cutler et al. 2012), but later development utilized other inorganic particles comprising silver, quantum dots, iron oxide, and silica. Among organic materials used in such assemblies included polymers, liposomes, macromolecules such as proteins and hybrid assemblies (Mirkin et al. 1996; Lee et al. 2007; Cutler et al. 2010, 2012). Besides these SNAs, hollow SNAs comprising cross-linked nucleotides that are like AuNP structures have been developed (Cutler et al. 2011).

SNAs have special characteristics that set them apart from other nanoparticles, such as muted response to immune attack, decreased vulnerability to nucleases, and enhanced binding affinities for complementary nucleotide sequences (Lytton-Jean and Mirkin 2005). Pertaining to the specific nature of DNA sequences it is conceivable to design SNAs that may indulge with the target molecules in the cell in a highly specific manner to cause interference. Furthermore, since most of the genetic information flow in a cell is mediated by DNA and or RNA sequences, highly specific SNAs can be developed that reduces the time to screen for

molecules that may interfere with critical cellular processes, thereby making SNAs reagents of choice to manipulate cell behavior (Mirkin et al. 1996; Rosi et al. 2006; Park et al. 2008).

### 8.7.2 Surprise; SNAs Can Cross Cell Membrane

Cell free DNA has been detected in cancer patients and used as a biomarker in cancer diagnostics. Thus, creating a nanoparticle with complementary DNA sequence may capture the cell free DNA in the peripheral blood of cancer subjects. However the challenge of DNA coated nanoparticle to cross the cell membrane remained the huge obstacle (Jensen et al. 2013; Radovic-Moreno et al. 2015). The breakthrough came in the SNAs design that carries a very large number of “spines” allowing strong interactions with the intended complementary nucleic acid sequences and as a lucky break, these spines like SNAs were able to enter the cells. We still know little about the mechanisms governing the entry of SNAs into living cells, however, one possibility is that these SNAs assemblies mimic scavenger receptors that are distributed over the cell surface, and thus SNAs make their way into the cells (Patel et al. 2010). Such scavenger receptors are known to regulate cell’s interaction with its environment. SNAs with multiple assemblies of nucleic acids are “hooked” on such scavenger receptors and get carried inside the cell (Song et al. 2009). Electron micrographs have revealed binding of SNAs to the scavenger receptors, as the adjoining cell membrane encased the SNAs allowing entry of SNAs into the cell cytoplasm. Proof of SNAs being able to block specific RNA came as the Mirkin’s group was able to inhibit expression of the mRNA encoding green fluorescent protein (GFP) by challenging cells with SNAs specific to inhibit the GFP mRNA and this approach can be used to deliver cancer drugs (Mirkin et al. 1996; Tan et al. 2016; Bousmail et al. 2017). Such attributes of SNA make them the vehicle of choice for immune system perturbations, controlling gene expression and chemotherapeutics (Anderson 2003; Yamankurt et al. 2019; Choi et al. 2013; Akinc et al. 2008; Jones et al. 2015; Sprangers et al. 2017).

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## 8.8 Concluding Remarks

Collectively, the new advances are vastly improving our understanding of biological processes and are propelling nanotechnology-based innovations and techniques towards clinical use in gene and cell therapies to combat cancer. However, like most of the previous technologies therapeutic approaches must overcome well known hurdles like heterogeneity of cancer tissue, specificity of drug delivery, stability of the drug and its retention, cell toxicity and adverse effects. Nevertheless, with setbacks opportunities also knock and nanotechnology offers a lot of potential to deliver the desired outcomes.

## 8.9 Glossary and Abbreviations

CAR-T:Chimeric antigen receptor—T cells  
Cas:CRISPR-associated protein  
CPI:Checkpoint inhibition  
CRISPR:Clustered regularly interspaced short palindromic repeats  
crRNA:CRISPR RNA  
CTLA-4:Cytotoxic T-lymphocyte-associated protein 4  
DSB:Double-strand break  
EPR:Enhanced permeability and retention  
HDR:Homology-directed repair  
miRNA:Micro RNA  
NGS:Next-generation sequencing  
NHEJ:Non-homologous end-joining  
NP:Nanoparticles  
PAM:Protospacer adjacent motif  
PD-1:Programmed death receptor 1  
PDL-1:Programmed death-ligand 1  
PET:Positron emission tomography  
PMSA:Prostate specific membrane antigen  
sgRNA:Single guide RNA  
siRNA:Small interfering RNA  
spCas9:*Streptococcus pyogenes Cas9*  
TME:Tumor microenvironment  
tracrRNA:Trans-activating crRNA

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# Application of Nanomaterials in Treatment of Microbial and Viral Infections

Adeeb Shehzad, Raheem Shahzad, Hanan Aldossary, and Ebtesam A. Al-Suhaimi

## Abstract

The rapid development of drug-resistant issues in pathogenic viral, bacterial, and fungal organisms and the consequent spread of infectious diseases are currently getting serious attention. Nanomaterials are the most capable therapeutic agents to cope with such issues and challenges. The extraordinary physio-chemical properties and remarkable antimicrobial capabilities of nanoparticles have triggered their application in biomedical fields. Nanomaterials from organic and inorganic nature have shown the proficiencies of disrupting microbial cells through different mechanisms. Besides with the direct effect on the microbial cell membrane, DNA, and proteins, these nanomaterials produce reactive oxygen species (ROS) that damage cell components of bacteria and viruses. Presently, a serious danger related with these antimicrobial nanomaterials is their toxicity to human and animal cells. Widespread studies have reported the amount, time, and cell-dependent toxicology of various nanomaterials. But

A. Shehzad · H. Aldossary

Institute for Research and Medical Consultations, Imam Abdulrahman bin Faisal University, Dammam, Saudi Arabia

R. Shahzad

Basic and Applied Scientific Research Center, Imam Abdulrahman bin Faisal University, Dammam, Saudi Arabia

Department of Biology, College of Science, Imam Abdulrahman bin Faisal University, Dammam, Saudi Arabia

E. A. Al-Suhaimi (✉)

Institute for Research and Medical Consultations, Imam Abdulrahman bin Faisal University, Dammam, Saudi Arabia

Department of Biology, College of Science, Imam Abdulrahman bin Faisal University, Dammam, Saudi Arabia

e-mail: [ealsuhaimi@iau.edu.sa](mailto:ealsuhaimi@iau.edu.sa)

some of them have shown excellent biocompatible properties. In this chapter, the antimicrobial activities of various nanomaterials have been described, exhibiting broad range of biological properties that are highly dependent upon their size, structure, quantity, and binding with receptor cell of different type.

### Keywords

Nanotechnology · Microbial · Oxidative stress · DNA · Biomedical applications

## 9.1 Introduction

The emergence of nanotechnology has generated a great deal of new research in various scientific fields. The exposure of new features of nanomaterials has led to a revolution in every field, including biomedicine and biotechnology (Arvizo et al. 2012). Nanomaterials (materials with a diameter <100 nm) have shown better properties than the bulk materials of the same nature (Li et al. 2012; Roduner 2006). New synthetic routes including chemical reactions in solution (sol-gel methods), synthesis in gaseous phases, and mechanical processing have been established to make nanoparticles (NPs) of various sizes and dimensions (Li et al. 2012). The small size of nanomaterials such as proteins are highly suitable for carrying out several functional biological operations (De et al. 2008). To progress improvements in biological systems and nanomaterials a new branch of science known as nanomedicine was recently developed (Kim et al. 2010). Nanomedicine provides several examples of effective utilization of nanotechnological tools in biomedical research. Currently, nanotechnology studies are exploring medical science using novel tools such as medical imaging, magnetic hyperthermia, sensing, targeted drug and gene delivery, antimicrobial wound healing, and artificial implants (Ding and Xu 2019; Li et al. 2012; Hubbell and Chilkoti 2012; Khoshnevisan et al. 2019; Singh et al. 2008).

The emergence of infectious diseases and development of resistant viral, bacterial, and fungal strains to available antibiotics is becoming a serious hazard to public health (Dodds 2017). Accordingly, there is a strong demand to develop innovative approaches for the identification of new materials that can cope with these serious problems. Precursors of currently identified nanomaterials such as silver (Ag), zinc (Zn), copper (Cu), titanium (Ti), iron (Fe), and silica ( $\text{SiO}_2$ ) have long been utilized as anti-biotic agents in wound healing and related medical conditions. NPs of these materials along with organic NPs have shown tremendous potential as bactericidal and fungicidal elements (Lima et al. 2013; Varghese et al. 2013; Das et al. 2011). Indeed, these nanomaterials are currently demonstrating the potential to be developed as antibiotics or drug carriers (Webster et al. 2013). For example, Ag, ZnO, and  $\text{TiO}_2$  NPs have shown effective results against cancer, polio, hepatitis B, and many other diseases of severe concern (Arvizo et al. 2012; Taccolla et al. 2011).

Most nanomaterials possess strong antibacterial activities; however, the strength of these activities varies with the NP's nature, size, shape, and concentration, as well as the degree of exposure time and characteristics of the treated microbes (Bardhan et al. 2019). A number of studies have quantified the levels of bacteriostatic and bactericidal activities of various nanomaterials against different microbial species (Lima et al. 2013; Varghese et al. 2013; Das et al. 2011). However, there have been fewer efforts to describe the mechanisms by which the nanomaterial kills the microbes. Although the exact mechanisms of the antimicrobial activities of NPs have yet to be confirmed, several mechanisms have been suggested. For example, it has been suggested that nanomaterials either directly contact the cell membrane and damage it or release metallic ions ( $\text{Ag}^+$ ,  $\text{Zn}^{++}$ , etc.) that destroy the cell bodies (Zhang et al. 2019; Kim et al. 1998). Another proposed mechanism is that NPs lead to the generation of reactive oxygen species (ROS) that ultimately cause cell death (Fu et al. 2014).

The release of these bactericidal nanomaterials will preferably affect the lowest trophic levels (e.g., bacteria) first. However, the extent to which these effects are shown to be lethal to higher organisms is unknown. With increasing utilization in biomedicine and other fields, the potential hazards for humans and ecosystems are also enhanced. Several bactericidal nanomaterials have shown serious toxic effects to higher cell lines. Specifically, the toxic effects of Ag NPs against humans, zebra fish, rats, and clams have been reported (Arora et al. 2009; Hussain et al. 2005; Sung et al. 2008). Moreover, somewhat similar effects with lower intensity have been observed for  $\text{ZnO}$  and  $\text{TiO}_2$  (Yeo and Yoon 2009; Kiss et al. 2008; Pan et al. 2009). Additionally, Ag NPs have been shown to cause damage to tissues of the liver, lungs, and heart (Arora et al. 2009; Hussain et al. 2005; Sung et al. 2008).

Despite many reports of the toxic effects of these NPs, there are still many studies explaining the nanotoxicological effects of nanomaterials on human and animal cells. Furthermore, some of the nanomaterials including  $\text{TiO}_2$  nanorods have shown good levels of biocompatible properties urging no toxicity to the animal cells (Giavaresi et al. 2003). Nevertheless, the debate will last until extensive conclusive mechanisms of the nanomaterial interactions with cells are identified. Currently there is a continuous demand to determine how nanomaterials can be safely prepared and to what extent they can be applied in medical fields concerned with humans and higher animals. This chapter describes the potential of various nanomaterials to be effectively used as antimicrobial agents. The mechanism involved in NP interactions with microbial cells that consequently leads toward cell death is comprehensively described herein, and a detailed discussion of toxic and biocompatible nanomaterials and their relative effects is also explained to provide insight toward a clear understanding of the potential properties of nanomaterials on human health.

## 9.2 Nanomaterials

Nanomaterials refer to materials with dimensions ranging from 0.2 to 100 nm. The properties of these materials vary when their size approaches the nanoscale. The relative abundance of atoms at the surface plays a role in the properties of such materials (Eustis and El-Sayed 2006). Especially, as size of nanomaterial decreases, the percentage of atoms on the surface increases comparative to the total atoms of materials, which result in astonishing properties. Moreover, nanomaterials possess a much high surface area, leading to large surface-to-volume ratios. As a result, the electronic energy states become discrete, resulting in exceptional electronic, magnetic, optical, and mechanical properties (Zhou et al. 2011; Rakkesh and Balakumar 2013).

The unique physical and chemical properties of nanomaterials have resulted in their receiving a great deal of attention in the medical field. Since they have a size similar to most biological molecules, NPs have been extensively applied in biomedical research. Moreover, most nanomaterials have been found to possess antimicrobial activities against various pathogenic viral and bacterial species. Furthermore, nanomaterials have shown reasonable biocompatibility when applied in generation of scaffold materials (Chen et al. 2012; Zhang et al. 2011). Accordingly, nanomaterials have been applied in medical fields in targeted drug delivery, artificial implants, biocompatible scaffold materials, and sensing, imaging, and antimicrobial materials (Hahn 2011).

Nanomaterials can be categorized differently based on their nature and origin. In general, there are naturally occurring nanomaterials of an organic and inorganic nature, metal and metal oxides, and newly synthesized nanomaterials. These NPs interact with microbial and animal cells through a variety of mechanisms. The NPs interact either directly with the living cells or generate secondary products that affect the cells (Li et al. 2008). While nanomaterials have pros and cons, it is important to determine their relative applicability in medical fields considering both aspects.

Nanomaterials have been used in many health-related industrial products owing to their excellent antimicrobial activities (Sung and Kim 2012; Xu et al. 2013). For instance, Ag NPs are used in medical products during wound healing (Sarkar et al. 2007; Shrivastava et al. 2007). The antimicrobial and photocatalytic activities of TiO<sub>2</sub> have resulted in its extensive use in drugs, cosmetics, food products, and environmental applications for water and air purification (Wist et al. 2002). Zinc oxide NPs have been used in sunscreens, coatings, paints, wallpapers, cosmetics, lotions, and ointments due to their high photocatalytic and antimicrobial activities (Smits and Pavel 2011).

### 9.3 Antimicrobial Properties of Nanomaterials

As mentioned above, NPs of different natures exert various levels of bactericidal activities against pathogenic and non-pathogenic microbes. Nanomaterials can be categorized into organic and inorganic nanomaterials. Both organic and inorganic nanomaterials have shown effectiveness against microbial growth. The relatively less stable nature of organic antibacterial materials, especially at higher temperature, results in barriers in the design of products (Sawai 2003), while inorganic materials are much more stable in harsh process conditions and have therefore been more frequently utilized in antimicrobial materials (Fu et al. 2005; Makhluf et al. 2005). Herein, we briefly describe various nanomaterials that have been extensively studied for bactericidal activities.

Metals and metal oxides such as Ag, Ti, Zn, Fe, Cu, and Au are the most widely studied NPs for bactericidal activities. Silver has long been used for the disinfecting agent in many medical devices and water purification systems (Bosetti et al. 2002; Chou et al. 2005). In medical fields, Ag compounds are usually used to treat burns, wounds, and a variety of infectious diseases (Mishra et al. 2008). Investigation of the exceptionally high bactericidal effect of nanosized Ag NPs has led to the synthesis of Ag nanocomposites (Dallas et al. 2011; Guidelli et al. 2012). Moreover, the effects of particle size on bactericidal properties have been studied through electron microscopy (Norman et al. 2008; Gupta et al. 2008). The nanomaterial cells interactions and the consequent effects on cell lysis or growth are discussed in detail below.

Titanium dioxide is another important metal oxide, and its antimicrobial and photocatalytic activities have been thoroughly investigated. The ability of  $\text{TiO}_2$  to kill both gram-positive and gram-negative bacteria has been well established (Wei et al. 1994). Further studies have shown its effectiveness against several viral species including polio, hepatitis B, MS2 bacteriophage, and herpes simplex viruses (Hajkova et al. 2007; Huang et al. 2007). Similar to  $\text{TiO}_2$ ,  $\text{ZnO}$  NPs have been shown to exhibit broad spectrum bactericidal and fungicidal activities. Some recent studies have also reported effective anticancer activities of  $\text{ZnO}$  NPs (Taccola et al. 2011). Similarly, Hahn (2011) reported the antibacterial activity of  $\text{ZnO}$  particles against *Salmonella typhimurium*, *Bacillus subtilis*, *Escherichia coli*, and *Staphylococcus aureus* found effective antibacterial activities of  $\text{ZnO}$  against both gram-positive and gram-negative bacteria. Copper oxide ( $\text{CuO}$ ), gold ( $\text{Au}$ ), and iron oxide ( $\text{FeO}$ ) are other the common inorganic NPs used for the same purpose. The antibacterial activities of  $\text{CuO}$  are relatively lower than those of Ag or  $\text{ZnO}$ ; therefore, a relatively higher concentration of nanocopper is required to achieve effective bactericidal results (Chang et al. 2012). The antimicrobial efficiency of  $\text{CuO}$  varies greatly with the nature of the bacterial species, indicating differences in the bactericidal mechanism of  $\text{CuO}$ . Additionally, since it is much less expensive than Ag and other bactericidal nanomaterials,  $\text{CuO}$  can be used more frequently in nanocomposite materials for such applications. Gold nanoparticles are generally considered biologically inert; however, they can be modified to introduce antimicrobial properties. Indeed, a number of studies have reported the bactericidal efficacy of

photothermally functionalized Au NPs and nanorods (Norman et al. 2008; Gupta et al. 2008; Huang et al. 2007).

Among organic nanomaterials, carbon nanotubes (CNT) have been most widely exploited as bactericidal materials. Some studies have shown the antibacterial activities of CNTs against gram-positive and gram-negative bacteria; however, they have not been widely applied in this capacity owing to their poor dispersion in water. Chitosan (Ch) NPs have also shown broad spectrum antibacterial, antiviral, and antifungal activities (Orellano et al. 2019). The extensive applications of Chitosan are mainly based on its antibacterial properties, biocompatibility, nontoxic nature, low immunogenicity, and as an absorption enhancer.

## 9.4 Mechanism of Nanomaterial Antibacterial Activity

The antibacterial effects of various nanomaterials are well known; however, the exact mechanism for their bactericidal nature is still not clear. Nevertheless, a number of mechanisms ranging from physical interaction with the microbial body to chemical disruption of the cells have been proposed. Herein, we describe the various proposed mechanisms in detail to signify the role of different nanomaterials in biological applications. The overall antimicrobial activities of the nanomaterials are attributed to three particular mechanisms:

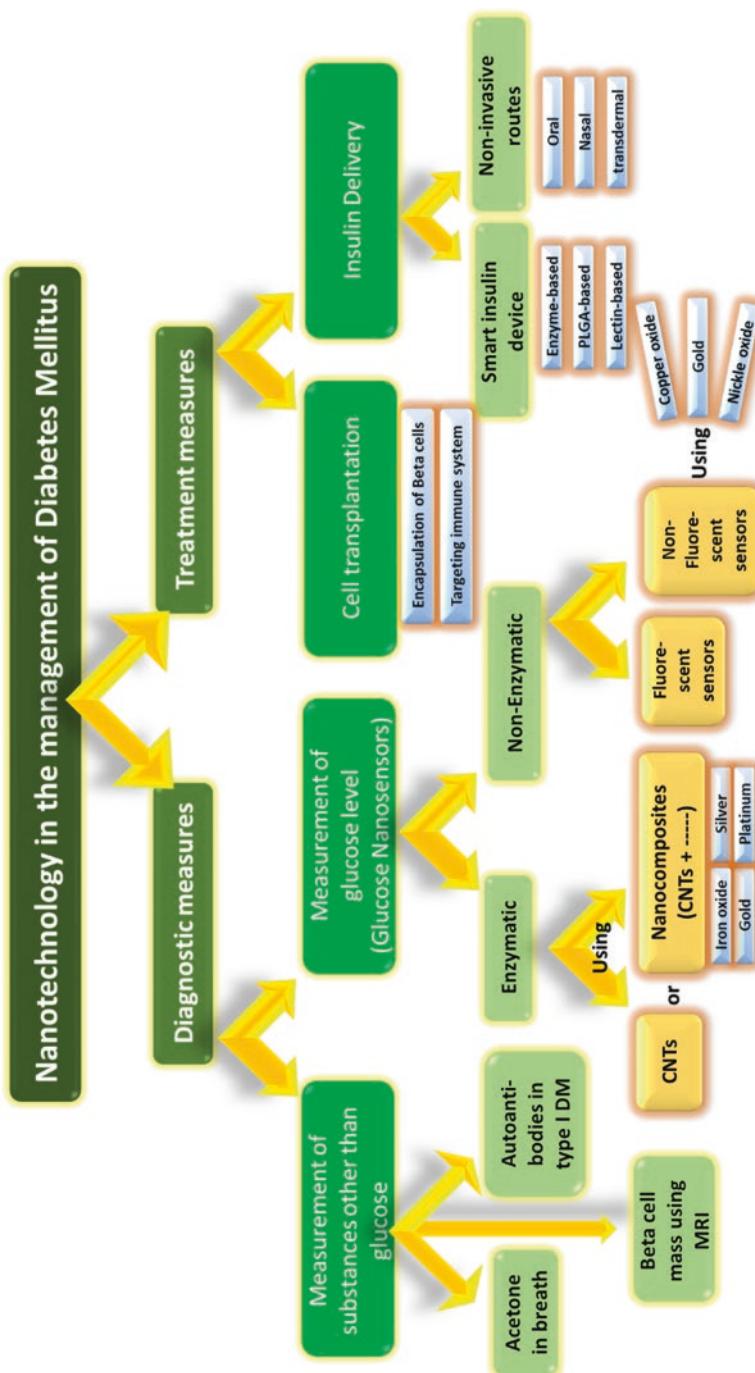
1. the interaction of nanomaterials with the cell membrane,
2. generation of ROS that damage the cells, and
3. uptake of free ions that damage the cells from the nanomaterials solution.

Figure 9.1 illustrates a generalized scheme of various mechanisms involved in the antimicrobial characteristics of NPs. As shown in Fig. 9.1, nanomaterials do not use a particular mechanism to cause cell death, but instead use a combination of possible mechanisms to destroy the cells. The complete description of these three proposed mechanisms with examples from the literature is given below.

### 9.4.1 Interaction of Nanomaterials with the Cell Membrane

It has been reported that NPs interact with the cell surface and penetrate the cells. For example, Ag NPs were observed inside bacterial cells (Rai et al. 2009; Chithrani 2011), while Smetana et al. (2008) reported the presence of holes in the surface of *E. coli* caused by the penetration of Ag NPs. The similar mechanism has also been suggested for ZnO NPs that enter the cell envelope and disintegrate the cell membrane (Fig. 9.1) (Jones et al. 2008; Sui et al. 2013). Furthermore, Pasquet et al. (2014) reported that after binding with the cell membrane, ZnO NPs prolong the lag phase of the microbial cell cycle.

The details of particle interaction with the cell membrane are not clear. The electrostatic interaction caused by the difference in charge on the positively charged



**Fig. 9.1** This algorithm summarizes the current state of research work in the management of diabetes mellitus using nanotechnology. This research field is still in the preclinical stage with promising results. CNT, carbon nanotubes

particle and negatively charged cell surface is considered to be one possible reason (Guzman et al. 2012). However, similar particle–cell interactions have been observed for negatively charged NPs, suggesting that some other mechanism is involved in the electrostatic interaction. A more generalized hypothesis is that the NPs (Ag) attach to the sulfur-containing proteins of the cell wall, causing perforation of the cell wall that leads to enhanced permeability of the cell membrane (Chithrani 2011; Smetana et al. 2008). The abundance of sulfur-containing proteins on the cell membrane surface and their high affinity toward Ag is the major cause of this interaction. Bioluminescence analysis has provided some information about how protein membrane damage occurs in response to Ag exposure (Marambio-Jones and Hoek 2010). The cytoplasmic material is released from the perforated and permeable membrane to the surrounding medium, which causes cell death (Pal et al. 2007). Li et al. (2010) studied the structure of *E. coli* through field emission scanning electron microscopy (FE-SEM) and transmission electron microscopy (TEM) after treatment with Ag NPs. They reported large gaps on the bacterial surface that ultimately led toward disintegration and disorganization of the bacterial cell. Moreover, they suggested that NPs initially enhance the permeability of the outer membrane and then penetrate the inner membrane, where they destroy respiratory chain dehydrogenases and inhibit cell growth.

#### **9.4.2 Bactericidal Effects through Reactive Oxygen Species**

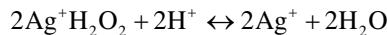
ROS are chemically reactive moieties containing oxygen that are produced as byproducts of metabolism during respiration. Environmental stresses lead to the excessive generation of ROS; however, cells possess antioxidant defense systems that can reduce or remove ROS. Nevertheless, when ROS levels become excessive, they generate oxidative stresses (Dutta et al. 2012). Additionally, ROS attack membrane lipids and cause the destruction of membrane and mitochondrial functions. The generation of ROS is the most common phenomena observed in response to treatment with NPs that leads to antibacterial activities (Song et al. 2019). It has been suggested that a few metallic NPs induce bactericidal effects through the generation of ROS. The photogeneration of ROS on the surface of metal oxides has been shown in various previous reports (Li et al. 2012). These oxides and hydroxides act as precursors for the generation of other ROS (Li et al. 2012). The photocatalytic production of  $H_2O_2$  from the  $ZnO$  surface has been shown to play an effective role in inhibiting bacterial growth. Photooxidized  $TiO_2$  generates hydroxyl radicals that are the primary reason for its antibacterial properties. Evidence of the generation of ROS in response to Ag NPs has been reported previously. Additionally, a recent study conducted by Li et al. (2012) showed the generation of ROS for various metal oxide NPs. Among seven different NPs,  $ZnO$ , and  $TiO_2$  were found to produce three types of ROS: superoxide radical, hydroxyl radical, and singlet oxygen. Other metal oxides ( $CuO$ ,  $Al_2O_3$ ,  $SiO_2$ ,  $Fe_2O_3$ , and  $CoO_2$ ) were also found to produce ROS under UV irradiation. *E. coli* cell viability was found to decrease linearly with increasing ROS quantities in aqueous suspension (Li et al. 2012). Taken

together, these findings demonstrate the specific role of ROS in the bactericidal activities of metallic NPs.

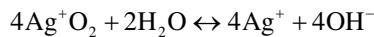
Another related study conducted by Zhang et al. (2011) revealed the generation of ROS from Ag, Au, Ni, and Si NPs in aqueous suspensions under UV irradiation. Among these, the Ag NPs generated superoxide and hydroxyl radicals, while the rest of the NPs generated singlet oxygen species. All of the NPs produced effective antibacterial activities against *E. coli* through the ROS generation mechanism. The role of ROS in the antibacterial activity of Ag was further confirmed by Dutta et al. (2012), who observed the concentration-dependent bacteriostatic and bactericidal effects of ZnO NPs against *E. coli*. The introduction of histidine, an antioxidant, to the culture media reduced the antibacterial effects, indicating the major role of ROS in the antibacterial activities of Ag NPs. Many previous reports have mentioned the effective role of ROS in the antibacterial activities of NPs. The production of super-oxides, hydroxyl radicals, and free oxygen species can severely damage membrane lipids, leading toward a breakdown of the membrane. Of course, it is more commonly accepted that the antibacterial activities of the NPs are the result of the combined effects of metal ions, superoxide ions, and hydroxyl ions (Rizzello and Pompa 2014).

#### 9.4.3 Uptake of Free Ions from Nanomaterial Solution

Suspended or dissolved NPs often result in the generation of metallic ions that are highly reactive and induce severe toxicity in microbes (Rehbock et al. 2014). The interaction of these ions with respiratory and transport proteins via thiol groups ultimately results in the cell death (Tiwari et al. 2011). The uptake of these metallic ions is therefore considered the third possible mechanism of bactericidal effects of nanomaterials. The bactericidal efficacy differs based on the nature and charge of the metallic ions. The bactericidal power of various metals occurs in the following order:  $\text{Ag}^+ > \text{Hg}^{2+} > \text{Cu}^{2+} > \text{Au}^{2+} > \text{Zn}^{2+} > \text{Ca}^{2+} > \text{Na}^+$ . Similarly, the antibacterial order for the same elements with different charges is  $\text{Ag}^{3+} > \text{Ag}^{2+} > \text{Ag}^{1+}$  (Ul-islam et al. 2014). Nanoparticle ions are produced from suspensions of NPs. Several mechanisms have been proposed for the oxidative dissolution of nanoparticles, which subsequently produce metallic ions. In a study of the toxicological activity of Ag NPs. Asharani et al. (2009) proposed the following mechanism for the generation of  $\text{Ag}^{2+}$  ions.



In another proposed mechanism it was shown that metallic ions produced the oxidative dissolution of Ag NPs in the presence of oxygen (Yang et al. 2013).



There are several possible mechanisms for the bactericidal activities of metallic ions, the most likely being that metallic ions such as  $\text{Zn}^{2+}$  and  $\text{Ag}^+$  interact with the

positively charged cell membrane through electrostatic interaction. After entering the cell wall, the metal ions attach to the SH groups of proteins and solidify the wall. This consequently breaks the proteins comprising the cell, resulting in the loss of division and multiplication capabilities. Upon lysis of the bacterial cell, these metallic ions are released and then come into contact with other bacteria to restart the cycle (Ul-Islam et al. 2014).

A slightly modified explanation of the bactericidal mechanism of metallic ions has been discussed by various researchers. Specifically, it has been reported that  $\text{Ag}^+$  interacts with respiratory enzymes such as NADH dehydrogenase, leading toward the inactivation of respiratory enzymes. When such ions are attached to transport proteins through the sulfur group, they cause the collapse of proton motive force (Lok et al. 2007; Marambio-Jones and Hoek 2010). The high affinity of  $\text{Ag}^+$  or  $\text{Zn}^{++}$  toward the thiol groups is the major reason for their interaction with various proteins (Lok et al. 2007). Finally, Prabhu and Poulose (2012) reported that  $\text{Ag}^+$  ions prevent DNA replication and cause its condensation and localization in electron light regions.

Based on the above discussion, NPs may exhibit antibacterial activity due to any of the three aforementioned possibilities. However, since these possibilities occur through a slightly different mechanism, it is more likely that all three or even more mechanisms are involved in the bactericidal process. Whatever the specified mechanism for a particular nanomaterial or microorganism, nanomaterials have been shown to have much higher antimicrobial activities than bulk materials. This has greatly extended the utilization of nanomaterials in various sectors, especially in the health industry.

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## 9.5 Toxic Effects of Nanomaterials Toward Animal Cells

Owing to the aforementioned effective antibacterial efficacy of nanomaterials, they have been used in a variety of medical applications. However, nanomaterials pose a definite risk of toxicity toward animal and human cells. Indeed, many studies have shown serious health hazards related to the *in vitro* and *in vivo* toxicological effects of nanomaterials (Sukhanova et al. 2018). Similar to their antibacterial activity, the cells toxicology of the nanomaterials is dependent on a variety of factors including shape, size, charge, agglomeration, dispersion, and surface coating. (Li et al. 2012). Moreover, the *in vivo* studies showed that toxicological effects of nanomaterials are much stronger than *in vitro* studies. These nanomaterials can penetrate and affect the major body organs. However, the exact toxicological mechanism of nanomaterials and the level of hazard they pose are still unknown. Previous studies have shown different levels of toxicity for various NPs. Prasad et al. (2006) found toxicity of  $\text{ZnO}$  against neuroblastoma cells. Specifically, they observed a 50% cell death in response to 100 g/ml of  $\text{ZnO}$ . Yuan et al. (2010) reported a 90% cell death of HELF cells in response to 20 mg/L of  $\text{ZnO}$ . Haase et al. (2011) found the cytotoxic effects of silver NPs on macrophages derived from human monocytic leukemia cell line THP-1. Cell

exposure to different size of NPs (20 nm and 40 nm) for 24 and 48 h revealed strong toxic effects for both type Ag NPs. The resultant IC (50) values found were 110 g/ml (Ag 20 nm) and 140 g/ml (40 nm) after 24 h that drastically decreased to 18 g/ml and 30 g/ml, respectively, after 48 h. Cell type-dependent TiO<sub>2</sub> toxicity affecting cellular functions such as cell proliferation, differentiation, mobility, and apoptosis has also been reported in *in vitro* studies (Zhang et al. 2011). Naqvi et al. (2010) studied the dose- and time-dependent role of superparamagnetic iron oxide nanoparticles (SPION) coated with Tween 80 on murine macrophage (J774) cells. They found that lower doses in the range of 25–200 g/mL showed negligible toxicity, and that the cell viability was >95% after three hours of exposure. However the toxicity increased drastically in response to higher concentrations (300–500 g/mL) and prolonged (six hours) exposure, and the cell viability decreased to 55%–65% (Naqvi et al. 2010).

The toxic effects of NPs could be endorsed to numerous factors, nonetheless, generation of ROS is the most effective reason for both their cytotoxicity. ROS is physiologically essential but possibly damaging to cells. The levels of ROS are generally optimum in the healthy cells, but when the level of ROS increases beyond certain limits they cause severe oxidative stress, resulting in cell death through oxidation of the lipids and modification of the DNA and proteins (Valko et al. 2007; Droege 2002; Gwinn and Vallyathan 2006). ROS generated from TiO<sub>2</sub> NPs induced oxidative stress that results in early inflammatory actions (Shi et al. 2013). Oxidative stress has been shown to be generated by CNTs in fish brain and pulmonary inflammation in rats (Handy et al. 2008; Oberdörster 2004). The disproportionate generation of ROS has also been reported to damage mitochondrial DNA (Atha et al. 2012; Sharifi et al. 2012) and the toxic effects generated by ROS are not limited to particular cells or organs, but also affect various body system and functions (Chow et al. 2003; Adibhatla and Hatcher 2008).

Potential interactions between endocrine system and the toxicity of nanomaterials are quite limited. Data indicated that several nanoparticles are able to alter normal and physiological functions of the endocrine system (Iavicoli et al. 2013). Exposure to engineered nanomaterials causes endocrine-disruption and evolution of T2DM which needs more investigation. *In vitro*, *in vivo*, and epidemiological studies related to exposure endocrine-disrupting chemicals with obesity, T2DM, and other metabolic diseases it was revealed that endocrine disruptors have an ability to interact with the cell receptors to mimic hormone's action or through blocking the action of hormones on the target cell (Priyam et al. 2018). Despite these reports, Simon et al. (2014) results revealed that multiwalled carbon-nanotubes stimulated ROS production in RTL-W1, T47Dluc, and H295R cells, indicated no cytotoxicity, and decreased the bioavailability as well as toxicity of the biocide triclocarban. Recently Dagar and Bagchi (2020) reported that NPs lead to cause hormonal disturbance, neurological and immune disorders, influence fertility, and work as endocrine disruptive chemicals (EDCs). EDCs disrupt normal functions as they can block or mimic a hormone's physiological functions. EDCs include industrial (bisphenol A, PCB, plasticizers, dioxins, etc.) as well as synthetic chemicals (pesticides, solvents, etc.). Exposure to EDCs is unavoidable, so, an urgent need to

specify EDCs that have high effects on human health especially those acts via endocrine system to change cell signaling and responses.

## 9.6 Biocompatibility of Nanomaterials

The biocompatibility of nanomaterials is a critical aspect for their effective biomedical applications. NPs generally come in direct contact with body tissues and cells, where they can cause positive or damaging effects on the body. A few inorganic and polymeric NPs have been shown to be biocompatible in their action. Herein, several such NPs and their effective roles are discussed.

Several studies have reported nontoxic and biocompatible performance of SPION in different human and animal cells. Jain et al. (2008) investigated the *in vivo* performance of SPION in rat liver and concluded that it did not influence liver function. Li et al. (2012) have stated the nontoxicity of dextran-coated (SPION) toward primary human monocyte-derived macrophages and dendritic cells. Furthermore, Sun et al. (2007) presented good biocompatibility of sodium oleate-coated iron oxide NPs.

The biocompatible and nontoxic characteristics of silica NPs have been well documented. Mesoporous silica NPs have been successfully used in a number of biological and biomedical applications, including gene and drug delivery (Horcajada et al. 2012; Veiseh et al. 2010). Both bare and coated silica NPs have been reported to have no effect on cell survival. Silica NPs have been shown to be nontoxic to human and animal systems when they have optimized structural features and when applied in the right dosage (Qhobosheane et al. 2001). It has been reported that the lipid coating of silica NPs increases the functionalization, introduces multiple properties, increases bioapplicability, and improves pharmacokinetics. (Tang et al. 2012).

The biocompatibility of Au NPs has been reported in many previous studies. In a MTT assessment test conducted to evaluate the biocompatibility of gold NPs, Chandran and co-workers reported more than 90% viability of the cells until 48 and 72 h, and that 70–80% of cells were viable even in the presence of very high concentrations (80 M) of Au NPs (Correard et al. 2014). Similarly, Liu et al. (2012) reported good blood compatibility of zwitterionic Au NPs. The safe nature of Au NPs has resulted in their receiving attention for application in combination with biocompatible polymers. Recently, Grant et al. prepared composite polymeric materials by cross-linking collagen with Au NPs and found that the composite scaffold materials demonstrated enhanced stability and biocompatibility (Tshikhudo et al. 2004). Pure Ti and  $TiO_2$  are used in dental and orthopedic implants (López-Huerta et al. 2014; Li et al. 2008). Titanium foils are enclosed by nanoporous surface of  $TiO_2$  which enhances the proliferation and mineralization of osteoblasts (Brammer et al. 2009). Giavaresi et al. (2003) found a positive effect of nanostructured  $TiO_2$  coating on cell proliferation and activity. While another study reported that the growth rate of osteoblast cells increased 3 to 4 times in response to treatment with  $TiO_2$  nanotubes (Nikolaichik et al. 2013). Furthermore, Czarnowska et al. reported a pronounced improvement in

biocompatibility and tissue-healing properties following oxinitriding of Ti alloy under glow discharge conditions (van Hove et al. 2015).

