MODULE-2

Nanoparticles in bone substitutes

Bone undergoes self-repair of small defects due to the synergistic actions of mesenchymal cells, osteogenic cells, and cells of the immune system

This self-repaired bone contains physico-chemical and mechanical properties that recapitulate the bone which was replaced. However, larger defects are unable to undergo the same level of self-healing, and **regenerative medicine** approaches are paramount in addressing these clinical challenges. Tissue engineering and regenerative medicine (TERM) aims to conjugate engineering and biological properties to create functional substitutes for damaged and diseased tissues. The strategy used on TERM research combines three essential elements - scaffolds, stem cells, and growth factors - to produce a tissue engineered construct

Tissue engineering

Tissue engineering is a process wherein fibroblasts (Fibroblasts secrete collagen proteins that **are used to maintain a structural framework for many tissues)** are extracted from a patient and then used to artificially grow cells and tissues in a lab setting using the principles of the body's own tissue growth.

This is done using "tissue scaffolds" which encourage cells to develop in a certain way, such as influencing them to grow into bio-artificial bone marrow when implanted under a patient's skin.

Using tissue engineering, scientists have been able to create a wide variety of artificial organs including bladders, pancreases, livers, and even whole bones.

Regenerative medicine

Regenerative medicine, which RegenerVate specializes in, is more focused on using **stem cell regenerative therapy** to kick start the patient's body's healing processes with their own stem cells, rather than utilizing lab-grown replacements and tissue scaffolds.

This natural process is less about replacing non-functioning tissue by growing new cells, and more about stimulating the body to replace them itself using stem cell therapy and stem cell treatments

While the body naturally loses its ability to replicate cells and replace tissue as it ages, the introduction of stem cell regenerative therapy and treatment effectively "turns back the clock" on those repair functions, thus helping the body heal as it did in youth.

Scaffolds provide the support for cell growth and tissue formation. For that, they are seeded with stem cells. Growth factors are also included as they regulate the differentiation and

proliferation processes. Bone tissue regeneration is one of the greatest challenges for TERM. The anatomical complexity of bone, allied with the high mechanical stress to which it is exposed, makes it unique, and almost impossible to replicate.

Nanotechnology has made it possible to create structures within the same size as those that constitute naturally occurring bone, opening a new era for TERM. Hence, nanoparticles (NPs) can be used to modify scaffolds properties, leading to enhanced characteristics such as superior mechanical properties and osteointegration, osteoconduction, and osteoinduction. Moreover, NPs can be applied to deliver drugs in a controlled and dependent manner, either systemically or locally. In another approach, NPs can be used to label cells, namely stem cells, enabling the continuous cell tracking and monitoring of its fate. Antibodies, labeling probes, hydrophobic or hydrophilic molecules, DNA, and/or igonucleotides are some of the molecules that can be linked to NPs, allowing a tailored application for the desired purpose.

BONES

Osteoinductive growth factors, in particular recombinant human bone morphogenetic protein-2 (rhBMP-2), have demonstrated remarkable efficacy, but a number of concerns and controversies exist regarding the safety of their clinical use and high cost.

Although numerous synthetic bone graft substitutes are available, the problem of delayed and/or compromised healing remains a significant clinical challenge

The ideal biomaterials for bone regeneration should not only be **biocompatible and osteoconductive but also osteoinductive.**

They should be able to leverage the self-healing capabilities of the bone by

(i) providing the main structural, compositional, and biochemical cues for the formation of new tissue;
(ii) engaging the host's resident immune cells in the regenerative response;
(iii) promoting the recruitment, proliferation, and differentiation of progenitor cells; and
(iv) recovering an adequate local blood supply to support healing and remodeling

Nanostructured biomaterials have proven superior at enhancing bone regeneration due to their unique chemical and physical properties (e.g., magnetic, electrical) that are uniquely different from their bulk counterparts.

In the rational design of regenerative nanotechnologies for bone regeneration, **four crucial elements of bone** should be considered and recapitulated as closely as possible:

(i) composition,.
(ii) physical stimuli,

(iii) architecture and
(iv) biochemical cues

Mimicking Bone Composition: Bioceramics and Composite Nanostructured Biomaterials

Bioceramics

Bone is a natural nanostructured composite, consisting of approximately 60% (dry weight) mineral, mostly nano-apatite—which is a calcium phosphate (CaP) ceramic.

Accordingly, a number of **bioceramics containing calcium and phosphorous** have been proposed for bone regeneration

Of these, CaP materials most closely mimic the mineral phase of bone and have demonstrated relatively greater osteoinductivity, making CaP a common material of choice for bone grafts. A number of bioceramics have been used clinically for several decades both for load- and non-load- bearing applications.

While conventional bioceramics had poor mechanical properties and unfavorable biodegradability and porosity the latest generation of bioceramics are structured at the nanoscale and have significantly improved bioactivity, biodegradation and mechanical properties

Hydroxyapatite-Based Ceramics

Among CaP ceramic phases, synthetic hydroxyapatite (HA) has been the one most extensively studied due to its biocompatibility and resemblance to the composition of natural bone mineral.

First generation materials were fabricated with stoichiometric HA [$Ca_{10}(PO_4)_6(OH)$], which has been successfully synthesized and mass produced through several synthesis strategies, including hydrothermal reactions, sol–gel syntheses, and mechanochemical syntheses

However, natural bone mineral is produced in a very dynamic environment with numerous ions present (e.g., Mg^{2+}, K^+, Na^+, CO2−332-, HPO2−442-), which frequently substitute ions in the apatite lattice. The apatite present in natural bone is calcium deficient and is characterized by a Ca/P ratio lower than the typical 1.67 of stoichiometric HA.

Various substituted nanostructured HAs have been proposed, some of which have been used as tools to fine-tune or stimulate specific biological functions. For example, Mg^{2+} plays a vital role in osteogenesis and is present in young and newly formed bone.

Mg-substituted HA showed enhanced cell adhesion, proliferation, and metabolic activity compared to HA.

Sr acts to enhance bone formation *in vivo* by inhibiting osteoclast-mediated bone resorption while upregulating osteoblast activity which is why Sr-based drugs have been long used to treat osteoporosis (e.g., strontium renelate. Thus, Sr-doped nano-HA has also been extensively used in bone regenerative strategies al.

Similarly, substitution with Zn has been shown to enhance osteogenic activity

A conceptually new type of nanostructured calcium-deficient HA, by substituting it with Fe^{2+} and Fe^{3+} to endow the HA with superparamagnetic properties. This magnetic behavior may potentially be exploited for bone regeneration purposes to enhance osteogenesis

Nanostructured Composites

Biomimicry is an increasingly popular strategy in regenerative medicine, aiming to engineer materials that closely resemble the target tissue. Since bone is a natural composite—made of an inorganic component (mostly multi-substituted HA) and an organic component (mostly type I collagen)—researchers have long focused on developing nanostructured ceramic/polymer composite materials with the purpose of recreating the composition and function of natural bone. Nanostructured composites for bone regeneration leverage the osteoconductivity of synthetic CaP ceramic phases and the unique mechanical properties of polymers. For example, both synthetic polymers like **poly(L-lactic acid) (PLLA; poly(e-caprolactone) (poly(lactic-co-glycolic acid) (PLGA; as well as naturally occurring polymers such as gelatin silK, chitosan alginate and coll**agen have been combined with HA and TCP to fabricate a plethora of composite materials over the past three decades

The major drawback, common to all these approaches in the manufacturing of porous structures is the inability of conventional methods to completely control the architecture of scaffolds, such as pore size and interconnections.

D-printing techniques have received much attention due to the capacity to fabricate specific and complex structures

Nanostructured Bio-Glasses

Bioactive glasses are mainly comprised of calcium oxide, silicate, borate, and phosphorous

By varying the relative amounts of these components, different bioactive glasses can be manufactured and, over the past three decades, many variants have been proposed for bone regenerative applications

Bioglasses can be prepared by melt–quench or sol–gel process . While the first generations of bioglasses were solid or macroporous, the latest nanostructured versions, synthesized through the sol–gel approach, have unique nanostructural features, including improved nanotextural properties, highly ordered structure, and controlled pore size and pore interconnectivity.

Such nano-features greatly enhance osseointegration compared to first generation bulk bioglasses. The graft-bone integration begins with the solubilization of surface ions resulting in a silica gel layer. A nanostructured calcium phosphate phase (i.e., hydroxyapatite) starts to nucleate on this layer, activating local osteoblasts to form new bone

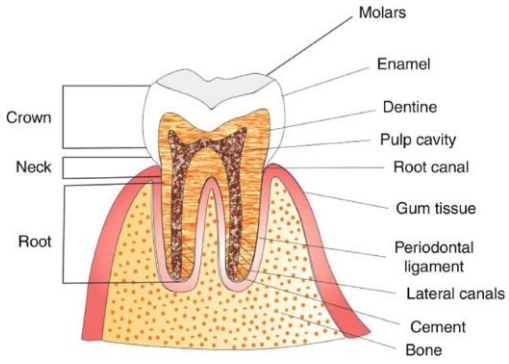
This mechanism contributes to the nano-bioglass degradation, while promoting bone formation. Even their degradation depends on their composition and nanostructure and can be tailored from days to months; for example, borate-based bioglasses have been shown to degrade much faster than silicate varieties

Recent studies showed that increasing the surface area and porosity of nanostructured bioglasses can greatly accelerate their biodegradation, as well as biointegration

Nanoparticles in dentistry

The tooth is mainly comprised of **four parts**: **enamel, dentin, cementum and dental pulp**. Of which, the hardest part is enamel which is highly mineralized part supported by dentin, which

occurs in between the enamel and the pulp chamber, with microscopic channels known as dentinal tubules. Cementum is a specialized bone-like structure that covers the dentin root whose principle role is to serve as a medium for attachment of periodontal ligaments .



With the evolution of dentistry, various dental treatment methods and materials such as temporary dressings, dental restorative materials (such as crowns, bridges and dental fillings), endodontic materials (resin composite and root canal treatment), a coating of the teeth and teeth polishing agents were developed.