As mentioned above, most inorganic NPs have toxic effects on both microbial and animal cells, and their comparative biocompatibility and toxicity are dose and cell-type dependent (Li et al. 2008). Additionally, the modification of NPs, effective levels of biocompatible properties have been witnessed in Ag (Ravindran et al. 2013). Likewise, Li et al. (2008) reported the biocompatibility and biosafety of ZnO nanowires (NWs) against Hela and L929 cells. They found that Hela cells showed full biocompatibility to ZnO nanowires (NWs) at all concentrations. However, the reproductive capability of L929 cells was good at lower levels of NWs, while the cell viability was reduced to 50% at higher levels (100 g/ml) of ZnO NWs (Li et al. 2008). Additionally, hemolytic behavior of less than 5% even at very high doses has been reported by Zhao et al. indicating biocompatibility of ZnO NPs (Zhou et al. 2006). In a study in which the surface of silver was modified and it was further reacted with modified thiol groups to produce nanoclusters of Ag, these particles showed no toxicity toward fibroblast cells (Atha et al. 2012). In a recent study, Von White et al. (2012) produced Ag NPs using garlic extract as a reducing agent. They reported that the NPs did not decrease the proliferation of vascular smooth muscle cells and 3T3 fibroblasts at a concentration of 25 g/mL.

Considering the combined cytotoxic and biocompatible effects of nanomaterials, it can be concluded that most nanomaterials have both properties. Moreover, these properties are extremely reliant on various parameters such as the dose quantity, cell type, size of nanomaterials, and the incubation duration. These properties can be tailored by slightly modifying the surface or charge properties of the nanomaterial. Nonetheless, a great deal of research is still required to intensively investigate the reasons for the varying properties of nanomaterials.

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## 9.7 Concluding Remarks

Nanomaterials, especially inorganic metals and metal oxides, are currently playing a vital role in reestablishing various research fields including biomedical fields. Moving toward narrower dimensions of nanomaterials has revealed some very interesting properties of much higher caliber than bulk materials. From a biological point of view, two important aspects of most nanomaterials are their antimicrobial and biocompatible properties. Nanomaterials are primarily found to possess antibacterial, antifungal, and antiviral properties, and microbes are attacked by nanomaterials either directly by attachment with their cell membrane followed by subsequent destruction of the proteins and DNA or by the generation of ROS that ultimately kill the cells.

The cytotoxic performance of nanomaterials is slightly dissimilar for higher animal cells, nonetheless still occurs. Nanomaterials such as TiO<sub>2</sub>, Ag, ZnO, and CNT show moderate to high levels of cytotoxicity in the animal cells. Moreover, nanomaterials like, Fe<sub>2</sub>O<sub>3</sub>, SiO<sub>2</sub>, Au, and TiO<sub>2</sub> have also shown good level of biocompatible properties. By the way cytotoxic nanomaterials have been transformed to

biocompatible materials through minor deviations in their surface structure. Consequently, it is established that nanomaterials keep a broad level of biological properties that are highly dependent upon their size, structure, quantity, and receptor cell type. Nevertheless, further studies are still essential to recognize added reasons for the performance of nanomaterials.

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# Application of Nanomaterials in Treatment of Endocrine Diseases

10

Khulood M. Al-Khater and Ebtesam A. Al-Suhaimi

## Abstract

The endocrine system is very essential to maintain body homeostasis. Disturbance of endocrine function leads to well-established diseases; such as diabetes mellitus, thyroid and parathyroid disorders, infertility, and obesity. There is no absolute cure for these diseases; however, current treatment aims to monitor them and prevent their further progression. Scientists are working hard to find better treatment strategies for endocrine disorders. Nanotechnology holds a great promise in finding solutions to these diseases, and this field is advancing very rapidly because of the targeted type of drug delivery, and hence, it reduced the side effects of the current medications. This chapter highlights the current state of researches concerning the use of nanotechnology in managing three examples of endocrine diseases: thyroid dysfunction, diabetes mellitus, and obesity. For example, nanotechnology has been implemented in finding non-invasive routes of insulin delivery such as oral, nasal, or transdermal routes. Glucose nanosen-

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K. M. Al-Khater (✉)

Department of Anatomy, College of Medicine, Imam Abdulrahman Bin Faisal University, Dammam, Saudi Arabia

Institute for Research and Medical Consultations, Imam Abdulrahman Bin Faisal University, Dammam, Saudi Arabia

e-mail: [kalkhater@iau.edu.sa](mailto:kalkhater@iau.edu.sa)

E. A. Al-Suhaimi

Institute for Research and Medical Consultations, Imam Abdulrahman Bin Faisal University, Dammam, Saudi Arabia

Department of Biology, College of Sciences, Imam Abdulrahman Bin Faisal University, Dammam, Saudi Arabia

e-mail: [ealsuhaimi@iau.edu.sa](mailto:ealsuhaimi@iau.edu.sa)

sors have been invented in order to improve the accuracy of detection of serum glucose. It is important to emphasize that this field of research is still in the pre-clinical stage and more work is needed to provide evidence of its safety. Immune response and toxicity are the main issues that concern researchers when using nanotechnology.

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### Keywords

Nanotechnology · Nanoparticles · Thyroid disease · Diabetes mellitus · Obesity · Toxicity · Glucose biosensor · Oral insulin · Smart insulin

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## 10.1 Introduction

The endocrine system consists of several glands (well-formed organs or scattered tissues and cells) that secrete hormones and, therefore, plays a key role in regulating various functions in the body. Disturbance of endocrine function leads to a wide range of well-established diseases; such as diabetes mellitus, thyroid dysfunction, Cushing's syndrome, and obesity. There is no absolute cure for these diseases, but the current medications help in monitoring these conditions and keeping the hormonal levels as close as possible to the physiological level in order to maintain body hemostasis.

The field of nanotechnology has very wide applications in science, including the biological systems. This field is advancing very rapidly, and very promising results have been documented in the literature. However, research work of nanotechnology in management of endocrine diseases is still in the infancy stage, being done only *in vitro* or on experimental animals. The goal of implementing nanotechnology in the treatment of endocrine diseases is to create endogenous nano-biosensors that detect minute changes in hormonal levels and respond spontaneously in a harmony in order to recover from this abnormal situation. Researchers describe this internal monitoring system as nanonetworks with chemical communication between nano-machines that form the unit of these networks (Ferreira et al. 2017).

This chapter is focusing on number of endocrine diseases; viz. thyroid dysfunction, diabetes mellitus, and obesity. Up-to-date information about the status of research work of nanotechnology to manage these disorders is presented.

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## 10.2 Thyroid Dysfunction

Thyroid gland is fundamental for maintaining an optimal level of metabolism in the body, in addition to other significant functions. Any disturbance of these functions, either hyper or hypothyroidism, is associated with disorder in metabolic activities of various organs. The traditional treatment for hypothyroidism involves provision of thyroid hormone as a compensatory measure. Whereas the current treatment of hyperthyroidism includes anti-thyroid drugs, radioactive iodine and, in some cases,

surgery. These treatment modalities have side effects that might limit their long-term use. Consequently, there is a need to improve these strategies and invent more target-specific methods that impose their effects on the thyroid only. The field of nanotechnology is very promising in this regard, and it carries a great potential to treat thyroid diseases.

Targeted drug delivery is one method by which the nanotechnology improved the efficacy of the therapeutics and decreased their possible side effects. Thyroid stimulating hormone (TSH) has been conjugated to nanoparticles (nanoliposomes) aiming to target the TSH receptors on thyroid cells (Paolino et al. 2014). This strategy proved that the uptake of the TSH-nanoliposomes (sometimes loaded with specific drugs) by the cells was much more than that of the unconjugated forms (Paolino et al. 2014). The trace element, selenium, is essential for normal activity of thyroid; mainly for the conversion of thyroxin (T4) into triiodothyronine (T3). Intramuscular injections of nano-selenium have been found to increase the activity of thyroid in growing rabbits (Eid et al. 2019).

Another research area of nanotechnology in thyroid diseases involves improving the surgical outcome of thyroid operations. Injections of carbon nanoparticles have been administered during thyroidectomy in order to make the operation more precise and prevent the inadvertence removal of the parathyroid glands (Su et al. 2018; Gu et al. 2015). This is accomplished by coloring the lymph nodes and thyroid gland, but not the parathyroid, with a black color. Therefore, the surgeon can safely remove the cervical lymph nodes along with the thyroid and keep the parathyroid glands intact. However, the significance of these carbon nanoparticles has been debated (Xue et al. 2018).

Nanotechnology has been also implemented in diagnosing thyroid diseases as it was found to detect very low levels of hormones. Nanoparticles were incorporated into electrochemical biosensors in order to improve their sensitivity to detect hormones in the body (Wang et al. 2013; Bendo et al. 2014; Cui et al. 2012; Salahvarzi et al. 2017). Europium (III) nanoparticles were used recently to detect low levels of TSH (Huhtinen et al. 2004; Näreöja et al. 2017). In an attempt to improve the detection of thyroid diseases, silver nanoparticles have been used to enhance the surface of spectroscopy, a tool that provides a molecular analysis of the biological sample (Huang et al. 2011). Enhanced detection of thyroid cancer has been reported by Chen et al. following the use of iodinated gold nanoparticles in the dual-modality fluorescence/CT investigation in a mouse model of thyroid cancer (Chen et al. 2017).

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### 10.3 Diabetes Mellitus

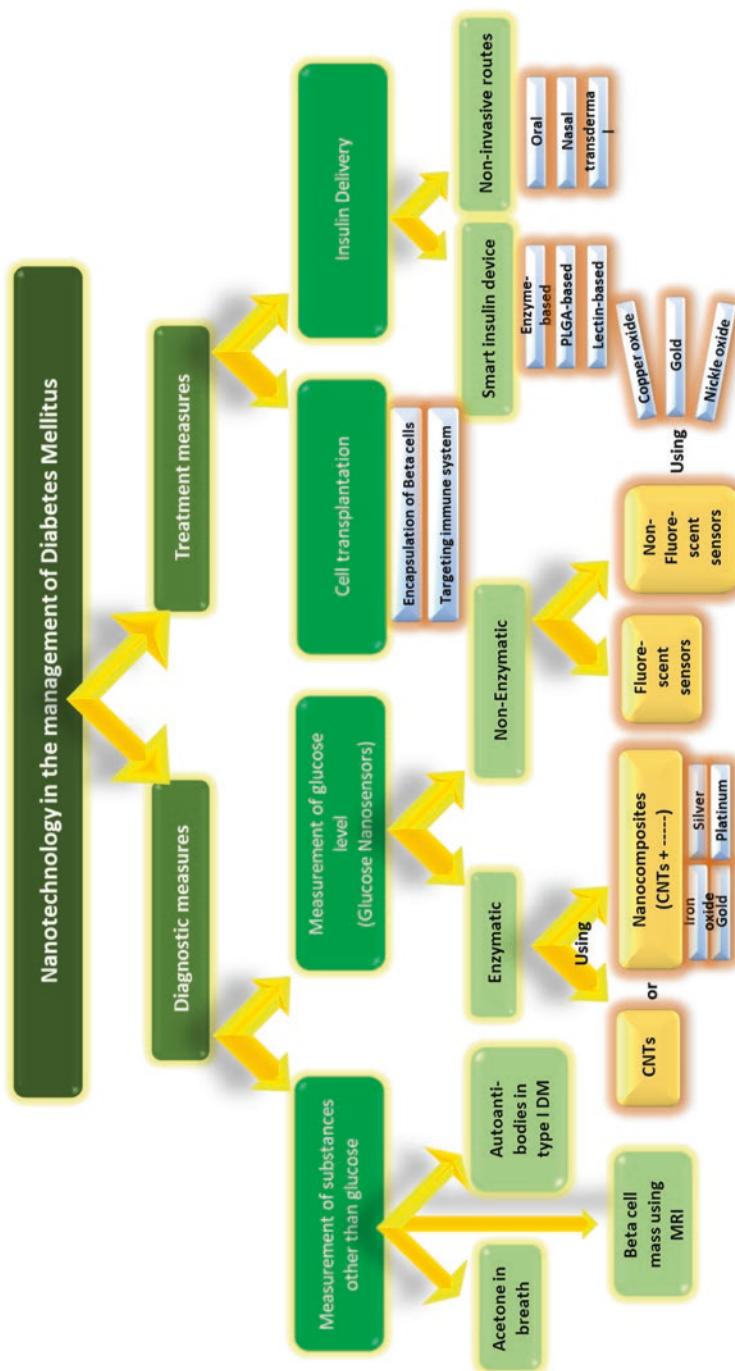
Diabetes mellitus (DM) is a worldwide chronic disease that affects variable percentages of populations in many countries. It has three main types: type I, or juvenile diabetes, type II, or adult diabetes, and gestational diabetes that occurs during pregnancy (Diabetes Overview—Symptoms, Causes, Treatment 2019). This chronic disease results from abnormality in glucose metabolism due to lack of (type I) or resistance to (type II) insulin, and this leads to increase in blood glucose level

(hyperglycemia). A study published by the International Diabetes Federation (IDF) reported that Gulf countries were among the top 10 in the prevalence of DM in 2011, and expected to continue with this high prevalence in 2030 (Whiting et al. 2011). According to the same study, the highest number of diabetic patients in the world in 2011 was in China, India, and the United States with 90, 61, and 23 million, respectively. In 2030, these numbers are expected to rise to 129, 101, and 29 million in these three countries, respectively (Whiting et al. 2011). The total number of diabetic patients in the world in 2030 is expected to exceed 550 million (Whiting et al. 2011). According to the American Diabetes Association, currently, there are more than 30 million American diabetic patients (Statistics | ADA 2019). In Saudi Arabia, death from DM accounts for 5% of the total deaths, and the average prevalence of diabetes was reported to be 14.4% (14.7% in males and 13.8% in females) of the total population (*WHO | Diabetes country profiles 2016* 2016). According to the WHO, Saudi Arabia is ranked as the second highest in the Middle East, and the seventh globally with regard to the prevalence of DM; about 7 million Saudis are diabetic and 3 million are pre-diabetic (Al Dawish et al. 2016). Therefore, a worldwide rise in the prevalence of DM is expected. This would put more burden on health institutions, especially that DM has very well-documented complications that might affect the heart, kidneys, vision, or peripheral nerves. The field of management of DM is very active in developing the best strategies to monitor and manage this disease and prevent its complications. The Ministry of Health in Saudi Arabia has approved the National Executive Plan of Diabetes Control (a national ten-year executive plan to control diabetes “2010–2020”), and one of its objectives is to create and develop the research tools and studies related to diabetes.

Nanotechnology holds a great promise in the management of DM. This basically involves improvements in the detection of blood glucose level (BGL) using very sensitive sensors, and precise monitoring of the release of insulin according to the exact needs of the body. Therefore, nanotechnology nowadays is applied in order to improve the techniques by which glucose level is maintained within its normal range, i.e., maintain the glucose homeostasis in the body (Kesharwani et al. 2018). Figure 10.1 presents a summary of the recent research work involving management of DM using nanotechnology.

### 10.3.1 Detection of BGL in Diabetic Patients

The daily glucose monitoring performed by the diabetic patients is principally using two main methods: self-monitoring (glucometers) and continuous monitoring. The *self-monitoring* method implies that a small finger prick is performed to obtain a drop of blood and place it over a strip inserted into a device, this gives a reading for the BGL. There are number of disadvantages in this method, one of them is the discomfort that is caused by the finger prick, in addition to the interrupted nature of checking the BGL that might miss dangerous fluctuations in its level. These disadvantages led to the invention of the *continuous monitoring* method that uses a special biosensor implanted subcutaneously and frequent (every 5–15 min)



**Fig. 10.1** This algorithm summarizes the current state of research work in the management of diabetes mellitus using nanotechnology. This research field is still in the preclinical stage with promising results. CNT: carbon nanotubes

measurements occur in order to estimate the BGL through the interstitial fluid (Cappon et al. 2019).

Over the last half a century, there has been a great advancement in the technology of detection of BGL by continuous glucose monitoring using the glucose biosensor technology (Yoo and Lee 2010). The basic structure of the biosensor consists of the *detector* (e.g., receptor, enzyme, or antibody), the *transducer* that converts the detected biological structure into a signal (magnetic, electrochemical, optical, or others), and the *reporter* that transforms the signal into a form that can be read. The popular detector used in these devices is the enzyme, and the most commonly used enzyme is the glucose oxidase because of its high specificity to the glucose molecules. The widely used type of enzymatic glucose biosensor uses the electrochemical type of transducers, mainly the amperometric one, which was invented by Clark and Lyons in 1962 (CLARK and LYONS 1962), and then modified by Updike and Hicks in 1967 (Yoo and Lee 2010). Further development of this type of glucose biosensors led to emergence of what have been known as second and third generations of biosensors (Cash and Clark 2010). Compared to these new generations, the first one used oxygen molecules that are reduced to  $H_2O_2$  and this creates the electrical signals. In the second generation of biosensors, the oxygen is replaced with a synthetic chemical mediator. Whereas, in the third generation, the potency of detection of glucose has been improved by using the nanomaterials that are incorporated into these sensors (glucose nanosensors), but these are still in the preclinical stage. The advantages of nanotechnology in glucose detection include the small size of the sensor that offers a larger surface area for catalytic activity, as well as the improved optical features and longer lifetime. Other important advantage of using nanotechnology in measuring the BGL is that it is painless and frequent measurements are taken; therefore, high fluctuations in BGL can be detected.

Various types of nanomaterials have been used in manufacturing these sensors, a very promising type is the carbon nanotubes (CNTs) because of their high catalytic activity (Saeideh and Golshan 2018). These CNTs consist of a single type of nanomaterials, other designs consist of CNTs in addition to other nanomaterials (nanocomposites) (Lin et al. 2009; Wang et al. 2009). These nanocomposites have much more improved catalytic activity, and this has been manufactured by coupling the CNTs with gold (Wang et al. 2009), silver (Lin et al. 2009), platinum (Wen et al. 2009), or magnetic (iron oxide) (Baby and Ramaprabhu 2010) nanoparticles. Other types of nanoscale sensors used nano-polymers (Santhosh et al. 2009).

Other type of glucose biosensors does not use enzyme to detect the glucose, but is designed in a way that glucose is detected directly by the electrode, and this gives more stability to the sensor (Cash and Clark 2010). Using nanoparticles with these “non-enzymatic sensors” rendered them more precise and increased their accuracy. Nickel oxide nanoparticles have been used in this type of glucose biosensors, and these were deposited on DNA modified glassy carbon electrodes (Sharifi et al. 2014). This study reported that glucose detection was highly improved, and levels as low as 7 nM were measured by these nano-technically improved sensors. Other studies used gold nanoparticles (Hussain et al. 2011) or copper oxide (Jiang and Zhang 2010; Yang et al. 2010). However, these biosensors require batteries to

operate and this renders them larger in size. In order to overcome this limitation, researchers designed sensors that produce fluorescence upon binding of glucose; the degree of fluorescence corresponds to the level of glucose in the blood (Veiseh et al. 2015). Attaching nanoparticles, like carbon nanotubes, to glucose-binding moieties, like lectins, has been reported in fluorescence-based sensors (Barone and Strano 2009). This is a very promising approach in designing glucose sensors and has reached the clinical trials stage (Veiseh et al. 2015). However, challenges still exist and concerns regarding the toxicity of carbon nanoparticles in the fluorescent sensors have been raised (Veiseh et al. 2015).

Apart from glucose, nanoscale sensors have been developed to detect acetone in breath of diabetic patients (Chakraborty et al. 2008) or autoantibodies in type I DM (Lee et al. 2007). These methods of diagnosing DM are non-invasive, cheap, and easy to perform compared to other more sophisticated ones. Acetone is one of the gases normally present in the breath, but in a very low concentration (<0.9 ppm or mg/L) (Croppord et al. 1977). However, its concentration increases in diabetic patients (Barnett et al. 1969). The routine method of detecting acetone lacks sensitivity and specificity; hence, researchers developed a more sensitive sensors using the nanoparticles, like  $\gamma$ -Fe<sub>2</sub>O<sub>3</sub>, to detect acetone (Chakraborty et al. 2008).

A very useful method to diagnose and manage patients with type I DM is the use of MRI to detect the mass of beta-cells in islets of Langerhans. The degree of detection of these beta-cells has been increased by using nanoparticles as contrast agents (Medarova and Moore 2009; Laurent et al. 2016; Medarova and Moore 2008). Several nanoparticles have been used, of which iron oxide attracted more interest because of its biocompatibility (Veiseh et al. 2010). This detection of beta-cells was possible either for the original cells of the patient or for the transplanted ones (Medarova et al. 2006).

## 10.3.2 Implementing Nanotechnology in Treating DM

### 10.3.2.1 Insulin Delivery Systems

One of the major concerns of researches in the field of diabetes management is to find a more patient-friendly method of insulin replacement therapy. The current one is the subcutaneous (SC) administration, which causes pain, and hence, reduces the compliance of patients. The other drawback of the SC injection is that it allows insulin to enter the peripheral circulation, not the portal vein like the physiological insulin. Therefore, scientists aim to develop methods that improve the bioavailability of insulin by non-invasive routes of administration like the oral, nasal, or transdermal routes (Sharma et al. 2015). The very promising field of nanotechnology has been also implemented to achieve this goal. Insulin, a 6 kDa polypeptide, faces two obstacles when taken by the oral route; degradation by the enzymes of the gastrointestinal (GI) tract, and the thick lining of this tract. Therefore, the bioavailability of insulin is very low following oral intake (Bakhru et al. 2013). Many studies addressed these obstacles and tried to improve the bioavailability using the nanoparticles (Bakhru et al. 2013). One of the earliest studies was published in 1997 by

Nature, and documented a normal level of glucose following feeding rats with insulin especially prepared to overcome the harsh GI environment (Mathiowitz et al. 1997). They used a technique known as “phase inversion nanoencapsulation” to encapsulate the insulin by biologically erodable polymers, and reported an enhancement in its absorption across the epithelial lining of the GI tract. In another study, feeding rats with these same nanoformulations led to normal fasting levels of glucose following glucose tolerance test compared to control rats fed with unencapsulated insulin or saline only (Carino et al. 2000). Promotion of oral absorption of insulin in rats using lectin-modified lipid nanoparticles (Zhang et al. 2006) or chitosan-insulin nanoparticles (Ma et al. 2005) has also been reported. The research group of Sung demonstrated positive results of increasing the bioavailability of insulin following oral intake of nanoparticles loaded with insulin in diabetic rats (Sung et al. 2012). They used chitosan and poly(gamma-glutamic acid) to prepare the nanoparticles and loaded them with insulin, feeding diabetic rats with this preparation led to reduction in the glucose level (Lin et al. 2007; Chuang et al. 2013). Other group of studies performed on mice and aimed to improve the passage of insulin across the epithelial lining of GI tract by designing nanoparticles that target the FC receptor in the intestinal cells, and therefore, facilitate the active transport across these cells (Pridgen et al. 2013). Several factors have been thought to play a role in the uptake of protein drug by the GI tract, of which are the size of the drug particles and the nature of the polymer that encapsulate the drug (Bakhru et al. 2013). The smaller the size of the particle, the higher is the rate of absorption through the GI lining, and bioadhesive polymers are more prone to absorption than other types because of the longer stay in the GI tract. Despite these promising results, the oral delivery of nanospheres of insulin is still in the experimental laboratories and not yet approved for clinical use. More attention will be directed towards engineering the insulin delivery vehicles in order to overcome the harsh GI environment. Developing an innovative nano-technique to deliver insulin orally was a research project in 2014 at the Center of Excellence for Nano-Biology in KASCT in Saudi Arabia (Functional Nanomaterials Lab—3SINC 2014 and Solid State Lighting Program Workshop 2019).

The other method of non-invasive insulin replacement therapy is the nasal spray. This method attracted interest of investigators because of its easy application that ensures better compliance of patients. Nasal insulin therapy has already reached clinical trials and even some formulations gained, or in the process of gaining, approval by the FDA (Veiseh et al. 2015). However, caution does still exist because of lack of evidence of safety records in long-term usage. Therefore, investigators wanted to implement nanotechnology in improving the nasal formulations of insulin therapy, as nanoparticles are documented to enhance the delivery of inhaled drugs (Tang et al. 2009). One of the earliest studies that investigated the application of nanoparticles as carriers for nasal administration of insulin was performed in 1999 on a rabbit model (Fernández-Urrusuno et al. 1999). They used chitosan nanoparticles and demonstrated that these particles were successful in causing efficient transport of insulin across the nasal mucosa. Solid lipid nanoparticles were designed as a carrier for insulin delivered to diabetic rats by a nebulizer (Liu et al.

2008). This study reported that homogenous distribution of the nanoparticles in the lung alveoli was observed, and a reduction in the fasting glucose level was also found. Furthermore, these authors reported that their method of delivery of insulin is not cytotoxic and this suggested its safe application on the airways. This was also confirmed by another study (Bi et al. 2009).

Another novel non-invasive method of administering insulin is through the skin, or transdermal application (Veiseh et al. 2015). This was investigated using different nanoparticles *in vivo* on mice (Higaki et al. 2006; Nose et al. 2012) or *in vitro* on pig (Lopez et al. 2011) or human cadavers (Choi et al. 2012), and showed promising results.

The glucose sensors, described above, have been modified to respond by releasing insulin upon the need of the patient, a technique known as “closed-loop” system or “smart insulin” device, which mimics the natural pancreas. In this technique, the response to any change in the BGL is immediate and auto-regulated, and this technique is considered the ideal treatment for type I diabetic patients. Currently, there are three well-documented strategies to detect glucose; enzymatically using glucose oxidase, binding to synthetic molecules, or binding to glucose-binding proteins (DiSanto et al. 2015). The nanoparticles used in these glucose sensors were designed in a way that makes them respond to changes in the surrounding environment, for example, change in the pH that occurs secondary to hyperglycemia (Fleige et al. 2012). As a result, the insulin will be released and the normoglycemic state will be restored. This was successfully demonstrated in models of diabetic mice, where a microgel nanocarrier system sensitive to pH was injected into the mice and this system swells in response to hyperglycemia, and therefore, releases the insulin (Gu et al. 2013b). A long-term response, up to 10 days, after injecting diabetic mice subcutaneously with glucose-mediated controlled release of insulin using a degradable nano-network was reported (Gu et al. 2013a). As stated above, enzymatic detection of glucose has a disadvantage of being unstable; therefore, researchers used some chemical moieties that bind to glucose, such as lectins or PLGA, in order to design these “smart insulin” devices. PLGA nanoparticles-based devices have been designed to secrete insulin upon exposure to ultrasound in a model of diabetic mice, and this was found to regulate BGL for up to 10 days (Di et al. 2014). The most commonly used lectin in glucose sensors is a natural one extracted from plant known as concanavalin A (ConA) (Brownlee and Cerami 1979). The binding of glucose to this lectin-based sensor leads to dissociation and release of the insulin. Nanoparticles have been used to design such types of ConA-based “closed-loop/smart insulin” devices, and one large pharmaceutical company, Merck, succeeded in reaching clinical trials with such devices (Veiseh et al. 2015). However, no further advancement has been reported by this company due to lack of efficiency of use of this invention in humans, probably due to immune system. Therefore, more research work is needed in order to eliminate this unwanted immune response.

### 10.3.2.2 Cell-Transplantation Therapy

The invention of what is known as “bioartificial pancreas” to treat type I DM is not recent in the field of treatment of diabetes mellitus. Beta-cell transplantation was

first introduced in 1972 by a study performed on diabetic mice (Gates et al. 1972). This was followed by studies that tried to modify the original technique by encapsulating the transplanted pancreatic cells (Lim and Sun 1980). However, many challenges faced the effective clinical application of this invention. Cytotoxicity and immune response to the implanted islets of pancreatic cells are examples of such challenges. Therefore, researchers tried, and are still trying, to reduce the immune response by various strategies; nanotechnology is one of them. Protecting the transplanted cell with nanoparticles has been reported (Lanza et al. 1996; Wilson et al. 2008). Recently, Kozlovskaya et al. reported a new strategy for encapsulation of islets cells using layer by layer deposition technique, but avoiding the use of the cytotoxic polyionic polymers (Kozlovskaya et al. 2012). They also reported that this new coating technique decreased the synthesis of inflammatory cytokines, and therefore, better protection of the islets against the immune response. Suppression of the immune response by targeting the genes of immunity using nanoparticles has also been reported (Ko et al. 2001). Another interesting method is to synthesize a vaccine that prevents the auto-destruction of beta-cells in type I DM (Tsai et al. 2010). This was investigated in diabetic mice by treating them with magnetic nanoparticles coated with specific peptides, and this led to reversal of diabetes in 75% of the mice (Tsai et al. 2010). An up-to-date review of recent advances in cell replacement therapy for type I DM is available (Ernst et al. 2019).

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## 10.4 Obesity

Obesity is an epidemic chronic disease that has shown a steady rise in its prevalence among different populations (Gregg and Shaw 2017; Malik et al. 2013). Body mass index (BMI) forms the basis for diagnosing obesity and differentiating it from overweight; BMI above  $30 \text{ kg m}^{-2}$  is regarded as obesity, whereas, BMI between 25 and 30 is considered overweight. The health hazard of obesity is reported as being a strong risk factor for number of chronic diseases, among which are type II DM, cardiovascular diseases, hypertension, and cancer (Friedman 2009; Afshin et al. 2017). A recent report issued from the WHO indicated that the average prevalence of obesity in adults, estimated during 41 years (between 1975 and 2016), was 36.2% in the United States, and 35.4% in Saudi Arabia (*WHO | Overweight and obesity* 2018). A local study in Saudi Arabia estimated the prevalence of obesity among adults to be 35.5% (Al-Nozha et al. 2005). Furthermore, the current estimation of worldwide obesity indicated that it will exceed one billion by 2030 (Kelly et al. 2008). Apart from its associated health consequences, obesity forms a burden on the economy of any country because of the cost of treating its complications (Cawley and Meyerhoefer 2012). Prevention and treatment of obesity are, therefore, considered very essential for the wellbeing of people in any community. In Saudi Arabia, Saudi Guidelines on the Prevention and Management of Obesity has been released in 2016 in its first edition. Nanotechnology, nowadays, forms one of the new promising strategies to treat obesity.

Research work in this area is not as developed as that in the field of cancer. Attention to this has started only during the last decade, and the results are still in the development/preclinical phase being performed mainly on animal models. The main challenge facing the advancement of this field is the poverty of information that aid in the specific targeting of the adipose tissue. Several trials to overcome these challenges are reported in the literature; like targeting the white adipose tissue (Kolonin et al. 2004), or the blood vessels nourishing the adipose tissue (Xue et al. 2016; Hossen et al. 2013).

Currently, clinicians use several strategies to treat obesity. Apart from diet control and lifestyle changes, there are two main approaches: drug therapy (pharmacological treatment) and surgery. Many of the drugs that have been licensed in the past were banned and withdrawn from the market because of their serious side effects. Therefore, there is a strong need to implement new methods to treat obesity, and the field of nanotechnology is a promising one.

The direction of nanotechnology research in management of obesity is going towards three main targets; (1) improving the quality of food, by a process known as nanoscale food processing, (2) enhancing drug delivery by encapsulation with nanoparticles, and (3) implementing genetic and epigenetic approaches.

#### 10.4.1 Improving Food Quality

The term “Nutraceuticals” has been applied to field of processing of food products that are healthy and used to prevent or treat some diseases (Ash et al. 2019). Nanotechnology has been applied to improve the absorption of some fat-soluble nutrient materials like vitamins and minerals by rendering them water-soluble; therefore, improving their bioavailability (Thiruvengadam et al. 2018; Sonkaria et al. 2012; Pradhan et al. 2015). Another example of food manipulation is the improvement of taste and flavor without adding sugar (Silva et al. 2012). Beneficial supplementation of dietary silver (Ag) nanoparticles has been reported (Saleh and El-Magd 2018). This latter study found that addition of Ag nanoparticles to the diet of chickens led to increase in muscle mass as well as reduction in the plasma levels of cholesterol, triglycerides, and glucose. However, this finding needs more supportive evidence by further investigations, as some other studies did not support such findings (Sawosz et al. 2007). However, concerns regarding the safety of such food processing have been raised (Ash et al. 2019). Authors of this latter review expressed their fear of potential health hazards due to lack of knowledge of details about the digestion and absorption of the nanoparticles used in food preparations, and due to presence of inorganic nanoparticles that might cause release of free radicals and reactive oxygen species (ROS) leading to inflammation in the cells.

### 10.4.2 Encapsulation of Anti-obesity Drugs with Nanoparticles

The second approach implies that known anti-obesity drugs are loaded with nanoparticles. Examples of such approach are many in the literature, the following account highlights some:

- The lipase inhibitor anti-obesity drug, orlistat, has a very low bioavailability because of its hydrophobic nature (Ballinger and Peikin 2002). Its bioavailability has been increased by encapsulation with nanoparticles, and therefore, rendering it water-soluble (Sangwai et al. 2014).
- One of the documented pharmacological methods in treatment of obesity is targeting an endothelial cell marker of blood vessels that nourish the white adipose tissue, this marker is called prohibitin (PHB) (Kolonin et al. 2004; Sibuyi et al. 2018). Incorporating the nanotechnology into such strategy is expected to improve its efficacy. Indeed, this has been investigated using different types of nanoparticles, such as liposomes (Hossen et al. 2010, 2013), gold nanoparticles (S Sibuyi et al. 2017), or polymeric nanoparticles (Xue et al. 2016). These studies confirmed the assumption and provided an evidence for the enhancement of the effectiveness of this strategy of treatment. However, a concern has arisen due to the harmful effect of lack of oxygen on adipose tissue and the subsequent development of chronic inflammation of the tissue (Cao 2013).
- Another treatment modality that has been reported recently is induction of angiogenesis in white adipose tissue (WAT) in order to transform it to brown adipose tissue (BAT). This type of tissue, BAT, is thermogenic, i.e., helps in production of energy, and therefore, its activity will help in weight loss. As a method of treating obesity, researchers started to develop what have been known as “browning agents” in order to convert WAT into BAT. The incorporation of nanotechnology into this method led to more targeted therapy and, as a result, reduced the undesirable side effects. An example of such work is that by Xue et al. (2016), who succeeded in targeting WAT using nanoparticles conjugated to the drug (rosiglitazone) and inducing angiogenesis. This prevented weight gain in the experimental mice. Jiang and coworkers also designed a targeted drug therapy that used the FDA-approved nanoparticles [poly (lactic-co-glycolic acid) (PLGA)-based nanoparticles] to transform WAT into BAT, but by using another strategy (Jiang et al. 2017). They incorporated these PLGA-based nanoparticles into a drug (dibenzazepine) that is known to inhibit the pathway of Notch signaling in fat cells (adipocytes). Local injection of these nanoparticles into the inguinal region was found to reduce the weight gain (i.e., reduce the obesity) in diet-induced obese mice. Zhang et al. implemented the nanotechnology in delivering browning agents using the microneedle skin patches (Zhang et al. 2017). This led to ~15% reduction in weight gain of mice after 4 weeks of application of these patches.
- Impaired mitochondrial function has been reported in many diseases including obesity through the effect on appetite and impaired weight regulatory mechanisms (Duchen 2004). Several studies reported drugs that are classified as

uncouplers of oxidative phosphorylation as a treatment modality of obesity (Blaikie et al. 2006; Liu et al. 2009). However, challenges were present in the effective delivery of this type of drugs to the mitochondria as toxicity occurs following high doses. Therefore, some researchers loaded the drug [2,4-dinitrophenol (2,4-DNP)] with nanoparticles that are very carefully developed to enhance the internalization by the cells and targeting the mitochondrial matrix (Marrache and Dhar 2012). These latter authors developed their new nanoparticles using PLGA-based nanoparticles. In order to enhance the bioavailability and prolong the circulation time of the drug, they incorporated the polyethylene glycol (PEG)-based polymer on the surface of PLGA- nanoparticles. Furthermore, to promote the internalization of the drug into the cells, they used a single terminal lipophilic triphenylphosphonium (TPP) cation as a copolymer. The resultant nanoparticle (PLGA-b-PEG-TPP) demonstrated its effectiveness in reducing the accumulation of lipid in the fat cells, and therefore, treating obesity. However, this has not yet reached the clinical trials stage, and some researchers warned of possible generation of ROS that might harm other tissues (heart and muscle) (Zhang et al. 2018).

#### 10.4.3 Genetic and Epigenetic Approaches

The third approach of nanotechnology in treating obesity is augmenting the gene delivery in particular types of therapy. In this approach, the therapeutic gene is encapsulated in a similar pattern to that of the drug. This method has an advantage over the traditional viral method of gene delivery since the immune system of the host will not be stimulated. Ash et al. presented a recent review in which they reported the up-to-date advances in genetic therapy of obesity and its related metabolic disorders (metabolic syndrome), and they suggested better results in case of delivery of these genes using the nanotechnology methods (Ash et al. 2019). Example of such genes is the GLP-1-FcmlgG-Leptin gene, whose increased expression in mice is found to increase insulin secretion and promote satiety feeling (Ye et al. 2013). A recent example of applying the nanotechnology is the study performed by Park et al. who used a polymeric vehicle, linear polyethylenimine (IPEI), to form IPEI-pDNA polyplexes nanoparticles (Park et al. 2015). These nanoparticles were used to deliver two known anti-obesity genes, islet amyloid polypeptide (IAPP) and leptin (LEP), into diet-induced obese mice. A weekly injection of this nano-gene therapy led to weight loss and reduction of plasma glucose and lipid levels.

Other promising approach to treat many diseases, including obesity, is therapeutics that target cellular processes that precede the level of genetic expression; these processes are known as epigenetic changes. One of these changes is the role of small non-coding RNAs, like miRNAs, on gene expression (Rupaimoole and Slack 2017). These miRNAs affect many biological metabolic processes, and are abnormally expressed in number of diseases including obesity. Therefore, targeting miRNAs is a potential treatment strategy for obesity. Encapsulating these miRNAs or the anti-miRNAs into nanoparticles would probably enhance their efficacy. Details

of recent advancement in the epigenetic therapy are presented by Ash et al. in their recent review (Ash et al. 2019).

As mentioned earlier, these researches are still in the development phase, and their transformation into the clinical trials has number of challenges. The most important of these, as mentioned by Zhang et al. (2018), is the importance of the clear understanding of the pathways involved in energy balance. Once this has been documented, the specific drug can then be encapsulated by the nanoparticles in order to enhance its efficacy. The other challenges are the appearance of unexpected side effects of the nanoparticles and the issue of biocompatibility; these must be carefully evaluated (Zhang et al. 2018; Lu et al. 2016). Other limitations of application of nanotechnology in the field of treatment of obesity are the ones mentioned by Ash et al. (2019), and these are related to the difficulty in controlling the size, shape, and mass of the nanoparticles, as well as the difficulty in designing a disease-specific nanoparticle whose release is triggered by specific mechanisms.

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## 10.5 Conclusion

The research field of nanotechnology, in general, and its biological application in treatment of diseases, in particular, are advancing very progressively in the literature. This is because of the highly targeted type of drug delivery that this technology can provide, a feature that would reduce the putative side effects of the current medications. Endocrine diseases are among the most widely occurring types of diseases in most communities. Application of nanotechnology in treating endocrine diseases holds a great promise in finding a cure, or at least improving the current treatment options. Among other endocrine conditions, the most widely investigated disease is diabetes mellitus. Researchers used two main approaches to manage diabetes by nanotechnology; they used it either to improve methods of detection of glucose in the blood or to enhance insulin delivery. Both of these approaches are currently still under investigations and not yet reached the clinical trials phase. Management of obesity has also been investigated using nanotechnology; however, the interest in this area is considered recent compared to that of diabetes. Three approaches have been described in the literature to treat obesity using nanoparticles; one involves manipulation of food to enhance its absorption or improve the taste, the second approach implies enhancing the drug delivery of anti-obesity medications by encapsulation with nanoparticles, the third one is through augmenting delivery of therapeutic genes (genetic) or miRNAs (epigenetic) via encapsulation with nanoparticles. Nanotechnology has also been implemented in management of thyroid diseases. This is either by conjugating the TSH with nanoparticles and improves its delivery to thyroid cells, by improving the precision of detection of thyroid hormones for diagnostic purposes, or to protect the parathyroid glands during thyroidectomy operations. At the end, it should be mentioned that despite all of these advantages of nanotechnology and the progress made so far, concern still exists regarding its safety and more research is needed to unravel this issue.

**Conflict of Interest** The authors declare that they have no conflict of interest.

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# Major Nano-based Products: Nanomedicine, Nanosensors, and Nanodiagnostics

11

Firdos Alam Khan

## Abstract

Nanomedicine is a very rapidly evolving field which has many biological, biomedical, and healthcare applications. Due to various beneficial properties of the nanomaterials, such as vast surface area, high biocompatibility, and biodistribution properties, these nanoparticles have been used to develop many useful products. Many of these nano-based products are under various developmental stages and many nanodrugs have been reported to have entered clinical phases of testing for various treatment conditions. In this chapter, we discuss some of the major nanoproducts which are developed for the treatment, diagnosis, and biosensing purposes.

## Keywords

Nanoproducts · Nanomedicine · Nanodiagnosis · Nanosensors · FDA-approved products

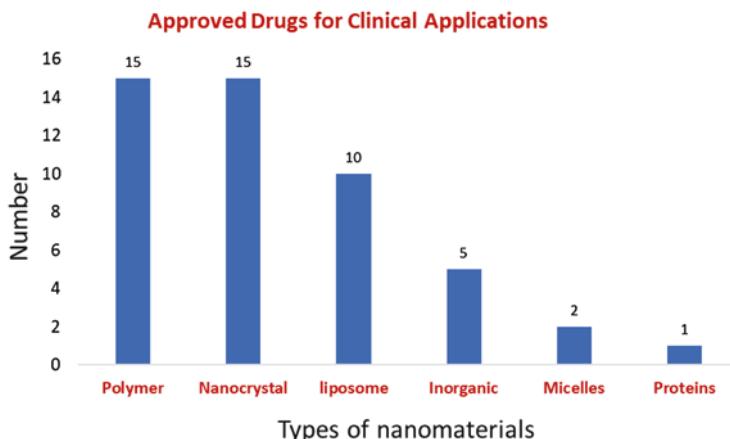
## 11.1 Introduction

The application of nanomaterials in the treatment of different diseases and detecting or diagnosis of different diseases is known as nanomedicine. The field of nanomedicine is growing with many useful applications. There are various useful applications of nanomaterials due to their physical and biological characteristics.

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F. A. Khan (✉)

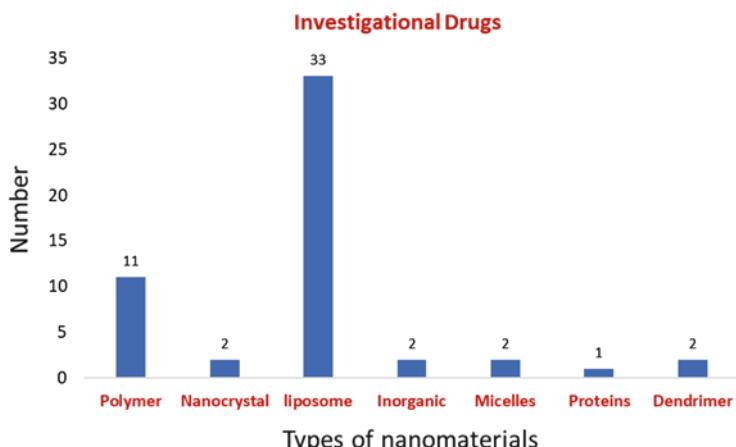
Department of Stem Cell Biology, Institute for Research and Medical Consultations, Imam Abdulrahman Bin Faisal University, Dammam, Saudi Arabia  
e-mail: [fakhan@iau.edu.sa](mailto:fakhan@iau.edu.sa)



**Fig. 11.1** List of approved nanodrugs synthetized from different types of nanomaterials

These characteristics make them highly attractive for many biological applications (Bobo et al. 2016; Caster et al. 2017; Ventola 2012a). Some of the advantages are higher biocompatibility, pharmacokinetics (PK) and with increased efficacy and decreased toxicity, and enhanced tissue selectivity, compared with conventional medicines (Bobo et al. 2016; Ventola 2012a, 2017; Havel 2016; Havel et al. 2016). To be clinically acceptable, all drugs must be thoroughly examined in different preclinical and clinical phases as per the Food and Drug Administration (FDA) of the USA. All materials to be injected in the human body must be cleared from safety, toxicity, and effectiveness profiling (Bobo et al. 2016; Ventola 2012a, 2017). The nanomaterials can be ranged from 1 to 100 nanometers, and because of their nanosize and better surface area, they show better biocompatibility. Other characteristics of nanomaterials are: they exhibit related dimension-dependent properties or phenomena which can change the biochemical, electronic, magnetic, or optical properties of a drug formulation in a way that can then be applied for therapeutic purposes (Ahn et al. 2014; Bobo et al. 2016; Ventola 2012a; Wolfram et al. 2015). Nanomaterials such as micelles, nanocrystals, liposomes, polymers, metals, metal oxides, other inorganic materials, and proteins are being used in the drug delivery as shown in the Fig. 11.1.

Other factors which are critical for effective therapy are the shapes and surface chemistry of the nanomaterials which are important for better adsorption, cellular uptake, accumulation and biodistribution patterns, and clearance mechanisms. Currently, 50 nano-based drugs have been approved and are available for use in clinical applications and many more are being studied in clinical trials for a wide range of disease indications (Fig. 11.2).



**Fig. 11.2** List of investigational nanodrugs synthetized from different types of nanomaterials

## 11.2 Advantages of Nano-Based Formulations

There are many advantages of the nano-based formulations and one of them is the conjugation techniques which allow linking of two or more molecules to the drugs molecules for better penetration and to alter its pharmacokinetics (Ventola 2012a, 2017; Havel et al. 2016). It has been found that nano-based formulations can overcome some of the shortcomings of traditional medicines by fostering better pharmacokinetics of the drugs and biodistribution. Moreover, these nano-based formulations can easily penetrate in the target sites with better longer drug circulation in the body.

The conjugation of nanomaterials with drugs molecules may be regulated with sustained manner for longer therapeutic impact on the targeted sites. Most of the currently tested nano-based conjugated formulations are normally improve the pharmacokinetic properties and they are normally involves in the non-specific accumulation of drugs in the tissues and tumors. Nevertheless, the active targeting can be achieved by conjugating ligands (proteins, antibodies, or small molecules) to the surface of the drug–nanomaterial conjugate that are designed to attach to receptors on specific cells. Interestingly, active targeting of drugs has shown an increase in intra-cellular drug accumulation and uptake by the cells of the targeted tissue.

Besides in vitro testing, preclinical and clinical studies are required to characterize the pharmacokinetics, pharmacodynamics, biodistribution, efficacy, and toxicity of nano-based formulations to understand how they differ from conventional dosage forms. In fact, these studies are needed because nano-based formulations can dramatically alter pharmacokinetics, for example, treatment of nano-based formulations (liposomal doxorubicin) ( $50 \text{ mg/m}^2$  dose of in humans) was found to increase the area under the curve (AUC) by 300-fold and reduce clearance by 250-fold compared to free drug.

### 11.3 Strategies to Improve Nano-Based Drug Formulations

To produce nano-based drugs, various strategies can be used to improve drug delivery and drug efficacy. Some of them are the exploitation of the small size of nanomaterials to circumvent important physiological barriers like the immune system, renal clearance, enzymatic and mechanical degradation. Also, to use nanomaterials to entrap drug molecules to protect them from physiologically hostile environments and for them for surface conjugation to target drugs to specific tissues, enabling higher therapeutic levels at a target site even with the use of lower doses (Ventola 2017; Wolfram et al. 2015; Zhu et al. 2014). Moreover, nanomaterials also have immunomodulatory effects that can impact the adaptive immune response. In addition, different types of nanomaterials such as polymeric nanomaterials, liposomes, and nano-emulsions have the ability to enter antigen-presenting cells which makes them to regulate the immune response (Gregory et al. 2013; Stanberry et al. 2012). For example, Poly-D, L-lactic acid-co-glycolic acid copolymer (PLGA) nanomaterials have been shown to deliver antigens to dendritic cells (Ventola 2017; Elamanchili et al. 2004). Because of an efficient delivery capability, nano-based formulations can increase the efficacy of vaccines.

Nano-based formulations also play a critical role in improving the efficacy of cancer immunotherapies because they can be designed to precisely deliver the drugs at the targeted signals to maximize a coordinated immune response against specific cells. In the recent past, many nano-based formulations are developed for the treatment of cancer, for example, nano-based formulations were used either passively or actively to target a tumor site. It has been found that passive targeting is frequently used to target solid tumors because the enhanced penetrability of blood vessels and weak lymphatic drainage enable the preferential accumulation of drug within the tumor microenvironment (Ventola 2012b, 2017). Whereas the use of active targeting relies on ligands conjugated to the nanomaterials that bind with tumor biomarkers and this potentially enhances the accumulation of nanomaterials at the tumor site and increases uptake by cells expressing the target receptor. Many preclinical and clinical studies have shown that nano-based formulations can passively improve tumor accumulation, decreasing normal tissue exposure. But, the clinical evaluations of active nano-based formulations are more limited and not as easily achieved.

To be an effective therapeutic molecule, nano-based formulations must be accumulated in the targeted tissues or diseased tissues, it has been found that an increased drug accumulation in the tumor can allow the efficient dose of a drug to be decreased, also diminishing side effects. It has been found that less than 0.01% of a treated dose of angstrom sized agents accumulates in a target region, compared to 1% to 5% for nanomaterials. Moreover, better accumulation of drug, as well as targeted release, can enable dose reduction, which also decreases side effects. In fact, the initial nano-based formulations/drugs were granted approval by the FDA based on lower toxicity compared with conventional formulation counterparts. For example, *Doxil* (doxorubicin hydrochloride, Janssen), the first nano-based formulation/drug to gain FDA approval, received an indication for the treatment of Kaposi's sarcoma

in patients with human immunodeficiency virus (HIV). Even though equally effective, its main benefit in comparison to conservatively formulated doxorubicin is considered by many to be reduced cardiotoxicity. But Doxil has been related with adverse events related to the nano-based formulations such as palmar-plantar erythrodysesthesia and complement activation-related pseudoallergy-like infusion reactions. Doxil with more than 20 years after its approval is still widely used for its original indication, as well as to treat the ovarian, metastatic breast cancer, and multiple myeloma.

The cancers are being treated with chemotherapeutic agents, and there are many reported side effects in the patients. The nano-based formulations can also be used to minimize the dose-limiting toxicities associated with conventional chemotherapeutic agents. It has been reported that many anti-cancer chemotherapeutic agents are hydrophobic in nature and are relatively insoluble in water solutions. These water-insoluble drugs are required to be supported by the toxic solubilizing agents for better solubility. With the advent of nanomaterials, there is no need for toxic solubilizing agents as nano-based formulations are viewed as a viable solution to the problems associated with administering poorly water-soluble drugs. Because of these reasons, nano-based formulations of many chemotherapies have been approved and more are in clinical development. Among all noted drugs is Abraxane (nab-paclitaxel, Celgene), which is a formulation of paclitaxel bound to albumin nanomaterials. The drug, Abraxane was approved by the FDA in the year 2005 for previously treated metastatic breast cancer and has since been granted indications for other cancers. Abraxane is more tolerable than conventional paclitaxel.

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## 11.4 Approved Nano-Based Formulations/Drugs

Over the past 25 years, approximately 50 nano-based formulations have received FDA approval and they are currently being used by patients (Ventola 2017; Centerwatch n.d.; Food and Drug Administration 2017). Most of these nano-based formulations are typically dispensed orally or intravenously, and occasionally by transdermal route. Among different types of nanomaterials, polymeric, liposomal, and nanocrystal nano-based formulations are mostly represented among approved nano-based formulations. Other types of nano-based formulations which have been used are micelles, metals, metal oxides, and other inorganic nanomaterials. These nano-based formulations/drugs have been approved for a variety of indications such as cancer. We have given a list of approved nano-based formulations/nanodrugs in Table 1 (Caster et al. 2017; Havel et al. 2016).

Most of the nano-based formulations or nanodrugs have shown a significant decrease in toxicity, while there is not much improvement as far as therapeutic improvement is concerned because many nano-based formulations and nanodrugs have not survived clinical development. However, nano-based formulations that are under clinical trials show exciting results about increased efficacy.

## 11.5 Nanodrugs under Developmental Stage

Every year many new nano-based formulations/drugs enter clinical phase of the drugs approval process and there are also many nano-based formulations and nanodrugs in the very early stages of development; details are unknown because this information is proprietary. Since 2007, the number of nano-based formulations and nanodrugs that have received (IND) investigational new drug approval from the US-FDA has steadily increased and during the year 2013–2015, there was the highest number of nano-based formulations that have entered clinical trials (Ventola 2017; ClinicalTrials.govn.d.). Among different diseases or conditions where nano-based formulations or nanodrugs have been used or applied are cancer and microbial conditions. Though, there are also many nano-based formulations being developed for other indications, such as autoimmune, anesthesia, metabolic disorders, ophthalmic conditions, neurological and psychiatric diseases, and others.

Most of the nano-based formulations belong to liposomes and polymers nano-materials, whereas few of them to micelles and dendrimers. However, nano-based formulations are less prevalent in investigational nano-based formulations/nanodrugs than approved nanodrugs. Interestingly, many new micelles, liposome, and protein-based nanoformulations still use synthetic polymer components.

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## 11.6 Major Approved and Investigational Nano-Based Formulations/Drugs

Over the past 25 years, several attempts have been taken to synthesize the clinical grade nanomaterials. We have listed all the nanomaterials synthesized in Table 1 along with nanomaterials type and their indications and benefits.

### 11.6.1 Liposomal Nanomaterials

Liposomes are the most frequently used nanomaterials in nano-based formulations due to their many unique properties. The special feature of liposome is its spherical shape which is basically composed of a lipid bilayer membrane. The use of liposomes was first described in the year 1965 and size usually ranges between 90 and 150 nm in diameter, and consequently is sometimes somewhat larger than conventional nanomaterials. It has been reported that liposomes can carry and deliver both hydrophilic and hydrophobic drug molecules at the targeted sites with great precision. These liposomes are also commonly created to carry different biomolecules such as monoclonal antibodies, antigens that are conjugated to their surfaces of the liposomes. Another striking advantage of the liposomes with compared with non-liposomal drugs, as they can circulate in the bloodstream for a prolonged duration of time, and thus providing a longer therapeutic effect. Moreover, liposomes can be easily accumulated at the site of a tumor or infection and help to effectively deliver the drug molecules. It is also possible to synthesize liposomes along with different

fatty-acids so that they can be temperature or pH-sensitive for effective drug delivery under diverse conditions.

The use of liposomal nano-based formulations for drug delivery has significantly impacted pharmacology. The use of nano-based formulations of existing drugs with low bioavailability or high toxicity has benefitted from the stability and improved biodistribution that liposomes provide. When delivered intravenously, traditional liposomes have short circulating half-lives in the bloodstream due to swift clearance. This is because the lipid bilayer structure of the liposome is identified by the immune system and cleared from circulation by macrophages. Though, in liposomal nano-based formulation, this clearance has been minimized by attachment of polyethylene glycol chains to a molecule of the liposome surface. For example, compared with free doxorubicin drug, pegylated liposomal doxorubicin has been reported to result in 4 to 16-fold enhancement of drug levels in cancer tumors. In addition, many approved liposomal nano-based formulations rely on passive targeting to get better drug distribution in the tissues. However, liposomes can be easily synthesized and can be linked with different targeting ligands to create new targeted combinations for drug delivery (Gabizon et al. 1994).

There are other methods to use liposomes for effective drug delivery and one such method is called encapsulation where drug can be encapsulated inside liposomes. The co-encapsulation of drugs in the nanomaterials can provide a novel means of drug delivery. More specifically, nanomaterials can be prepared to deliver drugs consecutively and at dose inside the tumor microenvironment, allowing for maximal synergy that is not possible with conventional drug delivery methods. For example, Vyxeos (a liposomal formulation) for the treatment of acute myeloid leukemia (AML) co-delivers cytarabine and daunorubicin and which demonstrated improved efficacy in phase 2 clinical trials, compared with a standard cytarabine and daunorubicin regimen (Ventola 2017; Lancet et al. 2014; Cortes et al. 2015). In 2017, the US-FDA has approved Vyxeos drug for AML treatment.

Recently, many nanodrugs incorporating liposomes have been approved for the treatments of cancer, and fungal infections and liposomes were the first nano-based drugs to be granted investigational drug status by the FDA for phase 1 clinical trials. Over the past 25 years, large number of nano-based formulations using liposomal delivery have been approved or are being investigated. In addition, there are many liposomal nano-based formulations of drugs under clinical investigation, for example, Arikayce (Insmed, Inc.) is an inhaled liposomal formulation of “amikacin” for the treatment of serious chronic lung infections. The drug Arikayce has significantly improved the drug half-life compared with conventionally formulated amikacin. This drug has successfully completed clinical phase 1–3 trials in patients suffering from chronic lung infections.

Another drug, Lipoquin (Aradigm Corp.) is a liposomal formulation of ciprofloxacin that permits prolonged drug release. Pulmaquin (Aradigm Corp.) combines liposomal and aqueous-phase ciprofloxacin to alter the amount of drug that undergoes delayed and rapid release. Both drugs have completed phase 2 trials in patients with either cystic fibrosis (CF) or non-CF bronchiectasis. Moreover, mitomycin C is a useful drug which has been approved drug for the treatment of anal squamous cell

carcinoma. Another drug, a pegylated liposomal formulation of a mitomycin C pro-drug, Promitil (LipoMedix Pharmaceutical, Inc.) is being investigated in preclinical studies, whereas a phase 1 study is expected to complete enrollment soon in advanced solid tumors (Ventola 2017; Clancy et al. 2013). Two novel liposomal nanoformulations of doxorubicin are being studied in clinical trials. The drug HER2-targeted MM-302 (Merrimack Pharmaceuticals, Inc.) is expected to improve efficacy compared to untargeted liposomal doxorubicin in patients with HER2-positive breast cancer.

There are also many advanced clinical trials being conducted with nanodrugs that incorporate complex targeted liposomal nanomaterials. Thermodox (Celsion Corp.) contains liposome-bound doxorubicin drug with thermally sensitive lipids that can be easily degraded when exposed to high heat and thus finally release the drug by disrupting the lipid bilayer. The coupling of this nanodrug with radiofrequency thermal ablation allows the drug to be released in a site-specific manner at the tumor. Several clinical trials are using combination drug therapy approach where Thermodox is combined with radiofrequency ablation in the treatment of hepatobiliary tumors. The use of nanoformulation of liposomal insulin in Hepatocyte-directed vesicular (HDV) is prolonged delivery of the drug directly to the liver. Several phase 1 and 2 clinical trials have demonstrated minor improvement in the peripheral glucose control with subcutaneous HDV compared with regular insulin (Clancy et al. 2013; Geho et al. 2009, 2014). In addition, an oral treatment of HDV insulin is under clinical trial stage. It has been found that HDV insulin can simultaneously improve the mechanism of action, pharmacokinetics, and routes of administration of a drug.

### 11.6.2 Polymer Nanomaterials

Polymer nanomaterials are widely used in nanomedical research because they can be easily synthesized in large amounts and secondly due to their high efficacy and good safety profiles. The polymers can be natural, synthetic, or pseudo-synthetic status and PEG is the most well-established polymer. These polymer nanomaterials can be engineered in a wide range of types and sizes ranging from 10 nm to 100 nm. They can range in size from a single polymer chain which is used directly as a therapeutic agent or diagnostic agent. Another striking feature of polymers is that these polymers can facilitate drug release for weeks without accumulating in the body. Consequently, polymeric nanomaterials are considered favorable carriers for numerous medications, including treatments for cancer, cardiovascular disease, and other diseases. In addition to therapeutic benefits, these polymers can also be used as a contrasting agent when conjugated to the surface of polymer nanomaterials, allowing them to be used in diagnostic imaging. These biodegradable polymers can be fully metabolized and can be easily removed from the body. For example, PLGA is a biodegradable polymer because relative proportions of polylactic acid (PLA) and polyglycolic acid can be used to improve the biodegradability of PLGA. There are many benefits for polymer-based nanoformulations especially for controlled release of the drug and better circulation of drug inside the body.

Over the past few years, many polymer-based nanodrugs have been approved by FDA and they were among the 10 best-selling drugs in the USA in the year 2013. For example, the polymeric drug Copaxone (Teva Pharmaceuticals) was approved in the year 1996 for the treatment of multiple sclerosis (MS) and another drug Neulasta (pegfilgrastim, Amgen) was approved in 2002 for chemotherapy-induced neutropenia, respectively. Moreover, additional pegylated biologic drugs have also been approved more recently. The nano-formulated drug Plegridy (peginterferon beta-1a, Biogen) was approved in the year 2014 for the treatment of relapsing forms of MS. In this nanodrug, pegylation of interferon gamma beta-1a improved drug half-life and exposure, compared with interferon alone (Ventola 2017; Hu et al. 2012). Because of a longer half-life, Plegridy can be administered once every two to four weeks, compared with other MS treatments that often need to be administered daily.

The nanodrug Adynovate (Baxalta U.S., Inc.) was approved in 2015 for bleeding prophylaxis and the treatment of acute bleeding in hemophilia A. This drug can be administered less frequently (compared to non-pegylated formulations of factor VIII) because of an increased half-life due to its pegylated nanoformulation; this may reduce anti-factor VIII antibody generation that reduces drug efficacy. Another nanodrug, Rebinyn (Novo Nordisk) was approved in 2017 for the treatment and control of bleeding episodes, and perioperative bleeding management in patients with hemophilia B. Glycopegulation increases the circulating half-life of recombinant factor IX, which allows for less frequent intravenous dosing and lower bleeding frequency. The nanodrug, Zilretta (Flexion Therapeutics) was approved in 2017 for the treatment of osteoarthritis knee pain and this drug is formulated as a suspension of microspheres within which small crystals of triamcinolone acetonide are embedded in a PLGA copolymer matrix (Ventola 2017; Flexion Therapeutics, Inc n.d.).

There are many polymer-containing nanodrugs which are under different phases of clinical trials are being used to improve passive tumor targeting by increasing the size of a drug. The Opaxio (Cell Therapeutic, Inc) is a nanodrug that contains poly-glutamic acid-conjugated (polioglumex) paclitaxel were not that much effective in the treatment of non-small-cell lung cancer (NSCLS) but was effective in the treatment of ovarian and fallopian tube cancers. The drug Opaxio is investigated as a maintenance therapy for ovarian cancer patients and has been reported to achieve a complete response after platinum and taxane therapy. Another nanodrug, NKTR-102 (Nektar Therapeutics) is a pegylated etirinotecan drug is under testing phase of 3 clinical trials. It has been found that extended exposure of tumor cells to this topoisomerase I inhibitor showed enhanced therapeutic response, which can be ascribed to the longer circulation of pegylated etirinotecan (Verschraegen et al. 2009; Morgan et al. 2008; Sabbatini et al. 2008; Awada et al. 2013).

Moreover, paclitaxel conjugated with poliglumex is also being tested in clinical trials for use as a radiosensitizer. In the case of chemotherapy treatment with 5-fluorouracil and cisplatin which is frequently given to sensitize tumor cells to radiation treatment but unfortunately, with this treatment, normal healthy tissues are also exposed to the toxic effects of radiation. Interestingly, nanoformulations can

potentially improve chemoradiotherapy treatment through tumor-specific delivery of the drugs, which increases efficacy while decreasing toxicity in normal tissues. The nanodrug Opaxio is also considered as a potential radiosensitizer which can be combined with drug (cisplatin) for the treatment of esophageal cancer and with drug (temozolomide) for advanced stage gliomas. In 2012, the FDA granted the orphan drug status to Opaxio for the treatment of glioblastoma (Ventola 2017; Dipetrillo et al. 2006, 2012; Jeyapalan et al. 2014).

The nanodrug, camptothecin (topoisomerase I inhibitor) possess potent anti-neoplastic activity, but its clinical use is rare because of significant systemic side effects. A nanoformulation of camptothecin can improve the safety profile of this drug. The drug CRLX101, is conjugated with camptothecin and a cyclodextran-PEG polymer, has been tested in phase 1 and 2 clinical trials in the treatment of lung cancers, gynecological malignancies, and solid tumors. In addition, clinical trials of drug (CRLX101) in the patients suffering from renal cell carcinoma and gastrointestinal cancers have been completed (Weiss et al. 2013). Interestingly, CRLX101 has shown promising results in clinical phase of testing and a polymer conjugate of docetaxel named (CRLX301) is also being studied in a phase 1 and 2 of clinical trial in patients with advanced solid tumors.

Moreover, polymer nanomaterials with antibacterial properties are also being investigated in the treatment of active infections. Quaternary ammonium polyethyleneimine-based polymers have potent activity that makes them particularly promising. Their highly charged nature can disrupt bacterial membranes in several gram-positive and gram-negative bacteria (Ventola 2017; Ortega et al. 2015). Polymeric formulations of doxycycline have also been developed to improve the treatment of chronic periodontitis (Ventola 2017; Valle et al. 2011). These nanoformulations have demonstrated a more sustained release and improved efficacy compared with free drug in treating this condition. Polymeric doxycycline is also being studied in phase 2 trials examining the potential benefits of the use of this nanodrug following mechanical debridement.

Two polymeric nanoformulations of antiretroviral agents were investigated in HIV patients. The drug (Efavirenz) which is a nonnucleoside reverse transcriptase inhibitor is often used as a first choice of treatment for HIV infections. Another drug (Lopinavir), a protease inhibitor, is commonly used in combination therapy in HIV patients. The nanodrugs (NANOefavirenz and NANOlopinavir) are used as antiretroviral agents that were produced with the specific aim to reduce the total dosage while maintaining clinical efficacy, thereby improving drug tolerability and reducing treatment costs. Interestingly, preclinical studies have demonstrated bioequivalent efficacy in suppressing HIV-1 HIV-IIIB and subtype A viruses.

### **11.6.3 Micelles Nanomaterials**

Micelles are self-assembling polymeric amphiphile nanomaterials that can be customized for the slow and controlled release of hydrophobic drugs. The composition and structure of micellar nanomaterials can be modified to achieve different particle

size, drug loading, and drug release characteristics. The micelles possess hydrophobic internal core, which can be exploited to encapsulate drugs that have poor aqueous solubility. Yet, the exterior surface of a micelle has enough polarity to allow dissolution in aqueous solutions.

The nanodrug Estrasorb (Novavax, Inc.) is an FDA approved based on the micellar formulation that is used for symptoms associated with menopause. It has been found that transdermal delivery of estradiol avoids first-pass metabolism and leads to stable serum levels for 8–14 days and this route of delivery also avoids gastrointestinal side effects. The nanodrug Paical (Oasmia Pharmaceutical) a micellar formulation of paclitaxel encapsulated in a proprietary retinoid compound (XR-17) is used in the treatment of ovarian cancer. Paical received the approval based on preclinical data that suggested it was less toxic than Kolliphor-based paclitaxel. Because of the broad applicability of micellar-based nanoformulations, new products are expected soon.

There are other micellar nanoformulations which are being used in the cancer treatments under different phases of clinical trials. The drugs oxaliplatin and cisplatin are frequently used platinum chemotherapies that have well-known dose-limiting nephrotoxicities and neurotoxicities. The nanoplatin (NanoCarrier Co., Ltd.) is a micellar formulation of cisplatin investigated in several phase 1 and 2 clinical trials examining its use alone and in combination with other chemotherapies. A micellar nanoformulation of SN-38 (an active metabolite of the topoisomerase inhibitor irinotecan) is also tested. Two phase 1 trials have been completed, as well as phase 2 trials in solid tumors, and triple negative breast cancer. The drugs Genexol-PM (Samyang Biopharm) is micellar formulation of paclitaxel that is being developed as an alternative to Kolliphor-based paclitaxel and this drug has been approved for the treatment of metastatic breast cancer and advanced lung cancer in South Korea. Several phase 1, 2 trials completed in patients with metastatic breast cancer have demonstrated low toxicity rates and favorable overall response rates ranging from 40% to 68% (Ventola 2017; Kim et al. 2004, 2007; Lee et al. 2008; Ahn et al. 2014).

#### 11.6.4 Nanocrystal Nanomaterials

The nanocrystals are versatile nanomaterials which can improve the pharmacokinetics and pharmacodynamic of some of the poorly soluble organic or inorganic materials by increasing their bioavailability and solubility (Ventola 2017; Bansal et al. 2012; Gao et al. 2013). In addition, nanocrystals hold a narrow, symmetric emission spectrum, tunable, and are photochemically stable (Bruchez et al. 1998) and they are composed of an optically active core surrounded by a shell that provides a physical barrier against the external environment, making them less sensitive to photo-oxidation changes.

The nanocrystal-based drugs are unique because they are composed entirely of drug compound. Furthermore, the increased surface area of nanomaterial enhanced dissolution speed and saturation solubility. The increase in saturation solubility increases the forces that drive diffusion-based mass transfer through the

gastrointestinal tract. However, the oral absorption mechanism for nanocrystal formulations is not fully understood, and their behavior after subcutaneous injection is not fully predictable. Solubility issues for many drug compounds have been solved through conversion into nanocrystals. The nanodrug (Rapamune), which is the first milled organic nanocrystal drug is approved by FDA in the year 2000. The active ingredient of this drug is sirolimus (a bacterial-derived macrocyclic immunosuppressant) used to prevent rejection after transplantation of an organ. The nanodrug, Rapamune is a nanocrystal-based formulation provides poorly soluble sirolimus with a continuous extended-release profile.

The Elan Nanosystems (now known as Alkermes) has proven to be useful for the preparation of other types of formulations such as oral suspensions, tablets, and intramuscular injections. Several drugs have been approved by FDA after Rapamune, which used the milling technique to produce several nanocrystal drug formulations, such as Tricor (fenofibrate, AbbVie) and Emend (aprepitant, Merck), respectively. This milling approach is found to be a potential solution for the wide range of solubility issues that occur with an estimated 70–90% of drug formulations. Whereas inorganic nanocrystal formulations which were approved by the FDA have limited option as nanocrystal forms of hydroxyapatite and calcium phosphate are used bone-graft substitutes.

The nanodrug, Matinas BioPharma has developed two lipid nanocrystal formulations as anti-microbial agents. The nanodrug (MAT2203) is an antifungal amphotericin B drug undergoing phase 2 trials in patients with chronic candidiasis who are intolerant to standard non-intravenous treatments. MAT2501 is a lipid nanocrystal formulation of amikacin. Traditional formulations of amikacin are linked with neurotoxicity and require careful monitoring. The targeted delivery of amikacin to designated areas by MAT2501 results in a reduction in total dose and an improved safety profile. The nanodrug, Matinas BioPharma is waiting for an IND designation from the FDA prior to commencing phase 1 trials with MAT2501.

### 11.6.5 Inorganic NPs

Many inorganic materials, such as metal oxide, metal, or silica, can be used to create NPs. Metal and metal oxide NPs are being investigated intensely for therapeutic and imaging applications. Iron oxide NPs have been studied in numerous clinical trials as contrasting enhancement reagents for magnetic resonance imaging (MRI). However, most of the FDA-approved iron oxide nanodrugs are indicated as iron substitution therapies. Some of these drugs Venofer (American Regent, Inc.), Ferrlecit (Sanofi-Aventis U.S.), Infed (Actavis Pharma), and Dexferrum (American Regent, Inc.) are all used in the treatment of anemia linked with chronic kidney disease (CKD). These nanoformulations contain an iron oxide core, coated with hydrophilic polymers (e.g., dextran, sucrose) that allow the iron to dissolve slowly after intravenous injection. The application of this formulation allows large doses to be dispensed rapidly without an increase in free iron levels in the blood and avoiding toxicity.

The superparamagnetic iron oxide nanoparticles (SPIONs) have low toxicity, remain in circulation for a long time, and are usually biodegradable. SPIONs particularly iron oxide and magnetite have long been used as non-targeted contrast agents for MRI. They also respond strongly when exposed to a magnetic field and therefore can be “functionalized” to target specific tumors. Hence, SPIONs are frequently used in the development of targeted MRI contrast agents and in drug delivery systems. Three SPION drug formulations Feraheme (ferumoxytol, AMAG Pharmaceuticals), Feridex, and GastroMARK have received FDA approval; however, the latter two have been withdrawn from the market. The drug (Feraheme) which is indicated for the treatment of anemia associated with CKD is still available in the market.

SPIONs form the energy that they release in a magnetic field, which can be used as hyperthermia agents for tumor diagnosis. Several SPIONS have been synthesized and many of them under different stages of preclinical and early clinical trials for a hyperthermia treatment against tumors. The nanodrug Nanotherm (MagForce AG) uses aminosilane-coated SPIONS for local hyperthermia treatment of glioblastoma tumors. After injection of Nanotherm drug directly into the tumors, an alternating magnetic field is applied to selectively heat the particles which caused the local heating of the tumor microenvironment to 40–45° C and finally caused the programmed and nonprogrammed cell death. During clinical trials, it has been found that treatment of Nanotherm in glioblastoma tumors demonstrated an OS increase of up to 12 months and this drug is presently waiting for FDA approval (Ventola 2017; Maier-Hauff et al. 2011).

Another metallic nanomaterial, gold nanoparticle (Au NPs) is showing a promising nanomaterial as anti-neoplastic agents. It has been found that AuNPs possess unique combination of thermal and optical properties and their size, shape, and/or surface chemistry can also modify for better activities. Moreover, the excitement of electrons in the AuNPs by electromagnetic radiation can generate a substantial amount of energy which makes them most suitable for cancer treatment. Moreover, colloidal AuNPs which are smaller than 5 nm are found to be excellent radiosensitizers for tumor diagnosis and therapy. In addition, AuNPs have also been used for the drug delivery vector for the extremely toxic antitumor agent tumor necrosis factor alpha (TNF $\alpha$ ), which can cause profound cardiovascular compromise. The AuNPs formulated with TNF $\alpha$  demonstrated decreased toxicity in preclinical studies, however, has low retention capability in the body, hence they were of little clinical value. However, a nanoformulation of AuNPs conjugated with PEG was found to significantly decrease clearance rates. The drug, Aurimune (CytImmune) is a recombinant human TNF attached to AuNPs by using a PEG linker that also acts as a biocompatible antifouling layer. In phase 1 of clinical study, Aurimune was shown to be well tolerated in patients with advanced cancer (Ventola 2017; Libutti et al. 2010). The PEG layer was also determined to decrease uptake by the mono-nuclear phagocyte system, which helped in the accumulation of drug in tumor. Till date, the FDA has not approved any AuNP-based drugs. Another metal such as silver is also known to be potent anti-microbials and these metal ions can easily penetrate bacterial cells and induce toxic effects.