These materials possess certain disadvantages such as the

- loss of teeth integrity and whiteness,
- formation of bacterial biofilm on dental coatings and
- inability to prevent dental erosion

which made people think for the development of better materials to serve the treatment purposes without losing the teeth integrity.

Nanoparticles used in Various Fields of Dentistry

Conventional dental materials often cause damage to the tooth properties, such as **tooth whiteness, integrity and promote biofilm formation over the teeth**. Taking the disadvantages of conventional materials into account, various nanomaterials have been developed for the application in the field of dentistry

Antibacterial nanotherapy

Several nanoparticles (eg, zinc oxide,,silver,and polyethylenimine have been incorporated into dental composites or dental adhesives to inhibit the bacterial growth through several mechanisms.

These mechanisms include disruption of the bacterial cell membrane, inhibition of the active transport as well as the metabolism of sugars, generation of reactive oxygen species, displacement of magnesium ions required for the enzymatic activity of oral biofilms, disturbance of the electron transportation across the bacterial membrane,and prevention of DNA replication. These nanoparticles were effective in reducing the *S. mutans and Lactobacillus acidophilus* biofilms in an in vitro model.

Coating tooth surfaces with antibacterial nanocoating was found to be effective in killing bacteria as well as inhibiting bacterial adhesion and maintaining its integrity in the presence of biological fluids (saliva).The antibacterial action of these nanoparticles was shown to be size dependent

Biomimetic remineralization

Different forms of nanocalcium phosphates were used as Ca2+ - and PO4 3− -releasing fillers, eg, dicalcium phosphate anhydrous, tetracalcium phosphate, monocalcium phosphate monohydrate, and carbonate hydroxyapatite.

The release of Ca and PO4 is dependent on degradability and volume fraction of CaP form.

Regardless of Ca and PO4 release, these nanocomposites could be still used for high-stress bearing applications since these nanoparticles are used in combination with other fillers, eg, whiskers fused with nanosized silica. The Ca2+ and PO4 3− can also be released on demand

Dental Implants

With the development of nanodentistry, the implant surface was modified mimicking nature .

Implant surface which imitates bone-like structures, properties and compositions at nanoscale level was made. Proper modification of the surface from macro to nanoscale may improve the process of implant healing.

Implant with bioactive coatings with micro hydroxyapatite (HAp) or micro β-tricalcium phosphate (β-TCP) surfaces appeared clinically undesirable.

The poor attachment of particles to the implant surface, which in turn resulted in their mechanical peel off due to shear forces, initiates an inflammatory response and in consequence removal of the implant.

The problem with conventional ceramics was replaced with nano counterparts with gradient micro nanosurfaces on the implant, which can guide tissue regeneration.

Nanostructured ceramics increase contact with surrounding tissues, comparing to their bulk forms, and do not cause any inflammatory response

Nanoparticles used in dentistry can be classified mainly into three classes

(1)*Polymeric nanomaterial*: chitosan, dendrimers, nanogels, polyethylene glycol and solid lipids

(2)*Metallic nanoparticles*: silver, gold and copper

(3)*Inorganic nanoparticles*: silica, zirconia, titanium dioxide, hydroxyapatite, zinc oxide and carbon nanotubes.

Nanocoatings on endosseous implants such as nanocrystalline diamond, carbon nanotubes, bioactive glass, poly (lactide-co-glycolide), hydroxyapatite nanocomposite or zinc-substituted n-HAp are the biomaterials of the future implant

Hydroxyapatite NPs

This is the main composition of mineralized tissues of the human body ($Ca_{10}(PO_4)_6{\cdot}2(OH)$). It is a natural calcium phosphate ceramic, predominant in 97% enamel.

Teeth are acellular in nature, thus it cannot be logically repaired like a bone. Thus regenerating the enamel surface is a significant challenge.
The nano sized HAp particles can easily integrate into the dental tubules. HAp helps to reduce dental hypersensitivity. HAp NPs can bind strongly with proteins as well as with bacterial and plaque fragments. Their high biological activity and reactivity enable them to bind to the dentin apatite and tooth enamel. Hydroxyl apatite nanoparticles can fit well with the very small cavities present in the enamel originated by acidic erosion. The HAp NPs are adsorbed robustly to the enamel of the teeth and thus retard auxiliary erosive demineralization.

Various toothpaste, mouth-rinsing solutions integrate these nanocrystals to repair the enamel surfaces. The biomimetic function of hydroxyapatite is to protect the teeth by making a film of artificial enamel around the tooth. The granular hydroxyapatite is employed in dental clinical rehearsal to reform periodontal shortcomings.

Zirconia NPs

The use Zirconia (Zirconium dioxide, ZrO2) has considerable significance in dental science.

It has similar metallic properties and color like tooth. Zirconia is a chemical oxide which is insoluble in water. Thus, it reduces the bacterial adhesion and has low cytotoxicity. Zirconia implants encompass glorious resistance against corrosion and carry, as well as sensible biocompatibility.