The “Cornell dots” are inorganic silica NPs which are produced by Cornell University as a diagnostic and therapeutic nanomaterial in the treatment of cancer. This Cornell dots were designed for lymph-node mapping in cancer patients and have also been found to use in the treatment of cancer tumors after in the animals. These dots are composed of an internal silica core labeled with a near-infrared fluorescent dye, a targeting moiety, and an antifouling polymer layer. This design has created an NP that is more stable and 20–30 times brighter than a conventional solution of the constituent dye.<sup>1</sup> A recent “first in human” trial ( $N = 5$ ) demonstrated a favorable PK/distribution and safety profile when used as a tumor imaging agent, allowing investigation in additional trials with humans in the near future (Ventola 2017; Fleischman 2016).

### 11.6.6 Protein Nanomaterials

Protein-based nanodrugs are the active therapeutic drug for the treatment. These protein-based nanomaterials are used in blood serum, which allow the smooth transport and dissolution of drugs during circulation. It has been found that natural proteins can be combined with conventional drugs in these agents to reduce toxicity. Over the past few years, albumin has gained attention as a drug carrier, leading to the investigation of numerous albumin-based nanoformulation in clinical trials. Like other nanoformulation, albumin particles alter the pharmacokinetics of a free drug, increasing passive accumulation in solid tumors via the EPR effect. In addition, after the protein nanoformulation dissociates into individual drug-loaded albumin molecules, cellular uptake mechanisms mediated by albumin-receptors are enabled (Ventola 2017; Fleischman 2016; Weissig et al. 2015).

The drug “Abraxane” which incorporates 130-nm albumin nanoformulation conjugated with paclitaxel is an early model of an albumin-based nanodrug (Ventola 2017; Weissig et al. 2015). This drug is approved in 2005 and was designed to eliminate the toxic solvent Kolliphor, which was necessary to solubilize paclitaxel (Desai et al. 2006). Albumin-linked paclitaxel nanoformulation improved infusion time and eliminated the need to concomitantly administer antihistamines and dexamethasone to avoid an immune reaction to Kolliphor. In addition to improving toxicity, Abraxane improved drug PK and efficacy compared to treatment with a conventional formulation of paclitaxel. After the success of Abraxane, several additional albumin-bound nanoformulations have entered clinical trials for the purpose of improving the efficacy and safety of these drugs (NAB-docetaxel, NAB-heat shock protein inhibitor, and NAB-rapamycin) (Fuentes et al. 2015).

After the successful approval of nanoformulation drug “Abraxane” attempts have made to use the unmodified proteins to engineered particle complexes for active targeting. The nanoformulation drug “Ontak” (Eisai, Inc.) was approved in 2008, which is the best example of an engineered fusion protein that combines targeting proteins with cytotoxic molecules. This drug is basically an interleukin (IL)-2 receptor antagonist that was initially designed to treat an aggressive form of

non-Hodgkin's peripheral T-cell lymphomas (PTCL) by targeting the cytoidal action of diphtheria toxin toward cells that overexpress the IL-2 receptor on T cells. In clinical trials, combination therapy with Ontak and cyclophosphamide or doxorubicin or vincristine or prednisone (CHOP), the first-line chemotherapy for PTCL, achieved an OS of 63.3%, compared with an OS of 32–35% with CHOP alone. Ontak was also not observed to be myelosuppressive, nor was it associated with significant organ toxicity (Ventola 2017; Fuentes et al. 2015). Ontak, representing the first actively targeted proteinaceous NP, may be effective for a range of hematological malignancies, many of which overexpress IL-2 (Foss 2006). RSV-F (Novavax) is a protein-based NP containing a respiratory syncytial virus (RSV) fusion protein that was developed to treat RSV in infants. Passive immunization enhances maternal RSV antibody transfer, offering a viable vaccination option. Preclinical data have shown that RSV-F and palivizumab (Synagis, MedImmune) demonstrate potential clinical significance when given in combination (Glenn et al. 2015). A phase 2 trial is assessing the safety and immunogenicity of different formulations of RSV-F NPs in healthy women of childbearing age.

### 11.6.7 Dendrimer Nanomaterials

Dendrimers are expected to be one of the more useful nanodrug platforms to date. They are composed of iterative monomers arranged concentrically around a central core (Svenson and Tomalia 2005). This configuration allows pharmacologically active substances to be encased within the interior cavity or to be connected to the NP surface. Precise control of many important NP properties is possible when forming dendrimers, including shape, size, charge, surface properties, and composition. Dendrimers can also consist of functional subunits that act as delivery vehicles or drugs. There is a wealth of information in the preclinical literature to support the use of dendrimer platforms in nanodrugs. Dendrimers have been successfully used for nanodrug formulations administered via many routes, including cutaneous, intravenously, orally, rectally, and vaginally. Many dendrimer-based drug compounds are expected to enter early phase clinical trials within the next few years. The many possible routes of administration for dendrimer-based nanodrugs make it likely that some of these agents will receive FDA approval.

A dendrimer-based cancer treatment, DTXSPL8783, is being investigated in clinical trials; a phase 1 study of this agent is underway in patients with advanced cancer. A dendrimeric antiviral/antibiotic compound, Vivagel (Starpharma), is in phase 3 clinical trials for bacterial vaginosis (BV). This unique nanodrug incorporates naphthalene disulfate groups on the surface of dendrimers. Phase 2 data have indicated high rates of clinical and pathologic cure of BV, as evidenced by symptomatic improvement and clear laboratory results, respectively. However, phase 3 data have been equivocal, with high rates of symptomatic improvement but lower rates of clinical laboratory cure being observed. Vivagel has also exhibited potent *in vitro* activity against HIV and herpes simplex virus. Phase 1 studies have

indicated that vaginal use of this nanoformulation is well tolerated and that antiviral activity is retained by cervicovaginal fluids in most patients up to 24 hours after administration (O'Loughlin et al. 2010; Price et al. 2011; Cohen et al. 2011).

## 11.7 Summary

During the past decade, nanodrug development has advanced significantly and over 50 different types of nanoformulations have been successfully approved for clinical applications. There are more than 100 nanoformulations currently under testing phases and many more are in early stages of development or in clinical trials. Most of the currently approved nanodrugs/nanoformulations are based on the conventional drugs that are approved and are composed of simple nanoformulations. However, nanodrug platforms are incorporating a broadening range of NP types and becoming more complex.<sup>1</sup> Even though few nanodrugs that are in the early stages of development will ultimately receive regulatory approval, the amount of work that is occurring in this field predicts that many new nanodrugs will eventually be available for clinical use. There are many challenges which nanodrugs or nanoformulations are facing such as toxicity, and long-term implications on human genetics need to be properly studied before claiming nanodrugs or nanoformulations are safe for human use.

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# NANOMATERIALS: Global Research Publication, Research Quality, and Patent Trends

12

Ayman Akil and Walid Hassan

## Abstract

Understanding the dynamics of research activities in nanomaterials for human health research area is a key to devise an informed strategy for researchers, decision-makers in addition to various players in the broader research community. This goes beyond researchers involved directly in such research activities as governments, funders, and specific industrial firms need also to allocate hot areas and centers of excellence within the nanomaterial for human health research area in order to act and devise plans accordingly. This chapter offers a comprehensive and thorough diagnosis of the research publications' output and identifies major countries, universities, and other stakeholders. In addition to this, an overview of the patenting activities inside the nanomaterial for human health research area is introduced. Having covered research articles and patents, this chapter serves as a good starting point that feeds into an optimized approach of this research area. The growing research output in the last 10 years demonstrates again the relevancy and importance of this research areas and urges for even more investment and funding of scientific projects that undertake topics of nanomaterials with human health approaches.

## Keywords

Nanomaterials · Publications · Patents · Research quality · Research impact · Citations

A. Akil · W. Hassan (✉)  
Clarivate Analytics, Dubai, United Arab Emirates  
e-mail: [Walid.Hassan@clarivate.com](mailto:Walid.Hassan@clarivate.com)

## 12.1 Introduction

In the course of this chapter, we explore the current state-of-the-art universal nanomaterials for human health research landscape. The significance of such a study stems from the very significance of the much-needed clear global informed strategy. It is generally accepted that the spike in the investments of research resources in a certain topic is an indicator of the importance and relevance of this topic. This rising interest could translate into similar trend in research output or impact. Having access to the current indexed nanomaterials for human health research output is an advantage if to explore the opportunities and challenges standing altogether in this research landscape. This is the first step to provide the decision-makers with smart tools to make informed decision on where and how much they should allocate resources or in which direction to grow. Moreover, this will equip the decision-makers in this context with actionable information rather than broad and irrelevant data.

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## 12.2 Methodology

In the course of this chapter, data on the global nanomaterials for human health research was extracted and analyzed in the web interface of *Clarivate Analytics*, formerly the IP and science business of Thomson Reuters, *Web of Science* (WOS) database.

We used Web of Science in order to analyze the research output during the last 10 years (2010–2019). Web of Science is a publisher-independent global citation database, founded by the inventor of the world's first citation index Dr Eugene Garfield. Web of Science is a multidisciplinary platform that connects regional, specialty, data, and patent indexes. The comprehensive platform allows you to track ideas across disciplines and time from over 1.7 billion cited references from over 159 million records, in addition to this, the curated collection of Web of Science Core Collection contains over 21,100 peer-reviewed scholarly journals published worldwide (including Open Access journals) in over 250 sciences, social sciences, and arts and humanities disciplines. Conference proceedings and book data are also available.

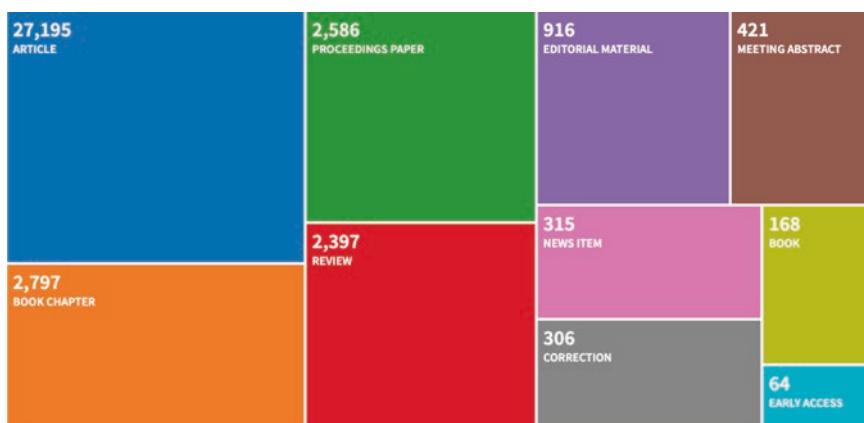
In 1960, Eugene Garfield established the Institution for Scientific Information (ISI). Later, ISI was acquired by Thomson Reuters and was merged in 2016 into Clarivate Analytics. Garfield founded the print edition of *Science Citation Index* (SCI) in 1964 and started the *Social Sciences Citation Index* (SSCI) and *Arts and Humanities Citation Index* (A&HCI) in 1973 and 1978, respectively. WOS is considered one of the remarkable science citation search discovery. It is widely used across academic libraries and research institutions as a search engine, benchmark tool, and as an analytical platform as well. The curated WOS database provides an objective and comprehensive overview of the research performance of entities, this serves as a typical starting point if to construct a remarkable national, regional, or international presence in the scientific community.

The usage of this tool in scientific search and analytical information extraction is widely accepted among the scientific Scientometrics community and has been a common practice of multitude of research and review scientific publications (Li 2018). The

platform enables a descriptive demonstration of the papers addressing certain topic across countries and research entities. In addition to research papers, *Derwent Innovation* (Clarivate Analytics 2018) was used to explore the global trend in filing phosphate-related patents across more than 90 patent office worldwide. These later tasks could be performed largely due to the WOS database which includes billions of citation connections, metadata fields, in addition to thousands of items updated practically daily. The application of the bibliometric data can highly help the research community working at subjects related to phosphate at better understanding the dynamics of the key players in various research areas connected to the research area lifecycle.

The indexed content in the core collection of WOS includes published items in more than 21,000 journals published worldwide (including Open Access journals) in over 250 sciences, social sciences, and arts and humanities disciplines in addition to the conference proceedings and book data, this offers a unique opportunity to scout the activities of the global research community in a certain research area or geographic entity. The conducted analysis was based on the records metadata, considered to be intellectual or geographical indicators, captured by WOS. Concerning the geographical and institutional information, we relied on the addresses mentioned in the bibliographic metadata of the publications. There are five major methods used broadly in bibliometric studies: citation, co-citation, bibliographic coupling, co-author, and co-word (Zupic 2015). Such approaches deal with the quantitative aspect of the scientific publications and the last method used in the bibliometric studies mentioned above was used in the course of our study.

We limited our search for the Web of Science “Nanoscience Nanotechnology” category for the time period 2010–2019 and this yielded more than 415,000 research and scientific indexed record. As we are interested in the field of Nanomaterials for the human health, then we limited the search to only to subcategories of Nanoscience-Nanotechnology related to human health and we got around 34,000 records. Figure 12.1 shows that the records’ types range from articles (around 81%), to book chapters (around 8%), conference proceedings (7.5%) in addition to reviews, editorial materials, etc.



**Fig. 12.1** Documents' types of the human health-related Nanomaterials records indexed in Web of Science (2010–2019)

## 12.3 Key Countries in Nanomaterials for Human Health

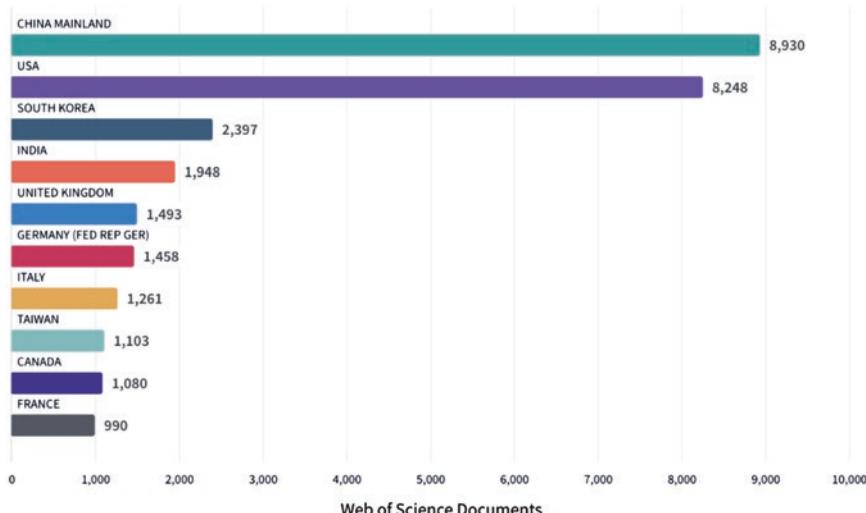
### 12.3.1 Research Volume

Moving to the key players in Nanomaterials for human health, Fig. 12.2 shows the top ten nations in terms of the number of research records indexed in Web of Science and related to nanoscience for human health. The data clearly shows that China is leading followed by the USA, and their research output in this research category constitutes around 60% of the rest of the top 10 list.

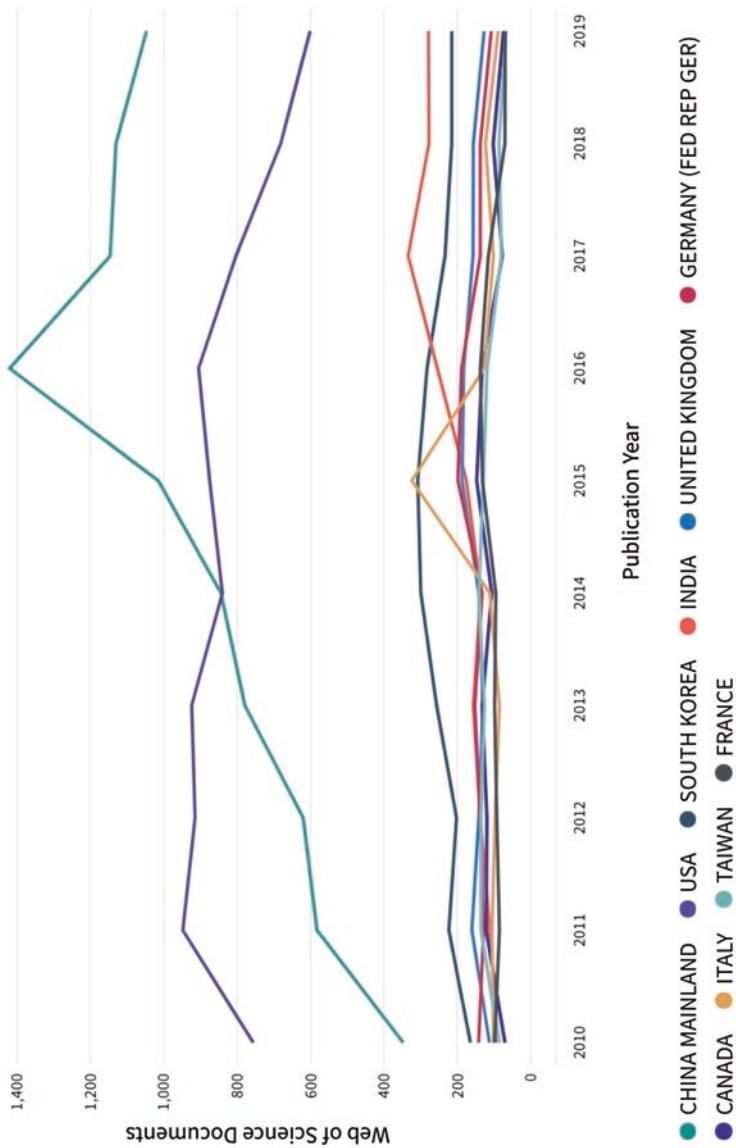
Figure 12.3 shows the dynamics of research output of the top ten nations in nanomaterials for human health. It shows that the USA output was roughly stable from 2010 until 2016 when it started to decline slowly. On the other hand, China surpassed the USA as the world largest research publishes in this area in 2014 and since then it is globally leading.

### 12.3.2 Research Quality

Trying to get into the quality of those research papers, we adopted one of the quality indicators highly adopted throughout the research community which is the category normalized citation index (CNCI). According to InCites (2018), the impact (CNCI) of a research document is “calculated by dividing the actual count of citing items by the expected citation rate for documents with the same document type, year of



**Fig. 12.2** Top ten nations in terms of the number of research records indexed in Web of Science and related to Nanoscience for human health (2010–2019)

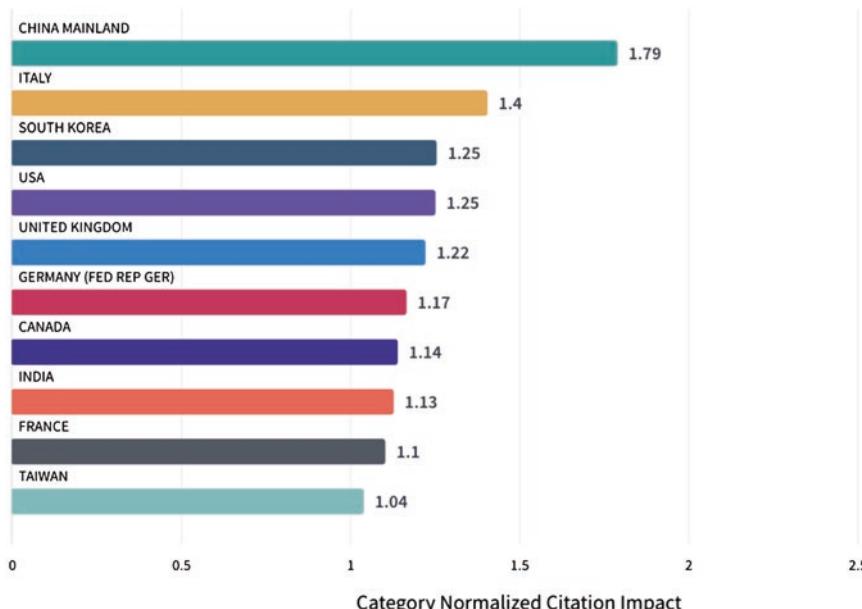


**Fig. 12.3** Dynamics of the research output of the top ten nations in terms of research papers in nanomaterials for human health category (2010–2019)

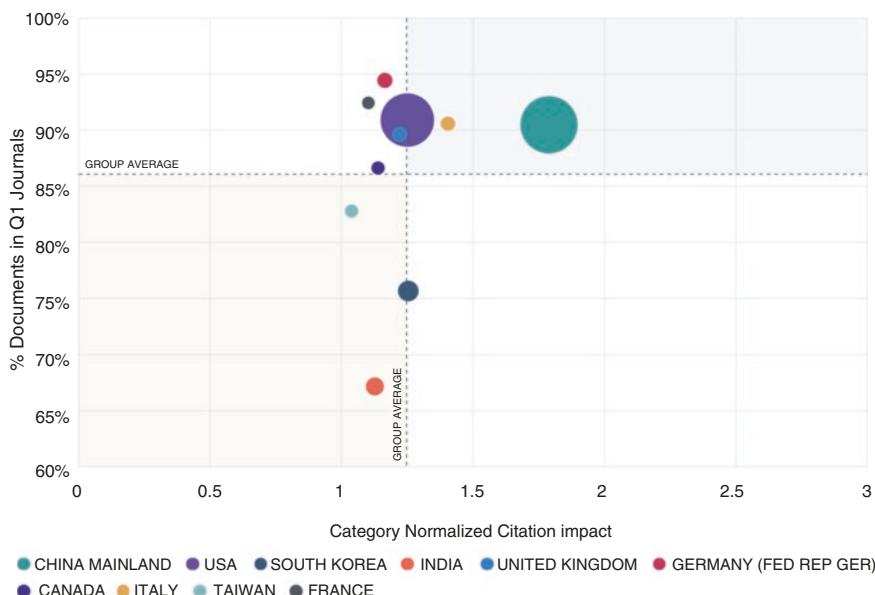
publication and subject area. When a document is assigned to more than one subject area an average of the ratios of the actual to expected citations is used. The CNCI of a set of documents, for example, the collected works of an individual, institution, or country/region, is the average of the CNCI values for all the documents in the set.” Figure 12.4 shows the research impact (CNCI) of the research output publishes by the top ten nations in terms of the number of research records indexed in Web of Science and related to Nanoscience for human health.

In order to better understand the quality of the research output of the top ten nations in terms of research volume in nanomaterials for human health, we introduced % Q1 quality indicator. % Q1 is the percentage of papers published in the first quartile journals indexed in Science Citation Index Expanded (SCIE) or Social Science Citation Index (SSCI), the journals in both databases, SCIE and SSCI, are ranked based on their Journal Impact Factor and the top 25% are the Q1 journals. Figure 12.5 shows the above-mentioned top ten nations against 2 quality indicators simultaneously, CNCI and % Q1 journals.

While China and the USA share almost the same % documents published in Q1 journals, it is worth noticing that the research output of China far exceeds that of the USA in category normalized citation index.



**Fig. 12.4** Research impact (CNCI) of the research output publishes by the top ten nations in terms of the number of research records related to Nanoscience for human health



**Fig. 12.5** Plot of the top ten nations in research output in nanomaterials for human health against two quality indicators simultaneously, CNCI and % Q1 journals. The circle area represents the volume of research of the given country (2010–2019)

## 12.4 Key Research Organizations and Academic Bodies

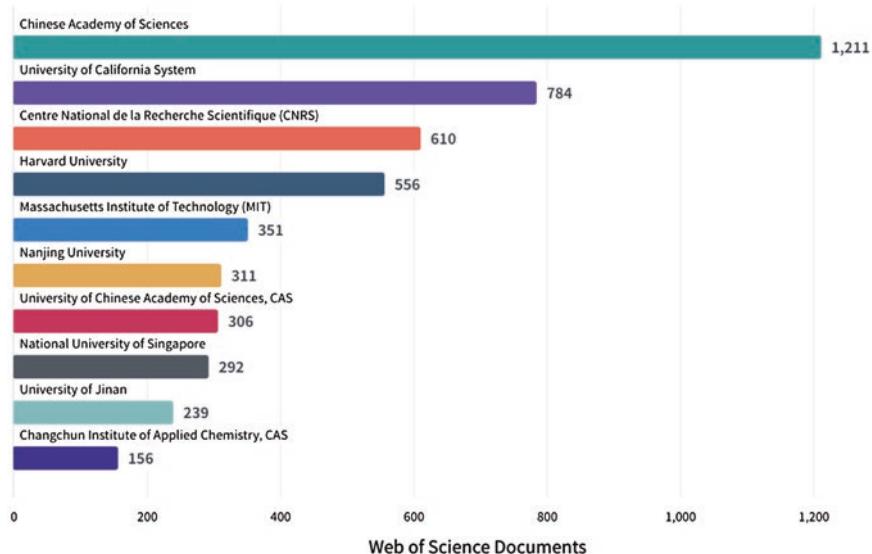
### 12.4.1 Research Volume

Delving into the key organization that is highly contributing to the research volume in nanomaterials for human health, Fig. 12.6 shows that the “Chinese Academy of Sciences” followed by “University of California System” are leading globally in terms of their Web of Science indexed publications in this particular research area.

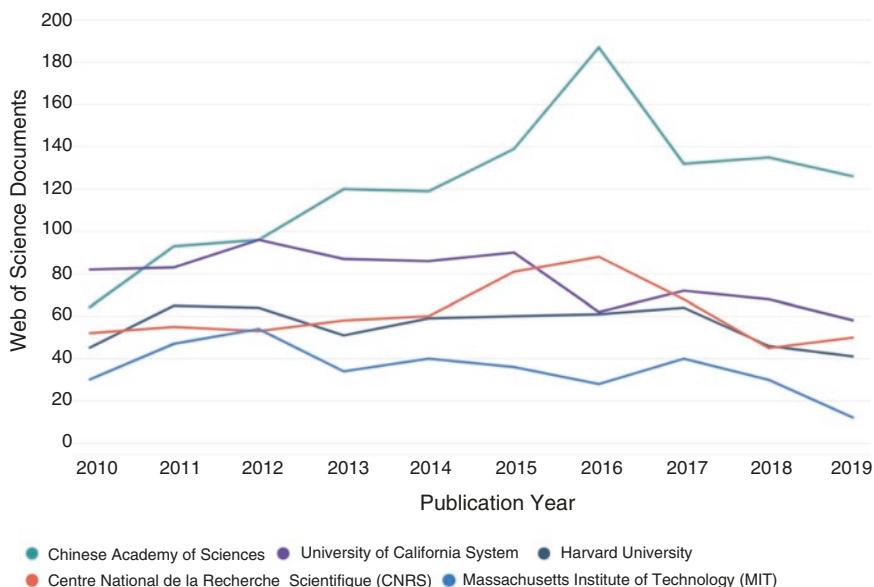
Figure 12.7 shows the trend of the research output of the top 5 organizations in the world in terms of nanomaterials for human health research output. It is worth mentioning that the “Chinese Academy of Sciences” outperformed the “University of California System” as the world’s top research publications publishes in nanomaterials for health sciences in 2012 and since then it is leading worldwide.

In addition to this, one notices an overall slowdown in the annual number of publications of the top 5 organizations since 2016. This negative trend needs data to better understand these dynamics though it is already an alerting signal for nanomaterials research community.

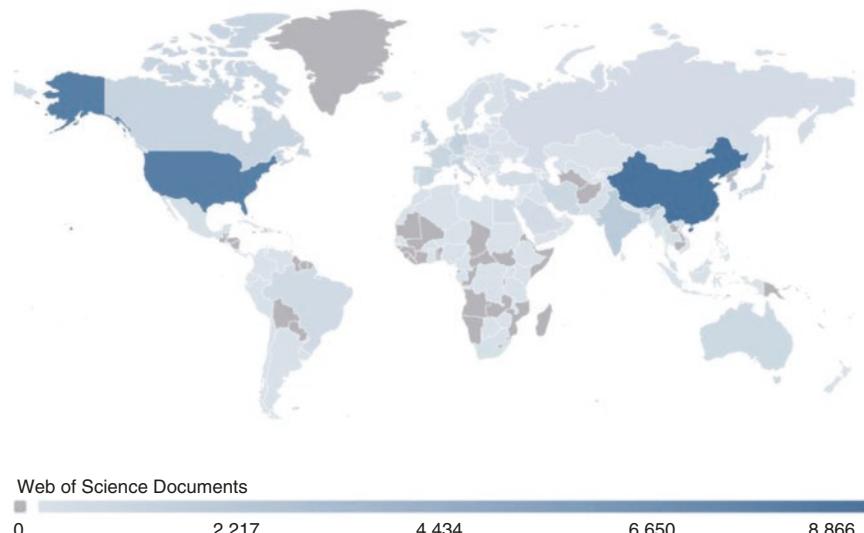
In order to have a broader look at the key organizations in terms of the number of publications in nanomaterials for human health, Fig. 12.8 shows the geographic distribution of these organizations. Clearly, we see a dense research output coming out from China and the USA.



**Fig. 12.6** Top 10 organizations in terms of number of publications in nanomaterials for human health (2010–2019)



**Fig. 12.7** Research output trend of the top 5 organizations in the world in terms of nanomaterials for human health research output (2010–2019)



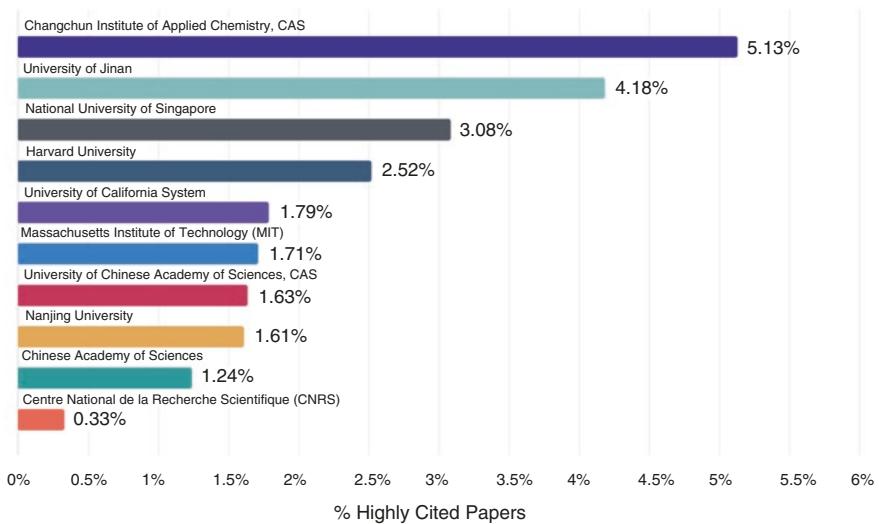
**Fig. 12.8** Geographic distribution of organizations contributing to the number of publications in nanomaterials for human health (2010–2019)

### 12.4.2 Research Quality

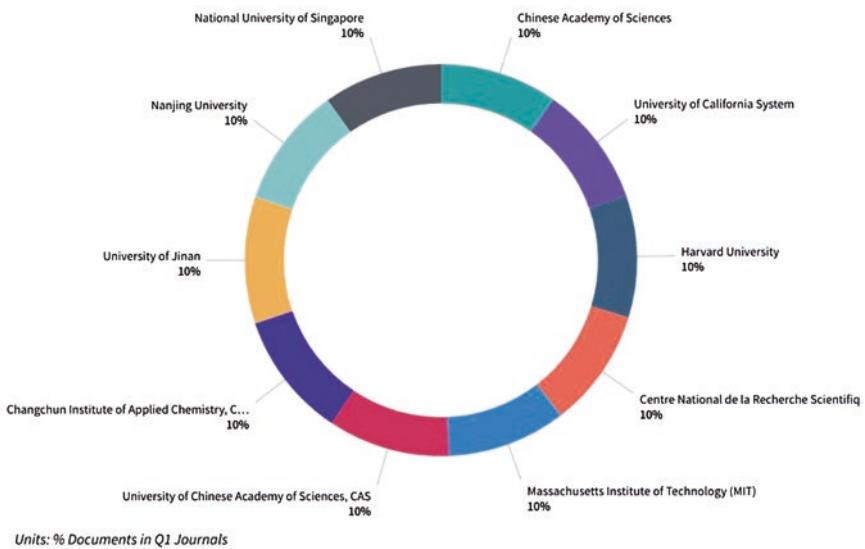
Moving to the quality indicators, the research community looks at “Highly Cited Papers” as a key impact and quality indicator. According to C. Analytics, “The Highly Cited Papers indicator shows the volume of papers that are classified as highly cited in Essential Science Indicators (ESI). ESI is a separate service also hosted on the InCites platform and should not be confused with the subject scheme of the same name. Highly cited papers are the top one percent in each of the 22 ESI subject areas per year. They are based on the most recent 10 years of publications. Highly Cited Papers are considered to be indicators of scientific excellence and top performance and can be used to benchmark research performance against field baselines worldwide. Although Highly Cited Papers are synonymous with % Documents in the Top 1% in InCites, they are not identical because of differences in subject scheme, time period, and document type.”

Figure 12.9 shows that the top 2 organizations in terms of percentage of highly cited papers come from China followed by “National University of Singapore.” This is a clear indication of a strong and impactful Asian presence in this research area.

Moving to another quality indicator, Fig. 12.10 shows the % publications in Quartile 1 (Q1) journals of the top 10 organizations in terms of number of nanomaterials for human health research publications, the journals being ranked according to their Journal Impact Factor (JIF). Historically, librarians used to evaluate Scientific journals for almost 75 years, and Gross and Gross (1927) wrote a study on the citations pattern in the early 1920s. JIF “is a measure of the frequency with which the average article in a journal has been cited in a particular



**Fig. 12.9** Top 10 organizations in terms of the % of Highly Cited Papers in nanomaterials for human health research publications (2010–2019)



**Fig. 12.10** % publications in Quartile 1 (Q1) journals of the top 10 organizations in terms of number of nanomaterials for human health research publications (2010–2019)

year or period. The annual *JCR* impact factor is a ratio between citations and recent citable items published. Thus, the impact factor of a journal is calculated by dividing the number of current year citations to the source items published in that journal during the previous two years.”

In order to have a better understanding of the quality of publications of the top 5 organizations in terms of number of nanomaterials for human health publications, Fig. 12.11 depicts 2 quality indicators, i.e. Category Normalized Citation Impact and Highly Cited Papers, in addition to the volume of publications in nanomaterials for human health. There is almost a linear relation which signals a consistency in the quality indicators.

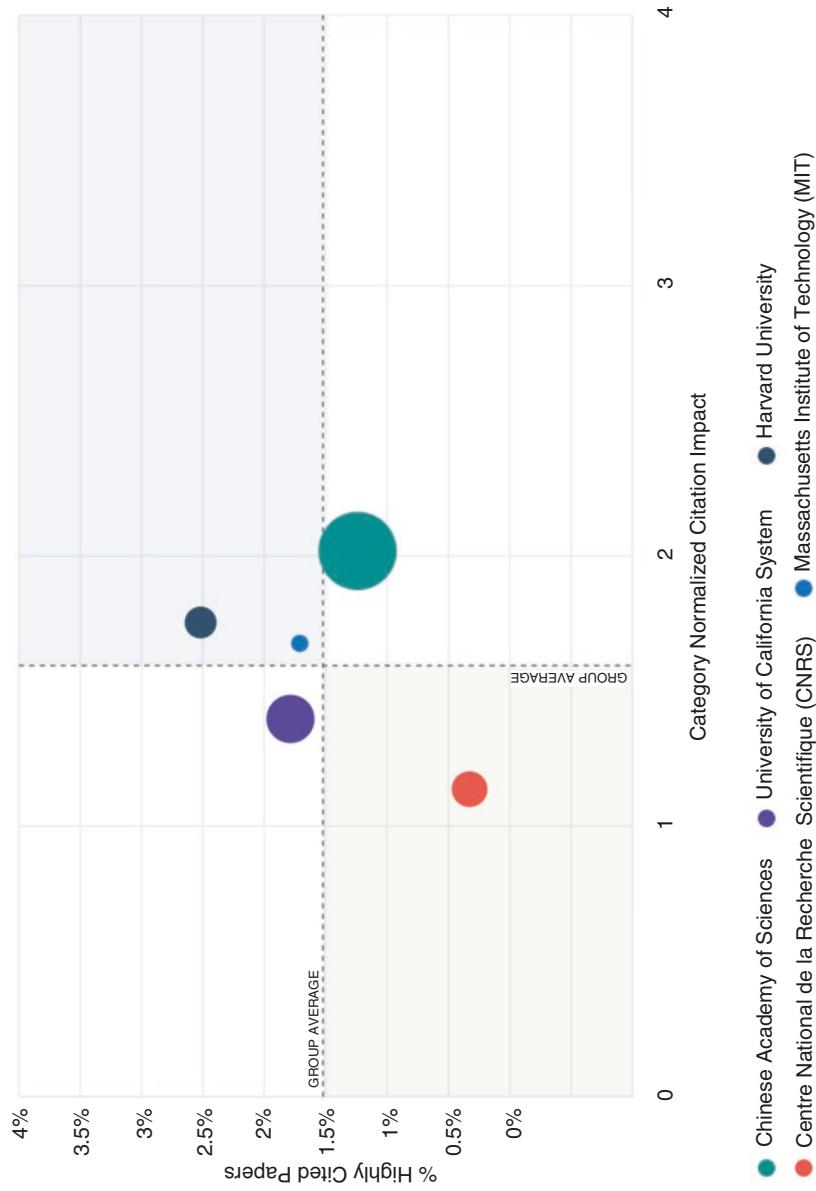
## 12.5 Organizational Research Collaborations

A common knowledge of the global research community is that research collaborations and partnerships improve the researchers’ performance and enable them to better achieve their research objectives (Confraria et al. 2020). We started by showing the international collaborations of the research organizations in nanomaterials for human health and then we shifted to a broader research area that is Nanotechnology (Nanoscience and Nanotechnology in Web of Science research categories). Figure 12.12 shows the percentage of international collaborations in the publications of the top 5 organizations in terms of number nanomaterials for human health research area. Clearly, Massachusetts Institute of Technology (MIT) and Harvard University are leading followed by Centre National de la Recherche Scientifique (CNRS). This hints to a great international networking in these research areas for the named research organizations.