Moreover, high fracture resistance can be acquired by ZrO2 because of energy retention property throughout the conversion of polygonally shaped molecules into monoclinic ones. Zirconia NPs is a bio inert material, the encapsulation by animal tissue is weak and also the unleash of remains virtually unnoticeable. Nano zirconia-alumina materials combine the physical and chemical properties of ceramic material. In these NPs, low percentage of tetragonal ZrO2 particles is in an aluminum oxide matrix. Thus, the toughness and longevity which are the principal interest in the dentistry are retained.

Alumina/zirconia nano composites are new implant materials which show better efficacy as compared to the ceramic materials. Zirconia oxide nanoparticles are found to have anti-biofilm activity against certain bacteria and therefore they can be effectively used as a polishing agent in dental practices

Silica

In the field of dentistry silica NPs used as dental filler. Various dental filler products developed to improve their mechanical properties. Tooth polishing is a conventional practice, which uses silica particles. Silica particles are used in polishing for their biocompatibility and low cost **Polishing of teeth surfaces** is often done to protect the enamel surfaces.

Thus, polishing prevents dental caries, which acts as a primary defense mechanism against the cariogenic bacteria. Modified silica nanoparticles are used to treat dental hypersensitivity. Enamel loss exposes dentinal tubules, thereby increasing the risk of dental hypersensitivity.
Over the years, a number of desensitizing agents are commercially available that aim at occluding dentinal tubules. Unfortunately, the products can penetrate only up to a small depth into the dentinal tubules which may not combat the daily adverse conditions.
mesoporous silicas have been widely researched over the past two decades.

Silver NPs

AgNPs have also been studied for use in several areas of dentistry which includes endodontics, dental restorative material, dental prosthetics, dental implants.
Incorporation of AgNPs decreases microbial colonization over dental parts and increases oral health.

As the nanoparticles possess small size having the larger surface area, they show the antimicrobial effect at very low level. Because of its minute size, AgNPs can able to penetrate easily the bacterial cell membrane resulting in rapid bactericidal activity.

Silver can interfere with DNA and proteins by interacting with —SH groups, and also alters the base pairing, DNA unwinding, cell wall synthesis and respiratory processes,-resulting in bacterial death

What Is Regenerative Medicine?

Regenerative medicine seeks to replace tissue or organs that have been damaged by disease, trauma, or congenital issues, vs. the current clinical strategy that focuses primarily on treating the symptoms. The tools used to realize these outcomes are tissue engineering, cellular therapies, and medical devices and artificial organs.
Combinations of these approaches can amplify our natural healing process in the places it is needed most, or take over the function of a permanently damaged organ.
Regenerative medicine is a relatively new field that brings together experts in biology, chemistry, computer science, engineering, genetics, medicine, robotics, and other fields to find solutions to some of the most challenging medical problems faced by humankind.

When injured or invaded by disease, our bodies have the innate response to heal and defend. What if it was possible to harness the power of the body to heal and then accelerate it in a clinically relevant way? What if we could help the body heal better?
The promising field of Regenerative Medicine is working to **restore structure and function of damaged tissues and organs**. It is also working to create solutions for organs that become permanently damaged. The goal of this approach is to find a way to cure previously untreatable injuries and diseases.

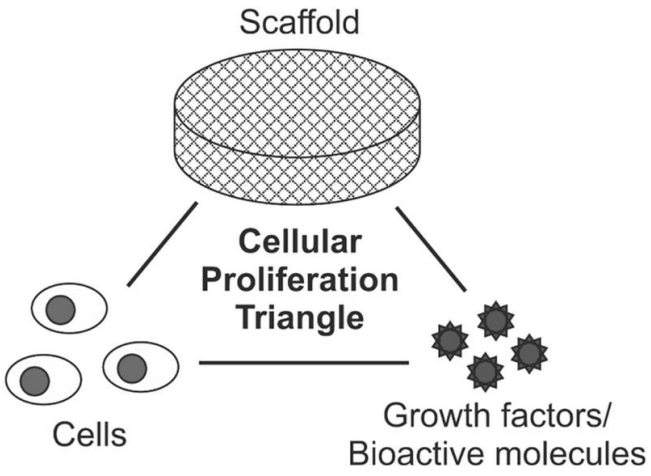
The concentrations in the field of regenerative medicine are:

1. **Tissue Engineering and Biomaterials**

Tissue engineering is a strategy where biologically compatible scaffolds are implanted in the body at the site where new tissue is to be formed. If the scaffold is in the geometric shape of the tissue that needs to be generated, and the scaffold attracts cells the outcome is new tissue in the shape desired. If the newly forming tissue is subjected to exercise as it forms, the outcome can be new functional engineered issue

2. Cellular Therapies

Many millions of adult stem cells are found in every human. Our body uses stem cells as one way of repairing itself. Studies have illustrated that if adult stem cells are harvested and then injected at the site of diseased or damaged tissue, reconstruction of the tissue is feasible under the right circumstances. These cells can be collected from blood, fat, bone marrow, dental pulp, skeletal muscle and other sources. Cord blood provides yet another source of adult stem cells. Scientists and clinicians are developing and refining their ability to prepare harvested stem cells to be injected into patients to repair diseased or damaged tissue.



3. Medical Devices and Artificial Organs

In cases where an organ fails, the predominant clinical strategy is to transplant a replacement organ from a donor. The principal challenges are the availability of donor organs, and the requirement that the donor take immuno suppression drugs—which have side effects. Further, there are many instances where the time to find a suitable donor organ requires an interim strategy to support or supplement the function of the failing organ until a transplantable organ is found. Using circulatory support as an example, there are technologies in various stages of maturity, initially using ventricular assist devices (VADs) as a bridge to a heart transplant, and now there are VADs that are used for long-term circulatory support (destination therapy).
Scientists and clinicians around the world are developing and evaluation devices to supplement or to replace the function of many organ systems including the heart, lung, liver and kidney

Scaffolds

Temporary or permanent artificial extracellular matrices to accommodate cells and support 3D tissue regenerations.

Types of Scaffolding

- *Naturally derived biomaterials*
- *Synthetic biomaterials*
- *Accelular Tissue Matrices*

Stem cells

Stem cells have emerged as a key element of regenerative medicine therapies due to their inherent ability to differentiate into a variety of cell phenotypes, thereby providing numerous potential cell therapies to treat an array of diseases.

What qualities of a stem cell are needed for successful repair of disorders ?

1: Clinically feasible stem cell population
2: Capable of multipotential differentiation
3: Anti-inflammatory

Use of Nanoparticles in Tissue Engineering and Regenerative Medicine

The impact of nanotechnology has altered traditional and simple approaches in TERM toward more complex and efficient systems. Along NPs, other products of nanoscale technology such as nanofibers and nanopatterned surfaces have been used for directing cell behavior in TERM field.

Utilizing simultaneous therapeutic and imaging systems, embedding novel biomaterials with superior spatiotemporal control within scaffolds, modulating release of multiple bioactive agents especially growth factors to direct fate of stem cells and morphogenesis, adjusting mechanical strength of scaffolds for hard tissue applications, and minimizing toxicity and increasing biocompatibility through tissue specific delivery are among various applications of NPs in TERM.

NPs can be prepared with various types of materials such as **ceramics, metals, natural and synthetic polymers**

Metallic Nanoparticles

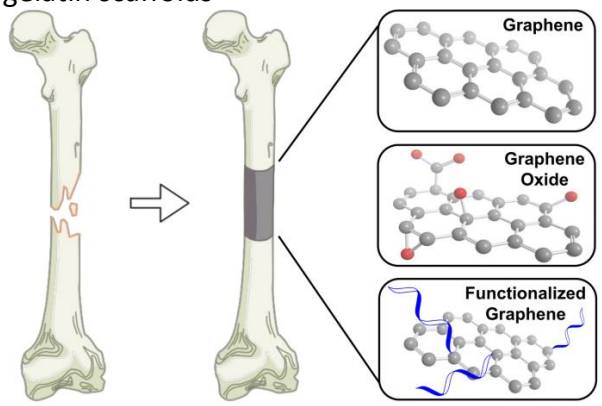
Gold nanoparticles (AuNPs) can be described as a colloid of nanometer sized particles of gold. Colloidal gold solutions present different properties compared to the bulk gold, for example, their optical property due to their unique interaction with light.

On gold surface it is possible to conjugate various ligands including polypeptide sequences, antibodies and proteins with various moieties such as phosphines, amines, and thiols, as their strong affinity to gold is known

One potential use of gold NPs in the context of regenerative medicine is as a safety measure if the implanted tissue is replacing a resected tissue/organ due to tumor growth. One example is the use of AuNPs for disturbing the cancer cell division by selectively transporting the particles into affected cells' nuclei. Kang and colleagues developed polyethylene glycol (PEG) coated

AuNPs (30_nm) through binding it with nuclear localization signal (NLS) peptides together with arginine—glycine—aspartic acid

Different NPs can be used for bone tissue engineering with emphasis on scaffolds' improvement and drug delivery. Among NPs (organic and inorganic), AuNPs have been used in scaffolds for enhancing bone regeneration, due to their potential to promote cell differentiation Heo et al. presented an enhanced bone regeneration by using a complex composed of AuNPs and gelatin scaffolds



Silver Nanoparticles

- Silver nanoparticles (AgNPs) can also be described as a colloid of nanometer sized particles of silver and are one the most widely used metallic NPs in biomedical field mainly for their antimicrobial properties.
- In an animal model, Tian et al. studied the impact of AgNPs treatment on burn and diabetic wounds as potential wound healing accelerator (Tian et al., 2007). They found that the delivery of AgNPs not only had an antimicrobial effect but also it has accelerated the rate of healing.
- AgNPs have been utilized to elaborate chitin/nanosilver composite antimicrobial scaffolds. It has been also observed that the blood clotting efficiency was increased with this scaffold due to the fact that silver can affect the pathway of coagulation by denaturing the anticoagulant proteins
- It was reported that a porous chitosan-alginate with biosynthesized AgNPs has been shown to have cytotoxic effects against MDA-MB-231 breast cancer cells

Ceramic Nanoparticles

Ceramic nanoparticles (CNPs) are basically comprised of inorganic compounds, besides metals, metal oxides, and metal sulfides and they can be used in production of nanoscale materials of various shape, size, and porosity (Singh et al., 2016).