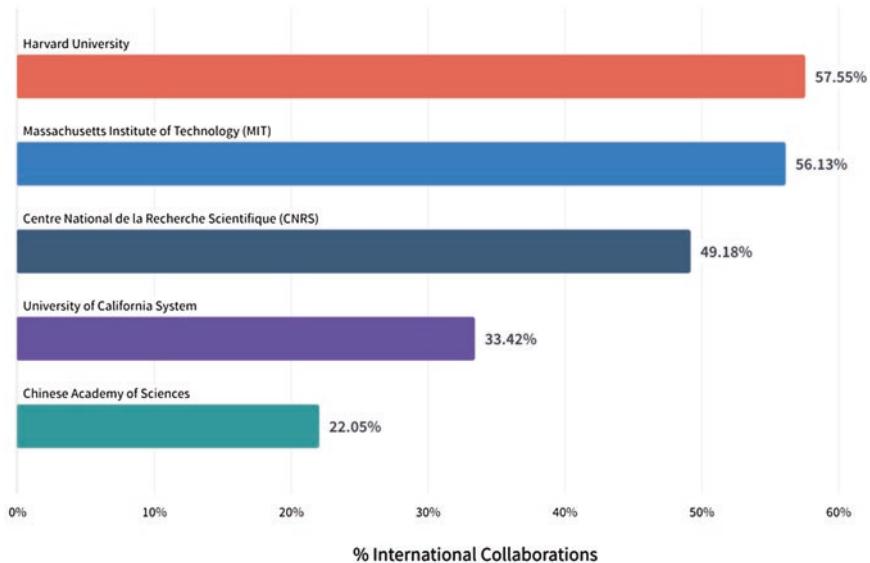
The research community has long ago looked at the collaboration between academia and industry as a positive sign that signals a research that can be commercialized and hence funded. Figure 12.13 shows the collaboration of the top 5 organizations in terms of number of nanomaterials for human health research with industrial organizations. Clearly, Harvard University is leading with 2.16% of its publications in the nanomaterials for human health research area being done in collaboration with industrial organization. This is quite an impressive portfolio for an academic body, and it hints at an advanced and go-to-market research done at Harvard University.

Shifting to the broader research area, Fig. 12.14 shows the percentage of international collaboration in indexed publications for the top 5 universities and research organizations in terms of number of publications in the Nanoscience and Nanotechnology research area.

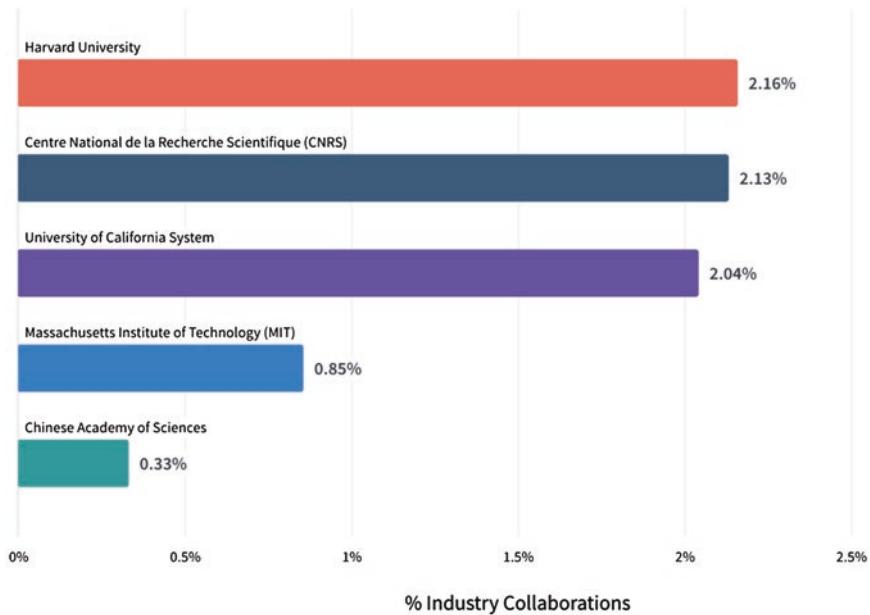
It is worth mentioning that the percentage of international collaboration of the top 5 looks very similar in the case of nanomaterials for human health research area and Nanoscience and Nanotechnology research area as well. Shifting to the percentage of collaboration with industry of the top 5 universities and research organizations in terms of number of publications in the Nanoscience and Nanotechnology research area, Fig. 12.15 shows a different trend as compared to Fig. 12.12. It is



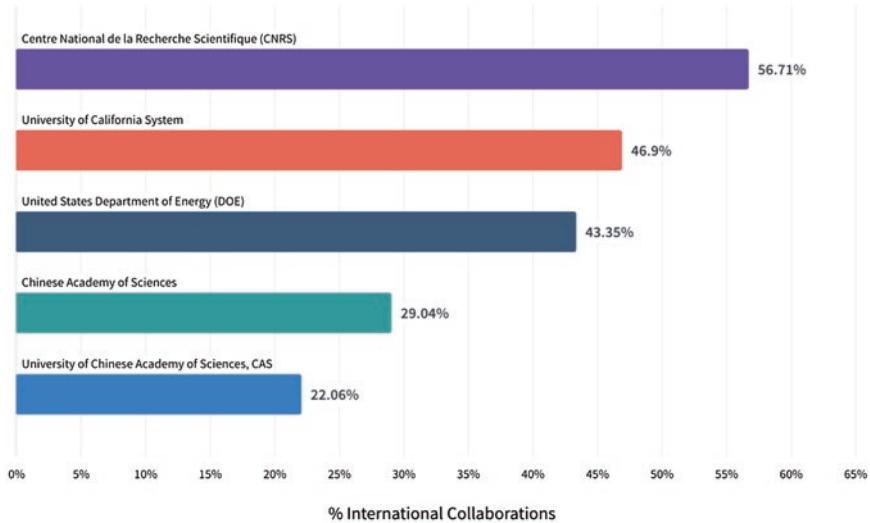
**Fig. 12.11** Depicts two quality indicators, i.e. Category Normalized Citation Index and Highly Cited Papers of the top 5 organization in terms of volume of nanomaterials for human health research. The size of the dot reflects the volume of publications (2010–2019)



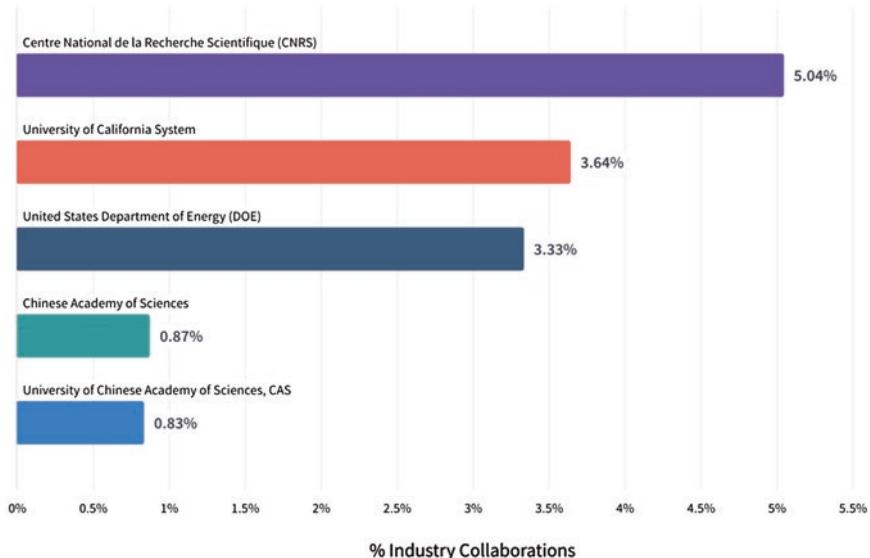
**Fig. 12.12** Percentage of international collaborations of the top 5 organizations in terms of number nanomaterials for human health publications (2010–2019)



**Fig. 12.13** Percentage of industrial collaboration of the top 5 organization in terms of number of nanomaterials for human health research (2010–2019)



**Fig. 12.14** Percentage of international collaboration of the top 5 organizations in terms of number of publications in Nanoscience and Nanotechnology research area (2010–2019)



**Fig. 12.15** Percentage of industrial collaboration of the top 5 organization in terms of number of publications in Nanoscience and Nanotechnology (2010–2019)

clear that when it comes to collaborations with industry, top productive universities and research organizations in Nanoscience and Nanotechnology tend to collaborate excessively with industry as compared to their peers in the nanomaterials for life sciences research area with the top publishing research entity in the first case publishes more than twice industry-collaborated publications as compared to the top publishes organization in the second case.

The relation between international and industry collaboration of the top 5 universities and research organizations in terms of research output volume is better depicted in Fig. 12.16, where we see a linear proportional relationship in the case of Nanoscience and Nanotechnology research area, while such linear relationship in the case of Nanomaterials for human health is absent.

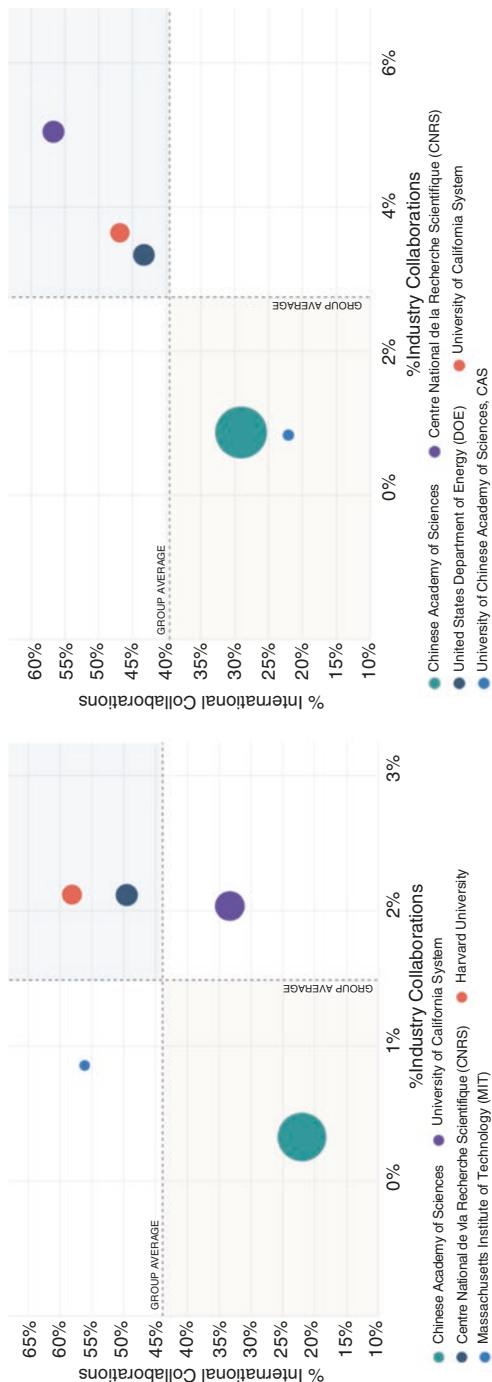
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## 12.6 Patents Landscape

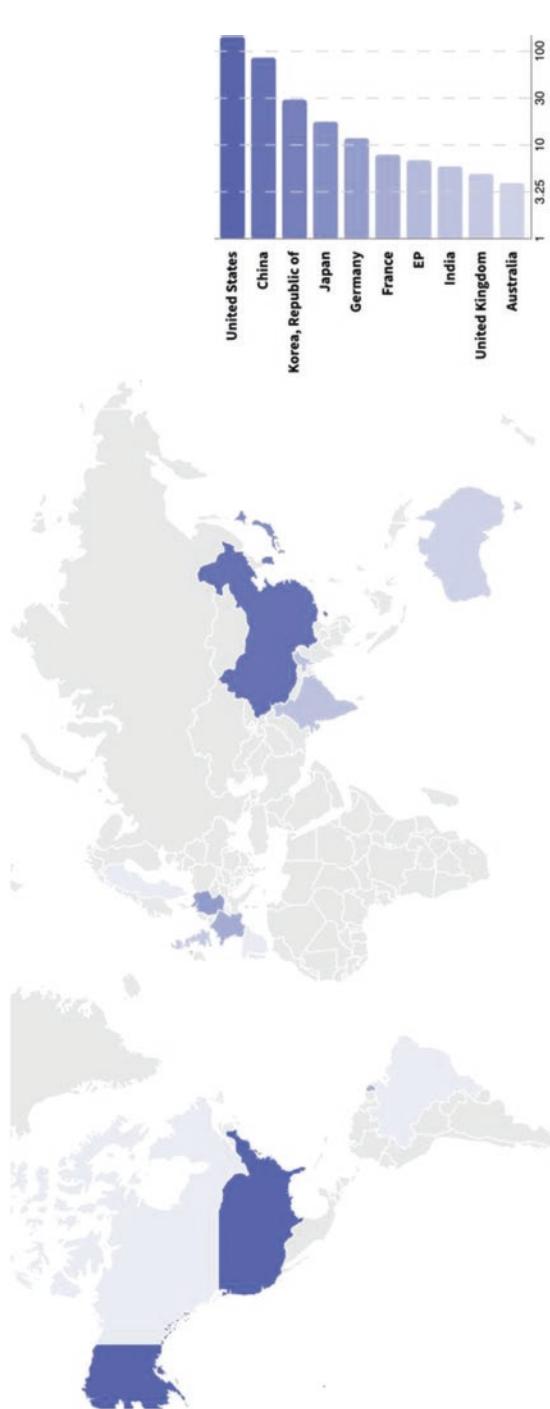
Moving from research publications, we used DI—Derwent Innovation (D. Innovation 2018) database in order to analyze the patenting activities in the field of nanomaterials for human health. Using patent and non-patent data sources, including the Derwent World Patents Index (DWPI) and Web of Science, enables us to have a comprehensive approach to the topic. In order to have accurate analysis counting inventions rather than individual patent documents, we have used Derwent Innovation that structures the patents into patent families based on the single innovation. The search retrieved 387 DWPIU family, Fig. 12.17 shows the geographic distribution on inventions that fit in the category nanomaterials for human health, working with DI we identified the latter category as the set of DWPI patents' families that fit into nanoscience with approaches to medical and health sciences. The USA is leading in terms followed by China and Republic of South Korea, respectively. It is worth mentioning that 53% of worldwide filings in these results are granted, which indicates protection for active (Alive) patents in the relevant markets. On the other hand, 47% of this result set is pending applications. It is known that higher percentages of applications point to a new or growing market, whereas lower application rates can point to already established markets or low growth areas.

In order to further investigate the stakeholders of patenting activities in the realm of the nanomaterials for human health technology, Fig. 12.18 shows the key players applying for patents in this category. It is clear that University of California and Texas University are leading with 8 patents each. Clearly, majority of the patents are still alive which hints into an active field of technology where inventors are still interested in investing in it.

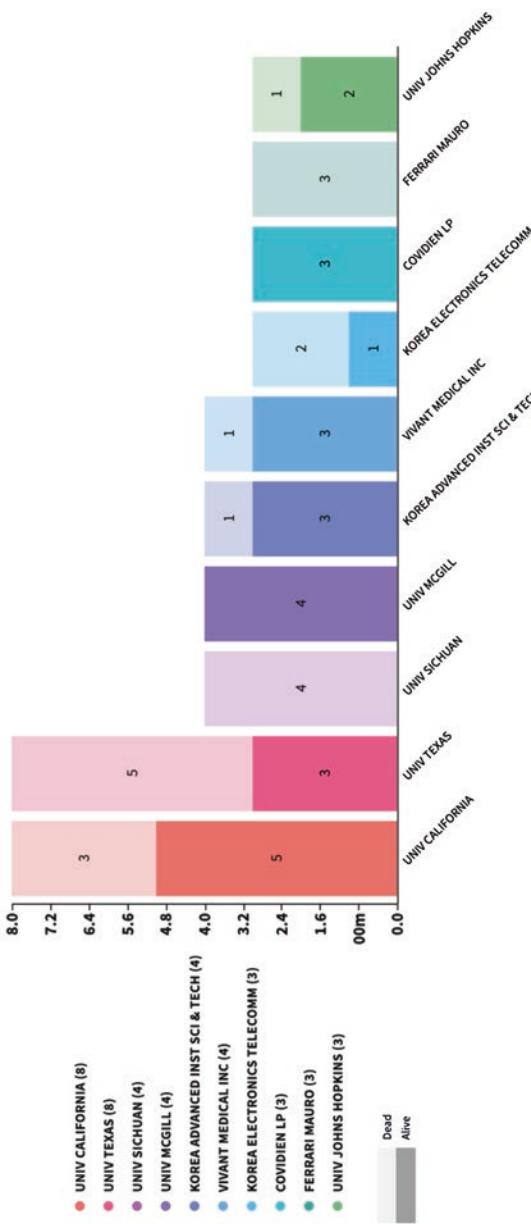
In order to have a better understanding of the patenting in this technology, we allocated the top technology niche in the broader area of nanomaterials for human health technologies which is labeled by DI as “SURGICAL, PATIENT, IMAGING, MEDICAL, ULTRASOUND, TISSUE.” Figure 12.19 shows the patenting trend in this niche technology. We see a clear growing which is a sign of growing interest in this area.



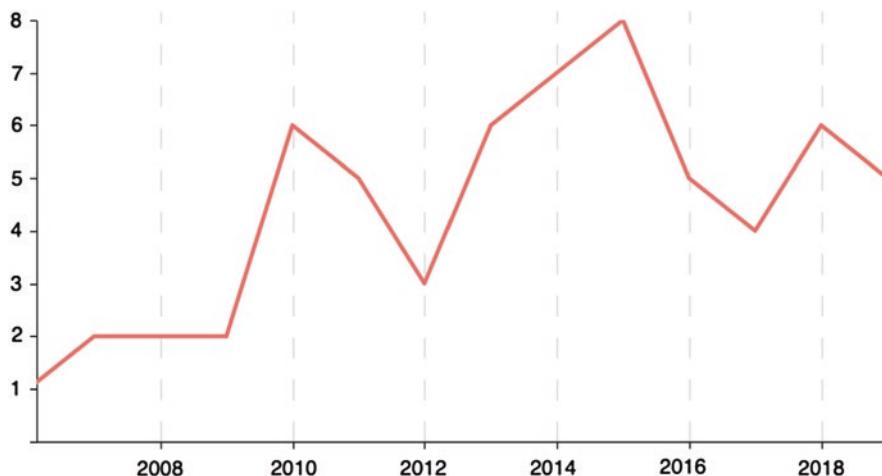
**Fig. 12.16** Relation between % international and % industry collaboration of the top 5 universities and research organizations in terms of research output volume in Nanomaterials for human health (left) and Nanoscience and Nanotechnology (left). The size of the points reflects the number of publications of the respective entity (2010–2019)



**Fig.12.17** Geographic distribution of the origin of technology development of patents' applications that fit in the category of nanomaterials for human health



**Fig. 12.18** Key players applying for patents in the nanomaterials for human health category (2010–2019)



**Fig. 12.19** Patenting trend in the top niche technology area of nanomaterials for human health technologies which is labeled by DI as “SURGICAL, PATIENT, IMAGING, MEDICAL, ULTRASOUND, TISSUE” (2006–2019)

## 12.7 Summary

Understanding the main factors influencing the performance of researchers and research institutions in a specific research area is imperative to build and fine-tune an informed research growth strategy. To address these issues, a systematic study that captures and analyzes the nanomaterials research dedicated for human health published articles was conducted, in addition to this, a breakdown of the volume of filled patents is introduced on the level of corporates and countries as well. This analysis uncovers the up-to-date nanomaterials for human health research and benchmarks the output of prominent universities, research institutions, and research entities against global peers in terms of volume and impact. The publication data, as well as the patent data, is showing that this field is still of great interest to the research community. Trends extracted from patenting and publication dynamics hint to the future overall direction of technology which is promising in terms of growing investment in the area of nanomaterials for human health technologies.

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# Nanomaterials and Their Negative Effects on Human Health

13

Vijaya Ravinayagam and B. Rabindran Jermy

## Abstract

Mesostructured silica, dendrimers, and allotropes of carbon were exhaustively used in biomedical, cosmetics, semiconductors, and food industry applications. Considering the huge prospect of nanomaterials, their potential hazards on exposure to humans and their related ecotoxicological effects needs to be summarized. Nanoparticles with size below 100 nm could pass into the lung and then to blood through inhalation, ingestion, and skin contact. As nanotechnology innovation is expected to achieve \$ 2231 million by 2025, humans will be exposed ever increasingly in day-to-day life and in industries. In this review, the latest synthetic methodology of silica, dendrimers, and CNTs, their biological applications (in vitro and in vivo) related to toxicity were discussed. In terms of structured silica, the toxic and non-toxic effect induced by specific templates (cetylpyridinium bromide, cetyltrimethylammonium bromide, dipalmitoylphosphatidylcholine, C16L-tryptophan, C16-L-histidine, and C16-L-poline) that are used to generate mesoporous silica, silica nanoparticle sizes (25, 50, 60, 115, and 500 nm), and silane functionalization ( $\text{NH}_2$  and  $\text{COOH}$ ) were discussed. The recent applications of different generations (G3, G4, G5, and G6) of amphiphilic Janus dendrimers were discussed along with toxicity effect of different charged dendrimers (cationic and anionic) and effect of PEGylation. Recent synthesis, advantages, and disadvantages of carbon nanotubes (CNTs) were presented for structures like single walled carbon nanotubes (SWCNTs) and multiwalled

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V. Ravinayagam (✉)

Deanship of Scientific Research & Department of Nano Medicine Research, Institute for Research and Medical Consultation (IRMC), Imam Abdulrahman Bin Faisal University, Dammam, Saudi Arabia

e-mail: [vrnayagam@iau.edu.sa](mailto:vrnayagam@iau.edu.sa)

B. R. Jermy

Department of Nano Medicine Research, Institute for Research and Medical Consultation (IRMC), Imam Abdulrahman Bin Faisal University, Dammam, Saudi Arabia

carbon nanotubes (MWCNTs). The influence of diameter of SWCNTs (linear and short), thickness (thin and thick), effect of oxidation, metal oxide species ( $\text{TiO}_2$ , Fe, and Au), and biocompatible polymers (polyethylene glycol, bisphosphonate, and alendronate) were shown in relation to molecular pathways in animal cells.

### Keywords

Nanomaterials · Negative effects · Human health · Animal studies · in vitro studies

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## 13.1 Introduction

Nanoparticles applications have expanded from general catalytic applications to biomedical uses and food industry. Nanomedicine represents a set of interdisciplinary research at the interface between biology, physics, and chemistry. In the current scenario, nanomedicine research has been reported using target-oriented drug therapy. Research consideration has been given to develop nanotechnology based therapeutic tools for treating chronic diseases such as cancer, cardiovascular, and diabetics. Structured silica nanoplatforms (MCM-41, SBA-15, MCM-48), carbon nanotubes (single walled and multiwalled), polymers (Chitosan, liposomes, dendrimers) are most widely reported for theranostic applications. The nanoparticle utilization has evolved from the monotonous role of drug delivery system to multi-functional role of labeling, drug and gene transports, detection of pathogens and proteins, as RNA and DNA probe, optical imaging enhancer, tissue designing, biomolecules and cell isolation, and as tumor destructor. Though they are best available drug carriers, they are still marred by certain critical issues like toxicity at high dose level, passivation due to multiple inorganics, and low pH sensitive. In order to reduce the toxicity, several modifications like using biocompatible polymers are reported. The benefit over conventional drug supply includes high stability, solubility, slow metabolism, less excretion of drug, and specific target oriented (Hare et al. 2017). This type of cargos is reported to be effective for several recently developed biomolecules based drugs such as proteins, peptides, oligonucleotides that showed poor biocompatibility (Hoffmann et al. 2018).

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## 13.2 Silica Nanomaterial

Recently, the different types of nano-sized silicas such as graphitized/mesoporous carbon, MOFs and metal oxides are very attractive due to their ordered atomic layers, high surface area, ordered pore size distributions and therefore used in drug delivery and diagnostic applications. Silicalite, the silica form of ZSM-5 nanozeolite has been widely used as adsorbent in industrial relevant process. The structure of silicalite is composed of MFI three-dimensional structure with a diameter of

0.56 nm. The unique properties of such silicalite include (1) high crystallinity, (2) homogeneous structure with high degree of silanol domain, (3) high ability for surface functional groups mobilization, and (4) less structural defects. The surface area is contributed by both internal and external surface area with MFI pore system. The chosen silicalite has 3D porous network, which can provide extensive diffusional access to drug molecules. The other added advantage of such kind support involves the presence of high number of silanol groups relevant for surface functionalization and ability to modification to meso form using several templates such as CTAB, F127 to high surface area support (up to 1100 m<sup>2</sup>/g) with large pore volume (1 cc/g) and pore diameter (4 nm). Overall, the ability to expand the pore system using mesoporous templates such as CTAB, brij-56, F127, and P123 and create combination of high surface area and ordered pores with uniform pore size distributions makes them attractive for the applications in nanomedicine. In particular, the high adsorption and desorption capacity of external and internal surface hydroxyl groups are expected to be the perfect host (cargo)/drug carriers in drug delivery study.

The attention is usually related to the superior textural characteristics such as high surface area, tunable pore sizes, and high chemical stability. Conventional microporous zeolites (such as ZSM-5) were prepared through sol-gel technique using tetrapropyl ammonium templates. The pores range between 0.5 and 2 nm. The micropores are reported to be in narrow size distribution with slightly high specific surface area between 100 and 500 m<sup>2</sup>/g, and pore volume and adsorption capacity for gaseous or liquid adsorption. Silicalite, the silica form of ZSM-5 nanozeolite has been widely used as adsorbent in industrial relevant process. The chosen silicalite has 3D porous network, which can provide extensive diffusional access to drug molecules. The other added advantage of such kind support involves the presence of high number of silanol groups relevant for surface functionalization and ability to modification to meso form using several templates such as CTAB, F127 to high surface area support (up to 1100 m<sup>2</sup>/g) with large pore volume (1 cc/g) and pore diameter (4 nm).

Due to nanotechnology revelation (nano biomedical technology), there is a renewed interest in the therapeutic and diagnostic utilization of structured silicas. The application of diagnostic nanosilicas has been used as new transfecting agents (Tieu et al. 2019) and immunoassay (Banerjee and Jaiswal 2018). The role of diagnostic nanosilica drug carrier is to respond to external field and thereby assist bio-imaging, magnetic targeting agent to carry drug and delivery (Tiwari et al. 2018). For example, the magnetic nanosilicas have already been shown to have the potential to develop cancer-based drug on commercial basis. The material has shown positive success in animal study and in clinical trials (Rainone et al. 2017).

Cisplatin/hierarchical mesosilicalite nanoformulation (IAUM-56) has been developed. The basic nanocarrier has been derived from silicalite, a zeotype material. The micropores of silicalite were turned into hierarchical pores through template mediated dissolution technique (top-down approach) The formation of micro- and mesopores (hierarchy) was established through several characterization techniques such as X-ray diffraction, BET surface area analysis, and transmission electron microscope. The cytotoxic effect of the designed in-house nanoformulation

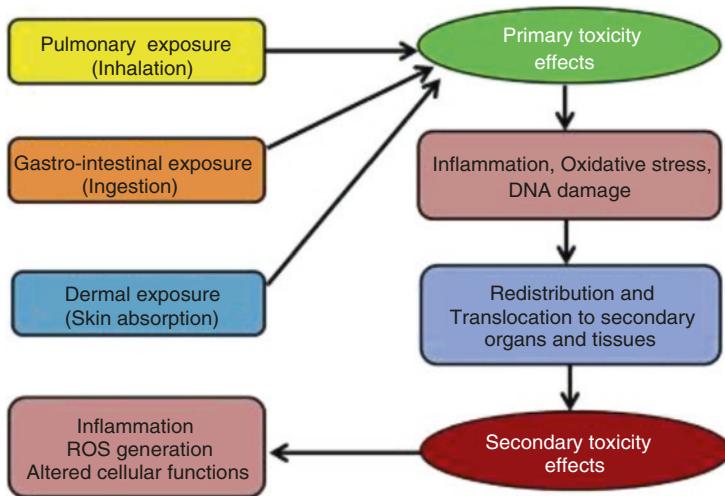
IAUM-56 against HeLa cells and MCF-7 cells exhibited a drastic reduction in cell proliferation. Drug cisplatin showed high toxicity against normal fibroblast cells, while IAUM-56 showed a lesser toxicity (Jermy et al. 2018). SPIONs/SBA-16 silica composite functionalized with silane and cisplatin has been reported (Jermy et al. 2019). The MTT cell viability assay shows that HeLa cells were most sensitive towards cisplatin bound with silane/SPIONs/SBA-16 nanoformulation. The LC<sub>50</sub> value shows that the formulation is 2.9 times more effective on HeLa cells compared to HCT 116 and HEK 293.

Multifunctional nanocomposite silica was reported involving natural antioxidant curcumin and polyethylene glycol. The textural features of mesoporous silica as analyzed by transmission electron microscope and dynamic light scattering techniques show monodispersed spherical silica with particle size of about 185 nm with polydispersity index value of 0.52. Zeta potential analysis of silica indicated positive surface charge of  $38.3 \pm 6.09$  mV. The loading of curcumin over silica reduced the surface charge positivity to  $36.2 \pm 3$  mV. With polyethylene glycol coating, further decrease in charge occurs to  $20.8 \pm 4.28$  mV. However, the presence of such negative potential increases the stability in between the particles. The release study of nanoformulation involving mesoporous silica, curcumin, and polyethylene was measured at tumor pH value of 5.5 and normal physiological pH condition of 7.4. The nanoformulation showed a controlled release, beginning with slow release of curcumin (<1%) until 192 h in normal pH of 7.4. In case of tumor pH 5.5, the polyethylene glycol disintegrates and followingly curcumin release was accelerated to 52%. The release efficiency was reciprocated in in vitro study, where the formulation exerted significant cytotoxicity against HeLa and HepG2 cells. The prime reason was attributed to the cell cycle arrest at G2/M (Elbaly et al. 2020).

### 13.2.1 Silica toxicity

The emergence of nano-revolution sparked a wave in interdisciplinary field of research from material to medicine. The market of nanotech and smart pill is expected to grow 125 billion US dollar and 650 million US dollar by the year 2025. Nevertheless, the health benefit of stable in humans remains an apprehensive and intimidating. In particular, the nanoparticle seems to affect the reproductive organ and lungs more severe followed by liver and skin (Elalfy et al. 2018). In case of silica nanoparticle (Fig. 13.1), the exposure route was reported to exert several kinds of toxicity (Shi et al. 2013; Missaoui et al. 2018; Inoue and Takano 2011).

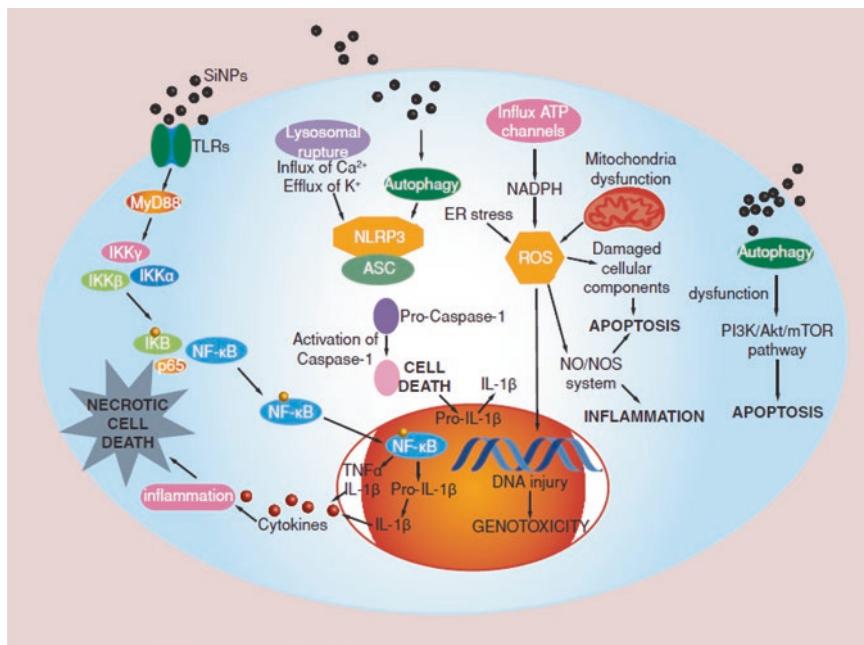
In cell regulation, protein kinase B (serine/threonine) plays an important role in cellular functions like survival of cells, multiplication, cellular moments, proinflammatory cytokines expressions (Szymonowicz et al. 2018). In molecular signaling pathway, Phosphoinositide 3-kinases (PI3Ks) enzyme is present in cell membrane. Tyrosine kinases are the cell surface receptors, it activates the PI3K and produces the phosphatidylinositol 3-phosphate (PI3P), which is a lipid mediator. In parallel, PI3P and Akt binding activate proteins lead to Akt activation by two phosphorylated amino acids. Various substrates were recognized by AKT. In cell cycle function,



**Fig. 13.1** Silica nanoparticle toxicity at different human body exposed sites (Shi et al. 2013; Missaoui et al. 2018; Inoue and Takano 2011)

proliferation and apoptosis occur with balance. Pro-apoptosis proteins (BAD, BAX) involved in the for Akt kinase activity as substrate (Koh et al. 2000). The biological effect of nanosized silica exhibited high internalization, elevates the inflammatory responses, initiates autophagy through PI3k/Akt/mTOR pathway (Duan et al. 2014). In addition, silica nanoparticle induced apoptotic pathway is reported to generate reactive oxygen species. This generated free radicals causes the stress and stimulates TNF  $\alpha$  and TNFR1 which leads to stimulation of FADD–procaspase-8–Caspase-8–BID resulted program cell death. The BCL-2 protein then shifts to mitochondria and activates the mitochondrial pathway related protein Caspase-3, -6, -7 leading to mitochondrial apoptosis (Asweto et al. 2017). Silica nanoparticle 30 (SNP30) induces reactive oxygen species production in KUP5 cells. P2X7 receptor is simulated by SNP30 and releases ATP. Increasing the reactive oxygen species simulates the inflammation pathway through caspase-1-dependent pathway (Kojima et al. 2014). The toxic effect of nanomaterial was studied using 3D human liver microtissue model. In vivo study involving nanomaterial dose at lower concentration for 3 weeks with liver's recovery tenure of 2 weeks was studied. Investigation revealed that cytokine profile involving IL6, IL8, IL10, TNF-alpha is effective in analyzing recovery period in pro-inflammatory response reduction (Kermanizadeh et al. 2019). Chen et al. (2018) have reviewed and reported that toxicity due to silica nanoparticle involves generation of reactive oxygen species and autophagy are critical mechanistic pathway to affect immune system (Fig. 13.2).

The different dose effect of mesoporous silica prepared using cetylpyridinium bromide as template, tetraethylorthosilicate as silica source was studied for heart and lung toxicity. The in vivo study was conducted by varying the dose of nanoparticle between 25 and 200 mg per kg body weight for the period of one month.



**Fig. 13.2** Silica nanoparticle induced toxicity (Chen et al. 2018)

The study revealed that silica nanoparticle elevated the cardiac makers, tumor necrosis factor alpha (TNF $\alpha$ ), and cholesterol. Mesoporous nanoparticles administrated rats showed abnormalities in hematological indices due to elevated levels of reactive oxygen species. Antioxidant levels were reduced leading to toxicity in cardiac and lungs. It was confirmed by histopathological examinations. Conclusively MSNs affect the cardiac, respiratory, hematological, and tissue (Hozayen et al. 2019).

Li et al. (2019c) explored the inhalation toxicity of silica nanoparticle in the presence and absence of surfactant dipalmitoylphosphatidylcholine. In vitro study indicated that at dose level of 128 microgram/milliliter, the nanoparticle without template exerted cytotoxicity in 16HBE human bronchial epithelial cells. However, the presence of template reduced the toxicity. In case of A549 adenocarcinomic cells, such toxicity of silica was not observed. The main cause of such adverse effect was found to be due to internalization effect and generation of reactive oxygen species. In 16HBE cells, ABC transporter (Calcein) plays a key role, induced by silica at high dose. Inhibitor of transporter (MK571) elevates the silica toxicity in 16HBE and A549 cells.