IN general, CNPs can be classified according to their tissue response as being inert, bioactive or resorbable ceramics and magnetic NPs.

Bioactive Glass Nanoceramics

Bioactive glass ceramic nanoparticles (n-BGC) with SiO2-CaO–P2O5-Na2O core structures were established by Larry Hench's team in 1969 (Jones, 2015). Bioglasses can be formed from various elements such as silicone, sodium, potassium, magnesium, phosphorous, oxygen, and calcium which can be absorbed by the cells.

Cancer Nanomedicine: Targeted Therapy

Cancer is considered as one of the most challenging health care problems.Though there are many approved drugs that can be used for cancer therapy, drug resistance and delivery are among of the barriers of the treatment. In addition, pathological characteristics of tumors and their abnormal blood vessel architecture and function also reduce the efficiency of the conventional cancer treatment.

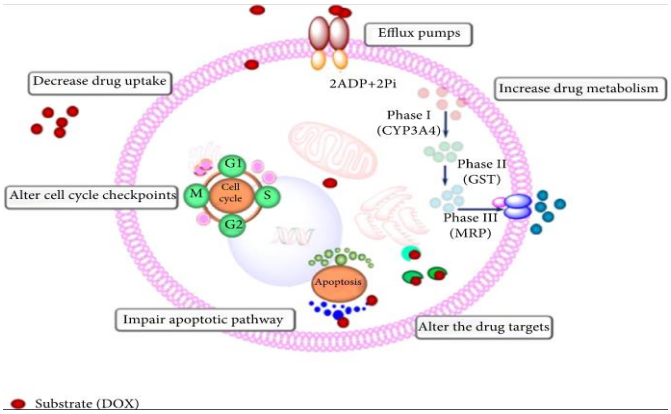
NPs have many properties such as their small size, ability to load various drugs and large surface area, and ability to increase the absorption of conjugated. Therefore, the **NPs have been considered as excellent tumor-targeting vehicles.** The recent nanoscale vehicles include liposomes, polymeric nanoparticles, magnetic nanoparticles, dendrimers, and nanoshells; lipid-based NPs have been used as conjugates. There are few examples of approved conjugated anticancer NPs including AmBisome (amphotericin B liposomal) and Doxil (liposomal doxorubicin).

There are several therapeutic methods that have been used to treat tumors and their surrounding environments. An example of these strategies is **chemotherapy, which was first tried in 1942** when Louis Goodman and his colleagues tested using nitrogen mustard in treatment of non-Hodgkin's lymphoma.

However, chemotherapy has helped in the improvement of cancer therapy of patients; in most cases, cancer with a more progressive stage normally occurs, and usually, multidrug resistance takes place. Targeting the surrounding environment of tumors also has been tried, since cancer cells depend primarily on oxygen and angiogenesis for survival and metastasis.

The failure of chemotherapy in the clinic is mainly due to different extents of multidrug resistance (MDR) results with approximately 90% of cancer patients died.

MDR occurs when tumor cells develop resistance to structurally and functionally unrelated classes of chemotherapeutic agents leading to drug inactivation and/or drug efflux from cancer cells leading to obstacle of the treatment.



There are several report hypotheses of the molecular mechanisms of MDR, mainly including increasing efflux of membrane transport proteins, detoxification by reducing the drug

activation and potentiating drug metabolism, alteration in drug targeting by enhancing the DNA repair mechanism, blocking apoptosis, and alteration of cell cycle regulation.

All these mechanisms synergistically interact together to produce MDR.

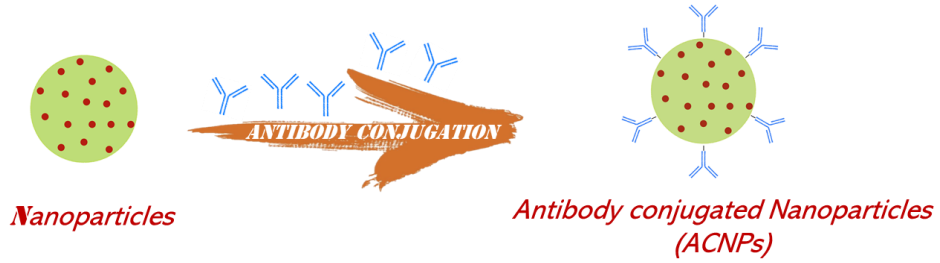
Cancer Targeting with Conjugated Nanoparticles

They prevent the degradation of the conjugated drug. They also improve its absorption through the epithelial diffusion that ultimately results in reaching the optimum concentration in a short time. NPs also alter the pharmacokinetic and distribution profile of the drug in the tissue and increase the intracellular efflux in cancer cells.

Enhancing the permeability and retention effects of anticancer drugs is considered as passive targeting of NPs to the tumors. However, actively **targeted NPs can be designed based on tumor microenvironment- and ligand-directed targeting to the tumor cells.**

Therefore, as a unique inherent property of NPs to the solid tumors, the nanoparticle is considered as an excellent tumor-targeting vehicle. This effect makes the accumulation of NPs preferable at the tumor site. In addition, the multifunction of NPs allows targeting the tumor site that is directly connected to the main blood circulation.