The biocompatibility, degradation, and toxic effect of silica mesoporous nanoparticles synthesized from sodium silicate (silica source) have been reported. The study indicated a less toxic effect of such nanoparticles. At normal blood pH condition, the silica determined to be degraded within 6 days to silicic acid form. In addition, nanoparticle found to be biocompatible (90% cell viability) with different cell lines (HEK-293, Caco-2, HepG2, and 3 T3). The silica concentration can be as high as

200 microgram per milliliter. Hematological and biochemical assays showed that mice have well tolerance level (40 mg/kg) and excreted as silicic acid (20 mg/kg) in 4 days (Bhavsar et al. 2019).

Silica nanoparticle toxic effect for particle size of 50 nm (small) and 500 nm (large) was explored through intravenous administration, single dose in male and female immune-competent inbred BALB/c mice. The particles were prepared following Stober synthesis using cetyltrimethylammonium bromide (template), ammonia (catalyst), ethanol (solvent), and tetraethylorthosilicate (silica source) at room temperature. The maximum tolerance dose study showed that nanoparticle with 50 nm size induces cytotoxicity for female of  $103 \pm 11$  mg/kg and male of  $100 \pm 6$  mg/kg. The toxic effect found to reduce with increase in particle size. However, the sex related toxicity reported to increase with large particle silica ( $40 \pm 2$  mg/kg for male;  $95 \pm 2$  mg/kg for female) (Mohammadpour et al. 2019).

Toxic effect of three types of mesoporous silica was reported using three different types of heterocyclic amino acid-based templates such as C16-L-tryptophan, C16-L-histidine, and C16-L-poline, respectively. The in vitro and in vivo study were studied evaluating the silica particle bioavailability, disintegration, which are primarily based on their wettability. The silica was reported to disintegrate within 2–13 weeks under simulated physiological condition. The study showed that silica synthesized with C16-L-tryptophan template contains largest hydroxyl groups and tends to disintegrate quickly due to high wettability. However, severe hemolysis and cell cycle arrest were observed. Therefore, caution must be taken by choosing the silica with apt wettability property (Li et al. 2019).

The nanoshell containing silica alone and iron-silica (Fe-SiO<sub>2</sub>) with size ranging between 497 and 455 nm was synthesized using tetramethyl orthosilicate (silica), iron ethoxide (iron source), and amino polystyrene (template). The thickness of shell involving silica was found to be about 33 nm, while Fe-SiO<sub>2</sub> of about 27 nm, respectively. The single dose acute toxicity study using 10–20 mg per kg dose showed that only very less amount of silica was present inside the body after 10 weeks and then nanoformulation showed no chronic toxicity (Mendez et al. 2017).

Lung inflammation in in vitro using human lung cancer epithelial cell line (A549), and in vivo using C57BL/6 mice was assessed using silica prepared by Stober method and silane functionalized silica (3-aminopropyltriethoxysilane). The nanoparticle in the absence and presence of silane group found to exhibit a less lethal effect at a concentration of 200 µg/ml. Respiratory toxicity study was reported by introduction of two types of silica directly into trachea (intratracheal instillation). Surprisingly, silica alone induced an elevated level of inflammation compared to silane functionalized silica. Each mouse treated with two doses of silica (0.1 and 0.5 mg) showed an increased level of leukocyte and protein level indicating the responses to infections. However, silane functionalized silica reduces lung infection and improves the bioavailability of silica (Morris et al. 2016).

Silica nanoparticle with sizes of 25 nm, 60 nm, and 115 nm, along with amino and carboxylic acid functionalized silica was studied for embryotoxic effect. Each animal was intravenously treated with dose concentration of 200 microgram at three gestational periods (5.5, 12.5, and 16.5 dpc). At gestational period of 5.5 and 12.5 dpc,

silica with particle size of 25 and 60 nm was observed in conceptuses. In later part, three nanoparticles were observed in placenta due to high permeability factor. At 5.5 dpc, silica particle with 25 nm with positive charge density ( $+30 \pm 5$  mV) and  $-COOH$  functionalized silica with 60 nm ( $-44 \pm 5$  mV) were observed in conceptuses. In stage of 12.5 dpc, both species were observed in placenta and fetuses. At 16.5 dpc, silica irrespective of particle sizes with positive charge was found to be accumulated in placentas. However, with negatively charged silica particles, 60 nm and 115 nm were observed in fetuses. The study conclusively determines that silica with 60 nm with negative charge density of  $-44 \pm 5$  mV was able to cross placenta and reach conceptus. Except silane functionalized with 25 nm size silica, other particles of different charge density were found to be non-toxic and safe for placental development (Pietrojusi et al. 2018). Silica nanoparticle induced an elevated level of oxidative degradation of lipids and inflammatory cytokines. Ultrafine silica particles were excreted in urine (Hong et al. 2017). Pure silica induced oxidative stress and toxicity in vitro lung cell line (A549) and animal model studies cells through the oxidant generation such as reactive oxygen generation and lipid peroxidation (18).

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### 13.3 Dendrimers

Dendrimers are supramolecular structured branched polymers. Branching of polymers occurs from perfect dendrimers and expands from dendrons layers in an ordered precise pattern. The assembly of chemical species is driven by phase separation leading to spatially arranged supramolecular domain (e.g., surfactant templates-based micelles, vesicles, and rods). The repeating layers were known as generation (G). The structure depends on the initiator core that can lead to helical tubules, supramolecular hydrogels, and spherical aggregates. Initiator core can be cystamine, ammonia, or ethylenediamine. Methyl acrylate addition to core followed by amidation leads to expand dendrimers. Based on the elements of organics (carbon, phosphorus, and nitrogen) in core and number of branching polymers, over 100 dendrimer families were prepared. Dendrimers at lower generations display open structure due to less branching patterns, while the surface density increases with branching leading to globular form. With each generation, an increase in the particle size occurs to about 1 nm. Precise control leads to formation of monodispersed dendrimers. The presence of active functional groups at the external end site promotes further functionalities.

Dendritic units containing hydrophilic and hydrophobic groups are used to prepare supramolecular structure known as amphiphilic Janus dendrimers. Controlling the chemical functionalities of such dendrimers in water leads to different structured morphologies known as dendrimersomes. Recently, a series of hybrid types of Janus dendrimers based on fluorinated, hydrogenated, and combination of both chemical components were reported by Xiao et al. (2017). Moreover, Ohta et al. (2019) have reported the Janus diblock hyperbranched copolyimide. The morphological structure of Janus dendrimers tuned from spherical aggregated to cylindrical and then to

dendritic shape with aqueous solution temperature variation from 25 °C to 50 °C and to 70 °C. Onion shaped Janus dendrimers have been reported to be influenced by the enthalpy rather than concentration that leads to lamellar, micelles, and vesicles (Hu et al. 2019). Dendrons are attached with one or two cell adherence motifs. Scaffolds coated with linear peptide coating (L-lysine) showed improved colonization of scaffold surface by human bladder smooth muscle cells. Dendrimersomes can be an effective drug delivery system due to their unique physical characteristics such as high stability in biological media, monodisperse and can load both hydro/hydrophilic type of drugs.

siRNA delivery using disulfide containing Janus dendrimer was reported by Du et al. (2018). The reported redox sensitive delivery system with particle size of about 40 nm and distribution of size value of 0.20 (polydispersity index) was constructed in aqueous solution using cationic polymer with hydrophilic head group and disulfide group containing hydrophobic dendron. Redox property was imbedded using hydrating thin film technique. Zeta potential measurement indicated the positive surface charge of about 47 mV, which effectively allows the binding of siRNA with negative charge. The siRNA release study showed advantage of disulfide containing dendrimer with high percentage of siRNA release of about 67% within 5 min and attains to maximum of about 97% in 4 h. Redox based dendrimer also reported effective for simultaneous drug and gene delivery. Diaminobutane core (DAB) based generation 3 dendrimer (diaminobutyric polypropylenimine) with particle size ranging between 150 and 200 nm coupled with polyethylene glycol and camptothecin (anticancer drug) was reported to be effective for delivering DNA along with drug to cancer cells (Laskar et al. 2019). Somani et al. (2018) have investigated generation 3- and 4- DAB based dendrimer for gene delivery. Polyethylene glycated (PEG ~2 kDa) DAB exhibited high gene expression on treatment with B16F10-Luc cells. The toxicity induced by DAB on B16F10-Luc cells was reduced significantly by three-fold compared to dendrimer without modifications and therefore effective for gene delivery and transfection efficacy. Nile Red encapsulated Janus dendrimers are reported using copper-based click reaction for drug release of propranolol. Morphological characterization using transmission electron microscope and dynamic light scattering techniques showed the formation of uni- to multilamellar dendrimersomes in size ranging between 90 and 200 nm suitable for biomedical uses (Nummelin et al. 2017).

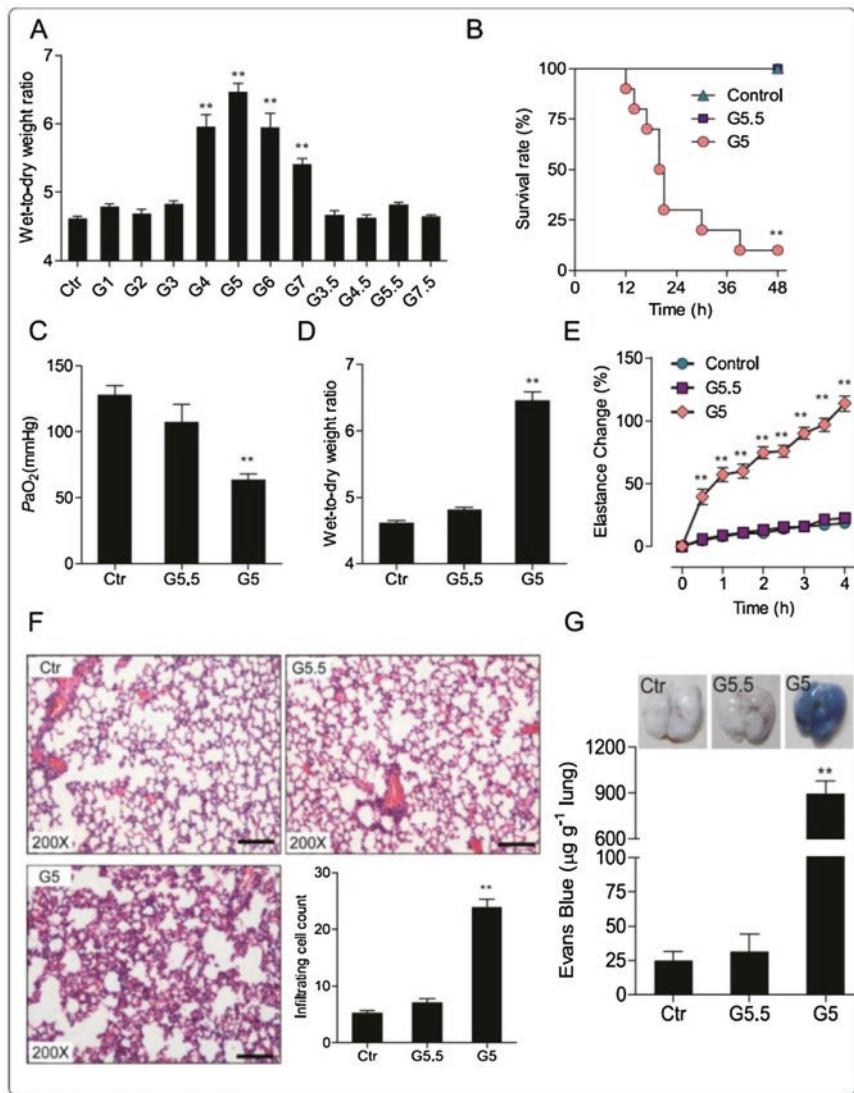
In case of dendrimers, the cytotoxicity depends on the type of dendrimers used in biomedical applications. There are reports suggesting that cationic dendrimers exhibit toxicity compared to anionic (Morris et al. 2017) and non-ionic dendrimers. The prime reason is attributed to the effective electrostatic interactions positively charged dendrimer with negatively charged cell membranes (Malik et al. 2000; Janaszewska et al. 2019). Dendrimers with primary amines at the terminal site like PAMAM and PPI were reported to exert dose dependent toxic effect (Mendes et al. 2017). Dendrimers with neutral or anionic terminal group showed less toxic effect (for example, carbosilane-poly(ethylene oxide)). The generation-based cytotoxicity induced by PAMAM was found to generate reactive oxygen species, enhance

lysosomal activity, apoptosis, and DNA disintegration (Mukherjee et al. 2010). PAMAM inhibited the growth of microbes significantly than G3 and G2. PPI with diaminobutane or diaminoethane core, poly(ethylene oxide) (PEO)-grafted carbosilane (CSi-PEO), and polyether dendrimers and surface functionalities were studied in *in vitro*. Li et al. (2009) have reported a molecular mechanistic based study about the PAMAM based dendrimer (Starburst polyamidoamine) effect in lung injury. *In vivo* study revealed that the nanoformulation triggers autophagic cell death by deregulating Akt-TSC2-mTOR signaling pathway. PAMAM induced lung injury can be suppressed by the addition of 3-methyladenine (autophagy inhibitor). Wang et al. indicated that cationic type of PAMAM dendrimers studied in human glioma cells promoted both cytotoxicity and autophagic degradation activity (autophagic flux). The autophagy was found to be initiated partly by intracellular reactive oxygen species and occur through Akt/mTOR pathway. Ultrastructural analysis clearly showed the accumulation of autophagic vacuoles caused by dendrimers. In addition, the Western blot analysis showed critical influence of dendrimers leading to autophagic flux effect at different PAMAM concentration for 20 h (Wang et al. 2014). It has been further reported that anionic PAMAM and CNTs remains as non-toxic and induce no toxic effect in mice. Whereas cationic PAMAM found to directly interact with angiotensin converting enzyme 2 (ACE2) leading to dysregulation of renin angiotensin system by reducing the activity and downregulation of its expression in lung tissue (Sun et al. 2015). It has been shown that modification of terminal surface of cationic dendrimer with less toxic anionic or neutral group can reduce the toxicity. Functionalization with biocompatible polymers such as polyethylene glycol and pyrrolidone can decrease the cytotoxicity (Fig. 13.3).

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### 13.4 Carbon Nanotubes

Carbon nanotubes (CNTs) belong to the carbon allotropes and are cylindrical in shape with hexagonal arrangements of carbon atoms. The CNTs were as strong as steel but thin like human hair. Based on their sidewalls and spatial arrangements, they are further differentiated as multiwalled carbon nanotubes (MWCNTs) and single walled carbon nanotubes (SWCNTs). Key advances in nanotechnology led CNTs into a wide variety of applications in electronics to energy sectors. Unique physico-chemical properties of CNTs are found to be an attractive material for drug and gene delivery applications. Numerous applications have been reported using CNTs as drug delivery system in chronic diseases. The transportation of CNTs was reported to occur by lymphatic circulation. SWCNTs and MWCNTs have been debated over toxicity due to their ability to interact with body organs. In particular, SWCNTs due to their dispersive and hydrophobic character reported to inflict more toxicity leading to apoptosis compared to MWCNTs (Cui et al. 2005). Inhalation of CNTs induces more toxicity than other mode of administration such as injection, dermal, and oral. Inside the body, the nanoparticle interaction occurs



**Fig. 13.3** G5 PAMAM dendrimers toxicity in lung (Sun et al. 2015)

with cells leading to distributions of particles to various organs and either remains localized or excreted depending on the chemical functionality. Importantly, the textural characteristics such as functional moieties and crystal size tend to play vital role in inducing toxicity. It has been proposed that toxicity occurs mainly due to extracellular matrix protein signaling leading to apoptosis.

### 13.4.1 Lungs Toxicity

SWCNTs were shown to exert respiratory toxicity of inflammation and cytotoxicity in rats through intratracheal deposition at the tissue walls and in alveolar leading to formation of different colored spots including black, brown, and gray (Honda et al. 2017). Particle transition of CNTs as clumps occurs during such deposition and then eventually transforms to tumor formation. SWCNT functionalized with carboxyl group treated animals showed respiratory embolization in lung. Also, inflammation was observed in lung histological examination. Due to oxidative stress and lung toxicity, a pro-inflammatory cytokines activation occurs and cause strong inflammation. This indicates that macrophage fails to phagocytize SWCNT and causes lung toxicity (Mohanta et al. 2019).

### 13.4.2 Liver Toxicity

Liver is one of the vital organs in the body, metabolic pathway is regulated by liver. Drug metabolism involves various enzymatic actions. An *in vivo* study using SWCNTs was evaluated to analyze the efficient bioavailability. SWCNTs were administrated for nine weeks. SWCNTs accumulation increases the uncontrolled cell growth, elevated the enzymatic action and inflammation (Principi et al. 2016). Aspartate aminotransferase, alanine aminotransferase, and bilirubin levels were elevated due to the liver toxic, which was evaluated by serum liver marker assays. Also, histopathological evaluation of SWCNT treated CD1 mice showed tissue inflammation and infiltration. Therefore, exposing CNTs without functionalization for long duration even with less dose imparts undesirable side effects such as inflammation and toxicity in liver.

Oxidized MWCNT nanomaterial was administrated in mice model. It leads to liver toxic and it was confirmed by serum liver function test such as aspartate aminotransferase and alanine aminotransferase. When it was treated by simvastatin, the toxic effect was reduced, and normal functions were restored (Qi et al. 2017). To evaluate the cytotoxic of Mitsui-7- MWCNTs, a studied was reported using various cells. Human alveolar type II epithelial cells A549 cells, the THP-1 The MRC-5 lung fibroblast cell line (ATCC CCL-171) were treated with different concentration of Mitsui-7- MWCNTs and exposed with different time durations. The exposure of MWCNTs to 24 h stimulates the proinflammatory response. Long term exposure leads to elevate profibrotic mediators but not in correlation with production of collagen or cell regulation (Chortarea et al. 2019). The cytotoxicity effect of various functionalized CNTs was studied in cell line of Caucasian colon adenocarcinoma grade II carcinoma (HT29). HT29 was used as experimental cell model in 2D (monolayer) and 3D (spheroid) system, along with *in vitro* 2D and 3D cancer cells (*in vitro*) and mice model (Balb2/c). Doxorubicin (DOX) was found to be effectively functionalized on the CNT surface and release in a controlled manner due to disintegration of peptide bonds with CNT surface. The DOX/CNT composite

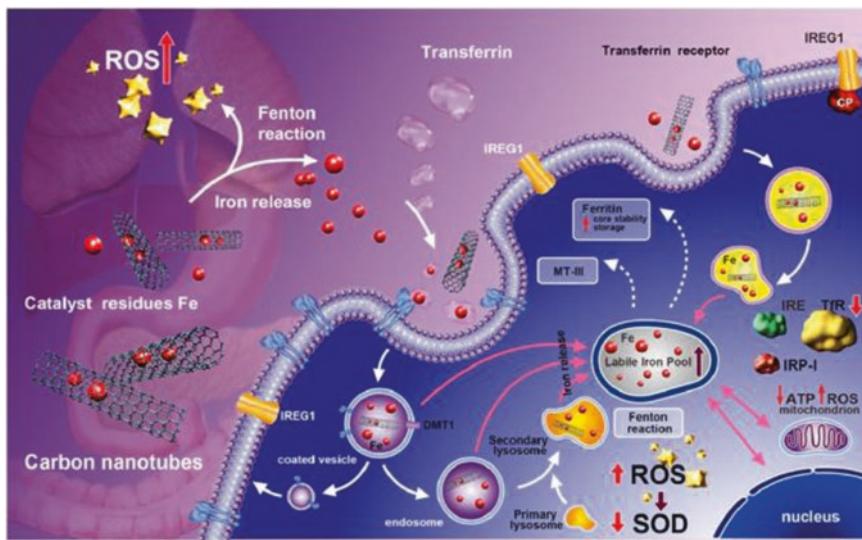
showed less cytotoxic effect compared to unmodified DOX. Liver marker enzyme ALT, AST, ALP Albumin, and total protein and blood profile confirmed the role of CNT in drug delivery. It reduces the DOX liver damage and other related complications (Perepelytsina et al. 2018). The effect of thin bundles (CNT-1) and thick bundles of SWCNTs (CNT-2) was studied for pulmonary toxicity. In vitro and in vivo study was conducted. CNT-1 and CNT-2 were administrated and tested for lung toxicity. The MIP-1 $\alpha$  protein, bronchoalveolar lavage fluid (BALF), and histotechnique indicated an inflammation in pulmonary cells caused by CNT-1. However, the toxicity induced by CNT-1 managed to recover in slow phase, while CNT-2 exerted significant acute toxicity in lung. In molecular level, various genes were altered by CNT-2 after treatment of 1 day. CNT-2 induces the reactive oxygen species (ROS), suppresses cell multiplications, while CNT-1 did not exert significant adverse effect. The study showed CNTs with thin bundle inflict less inflammation than thick shaped CNT-2 (Fujita et al. 2015).

Zhang et al. (2019) reported the engineering of MWCNT functionalized with thermosensitive block copolymer that act as gatekeeper for DOX. Based on the pH conditions, the release of drug is manipulated (tri-stimuli). A composite involving MWCNT and mesoporous silica grafted with Schiff base linked with DOX has been reported to be active against Hela cells with concentration less than 10 microgram/ml. The nanoformulation without DOX shown to be less toxic, while DOX loading with maximum capacity of 51.8% showed increased toxicity inside cancer cells.

Karthika et al. (2018) used de-toxification of MWCNT using metal oxide nanoparticles such as titanium dioxide along with gold. The coating over MWCNT was carried out using solvothermal technique. The bark extract of Guazuma ulmifolia Lam that belongs to Sterculiaceae family was used as reducing agent. DOX was used as anticancer drug with concentration of 0.5 mg/ml. The study showed that the presence of high surface area of CNT, enable the loading of TiO<sub>2</sub> and Au and increase the biocompatibility of nanoformulation. The linkage of DOX with CNT was attributed to the presence of hydrogen bonds and  $\pi-\pi$  stacking. The presence of such bonding seems to control the pH based DOX release of about 90% at cancer condition (pH 5.5) and of about 11% normal condition (pH 7.4) for a period of 10 h, respectively. In vivo study on Zebrafish embryos showed that nanoformulation involving TiO<sub>2</sub>-Au-MWCNT had reduced the toxicity not affecting the hatching of embryos. CNTs with iron metal residue have shown to generate hydroxyl radicals and their dissolution in biological media tends to affect biological targets (Ge et al. 2012; Fig. 13.4).

The cytotoxicity study of MWCNT has been studied with residual iron and iron extracted pure form of MWCNT (heat treated) in A549 human lung epithelial cells. Interestingly, the cytotoxic effect found to depend on the lack of surface defects of MWCNT caused by heat treatment due to free radical generation and interference inside the cell cycle. The presence of residual iron exerted less toxicity (Requardt et al. 2019).

The drug delivery application of Buckypapers and aggregated forms of CNT was reported using different kinds of drugs (acidic and basic characters). The study



**Fig. 13.4** Transport pathway, cellular and molecular mechanisms of toxicity associated with cellular exposure to carbon nanotubes and leached metal (Ge et al. 2012)

reveals the sonication effectiveness for conductive property and drug release experiments. The drug release pattern (passive) was found to depend on the non-covalent bonding. The release can be accelerated or decreased by adjusting the predetermined voltage or current of required polarity (Schwengber et al. 2017).

Modified CNTs were reported by functionalizing with biocompatible polymer polyethylene glycol and bone targeting groups (bisphosphonate and alendronate) for organ specific DOX drug delivery application. The nanocomposite was reported to be effective for DOX loading (35% wt/wt) and release ability (about 51% after 2 days). Biocompatibility of nanoformulation was proved with dispersibility (neoplastic transformation), chromosomal aberration, and cytotoxicity assays. In vivo study showed that compared to free DOX, DOX bound functionalized CNTs (PEG-dMWCNTs and BT-PEG-dMWCNTs) effectively decrease the tumor size and increase the survival period of mice (Falank et al. 2019).

Bisphosphonates were used as effective drugs against osteoporosis. It has been reported that MWCNTs can be functionalized with bisphosphonates such as neridronate, pamidronate, and alendronate. The effective conjugation of bisphosphonates with oxidized MWCNTs was established using thionyl chloride-DMF mixture solution under refluxing condition. After washing with THF, the prepared bisphosphonate along with glucosamine in THF was added and again refluxed. After conjugation, the release of neridronate was studied at three different pH conditions 1.2, 5.5, and 7.4. The high neridronate release at low pH 1.2 than pH 5.5 and pH 7.4 shows the important influence of amine protonation with subsequent dissociation of hydrogen bonding leading to controlled release (Dlamini et al. 2019).

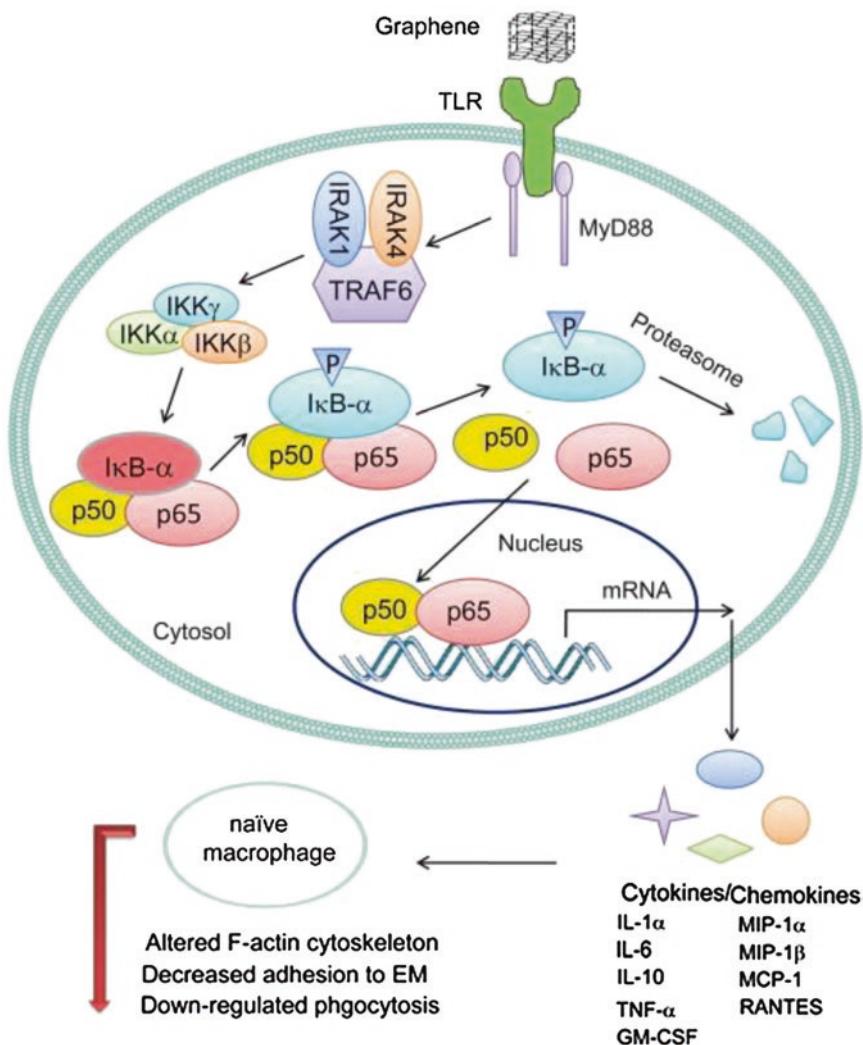
Multifunctional CNT (Fe) in composite with hydroxyapatite was synthesized using chemical vapor deposition technique for pH responsive DOX release. The formulation showed magnetic saturation value of 0.88 emu/g. The composite was fabricated with biocompatible polymer such as chitosan using acid treatment of CNT in the presence of sulfuric acid/nitric acid mixture. Folic acid was further functionalized using EDC chemistry (N-(3-dimethylaminopropyl)-N'-ethylcarbodiimide (EDC).HCl). In the presence of  $\pi$ - $\pi$  stacking and folic acid, high adsorption of DOX was reported (up to 130 wt%). In addition, pH sensitive DOX release (52 wt%) was observed at tumor acidic condition of pH 5.5 after 3 days. The formulation showed very less drug release of about 8 wt% at normal pH condition (pH 7.4) (Li et al. 2019).

Nervous necrosis virus attacks the protected central nervous system (CNS) of fish leading to mass mortality. The presence of barrier blood cerebral spinal fluid in CNS limits the conventional drugs reaching the target site and therefore reduces the therapeutic efficiency against infection such as necrosis and vacuolation. Single walled carbon nanotubes are (SWCNTs) reported to be an effective nanocarrier for tsoprinosine that contains inosine acedoben dimepranol active for antiviral effect. The drug activates T-helper cells to kill infected cells. The study shows an enhanced antiviral effect of SWCNTs functionalized isoprinosine with low concentration of 200 mg per liter for period of 3 and 5 days than pure form of Isoprinosine (Zhu et al. 2019). Graphene, allotrope of carbon was reported to simulate macrophages functions (Th1/Th2 cytokines) (Zhou et al. 2012; Fig. 13.5).

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### 13.5 Conclusion

Structured silica, dendrimers, and carbon nanotubes were important nanomaterials that are expected to play a major component in future biomedical relevant applications. The review shows that templates, solvents, silica source, functionalization silanes, and synthesis conditions of nanoparticles play a critical role in inducing lung inflammation. Mesoporous silica synthesized using sodium silicate as silica source, nanoshell based Fe-SiO<sub>2</sub>, C<sub>16</sub>-L-tryptophan template-based silica, dipalmitoyl phosphatidylcholine template-based silica were reported to be non-toxic and effectively disintegrate in biological medium. Silica nanoparticle synthesized using templates like cetylpyridinium bromide and heterocyclic amino acid-based templates such as C<sub>16</sub>-L-histidine and C16-L-poline were reported to be toxic. Mesoporous silica prepared by Stober method in the presence of template cetyltrimethylammonium bromide was reported to be toxic, while in the absence of template led to non-toxic silica nanoparticle. Non-porous silica prepared by Stober method in the presence of silanes exhibited reduced toxicity in lungs compared to unmodified silica. Dendrimers can be an effective nanocarrier by controlling the surface charge density of cationic dendrimers or using anionic or non-ionic dendrimers. The biocompatible can be improvised using polymers like polyethylene glycol and components like bisphosphonate and alendronate. In case of single



**Fig. 13.5** Graphene induced activation of macrophage signaling pathway (Zhou et al. 2012)

walled carbon nanotubes (SWCNTs), short and long form of SWCNTs are reported to induce inflammation and respiratory toxicity, though no genotoxicity was observed. Multiwalled carbon nanotubes (MWCNTs) have shown to induce proinflammatory response and elevate profibrotic mediators. However, the cytotoxicity of carbon nanotubes in lungs can be reduced significantly by using biocompatible polymers such as polyethylene glycol, chitosan, insertion of metal oxides (iron, TiO<sub>2</sub>, Au), oxidized form of nanotube with drug like Simvastatin, and by using thin bundles CNTs (Table 13.1).

**Table 13.1** Impact of nanomaterials on organs/cells/tissues

Sl. No	Material	Material source	Study type	Organ toxicity	Biocompatibility	References
1.	<i>Silica</i>	Cetylpyridinium bromide (template), urea, cyclohexane, isopropanol, tetraethylorthosilicate	Albino Wistar rats	Reactive oxygen species generation in heart and lung, inflammation, anemia, thrombocytopenia	–	Hozayen et al. (2019)
2.	MSN	Silica obtained from Nanjing XFNANO Materials Tech Co., Ltd. (prepared using Stober method)	A549 adenocarcinomic cells and 16HBE human bronchial epithelial cells	Without template-dipalmitoyl phosphatidylcholine exhibits toxicity in 16HBE human bronchial epithelial cells	With template-dipalmitoyl phosphatidylcholine induces less toxicity and high compatibility	Li et al. (2019)
3.	MSN	Cetyltrimethylammonium bromide (CTAB), Na <sub>2</sub> SiO <sub>3</sub> (silica source), ethyl acetate	HEK-293, Caco-2, HepG2, 3 T3	–	Exhibits high biocompatible (90% cell viability) with different cell lines (HEK-293, Caco-2, HepG2, and 3 T3). Degraded within 6 days	Bhavasar et al. (2019)
4.	MSN-50 nm (size) MSN-500 nm	Cetyltrimethylammonium bromide (template), ammonia (catalyst), ethanol (solvent) and tetraethylorthosilicate (silica source)-Stober method	Intravenous administration, single dose in male and female immune-competent inbred BALB/c mice	Cytotoxicity exhibited by both size nanoparticles under acute conditions	Exhibits less subchronic toxicity compared to non-porous silica on 60 and 180 days	Mohammadpour et al. (2019)

(continued)

**Table 13.1** (continued)

Sl. No	Material	Material source	Study type	Organ toxicity	Biocompatibility	References
5.	MSN	Different types of heterocyclic amino acid-based templates such as C16-L-tryptophan, C16-L-histidine, and C16-L-poline, respectively	In vitro and in vivo study	Severe hemolysis and cell cycle arrest were observed	Silica synthesized with C16-L-tryptophan disintegrates quickly due to high wettability, reduce toxicity	Li et al. (2019)
6.	Nanoshell SiO <sub>2</sub> and Fe-SiO <sub>2</sub>	Using tetramethyl orthosilicate (silica), iron ethoxide (iron source) and amino polystyrene (template)	Single dose acute toxicity study using 10–20 mg per kg dose	—	Trace amount SiO <sub>2</sub> nanoparticles observed in lung and no inflammatory response observed in lungs	Mendez et al. (2017)
7.	MSN	Cetyltrimethylammonium bromide (template), ammonia (catalyst), ethanol (solvent), and tetraethylorthosilicate (silica source)	Lung inflammation in in vitro using human lung cancer epithelial cell line (A549), and in vivo using C57BL/6 mice	Respiratory toxicity	—	Morris et al. (2016)
8.	MSN-Silane	Non-porous silica, no template used, ammonia (catalyst), ethanol (solvent), and tetraethylorthosilicate (silica source) 3-amino propyltriethoxysilane	Lung inflammation in in vitro using human lung cancer epithelial cell line (A549) and in vivo using C57BL/6 mice	—	Amine functionalized silica showed reduced toxicity, less reactive oxygen species in lungs compared to unmodified silica	Morris et al. (2016)

9.	Silica -25 nm, 60 nm, and 115 nm	NH2 and COOH functionalized silica nanoparticles	Embryotoxic effect In vitro lung cell line (A549) and animal model	Silica-25 nm size silica 3-aminopropyltriethoxysilane Oxidative stress and toxicity studies cells through the oxidant generation such as reactive oxygen generation and lipid peroxidation	Silica 60 nm and 115 nm Non-toxic and safe for placental development	Pietrojuti et al. (2018)
10.	Ultrafine silica	Silica nano and silica microscale obtained from Zhejiang Hongshen Material Tech. Ltd. Company, China	In vitro lung cell line (A549) and animal model	Generation such as reactive oxygen generation and lipid peroxidation	—	Hong et al. (2017)
<i>Dendrimers</i>						
1.	Diaminobutane core (DAB) based generation 3, 4, and 5 dendrimer (diaminobutyric polypropylenimine) for gene delivery	Diaminobutyric polypropylenimine (3-, 4-, and 5-), methoxy PEG (~2 kDa) succinimidyl carboxymethyl esters (diaminobutyric polypropylenimine)	Bioware PC-3 M-luc-C6 human prostate adenocarcinoma, bioware B16F10-Luc cells, A431 human epidermoid carcinoma, T98G human glioblastoma, DU145 human prostate carcinoma	—	PEGylated G3 and G4-DAB reduced cytotoxicity of dendrimer. G4-DAB with PEG (2 and 5 kDa) reduced toxicity of dendrimer at lower dose of 20 µg/ml	Soman et al. (2018)
2.	Cationic dendrimers polyamidoamine dendrimers G4, G5, and G6 (PAMAM)	Ethylenediamine core based polyamidoamine dendrimers G4 (14,215 Da), G5 (28,826 Da), and G6 (58,048 Da)	HaCaT cells, SW480 cells	Dendrimer charge density is correlated to chronic responses. Hormetic response at lower concentration, while toxicity observed at higher concentration	Adenocarcinoma of colon most sensitive to toxicity induced by dendrimers	Mukherjee et al. (2010)

(continued)

**Table 13.1** (continued)

Sl. No	Material	Material source	Study type	Organ toxicity	Biocompatibility	References
3.	Cationic type of PAMAM dendrimers	PAMAM G5 obtained from Sigma-Aldrich. Methanol removed and dissolved in PBS 7.4 (10 mg/ml)	Human glioma cells (U87MG, U118, U251MG, A172)	Cationic dendrimer induced cytotoxicity and autophagic degradation activity (autophagic flux)	–	Wang et al. (2014)
4.	Cationic PAMAM	PAMAM obtained from Sigma-Aldrich, methanol removed and mixed with PBS	C57BL/6 J mice	Dysregulation of renin angiotensin system Downregulation of its expression in lung tissue	–	Sun et al. (2015)
5.	Anionic PAMAM	PAMAM 1.5 (2935 Mw of Na salt) PAMAM 3.5 (12,931 Mw of Na salt) PAMAM 5.5 (52,901 Mw of Na salt)	Rat	–	Non-toxic, exhibited high biocompatibility, can be effective drug nanocarrier	Morris et al. (2017)
<i>Carbon nanotubes</i>						
1.	SWCNTs	SWCNTs (carbon nanotechnologies, Inc)	Human embryonic kidney 293 cells (HEK 293)	SWCNTs upregulate apoptosis associated genes (p16, Rb, and p53)	–	Cui et al. (2005)
2.	SWCNTs (short and linear type)	SWCNTs with 8.6 and 0.55 µm, ten fold diluted in PBS containing 1% salmon serum deoxyribonucleic acid	F344/DuCrIcrj male rats	Long SWCNTs deposited at terminal bronchioles, short SWCNTs in alveolus, respiratory toxicity, inflammation	No genotoxicity was observed in lungs	Honda et al. (2017)
3.	SWCNTs	Crude SWCNTs/ DMEM/10%(vol/vol) FBS	MITO-Luc and CD1 mice	Induces uncontrolled cell growth, elevated the enzymatic action and inflammation, liver toxic	–	Principi et al. (2016)

4.	Oxidized MWCNT	MWCNTs treated with 3 mol/L HNO <sub>3</sub> /calcined at 450 °C	Kunming mice (female)		Simvastatin reduce toxic effect of oMWCNTs	(Qi et al. 2017)
5.	Mitsui-7-MWCNTs	MWCNTs/bovine serum albumin (1 mg/ml in H <sub>2</sub> O)	Human alveolar type II epithelial cells A549 cells, the THP-1 the MRC-5 lung fibroblast cell line (ATCC CCL-171)	Exhibits proinflammatory response, elevates profibrotic mediators	–	Chortarea et al. (2019)
6.	Doxorubicin/fluorescein/CNTs	Oxidized CNTs/DOX-N-cylohexyl-N'-(2-morpholinoethyl) carbodiimide metho-o-toluenesulfonate/FITC/ultrasonic treatment in PBS	Cell line of Caucasian colon adenocarcinoma grade II carcinoma (HT29) in 2D (monolayer) and 3D (spheroid) system in vitro and mice model (Balb2/c)	–	DOX/fluorescein/CNT exhibits less cytotoxic effect compared to unmodified DOX	Perepelytsina et al. (2018)
7.	Thin bundles (CNT-1) And thick bundles of SWCNTs (CNT-2)	SWCNTs/bovine serum albumin (1 mg/ml; 10 mg/ml)	In vitro and in vivo study	Lung toxicity CNT-2 exerted significant acute toxicity in lung	CNT-1 toxicity managed to recover in slow phase	Fujita et al. (2015)
8.	DOX-MWCNT functionalized with thermosensitive block copolymer	MWCNT @ MSN-s-g-PNIPAM-b-PFBEMA-DOX	In vitro, HeLa cells	Cytotoxicity, DOX loading with a maximum capacity of 51.8% showed increased toxicity inside cancer cells	Biocompatible nanocarrier	Zhang et al. (2019)

(continued)

**Table 13.1** (continued)

Sl. No	Material	Material source	Study type	Organ toxicity	Biocompatibility	References
9.	DOX, MWCNT, titanium dioxide, gold	MWCNTs/TiO <sub>2</sub> /Au (dark extract) (1:1 and 1:3)	In vivo study on Zebrafish embryos	–	TiO <sub>2</sub> and Au increase the biocompatibility of MWCNT, toxicity not affecting the hatching of embryos	Karthika et al. (2018)
10.	Iron and iron extracted pure form of MWCNT	MWCNTs obtained from cyclohexane precursor, ferrocene as catalyst	A549 human lung epithelial cells and HepG2 cells	Iron extracted pure form of MWCNT showed cytotoxicity	Residual iron exerted less CNT toxicity	Requardt et al. (2019)
11.	DOX, CNTs, polyethylene glycol, glycol, bisphosphonate, and alendronate	Discrete MWCNT's, DMF, thionyl chloride, PEGylation, NHS, EDC, alendronate, Cy5, DMSO, DOTA label	In vivo study CD-1 mice	–	DOX bound functionalized CNTs (PEG- dMWCNTs and BT-PEG- dMWCNTs) effectively decrease the tumor size and increase the survival period of mice	Falank et al. (2019)

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# Nanomaterials and Ethical Issues

14

Oodhimalai Elango Santhini and Selvaraj Dinesh Kirupha

## Abstract

Even after many discoveries and inventions of new nanomaterial devices for biomedical applications, still there were no stringent rules and regulations framed for the pharmaceutical products or the biomedicines developed from it. As a result, there was a bridge gap between biomedical advancement and clinical trials. This chapter discusses about the nanomaterial's advancement, importance and its key role in the development of novel and improvised biomedical devices for many health-related issues in human body. There was a sharp edge of advancement with many novel inventions, at the same time there was a lack in discipline in the ethical issues and advancement related to it. Hence this chapter clearly connects the bridge between the issues and presented some improvisations for the development of nanobiomedical devices and their ethical issues.