A nanoparticle–biomolecule conjugate is **a nanoparticle with biomolecules attached to its surface**. The **conjugation** of **nanoparticles** with antibodies combines the properties of the **nanoparticles** themselves with the specific and selective recognition ability



An Overview of Conjugation Strategies for Clinical Implementation of Polymeric ACNPs

Barriers for the Treatment of Tumors Overcome by Nanoparticles

At the cellular level, the drug resistance is considered as a physiological barrier to the success of the anticancer drug.

The penetration of chemotherapy to the solid tumor is difficult due to the pathological characteristics of the solid tumors that include abnormal blood vessel architecture and function, interstitial hypertension, lack of lymphatics (a network of tissues, vessels and organs that work together to move a colorless, watery fluid called lymph back into your circulatory system), and dilated angiogenesis(the formation of new blood vessels)

This microenvironment to certain extent contributes to the drug resistance results in decreasing in the drug accumulation and/or penetration to the solid tumor.

Nevertheless, chemotherapy encounters another major barrier, i.e., **multidrug resistance** even after its penetration to the tumors.

Pharmaceutical history of the development of nanoparticle started with the first discovery of liposome. this ultimately allows **increasing the specificity of effective drugs and overcoming the resistance of tumors.**

The therapeutic index of NPs has improved the potential of commonly used drugs through increasing the efficacy and decreasing the toxicity of the drug and keeping its concentration in the steady state over a long period of time.

Thus, drug-coated NPs should have long half-life to give the maximum effect. The targeting of active sites of transporters or receptors is the main character of NPs because of their flexible surface chemistry that allows for potential conjugation of targeting ligands.

On the other hand, several **anticancer agents exhibit low specificity** towards cancer cells. Therefore, the delivery of drugs to the solid tumors is still a difficult approach The reticuloendothelial system (RES) that known as "mononuclear phagocytes" is the major defense system in the bloodstream of the body that rapidly removes NPs from the blood.

RES recognizes the NPs as foreign bodies. Hydrophilic and flexible polymers can coat the NPs from the opsonins hence avoid the uptake of NPs by the RES

Biomarkers

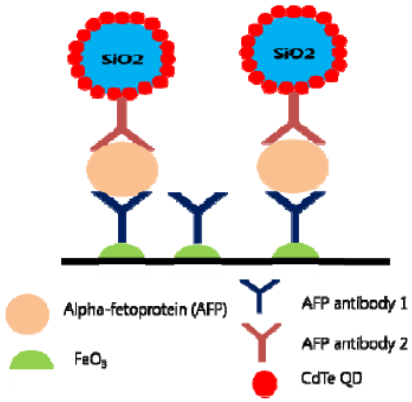
- A biomarker is an indicator of a biological state of disease. It is characteristic of a specific state and therefore can be used as a marker for a target disease.
- These biomarkers can be used to study cellular processes, and monitor or recognize disruption or alterations in the cellular processes of cancer cells.
- A biomarker can be a protein, a fragment of a protein, DNA, or RNA-based. Biomarkers, specifically cancer biomarkers, are **an indication of cancer** and by detecting them the existence of that specific cancer can be verified.
- Alongside the development of proteomic technologies, many protein biomarkers have been discovered for many types of cancer.
- As well, with DNA methylation analysis researchers have also been able to discover DNA biomarkers for some of the widely spread cancers.
- **Biomarkers in relation with nanotechnology and biosensors have opened up a new era of early cancer diagnosis and precise drug delivery**.

Gold Nanoparticles

- Gold nanoparticles (GNPs) have been in the bio-imaging spotlight due to their special optical properties.
- GNPs with strong surface-plasmon-enhanced absorption and scattering have allowed them to emerge as powerful imaging labels and contrast agents.
- Furthermore, GNPs have been proven to be **more biocompatible, less cytotoxic, and resistant to photobleaching.**
- According to their size and shape, GNPs can absorb and scatter light from the visible to near-infrared (NIR) region.
- GNPs have been extensively studied, especially in the medical area, and have been used as colorimetric biosensors, cancer imaging, cancer therapy, and drug delivery.
- They have been found to amplify the efficiency of Raman scattering and thus have been proposed as a novel tag.

Quantum dots (QDs)

- QDs are an exciting material to work with due to their unique optical properties compared to traditional organic fluorescent labels.
- Organic fluorescent dyes have several drawbacks that have limited their usefulness as molecular imaging tags.
- Their low photobleaching threshold and broad absorption/emission peak width have hindered their use in long term imaging and multiplexing (detecting multiple labels simultaneously).
- QDs have properties that overcome these limitations of the organic fluorescent dyes including high resistance to photobleaching, broad-band absorption with narrow emission bands ranging from UV to NIR, and size tunable emission bands.
- QDs can be used as **signal amplifying agents** in ultrasensitive cancer biomarker detection . A recent study has been conducted with QD functionalized nanoparticles in immunoassays, targeting alpha-fetoproteins (AFPs).
- CdTe QDs have been coated on SiO2 particles and through anodic stripping. Si/QD/antibody showed increased oxidation current of Cd2+ proving its signal amplifying ability.
- Increased amount of QDs per biomarker make the detection more sensitive, thus enabling detection even at low concentration
- Magnetic particles have also been functionalized with QDs for cancer targeting, separation and imaging.
- A high fluorescent multi-labeling could be achieved with this conjugation, providing both magnetic manipulation and multicolor fluorescent images.
- By immobilizing anti-epithelial cell adhesion molecule (EpCAM) antibody, this conjugate targeted tumor cells circulating tumor cells.