## Keywords

Nanomaterials · Biomedical devices · Ethical issues · Regulations · Guidelines

## 14.1 Introduction

Nanomaterials, since their discovery in 1959 by Richard Feynman (American physicist, 1918–1988), they have been intensively studied for various applications. In the case of health care sector, the nanomaterial has witnessed rapid technological advancements and has been used as a vehicle for delivering drugs, drug therapy, *in vitro* diagnostics, *in vivo* imaging and as active implants. In the last 5 years, the

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O. E. Santhini (✉) · S. D. Kirupha

Centre of Excellence for Medical Textiles, The South India Textiles Research Association (SITRA), Coimbatore, India

**Table 14.1** List of international standards for the evaluation of safety of nanomaterials

S. No	Name of test method	Scope or description	End result	References
ASTM, E56.0: 3: Environment Health and Safety				
1	Method for analysis of hemolytic properties of nanoparticles.	Describes the effect of nanoparticulate materials on the integrity of red blood cells.	Hemolytic property.	ASTM E2524-08 (2013)
2	Method for evaluation of the effect of nanomaterials on the formation of mouse granulocyte-macrophage colonies.	It is a part of the in vitro preclinical characterization cascade for nanomaterials for systemic administration in medical applications.	Immunological response.	ASTM E3143-18b
3	Method for evaluation of cytotoxicity of nanomaterials in porcine kidney cells and human hepatocarcinoma cells.	Assess the propensity of a nanomaterial to cause cytotoxicity to the cells of a target organ. This test assists in preclinical development.	Evaluates cytotoxicity through MTT reduction and Idh leakage.	ASTM WK54872 (under development)
4	Standard guide for measurement of electrophoretic mobility and zeta potential of nanosized biological materials	Measurement of mobility and zeta potential in systems containing biological material (e.g., proteins, DNA, liposomes and other similar organic materials) that possess particle sizes in the nanometer scale.	Electrophoretic mobility and zeta potential.	ASTM E2865-12 (2018)
5	Standard practice for performing cryo-transmission electron microscopy of liposomes	Covers procedures for vitrifying and recording images of a suspension of liposomes with a cryo-transmission electron microscope for quality assessment.	Shape, size distribution of liposomes.	ASTM E3143-18b
6	New test method for measuring the size of nanoparticles in aqueous media using dynamic light scattering	Facilitates regulatory review and oversight of nanoparticle-based biomedical products.	Size of nanoparticles	ASTM WK54872 (under development)

number of clinical trials involving nanomaterials has increased multiple folds. Since 1995, 50 nanopharmaceuticals, particularly polymeric, liposomal, and nanocrystal-based formulations have been approved by US FDA and are currently being used for various clinical indications (Bobo et al. 2016; Caster et al. 2017; Centerwatch 2017; Food and Drug Administration 2017).

Maximum number of the nanodrugs which are approved to-date have revealed reduced toxicity rather than improved efficacy compared to conservative

**Table 14.2** List of international standards for the evaluation of safety of nanomaterials

S. No	Name of test method	Scope or description	End result	References
International Standardization Organization (ISO)				
1	Nanotechnologies	Comprises 110 standards covering the characterization of physicochemical properties of different nanomaterials like carbon nanotubes, multi-walled carbon nanotubes, gold NPs, quantum dots, inorganic NPs, colloidal systems, etc.	Particle size, shape, concentration, morphology, specific surface area, zeta potential, etc.	ISO/TC 229
2	Biological evaluation of medical devices: Part22: Guidance on nanomaterials.	The standard provides guidance for sample preparation, characterization, release and toxicokinetic of nano-objects, biological evaluation, risk assessment in the context of medical device evaluation.	NA	ISO/TR 10993-22:2017
3	Nanotechnologies: Plain language explanation of selected terms from the ISO/IEC 80004 series.	Explanations for selected nanotechnology terms and the understanding of the use and applications of nanotechnology.	NA	ISO/TR 18401:2017(en)
4	Nanotechnologies: Methodology for the classification and categorization of nanomaterials	Provides nano-tree, where the wide range of nanomaterials including nano-objects, nanostructures and nanocomposites of various dimensionality of different properties are categorized.	NA	ISO/TR 11360:2010

preparations. In fact, many nanodrugs have not survived clinical development because they were unable to establish a significant improvement in efficacy (Caster et al. 2017). These boundaries are mainly due to the limited or unavailability of appropriate standards for the characterization of nanomaterials, associated toxicity issues and lack of regulatory guidelines for conducting clinical trials. Recently, various standard formulating committees such as ASTM, ISO and OECD have come up with different standard protocols for the evaluation of safety of nanomaterials for

**Table 14.3** List of international standards for the evaluation of safety of nanomaterials

S. No	Name of test method	Scope or description	End result	References
OECD Guidelines for the evaluation of nanomaterials				
1	Daphnia species acute immobilization test	To assess the effects of chemicals towards Daphnia magna.	EC50	Test No. 209 (OECD 2004b) (adopted for TiO <sub>2</sub> )
2	Fish embryo acute toxicity test	To determine the acute or lethal toxicity of chemicals on embryonic stages of fish—Danio rerio	LC50	Test No. 236 (OECD 2013) (adopted for TiO <sub>2</sub> )
3	Respiration inhibition test	To assess the effects of a substance on micro-organisms from activated sludge of waste-water treatment plants	Oxygen consumption	Test No. 209 (OECD 2010) (adopted for TiO <sub>2</sub> )
4	Earthworm-acute toxicity test	To test substances on moist filter paper in order to identify potentially toxic chemicals to earthworms in soil	Mortality	Test No. 207 (OECD 1984) (adopted for TiO <sub>2</sub> )
5	Earthworm, reproduction test	Assessing the effects of chemicals in soil on the reproductive output (and other sub-lethal end points) of the earthworm species Eisenia fetida or Eisenia andrei.	Mortality (LC <sub>x</sub> ), weight change and reduction in reproduction.	Test No. 222 (OECD 2004a) (adopted for TiO <sub>2</sub> )
6	Bioaccumulation in Fish: Aqueous and Dietary Exposure	A procedure for characterizing the bioconcentration potential of substances in fish, using an aqueous (standard and minimized tests) or dietary exposure, under flow-through conditions (but semi-static regimes are permissible).	Bioconcentration factor (BCF) and biomagnification factor (BMF).	Test No. 305

clinical applications (Tables 14.1, 14.2, 14.3, and 14.4). However, close collaboration among different regulatory agencies is still needed.

## 14.2 Regulatory Requirements

Currently, there are no specific regulations or guidelines available for approving nanomaterials for medical applications. European Union is regulating nanomaterials-based products under existing regulations for ‘General Medicinal Product

**Table 14.4** List of international standards for the evaluation of safety of nanomaterials

S. No	Name of test method	Scope or description	End result	References
Other standards/guidelines				
1	Drug products containing nanomaterials	Guidance on the composition and attributes of nanomaterials based on their impact on the quality and performance of drug product.	NA	USP Chapter <1153> (proposed)
2	In vitro assessments of nanomaterial toxicity	A review article discussing the current methodologies used for the assessment of physicochemical properties and in vitro effects of nanomaterials.	Cell-based in vitro systems to study the particle internalization, wide range of techniques for the detection of ROS and RNS and the list of inflammatory and healing cytokines related to nanotoxicity.	Jones and Grainger (2009)
3	In vivo assessment of nanomaterials toxicity	The mechanisms involved in nanomaterials toxicity including oxidative stress and inflammation are detailed.	Different routes of administration, exposure time (short-term or a long-term), interaction between the nanomaterial and the organism, physicochemical characteristics of nanomaterials in relation to their toxicity are discussed with the specific emphasis on the toxicity of dendrimers, silver NPs, gold NPs and carbon nanotubes.	Clichici and Filip (2015)
4	A decision-making framework for the grouping and testing of nanomaterials (DF4nanoGrouping)	The DF4nanoGrouping is a hazard and risk assessment tool that applies modern toxicology and contributes to the sustainable development of nanotechnological products. It ensures that no studies are performed that do not provide crucial data and therefore saves animals and resources.	Assigned nanomaterials to 4 main groups such as soluble nanomaterials, bio persistent high aspect ratio nanomaterials, passive nanomaterials, and active nanomaterials based on the functionality of the nanomaterials rather than its intrinsic properties alone. The division is to perform sub-grouping within the main groups, to determine and refine specific information needs.	Arts et al. (2015)

(continued)

**Table 14.4** (continued)

S. No	Name of test method	Scope or description	End result	References
Other standards/guidelines				
5	Antimicrobial property detection methods for nano-inorganic materials. (AQSIQ)	This standard applies to nano-powders and nano-antibacterial, functional components (structural units) of the material, such as fibres, fabrics, plastics, coatings and ceramics, etc.	Antibacterial activity.	GB/T 21510-2008

legislation'. Similarly, US FDA is regulating nanotechnology products under existing statutory authorities, in accordance with the specific legal standards applicable to each type of product under its jurisdiction. In the case of Japan, nanomedicines are regulated within the framework of the Pharmaceutical Affairs law on a product-by-product basis (Kuzma and Priest 2010) (Tables 14.5). The major issues faced by the regulatory authorities for the regulation of nanomedicines are the lack of scientific expertise and difficulty in classifying the nanomedicine. So far, the US FDA could regulate the nanoproducts under existing legislation but not the technology.

### 14.3 Ethical Issues

Despite the availability of high-end technologies, we still lack appropriate standards to evaluate the safety/toxicity of nanomaterials. Moreover, currently there are no hard-core regulations for regulating medicines based on nanomaterials. All these lead to various issues at ethical level as the *in vitro*/preclinical results are not matching at clinical levels. Nanomedicine is the one of the most developing areas of research with potential applications. But without proper rules and legislations it is impossible to regularize the area of interest into proper profound manner. Hence a set of rules for majorly affecting concerns such as equity, discrimination, intense change in human body with respect to ethical and non-ethical issues were explained deep in manner. The increase in population may lead to increase in the number of patients and which can be considered as one of the major concerns. Among many developing technologies to eradicate such infectious, widely spread and intensely affected diseases, nanotechnology was considered as one of the prime methodologies due to some advantageous benefits over other techniques. Quick delivery, less side effects and predetermined drug release under calculated time were some advantageous.

Some medicines under molecular level were found to show different nature under nanostructured dimensions. Over the past two decades there was steady regular increase in the development of nanostructures, with tremendous growth in

**Table 14.5** List of regulatory bodies and the status of the formulation of regulations

S. No	Region	Description of nanomaterials/Nanomedicine required for approval	Details of guidance documents
1	USA	'Engineered nanoscale material' is any particle, substance, or material that has been engineered ((1) purposefully produced and (2) purposefully designed to be a nanoscale material) to have one or more dimensions in the nanoscale (the scale measured in nanometers). (Concept Paper for the Nano-scale Materials Stewardship Program under TSCA).	<ul style="list-style-type: none"> <li>Final guidance for industry—considering whether an FDA-regulated product involves the application of nanotechnology</li> <li>Final guidance for industry—safety of nanomaterials in cosmetic products</li> <li>Final guidance for industry—assessing the effects of significant manufacturing process changes, including emerging technologies, on the safety and regulatory status of food ingredients and food contact substances, including food ingredients that are color additives</li> <li>Final guidance for industry—use of nanomaterials in food for animals.</li> <li>Draft guidance for industry—drug products, including biological products that contain nanomaterials (2017).</li> </ul>
2	European Union	a. 'Nanomaterial' means a natural, incidental or manufactured material containing particles, in an unbound state or as an aggregate or as an agglomerate and where, for 50% or more of the particles in the number size distribution, one or more external dimensions are in the size range 1 nm–100 nm. (Commission Recommendation of 18 October 2011 on the definition of nanomaterial) b. 'Nanomaterial' means an insoluble or bio-persistent and intentionally manufactured material with one or more external dimensions, or an internal structure, on the scale from 1 to 100 nm. (Regulation (EC) No 1223/2009 of the European Parliament and of the council of 30 November 2009 on cosmetic products)	<p>REACH guidance for nanomaterials:</p> <ul style="list-style-type: none"> <li>Nano-specific appendix to Chapter R.6 of the Guidance on Information Requirements and Chemical Safety Assessment (QSARs and grouping of chemicals):</li> <li>How to prepare registration dossiers that cover nanoforms <ul style="list-style-type: none"> <li>Updating of existing guidance</li> <li>Chapter R.7.a, R.7.b and R.7.c of the Guidance on IR&amp;CSA (Endpoint specific guidance).</li> </ul> </li> </ul>

(continued)

**Table 14.5** (continued)

S. No	Region	Description of nanomaterials/Nanomedicine required for approval	Details of guidance documents
3	Japan	No definition	<i>Japan Policy Document</i> Guide to Evaluating Emission and Exposure of Carbon Nanomaterials (carbon nanotubes and graphene) 2018. General Procedures for Safety Tests on Carbon Nanomaterials 2017. Published by National Institute of Advanced Industrial Science and Technology
4	China	Nanomaterials is the material which has a structure in the three-dimensional space in at least one dimension in the nanometer scale (from 1 nm to 100 nm ( $1\text{ nm} = 10^{-9}\text{ m}$ ) range of geometric dimensions), or constituted by the nanostructure unit and a material with special properties (GB/T19619-2004; (Terminology for nanomaterials) (AQSIQ) (Effective on 1 April 2005)	Not available
5	South Korea	Follows EU definition: ‘a natural or manufactured material containing particles, in an unbound state or as an aggregate or as an agglomerate and where, for 50% or more of the particles in the number size distribution, one or more external dimensions are in the size range 1 nm–100 nm’.	Not available.  South Korea Policy Document: Nanotechnology Development Plan (2018) 2018–2019 Published by Presidential Advisory Council on Science and Technology.

6	Canada	<p>Policy Statement on Health Canada's (HC) Working Definition for Nanomaterial. HC considers any manufactured substance or product and any component material, ingredient, device, or structure if it is:</p> <ul style="list-style-type: none"> <li>• at or within the nanoscale (one to 100 nm) in at least one external dimension, or has internal or surface structure at the nanoscale; or</li> <li>• it is smaller or larger than the nanoscale in all dimensions and exhibits one or more nanoscale properties/phenomena.</li> </ul>	<ul style="list-style-type: none"> <li>• Guidance on Sample Preparation and Dosimetry for the Safety Testing of Manufactured Nanomaterials. OECD Series on the Safety of Manufactured Nanomaterials. No. 36, ENV/JM/MONO (2012) 40</li> <li>• Important Issues on Risk Assessment of Manufactured Nanomaterials. OECD Series on the Safety of Manufactured Nanomaterials. No. 33, ENV/JM/MONO (2012) 8</li> <li>• Preliminary Review of OECD Test Guidelines for their Applicability to Manufactured Nanomaterials. OECD Series on the Safety of Manufactured Nanomaterials. No. 15, ENV/JM/MONO (2009) 21</li> </ul>	Not available. HC relies on authorities within existing legislative and regulatory frameworks.
7.	Switzerland	<p>The nanoparticles have at least one nanoscale dimension (1–1000 nm) plus a function and/or mode of action based on nanotechnology characteristics.</p>	<p>Not available.</p>	Not available. But general therapeutic products legislation is applicable.

biomedical field. Nanotechnology deals with multidisciplinary science with the combination of biology, science, physics and engineering. To construct a biomedical device under nanostructured dimensions is a tedious process, since it needs constant monitoring and thought-provoking ideas. For the past decade, nanotechnology played a vital role in health care products and medical implants, drug delivery devices and nanoscaffolds. There are also other ethical issues affecting the human as implants which results beyond healing purpose and impact on preservation of human identity.

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#### **14.4 Biocompatibility and Toxicity of Nanostructures**

Nanodrug delivery systems are the most well-established segment in nanomedicine which operates to deliver active agents to different organs of the body. The performance is erratic and they behave completely different both in vitro and in vivo studies. The nanoparticles disintegrate into smaller particles which cause serious harmful side effects to human body. It is ethically desirable to study the short term and long term impacts of nanoparticles to estimate the effectiveness in humans than other conventional drugs.

Nanotechnology research enjoying an astounding progress recently which has been previously achieved by pharmaceutical companies in drug delivery studies. About 78% of global sales in nanomedicine cover around drug delivery devices with a new level of research, pharmacogenetics and pharmacogenomics. Expensive nanomedical drug treatments and impact devices are still a human identity and ethical principles pertaining to human dignity.

The European Commission emphasizes that a specific method should be used to risk evaluation by using different approaches considering the exposure or hazardous condition. Recently, a legislative work has been undertaken to regulate the use of nanomaterials by many members of the European commission. There are two different types of regulations, which are related to the application of nanomaterials, such as REACH [Registration, Evaluation, Authorisation and Restriction of Chemicals; EC No. 1907/2006] and CLP [Classification, Labelling and Packaging; EC No 1272/2008].

Additionally, the regulations related to the use of biocides and cosmetic products are described under EC No. 1223/2009, whereas regulations about food and food additives have been described under EC No. 1169/2011. As nanotechnologies are also utilized in medicine, a directive on the community code relating to medicinal products for human use is described in directive 2001/83/EC.

There are no specific laws or regulations that have been defined for the use of nanotechnology application in many countries. The countries such as Japan, China, Korea and Taiwan, which are highly involved in nanotechnology have produced various guidelines. There is a need for the precautionary principle to face the risks related to the use or exposure of nanomaterials.

## 14.5 Conclusion

There was a clear confusion related to nanomaterials related biomedical devices due to the binding and non-binding acts for conducting nanomaterials research and their applications towards mankind. With wide inventions of nanomaterials and their applications research by the researchers for the past two decades there was a lacking in doctrine discipline. Since it is not so easy to regulate and facilitate a set of guidelines and regulations for the nanomaterial's inventions, the problem still prevails. The rules must be uniformly maintained from the selection of raw materials and must be followed till the completion of product for human trials. There exists another problem when we look upon the detections of materials, physical, chemical and biological characteristic analysis of the raw material and the developed products. This chapter emphasized the general problems faced during the product development and their economical value when it is presented in market. Even after several debates on the ethical and legislative issues associated with the nanomaterials product developments and their legal issues, still there were no clear rules and guidelines framed which was another blockage faced by the researchers in justifying their novel products with expected positive results.

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# Next Generation Nanomaterials: Smart Nanomaterials, Significance, and Biomedical Applications

15

Suresh Thangudu

## Abstract

Over the advancement of nanotechnology, a vast number of nanomaterials have been developed and successfully utilized in various applications. Specifically in biomedical applications, still it is a challenging to fabricate nanomaterials with good functional properties for achieving better therapeutics. To overcome the limitations of common nanomaterials, smart materials are grabbing more significant attention recently. In earlier days, these smart materials are often defined as a material which can respond in a timely manner to the surrounding environment. Thereafter, definition of smart materials has been expanded that the material that can stimuli by external factors and results a new kind of functional properties. Stimuli agents are further classified as light, temperature, electric, magnetic field, stress, pressure, pH, etc. These controlled abilities of smart materials make them particularly interesting to utilize in various applications such as controlled release of drugs, treatment of various diseases, biosensors, etc. So it is very important to know the various kind of smart nanomaterials and their unique properties under specific stimulating agents. Therefore, in the present chapter, we aim to show various classification of smart nanomaterials and its beneficial advantages in biomedical applications in the past to the future.

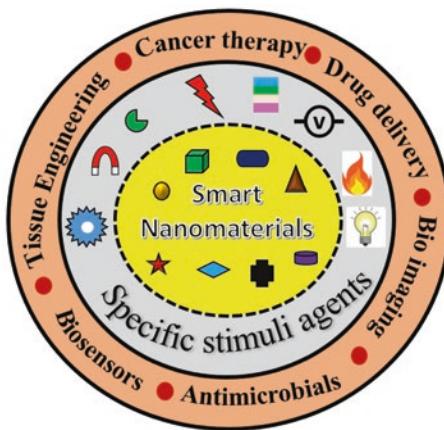
## Keywords

Next-generation nanomaterials · Smart nanomaterials · Significance and biomedical applications

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S. Thangudu (✉)

Department of Chemistry, National Tsing Hua University,  
Hsinchu, Taiwan, Republic of China

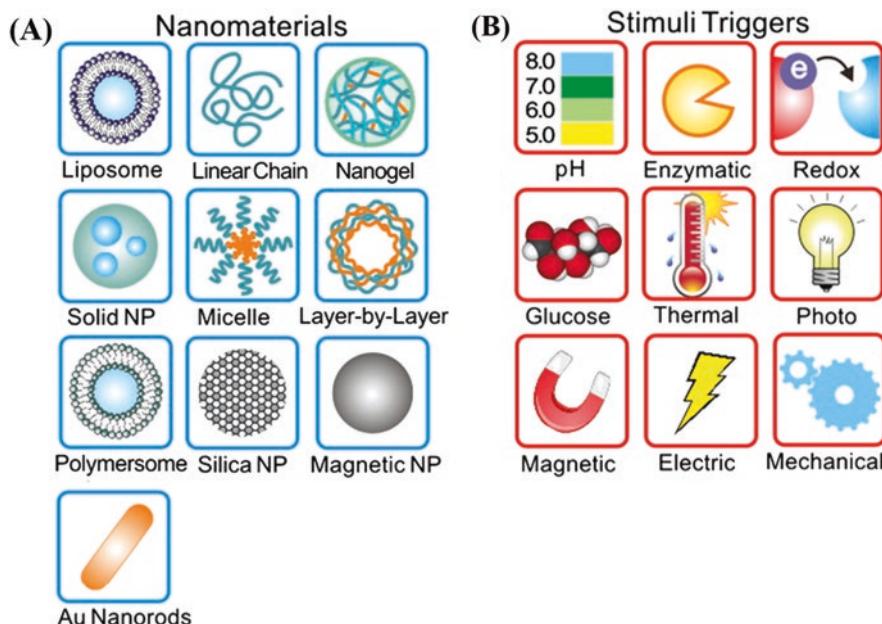


## 15.1 Introduction

Over the decades, nanotechnology and nanomaterials played a vital role in the development of scientific and technological disciplines. Specifically, nanomaterials such as graphene, nanotubes, metal, and polymer nanoparticles have been gaining significant attention. These nanomaterials (smaller size,  $\leq 100$  nm) exhibits an outstanding physio-chemical property such as higher molar extinction coefficients, superior reactivity, higher absorption and surface area, tunable plasmonic properties, photo and magnetic properties, and quantum effects due to their dimensions. Therefore, over the years several kinds of nanomaterials were successfully explored in various fields. Particularly, significant attention has been drawn in biomedical human health applications, where small molecules therapeutics exhibit serious disadvantages including poor photostabilities, non-biocompatible, side effects to other organs, fast renal clearance, lower retention time in blood fluids, poor targeting ability, and insufficient cellular uptake. Subsequently nanomaterials were developed and explored, even though nanomaterials offer an advantage over the small molecules still the non-controlled therapeutics make them as limiting factor. To overcome the existing challenges, recently stimuli responsive materials have drawn significant attention due to their controlled properties under specific stimuli agents and these materials are often called as smart materials.

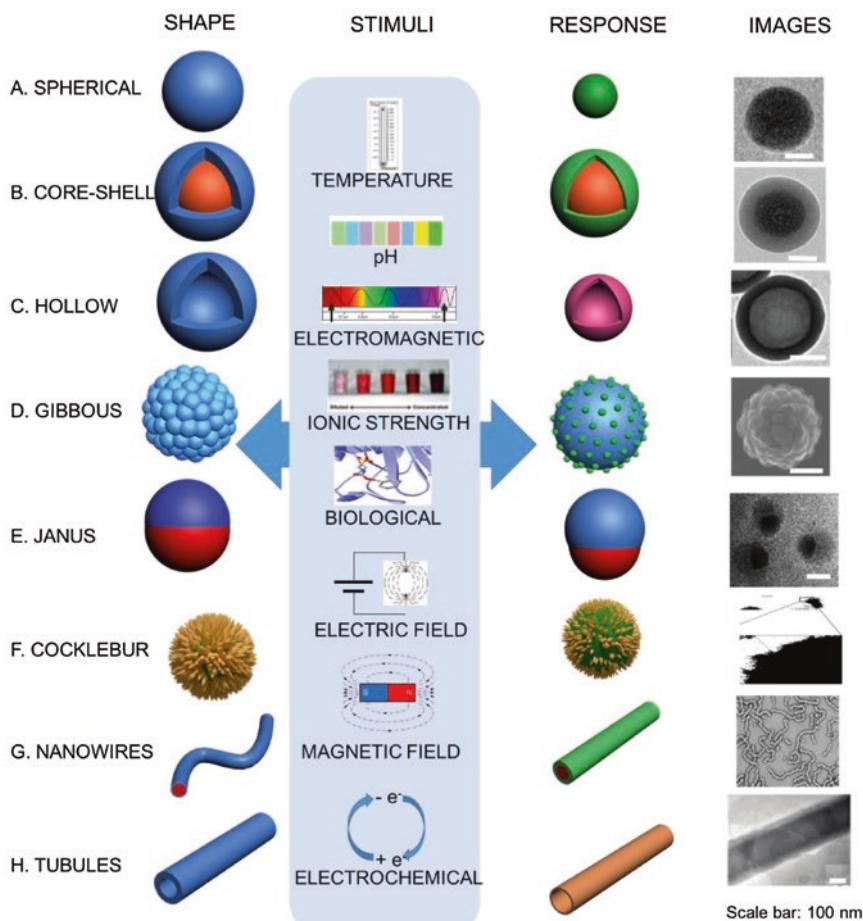
### 15.1.1 What Are Smart Materials

Smart material can be defined as “materials that can change their properties according to the specific stimuli.” In other words, “Materials that can change their shape, density, texture, color, modulus, rigidity, and toughness all on demand in response to specific stimuli.” In earlier days, these smart materials are often defined as a material which can respond in a timely manner to the surrounding environment.



**Fig. 15.1** Schematic (a) various classes of nanomaterials and (b) specific stimulating agents. Reproduced with the permission from Lu et al. (2014)

Thereafter, definition of smart materials has been expanded that the material that can be stimuli by external factors and exhibit a new kind of functional properties. Specific stimuli agents can be either temperature changes, wavelengths of light, pressure, stress, electric, magnetic field, chemical concentration, etc., while output produced can be color, heat, hyperthermia, magnetic, deformation, etc., Fig. 15.1 shows the schematic representation of various nanomaterials and corresponding stimulating agents. Under the specific stimuli, these materials can change their own properties such as changes in size, optical, mechanical properties, surface area, permeability, solubility, shape, among other nanomaterials. Basically, most of the smart materials exhibit five characteristic properties such as immediacy, transiency, self-actuation, directness, and selectivity. The immediacy is nothing but a material that can respond quickly once the stimuli appear, whereas transiency means it can react to more than one environment, and the properties depending on the specific environment. Some materials exhibit its own special internal properties, and which are not induced or produced by external actions is called as Self-actuation. Directness is that the output produced at the point of input given, so this response is local. Finally, selectivity is predictable and repeatable characteristic of the response, so a single environmental state can only lead to a unique and constant response of the material. Figure 15.2 shows the schematic representation of the response of the smart materials under specific stimuli. These results clearly reveal that how a specific morphology of a nanomaterial can change under specific stimulating agents.



**Fig. 15.2** Morphological changes of various nano-objects under specific physical or chemical stimulating agent. Reproduced with the permission from Lu and Urban (2018)

### 15.1.2 Smart Materials vs Common Materials

Unique, controllable and functional properties of smart materials make them fundamentally different from common materials. Most of the common materials exhibit fixed properties and change in the properties might occur by adding the new functional groups. Whereas in smart materials, these properties become viable. The materials can respond to the particular stimuli and eventually exhibit new functional properties. Another beautiful advantage of smart materials is the response that is simple and immediate, whereas response in common materials is complex, time-consuming, and complicated. However, the practical utility of smart materials is not yet studied well. Some advantages and disadvantages of smart materials are listed below.

Advantages	Disadvantages
• High energy density	• Very expensive
• Better durability and reliability	• Very sensible, proper storage needed
• Excellent bandwidth	• Not readily available
• Reduces the production cost	• Proper skill to recognize it among the other materials
• Ability to control the shape and size	• Long-term effects unknown
• Easily cooled with nanofluids	• Dropping people out of the labor
• Extensively used in textile industries	
• Reduces weight of component in mechanical and electrical industries	
• Real time health monitoring	
• Self-repairing if damage occurs	
• Simplified packing	
• Huge volume changes with respect to temperature	

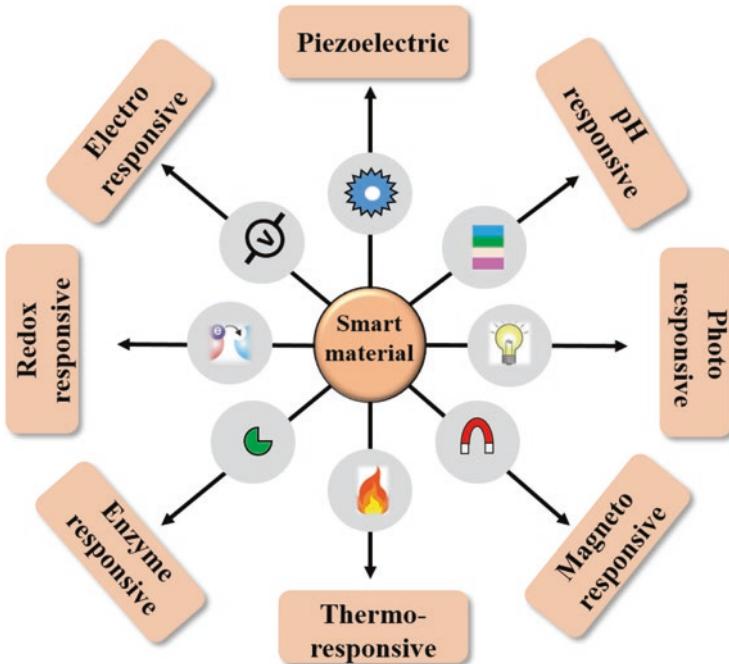
Interesting and controlling properties of smart materials make them different from common materials. Further, these smart materials were classified into various groups according to the specific stimuli agents. The detailed classification is discussed below.

## 15.2 Classification of Smart Nanomaterials Based on Under Specific Stimuli

Smart nanomaterials are classified as various subgroups with respect to the specific stimuli response. Properties of smart materials can be significantly altered by external inputs, such as stress, temperature, moisture, pH, electric and magnetic field in a controlled manner. According to their different properties, different types of smart materials are available and some of them are discussed below: A schematic representation of various types of smart nanomaterials under different external stimuli agents is shown in Fig. 15.3.

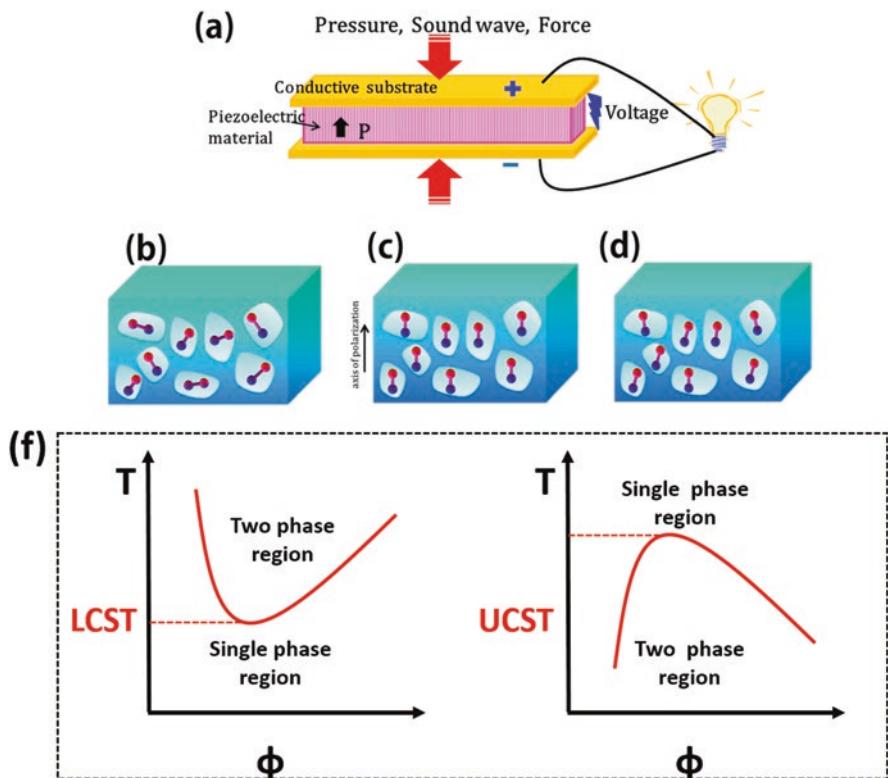
### 15.2.1 Piezoelectric Smart Materials

The word “Piezoelectricity” comes from the Greek words meaning “pressure electricity.” Pierre and Jacques Curie first discovered the effect of piezoelectricity in 1880. They identified the generation of spark from certain materials such as quartz. The piezoelectric material can be defined as “The materials can convert the mechanical energy to electrical energy directly under applied mechanical stress.” This effect is reversible, so the electric charge also can help to generate mechanical stress and vice versa. Under the specific stimuli such as mechanical stress or electric charge, material causes asymmetric nature or changes its shape, respectively. Most



**Fig. 15.3** Classification of smart materials with respect to specific stimuli

predominant materials in which we can observe the piezo-electric effects are non-conductive materials, such as  $\text{SiO}_2$  (Quartz). Besides, ceramics also exhibit excellent piezoelectric effects. Mostly used ceramic piezoelectric material is lead zirconium titanate (PZT). These PZT materials exhibit high piezoelectric coefficients and it is very cheap to manufacture. By the principle of piezoelectricity, when a piezoelectric material is placed under stress, it creates a dipole moment by changing the balance of ions in the crystal structure (Fig. 15.4(a-d)). Most importantly, the net dipole should not be cancelled by other dipoles in the unit cell. To achieve this the atomic structure of piezoelectric crystal should be non-centrosymmetric (Chorsi et al. 2019). Furthermore, controlled properties of piezoelectricity smart nanomaterials can be useful for various industrial applications particularly those related to vibrational generation and actuation. Thereafter, many commercialized products were developed based on piezoelectricity principle that include timekeeping using quartz resonance, microphones, radio antenna oscillators, speakers, hydrophones, and fuel injection (Topolov et al. 2015). Subsequently, later the development of advanced nanotechnology, a wide variety of smart nanomaterials stimuli by piezoelectric property were developed and can be fabricated as thin films, discs, or stacked sheets. However, the serious drawback of these piezoelectric materials is degradation due to the applying of repeated forces on the piezoelectric material. However, applying repeated forces on piezoelectric devices causes degradation



**Fig. 15.4** (a) Working principle of piezoelectric smart material. Polarization ( $P$ ) condition and directions of electrons (b) under without applied forces or strain (c) during the applied force (d) after the applied force removed. (e–f) The working principle of thermoresponsive smart materials of piezoelectric and thermoresponsive materials, respectively. (a–d) Reproduced with the permission from Chandrasekaran et al. (2019)

which is the main limitation so far. Thus, the design of the well-matured piezoelectric materials needs to be highly considered in future applications.

Over the advancement of nanotechnology, smart piezoelectric materials are extensively explored in human health applications. In early 400 B.C, electrotherapy is a commonly used technique in medicine to cure the neurological diseases based on electrical stimulations. As well, torpedo fishes are commonly exploited in reducing or controlling the pain on human body by producing electric shocks (of ~100 to 150 V). Subsequently, the advancement of knowledge and nanotechnology is able to store the electricity in batteries. Therefore, electrical stimulation of tissues gained more attention in the 1800s and development of piezoelectric materials was advanced. Notably, cell and tissues highly responsive to the applied electric fields even inside the cells. Thus, this uniqueness is extremely exciting and opening the new era to develop piezoelectric materials for clinical use. Subsequently, nanostructured piezoelectric interfaces play a vital role in nanomedicine for cell and tissue stimulation. Rather

**Table 15.1** Applications and characteristics of some Piezoelectric materials in biomedical applications

S. no.	Smart material	Characteristics	Application	Ref.
1	Poly(vinylidene fluoride) (PVDF)	Flexible, cheap, respiratory sensor	Monitoring human respiratory conditions	Liu et al. (2017b)
2	PDMS-SWNT	Fast response time, high stability, low detectable pressure	Monitor wrist pulse, muscle movement	Wang et al. (2014)
3	FSKNG	Highly durable, ultrasensitive, fast response	Monitor arterial pulses, vocal cord vibration, and gentle wrist movements	Ghosh and Mandal (2017)
4	Collagen films	Biocompatible, biodegradable protein	Temperature sensor, strain gauge, etc.	Moreno et al. (2015)
5	rGO/PVDF microdome array	Tactile sensitivities ultra-minute detections	Dynamic pressure sensor, temperature sensor	Park et al. (2015)
6	Fibrous scaffolds	Promoting chondrogenic and osteogenic differentiation	Human mesenchymal stem cell differentiation and tissue formation	Damaraju et al. (2017)
7	Boron nitride nanotube	Superior resistance to chemical and temperature	Excitation of neuron-like cells	Ciofani et al. (2010)
8	Barium titanate NPs	Biocompatibility, high dielectric constant	Stimulation of SH-SY5Y-derived neurons	Marino et al. (2015)
9	PZT nanoribbons	Minimally invasive, sensitive, scalable platform	To detect minute cellular deformations	Nguyen et al. (2012)
10	Enzyme/ZnO nanoarrays	Real time, sensitive	Biosensing	Han et al. (2017)

than passive structural units or carriers for medications, smart properties of piezoelectric devices are gaining more attention. Besides, stretchable, flexible, and cost-effective wearable e-skins of Piezoelectric smart materials are promising tools for the prevention of illnesses, health monitoring, Human physiological monitoring, and the prediction of early diseases. In addition, piezoelectric materials also can be used to promote disease healing by systematic studies of these materials on body. At the present stage, exact reason and mechanism of piezoelectric materials are not yet fully discovered but studies into the bone related injuries show that induced electric fields can accelerate the bone repair and growth of neurons (Rajabi et al. 2015). Table 15.1 shows the utilization of some piezoelectric polymer smart nanomaterials in various biomedical applications. “Despite the impressive potential of piezoelectric nanostructures in biomedical field, further research efforts are still necessary for the evaluation of the nanomaterial biocompatibility, retention, degradability, accumulation in complex *in vivo* systems before actual exploitation in clinical context.”

### 15.2.2 Electrochromic and Photochromic Smart Materials

At first, electrochromism is the phenomenon where the color or opacity of a material changes when a voltage is applied. Mainly reversible change in optical properties (such as absorbance, reflectance, transmittance, etc.) of material was observed under applied voltage. Since the discovery of electrochromism (EC), synthesis of EC materials gained considerable progress and the principle mechanism is as follows. It mainly involves the ions insertion/extraction into/out of EC materials. As expected, an efficient ion insertion/extraction process takes place in nanostructured materials due to the larger surface area. Therefore, several organic, inorganic, and polymer materials exhibit electrochromism properties which include viologens (Cinnsealach et al. 1998), polyaniline (PANI) (Gospodinova and Terlemezian 1998), organic polymers and metal oxides materials (Yoo et al. 2007; Wu and Yang 2007; Cheng et al. 2006). In inorganic materials, cathodic coloration under negative potentials and bleaching states under positive potentials were observed ( $\text{WO}_3$ ,  $\text{TiO}_2$ ,  $\text{V}_2\text{O}_5$  films, etc.). It is mainly due to the charge balancing ions ( $\text{H}^+$ ,  $\text{Li}^+$ ,  $\text{Na}^+$ ,  $\text{K}^+$  ions) and insertion/extraction of electrons accompanied by the reduction/oxidation reactions (Niklasson and Granqvist 2007). Whereas coloration under positive potentials was observed in case of nickel iridium oxide. Further, unique advantages of EC materials have been extended in various interesting applications that include anti-glare mirrors, smart windows, displays, and active camouflages (Wang et al. 2010). Among all, application of EC materials in smart windows stands as a potential application where they can save energy and provide indoor comfort by reversible color changes. These electrochromic materials in a biomedical application are rarely reported.

In a similar way like electrochromism, photochromism (PC) can be defined as color change of materials that occur under photon energy. Notably, these photochromic materials exhibit a reversible change in their color with respect to the specific photon energy. It was discovered in the late 1880s by Markwald and they discovered in reversible change of color of 2,3,4,4-tetrachloronaphthalen-1(4H)-one in the solid state. In the early days this new phenomenon labeled as phototropy and this name was used until the 1950s. Subsequently, the name was changed to photochromism. Photochromism can take place in both organic, inorganic compounds and also in biological systems. Owing to its phenomenal properties of photochromic materials, further explored in sensors and fast optical shutters applications. In health applications, PC materials are extensively used in ophthalmic sun screening applications and in UV light protection glasses. As well, in cooling glasses these PC materials were used and when exposed to sunlight these materials turn to darken and reverse back to colorless for personal comfort and safety (Christie 2013).

### 15.2.3 Thermoresponsive Smart Materials

Thermoresponsive polymers are a class of “smart” materials that can respond to a change in temperature; a property that makes them useful materials in a wide range

of applications and consequently attracts much scientific interest. The theory of thermoresponsive polymer (similarly, microgels) began in the 1940s with work from Flory and Huggins who both independently produced similar theoretical expectations for polymer in solution with varying temperature.

The main principle involved in the thermoresponsive materials are as follows and these types of thermoresponsive properties are mostly observed in polymeric materials. According to the principle, there are two main types of polymers such as lower critical solution temperature (LCST) and the second one is an upper critical solution temperature (UCST). In fact, the solubility of polymer in aqueous solution depends on several factors such as temperature, molecular weight, co-solvent, etc. As shown in Fig. 15.4(f), we can identify the critical solution temperature easily if the phase diagram of polymer mixture vs temperature exhibits the one- or two-phase region. Critical temperature points of LCST and UCST are shown below and above, respectively, in where the solvent and polymer are completely miscible. So therefore, if the polymer solution below the LCST is a clear and homogeneous solution whereas above the LCST is cloudy (which also called cloud point). It mainly happens because it is energetically favorable (Ward and Georgiou 2011). Importantly, the main reason for favorable phase separation by increasing the temperature is mainly because of entropy of the system according to the Gibbs equation  $\Delta G = \Delta H - T\Delta S$  ( $G$ : Gibbs free energy,  $H$ : enthalpy, and  $S$ : entropy).

As a proof of concept, several synthetic methodologies are successfully employed to produce various kinds of thermoresponsive smart materials that include free radical copolymerization, end-group functionalization, sol-gel transition phase method, copolymerization, self-assembly method, photopolymerization, etc. (Kim and Matsunaga 2017). By utilizing the unique advantages of smart materials, further explored in biomedical applications such as drug delivery, gene delivery and tissue engineering, etc. In drug delivery, numerous polymer/nanomaterials-based platforms were successfully utilized in the delivery of cancer drugs on the target site to achieve a massive therapeutic effect. The main key factors in sustainable delivery of drugs are needed to deliver drugs on right time, at the right area, and at right concentrations where many systems have a serious problem to achieve this. The main problems associated with the existing systems are enzyme degradations, low solubility of drugs, non-specific toxicity, fast clearance rates from the body, and inability to cross the biological barriers (Juillerat-Jeanneret 2008). To overcome this obstacle several nanocarriers-based polymers have been used (Liechty et al. 2010). However, it is very difficult to control the drug release rate on that specific nanocarriers because the concentration of drug on target site is either lower or higher. Subsequently, controlled properties of the thermoresponsive smart materials are used to deliver the drugs. These smart carriers can be able to deliver the drugs at a right time and right concentration by simply controlling with external stimuli. Table 15.2 shows some thermoresponsive smart materials in biomedical applications. On the other hand, Gene therapy aims at the treatment of many genetic diseases by correcting the defective genes that are responsible for diseases. To this end, several strategies have been employed to deliver the specific genes such as viruses, polymer carriers, etc. Even though having its own merits, serious stability issues

**Table 15.2** Applications and characteristics of some thermoresponsive materials in biomedical applications

S. no.	Smart material	Characteristics	Application	Ref.
1	PCEC	High gel strength, slow degradation	Drug release	Zhou et al. (2017)
2	PNIPAM-PDMA-PAA	Tunable properties	Drug delivery	Chen et al. (2018)
3	AuNPs-PF127-HPMC	Extended release, biodegradability, excellent safety profile	Drug delivery, photothermal platform, skin wound healing	Arafa et al. (2018)
4	PEO-PPO-PEO	Solubility, stability, release, and bioavailability of drugs	Sustained release of drugs, oral drug delivery. Release of nitric oxide for accelerating wound healing	Liu et al. (2017a)
5	Poly(NAGA-co-BA)	Loading of doxorubicin and IgG	Drug delivery, bioimaging	Hui et al. (2016)
6	Pluronic	Injectable, thermos responsive	Drug delivery	Jung et al. (2017)
7	NiPAAm	Injectable, long-term absorption, multifunctional sensing	Biomolecule carriers wound healing, sensing, imaging	Pentlavalli et al. (2017), Wu et al. (2018), Wang et al. (2011)
8	Poly( <i>N</i> -isopropylacrylamide) s (PNIPAAm)	Biocompatibility, low levels of toxicity	Oral and insulin delivery, hemostasis	Gandhi et al. (2015)
9	TMC-g-PNIPAAm	Minimal cytotoxicity	Gene carrier	Mao et al. (2007)
10	PDMAPAAm–PNIPAM 4	High payloads of DNA	Gene transfection	Zhou et al. (2007)

of polymers makes them as a limited for practical applications. So therefore, developing a new carrier systems for successful gene delivery is highly desired. Interestingly, thermoresponsive polymers stand as potential candidates to enhance the gene transfection efficiency by changing the temperature either during the complexation and/or during the incubation or transfection period defective genes that are responsible for these genetic diseases. In particular, some studies were revealed that polymer based PNIPAAm, linear and branched NIPAAm, DMAEMA, and PEI polymers exhibits better and enhanced transfection efficiency. As well, tissue engineering is an emerging topic towards restoring or improvement of tissue function by developing the biological substitutes (Sponchioni et al. 2019). Thermoresponsive smart polymer materials are commonly used in tissue engineering to enable cell growth and proliferation. Especially, these thermoresponsive materials can facilitate cell–cell interactions

and further facilitate their use for regenerative therapies by creating thick tissues with stacking methods. Whereas three-dimensional thermoresponsive platforms give the opportunity to control tissue geometries in a 3-D manner by mimicking the native tissue architecture and enable their further retrieval. It is still challenging to preserve their functionalities for a long time (Tekin et al. 2011). Table 15.2 shows the utilization of some thermoresponsive polymer smart nanomaterials in various biomedical applications.

#### 15.2.4 pH Dependent Smart Materials

pH stimuli smart materials can be defined as the material which can respond to the pH and exhibit new functional properties. Over the last decade, tremendous efforts have been made in the development of pH-responsive biomaterials along with other stimuli (temperature, etc.) responsive materials for targeted site-specific therapeutic applications. In general, different components of human body have different pH levels, for example, the pH of saliva is 6.5–7.5; whereas upper and lower parts of stomach have pH values of 4–6.5 and 1.5–4.0, respectively. Also, pathological state exhibits abnormal pH values compared to physiological state pH, for instance, bacterial infections contain acidic pus with pH of 6.0–6.6, the tumor microenvironment exhibits a lower extracellular pH in the range of 6.5–6.9, and an inflamed tissue exhibits pH in the range of 6–7. Based on these unique pH variations, various pH-responsive materials have been developed so far. As a proof of concept, several pH-responsive materials can be synthesized by incorporating (a) protonatable groups (amino, sulfonates, imidazolyl, and carboxyl groups) containing polymers such as polysaccharides (e.g., chitosan, etc.) and polypeptides (e.g., poly(*l*-histidine, etc.) that undergo pH-responsive solubility and/or conformational changes or (b) polymers carrying acid labile bonds (e.g., hydrazine, imine, oxime, ketal, acetal, orthoester, thiopropionate, vinyl ester, etc.) whose cleavage allows the release of molecules or the modification of the surface charge.

Several pH dependent polymers were addressed. For example, Chitosan, a polysaccharide with many primary amino groups in its polymeric backbone, is widely used for the development of pH-responsive biomaterials (Sultankulov et al. 2019). It possesses amino groups with pKa of 6.5; therefore, chitosan and its-derivative based biomaterials exhibit positive surface charge under pathological milieu (acidic condition) due to protonation of amino groups and neutral/negative surface charge in physiological milieu (neutral pH). Under pathological conditions, the positively charged chitosan materials readily adhere to negatively charged cell surfaces through electrostatic interactions so they can be used to target and provide site-specific therapeutic efficacy thereby excrete possible adverse effects. Recently, Yan et al. developed a pH-responsive glycol chitosan coated liposome system for tumor specific drug delivery. The coated liposomes exhibited negative-to-positive charge reversion from pH 7.4 to pH 6.5 which mediated cellular uptake, and thus higher antitumor efficiency was observed due to the more accumulation of DOX in the tumor cells compared to the free drug or conventional liposomes (Yan et al. 2015).

More recently, polyaniline conjugated glycol chitosan nanoparticles were developed to cause aggregation of bacteria in pathological milieu and by this means confine therapeutic effect to the focal infection (Korupalli et al. 2017). Another example, Poly-*l*-histidine (PLH), a pH-responsive imidazole groups ( $pK_a = 6.0\text{--}6.5$ ) containing cationic polypeptide, has also been widely used in the design of pH-responsive materials for drug delivery and other biomedical applications (Jiang et al. 2012). Under acidic conditions the protonated imidazole moiety leads to the phase transition from lipophilic to hydrophilic and causes the release of encapsulated drugs via the destabilization of PLH containing polymeric nanocarriers. Otherwise, the positively charged PLH produces a positive surface charge on the surface of PLH based materials and facilitates strong interactions with the negatively charged cell walls/tissues through electrostatic-mediated binding. The aforementioned advantages of PLH guided to the development of pH-responsive surface charge switching poly(*d,l*-lactic-co-glycolic acid)-*b*-PLH-*b*-poly(ethylene glycol) (PLGA-PLH-PEG) micelle nanoparticles for bacteria targeting antibiotic drug delivery. The results of NP binding studies demonstrated that PLGA-PLH-PEG NPs adhered to bacteria under conditions of acidity (Radovic-Moreno et al. 2012).

Likewise, insertion of acid labile chemical bonds in the polymer is another strategy to design pH-responsive materials. The cleavage of the chemical bonds under pathological acidic milieu has gained much attention in biomedical applications particularly in the field of the drug delivery systems for targeted and site-specific drug delivery (Kanamala et al. 2016). The rate-determining step for pH-responsive bond cleavage is acid-catalyzed hydrolysis, which can be modulated by choosing appropriate linkers. The most used pH-sensitive linkers for this purpose include imine, hydrazone, Polyacetal and polyketal, oxime, amide, ether, and orthoester bonds. In one of the studies, Yang et al. synthesized pH-responsive polymeric conjugates by covalently bonding between PEG with  $4\beta$ -aminopodophyllotoxin (NPOD) via imine linkage for tumor cell specific paclitaxel delivery (Kang et al. 2014). The mPEG-NPOD conjugates exhibited faster release of NPOD under acidic conditions compared to physiological pH conditions. Sun et al. conjugated Doxorubicin (DOX) to gold nanoparticles via hydrazone bonds to facilitate pH-responsive DOX release in tumor cells (Sun et al. 2014). In vitro studies demonstrated that the acid labile bond cleavage enabled the intracellular DOX release to cancer cells. Tomlinson et al. synthesized amino-functionalized linear pH-sensitive polyacetals to form water soluble polyacetal-DOX conjugates (Tomlinson et al. 2003). In vivo biodistribution studies in mice bearing tumors confirmed two-fold enhancement in the half-life of polyacetal-DOX conjugates compared to control (HPMA-DOX conjugates). These pH-sensitive conjugates not only exhibited less deposition of DOX in the liver and spleen and increased accumulation in tumor.

Following concerns are necessary to improve the better pH dependent smart nanomaterials for an efficient therapeutics and diagnosis, as follows:

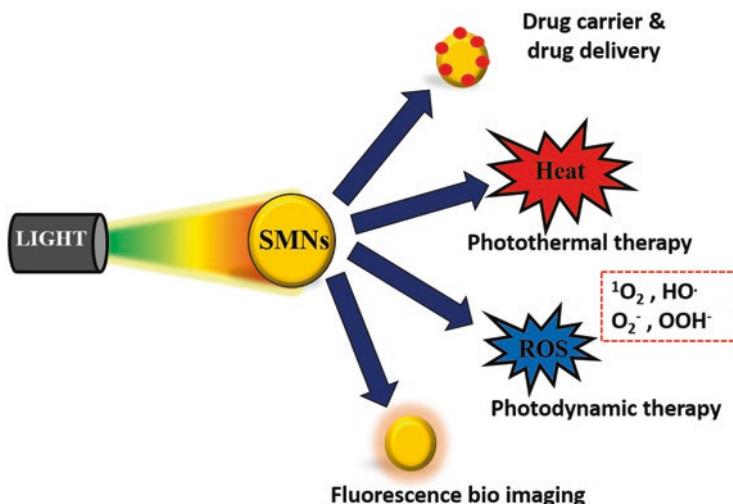
1. Appropriate sizes of nanocarriers are necessary for enhancing the retention time, avoiding undesirable clearance and accumulation in tumor specific cells via EPR effect.

**Table 15.3** Applications and characteristics of some pH dependent materials in biomedical applications

S. no.	Smart material	Characteristics	Application	Ref.
1	HyUPS nanotransistors	Multi-pH sensing capability	Receptor-mediated endocytosis in tumor cells	Wang et al. (2017)
2	LDH-ZnPcS8 nanohybrid	Photosensitizer delivery, minimal phototoxicity	PDT	Li et al. (2017)
3	AuNPs	Fast, ultrasensitive, quick aggregation, more uptake	PTT	Liu et al. (2013)
4	PDPA-b-PAMA/ SA	Targeting, high retention	Drug release	Wu et al. (2014b)
5	MelNP	Physical aggregation	Photoacoustic (PA) imaging	Ju et al. (2016)
6	PPC-Hyd-DOX-DA	Dual pH sensor	Drug delivery	Du et al. (2011)
7	PDPA-b-PAMA/ DMMA	Rapid cell endocytosis, long blood circulation, targeting	Drug release	Wu et al. (2014a)
8	LNPde <sub>PEG-FA</sub>	Nucleus targeting	Drug delivery	Fan et al. (2016)
9	PEG[(PLG/PEI)/ DNA]	Dual charge/size, long retention	Gene delivery	Guan et al. (2016)
10	cRGD-PCM/DOX NPs	Quick drug release, long circulation, targeting	Drug delivery	Liu et al. (2016b)

2. In some of the tumors such as CT26 colon tumor which has high permeability nature so that pH-responsive aggregation-based nanocarriers in slightly acidic pH tumor environment can promote more uptake.
3. Whereas for poorly permeable tumors such as the BXPC-3 pancreatic tumor, cellular uptake can be accelerated by using pH dependent size shrinkage nanocarriers via escaping from lysosomes and endosomes to deliver the drug to nucleus.
4. After successful internalization of nanocarriers by tumor cells, required dose of drug need to be released from pH-responsive nanocarrier to induce the cell apoptosis.
5. Programmable size changes of pH-dependent nanocarriers are useful for tumor imaging and diagnosis.

Utilization of some pH-responsive smart nanomaterials in various biomedical applications is shown in Table 15.3. Even though significant efforts have been made in pH-responsive materials still some challenges exist. Some of the challenges are poor penetration in deep tumors, nano carrier's aggregation in tumor microenvironment, varying tumor vascular leakage by changing pore diameter, unpredictable tumor environment due to the rapid mutations, etc.



**Fig. 15.5** Mechanism of light responsive smart nanomaterials

### 15.2.5 Photoresponsive Smart Materials

Photoresponsive smart materials can be defined as materials that can respond to the external light. Specifically, controlled properties and non-invasiveness of photo-triggered smart nanomaterials are playing very important role to achieve efficient therapeutics. Whereas other internal stimuli such as temperature, pH dependent, enzyme activity, glutathione concentration have some serious limitations in tumor biology. For example, most of the pH in tumor environment is 6.5 so that we cannot use beyond that and high temperature is 40–42 °C, overexpression of antioxidants as well as various enzyme overexpression (e.g., cathepsin, matrix metalloproteinases, plasminogen activators) compared to normal tissue can effectively minimize the therapeutic effects. To overcome these limitations, external stimuli agents such as ultrasound, magnetic field, and light responsive drug delivery systems stand as a potential candidate. To the proof of concept, many drug delivery systems were successfully utilized and proved that can effectively control the drug release by external stimuli when compared to internal stimuli (Raza et al. 2019). Among the all external stimuli, light is considered as an attractive external stimulus due to its tunable and controllable properties. In addition, light responsive smart materials also can be used as a biomarker to track the targeting ability, location of drugs, and visualize the tumors by optical imaging techniques. As shown in Fig. 15.5, there are three main types of photo-triggered theranostics, namely photoactivation of chemotherapeutics, photodynamic, and photothermal activation. First, photoactivation of small molecule to deliver the drugs to cure the non-curable diseases is a fascinating challenge. However, difficulties in the synthesis of drug carriers, non-controlled drug release and inter-individual variability as a limiting factor in many existing drug delivery systems(Liu et al. 2016a). To conquer the problems associated with the

drug delivery systems, light mediated chemotherapeutics by using smart nanomaterials such as polymers/nanomaterials is an alternative way where we can control the drug delivery on target sites and can be able to deliver the drugs with right concentration in a right time. Subsequently, several light responsive smart materials were successfully employed for drug delivery but unfortunately most of them respond to UV light which has few disadvantages like poor tissue penetration and harmful to normal tissues. Therfore, UV light responsive drug delivery systems are not useful for practical applications. To overcome the limitations, further NIR light responsive smart materials for drug delivery systems were developed which have deep tissue penetration due to lesser attenuation and also safer for tissues. Second, photodynamic therapy uses light and photoabsorbing chemical substance to produce the reactive oxygen species such as peroxide, hydroxyl, singlet oxygen, etc. which can effectively stimulate the cellular mediations to kill cancer/bacteria cell. Noticeably, photodynamic therapeutic systems require low levels of laser powers to generate the cytotoxic reactive oxygen species at interested area/regions is pivotal for its efficiency. In a conventional way, many photodynamic systems were successfully utilized the organic photosensitizers (such as Porphyrin, chlorin Ce<sub>6</sub>, etc.) to mediate the photodynamic effects but unfortunately photobleaching, poor solubility, lower molar extinction coefficients make limiting factors (Lan et al. 2019). As well, most of the photosensitizers are only activated in UV/vis region which may cause the potential light toxicity and limited tissue penetration. To overcome these challenges, photoresponsive smart nanomaterials drag a significant attention where these materials can effectively generate the reactive oxygen species, good water solubility, targeting ability as well as near infrared light activation makes them as potential agents to overcome deep tissue problems (Vankayala and Hwang 2018). Third, photothermal therapy is killing of cancer/bacteria cells by local hyperthermia generated on light activated photoabsorbing materials. In biological environments, the generated overheated media may cause several hazardous effects such as protein aggregation and denaturation, evaporation of cytosol, and cell lysis for living cells. As mentioned earlier, most of the recent phototherapeutic platforms effectively utilize the NIR responsive smart nanomaterials/polymers to overcome the drawbacks associated with photosensitizers as well as UV/vis light responsive fluorophores (Thangudu et al. 2020). Besides, recently there are many efforts made to visualize the deep tissue cancer tumors by NIR fluorescence imaging in a broad biological window region where several NIR fluorophores and nanomaterials were explored and still exploring rapidly (Deng et al. 2018). Table 15.4 shows the utilization of some photoresponsive smart nanomaterials in various biomedical applications.

In the future, in order to achieve an efficient phototherapeutic platform on nanomaterials-based systems, following requirements are very important to create a better therapeutic platform:

1. Light absorption: Particularly in phototherapeutic platforms, photocatalyst should absorb light absorption cross sections in broad biological window wave-

**Table 15.4** Applications and characteristics of some photoresponsive smart materials in biomedical applications

S. no.	Smart material	Characteristics	Application	Ref.
1	Ce <sub>6</sub> -MNPs	ROS species under 632 nm light	In vivo PDT	Huang et al. (2011)
2	Poly-Ru	ROS species under 660 nm	In vivo PDT and Photochemotherapy	Sun et al. (2017)
3	DPP-TI, DPP-TIH and DPP-r-TI.	ROS and heat generation under 660 nm	In vivo PDT and PTT	Yang et al. (2017)
4	Ultrathin black phosphorus nanosheets	ROS under 660 nm	In vivo PDT	Wang et al. (2015)
5	NaYF <sub>4</sub> :Yb, Er@ mSiO <sub>2</sub> @ Fe <sub>3</sub> O <sub>4</sub> -PEG NPs	Fluorescence imaging under 980 nm, drug carrier	In vivo bioimaging drug release	Liu et al. (2015)
6	EuGdOx@MSF	ROS, drug carrier under 980 nm	In vivo PDT, drug delivery, fluorescence imaging	Kalluru et al. (2016)
7	Copper sulfide	Heat under NIR lasers	In vivo PTT	Marin et al. (2018)
8	SWCNTs	Imaging under 785 nm	In vivo NIR fluorescence imaging	Yudasaka et al. (2017)
9	Au NEs	ROS and heat under 808, 980, 1064 nm	In vivo PTT and PDT	Vijayaraghavan et al. (2014)
10	Polymers	Heat under NIR lasers	In vivo PTT	Pierini et al. (2018)

length regions. Therefore, it would facilitate the large light to heat conversion efficiencies (Albota et al. 1998).

2. Low toxicity: Nanomaterials should not be toxic to the healthy cells. This is necessary to achieve selective treatments with minimum side effects (Teeguarden et al. 2006).
3. Easy functionalization: Surface functionalization is necessary to improve the cancer specific cell targeting which allows accumulation of high load of nanomaterials drug to improve the phototherapeutics (Nikolic et al. 2006).
4. Biocompatibility: It is necessary to minimize the side effects on other organs. So the nanomaterials must have long circulation times and high retention time in the bloodstream (Choi et al. 2006).

### 15.2.6 Electroresponsive Smart Materials

Electroresponsive materials are materials that respond to an applied electric field by changing their size or shape. These new class of materials attracted rapidly due to their potential applications in various fields such as sensors, optical systems,

actuators, robotics, artificial muscles, drug delivery, and energy harvesting applications. Briefly, electroresponsive materials were divided into two types mainly. First one is the ionic electroresponsive materials in which mobility of ions induced by electric field to create a change in the local concentration of the ions in materials or solution. It mainly occurs in conductive polymers, ion polymers, and polymer-metal composites. Second one includes the dielectric elastomers and electrostrictive polymers. Particularly in biomedical applications, controlled release of drugs and accumulation on electroresponsive nanoparticle drug carries such as polymers and microgels are interesting. For example, electroresponsive materials were assembled with poly(ethylene imine) with a ferrocene end-group and encapsulated pyrene. On applying a small electric field, oxidation of ferrocene and further hydrophobic nature to hydrophilic nature transition results from the release of encapsulated drug (Sun et al. 2013). The unique redox properties of the electroresponsive material can be a potential candidate for an electrocontrolled release of drugs to treat the various diseases. Importantly, release of drugs can control the erosion of gels and electrochemical treatment. Even though having its own merits, relatively high voltage and a long-time electrical potential treatment limit their applications.

### 15.2.7 Magnetoresponsive Smart Materials

The development of magnetic nanomaterials has been proved to be extremely beneficial advantages in whole industrial and commercial applications such as photonic, electronic devices, magnetic storage, and biomedical theranostics. It can be defined as “Magnetoresponsive materials are materials that can respond to an applied magnetic field as a stimuli agent.” Particularly, magnetic NPs (MNPs) are an emerging platform and paying more attention for their magnetic responsive based applications in biomedical field such as magnetic hyperthermia, magnetic resonance imaging (MRI) and magnetic guided drug delivery owing to its unique intrinsic chemical and physical properties. These magnetic responsive materials have been successfully synthesized by various techniques includes hydrothermal process, co-precipitation method, chemical vapor deposition, combustion, carbon arc, laser pyrolysis, electrochemical synthesis, high temperature thermal decomposition, microbial synthesis, etc. (Cardoso et al. 2018). First in magnetic hyperthermia, traditionally thermotherapy used to kill the cancer cells by increasing the local region/whole body temperature to 42–45 °C using microwaves, ultrasounds. Other way is by thermal ablation, applying a temperature above 45 °C to the diseased area. However, these traditional methods suffer from poor targeting ability and deep tissue penetration (Habash et al. 2007). Subsequently, magnetic NPs mediated hyperthermia for targeting the specific cancers have been developed (Mahmoudi et al. 2018). Where the magnetic properties of the MNPs in the fluid take the advantages of applied magnetic field and efficiently convert into heat. Even though development of promising magnetic hyperthermia therapies for treatment of cancer but still in infancy and more preclinical and clinical developments needed for before the practical use. Second, Magnetic Resonance Imaging (MRI) is a versatile tool and

useful in clinical diagnostics for many diseases which offers a real time spatial resolution and higher contrast of soft tissues without side effects. The principle relies on alignment of protons within a sample under applied external magnetic field. Clinically Gadolinium based chelating agents are extensively utilized as a MRI contrast agent to visualize the tumor diagnosis (Shen et al. 2015). However, economic, high contrast ability agents needed to replace the existing agents. Therefore, to further improve the signal to background noise ratio, various T1 weighted and T2 weighted contrast agents were developed. For example, Several MNPs such as gadolinium, iron, and non-iron based NPs systems were successfully employed as a MRI contrast agent (Perlman and Azhari 2018). Despite the several advantages of MRI, delayed imaging rate and accuracy, sensitivity and toxicity in some contrast media is still a serious issues. Therefore, more attention and efforts are required to improve the current MRI technologies for future biomedical applications. Third in magnetic induced drug delivery, several magnetic NPs were successfully utilized in controlled release of drugs to target tissue. In the future, the following requirements are very important to develop a magnetoresponsive material for the better therapeutic platform:

1. Degradation of some Nobel metal contrast agent's results decreases the image quality. Therefore, stable nanomaterials need to be developed.
2. High contrast ability with less cytotoxicity materials should be needed
3. None of the Radiation techniques are specific to tumors alone which may cause critical damage to other organs
4. In addition, advancement of the instrumentation that is capable to enhance and control the magnetic field is highly needed to increase the utility of magnetic responsive smart nanomaterials.

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### 15.3 Current Trends in Smart Nanomaterials

As discussed in the chapter, significant advances achieved on design and synthesis of various smart materials with respective to specific stimuli but still some concerns need to be improved for better future applications. First, serious drawback of piezoelectric materials is degradation due to the applying of repeated forces on the piezoelectric material. Thus, design of the well matured piezoelectric materials needs to be highly considered in future applications. As well, future thermoresponsive smart materials could be applied into chronic diseases such as diabetes which needed regular doses of drug. Programmable size changes of pH-dependent nanocarriers are useful for tumor imaging and diagnosis in future biomedical applications. In photoresponsive smart materials, broad biological window region NIR absorption photoresponsive smart nanomaterials are needed to develop for deep tumor therapeutics and fluorescence imaging. Over the advancement of smart materials, recently dual or multiresponsive stimuli smart materials for efficient therapeutics have gained significant attention. As compared to single stimuli responsive smart materials, multi-stimuli responsive materials offer more functions and finer

**Table 15.5** Dual or multistimuli responsive smart materials and applications

S1. No.	Smart material	Stimuli response	Application	Ref.
1	PLA-g-P(NIPAAm-co-MAA) NPs	pH and thermo	Drug carriers and release	Lo et al. (2005)
2	DS-g-PEG/cRGD nanoparticles	pH and redox	Nucleus targeted drug delivery	K. C et al. (2012)
3	Fe <sub>3</sub> O <sub>4</sub> -capped MSNs	pH and magnetic	Drug delivery	Gan et al. (2011)
4	DNA-capped MSNs	T and enzyme	Controlled drug delivery	Chen et al. (2011)
5	Azo-PDMAEMA	Light/pH/T	Controlled drug release	Tang et al. (2010)
6	PMAAS-S@Fe <sub>3</sub> O <sub>4</sub> microcontainers	pH/redox/magnetic	Drug carrier, hyperthermia, imaging	Bilalis et al. (2012)
7	P(NIPAAm-co-MAA) coated magnetic MSNs	T/pH/magnetic	Drug delivery	Chang et al. (2011)
8	S-NPs@DOX	pH/redox/T	Drug delivery	Yu et al. (2018)
9	MFNPs	pH/magnetic,	Targeting, drug delivery, MR imaging	Bhattacharya et al. (2016)
10	rGO-PDA Nano sheets	Light /pH	Drug delivery, phototherapy	Jiang et al. (2018)

modulations can be achieved through more parameters. As a result, utilization of some dual and multistimuli responsive smart nanomaterials in various biomedical applications were employed with effective therapeutic outcomes (Table 15.5). Importantly, clearance and accumulation of nanomaterials in non-targeted locations such as liver, kidney, and spleen are major problems in therapeutic diagnostics. It is mainly due to the fact that most of the nanomaterials fail to adequately overcome biological barriers. Therefore, more focus should be needed to develop nanomaterials to overcome the biological barriers efficiently to minimize the side effects of nanodrugs. Overall, smart, flexible, efficient and multiresponsive stimuli on a single site smart nanomaterial (all in one platform) are highly interested in future applications.

## 15.4 Conclusions

In conclusion, overall significant efforts have been achieved in the design and synthesis of the various kind of smart nanomaterials for biomedical applications such as drug delivery, bioimaging of cancer specific cells, tissue engineering and cancer, bacteria therapeutics, etc. Present smart materials can offer beautiful abilities like controlled therapeutics by tuning the specific stimuli agents to enhance the therapeutic efficiencies. To further improve the disease diagnosis dual or multiresponsive smart materials are paying more attention recently where we can trigger the multiple functions on a single smart nanomaterial. As well, in the present chapter we also

discussed the advantages and limitations of smart nanomaterials in biological applications which helps to construct the novel, stable, and efficient smart materials for future applications. Finally, we strongly envision that present discussions in this chapter will help to understand the abilities of various kinds of smart nanomaterials and develop a more advanced smart material platform for future biomedical applications for better human health.

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