- QDs have also been integrated into nano-bio-chips (NBCs) for detecting multiple cancer biomarkers.
- QD-labeled antibodies were used for multiple-color-fluorescence transduction signaling NBCs in combination with antigen capture by a microporous agarose bead array held in microfluidics.
- Cancer biomarkers of interest were carcinoembryonic antigen (CEA), cancer antigen 125 (CA125), and Her-2/*neu* in serum and saliva samples.
- This type of miniaturized chip proved to be superior to traditional enzyme-linked immunosorbent assay (ELISA), reducing the detection limit by nearly two orders of magnitude.

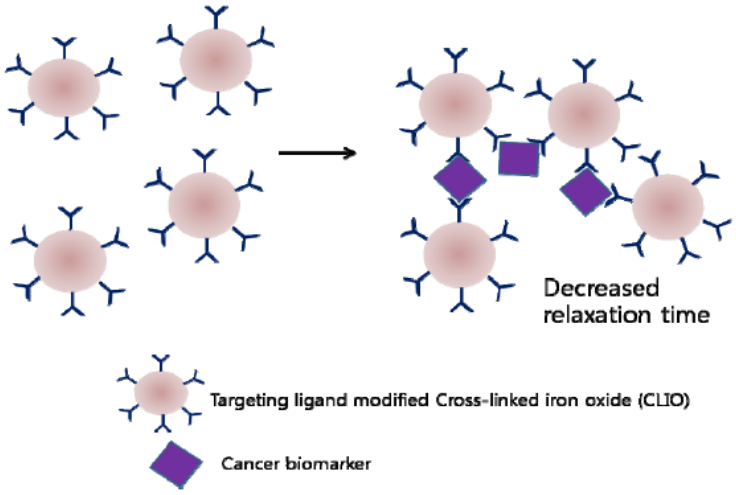
Silica nanoparticles

Silica nanoparticles doped with fluorescence resonance energy transfer (FRET) dyes have been investigated as simultaneous and multiplexed detection. Recently, a study has been conducted using these FRET nanoparticles to monitor cancer cells. By modifying the nanoparticles with aptamers targeting T-cell leukemia and B-cell lymphoma and by changing doping ratio of the dyes trapped inside the silica shell, a variety of fluorescent emission spectra could be obtained with a single excitation wavelength.

Magnetic nanoparticles

- By using the principle of decrease in transverse relaxation time due to aggregation of magnetic nanoparticles in presence of target molecules, concentration of cancer biomarkers could be measured.

- This device allowed *in vivo*, local environment monitoring for cancer biomarkers and could be left implanted after tumor surgery.
- A similar approached has also been used in the past to develop a biosensor to detect cancer biomarkers in turbid samples (blood, urine, and sputum).
- When magnetic particles aggregate through affinity ligands to the molecular target, a decrease in the bulk spin-spin relaxation time of surrounding water molecules occurs.
- This could be used as a chip-based nuclear magnetic resonance (NMR) system, and with miniaturization and multiplexing, detection of various biomarkers within a small sample volume could be achieved



NANOROBOTICS IN SURGERY

Robotic systems have markedly extended the reach of human beings in sensing, interacting, manipulating, and transforming the world around us. In particular, the union of diverse technologies has enabled a revolution in medical applications of robotic technologies toward improving health care. Industrial robots were developed primarily to automate routine and dangerous macroscale manufacturing tasks, medical robotic devices are designed for entirely different environments and operations relevant to the treatment and prevention of diseases. The rapid growth in medical robotics has been driven by a combination of technological advances in motors, control theory, materials, and medical imaging and increase in surgeon/patient acceptance.

For example, robotic surgical systems, such as the da Vinci system, allow translation of the surgeon's hand movements into smaller, precise movements of tiny instruments within the patient's body. In particular, the mechanical parts of existing medical robotic devices are still relatively large and rigid to access and treat major previously inaccessible parts of the human body.

Designing miniaturized and versatile robots of a few micrometers or less would allow access throughout the whole human body, leading to new procedures down to the cellular level and offering localized diagnosis and treatment with greater precision and efficiency.
Locomotion represents the first challenge for the miniaturization of robots into micro- and nanoscales. The design of an efficient nano/microscale machine requires a swimming strategy that operates under these low Reynolds number constraints and a navigation strategy for overcoming the Brownian motion.

Actuation mechanisms and potential biomedical applications of various types of micro/nanorobots

Typically, these tiny machines rely on either chemically powered motors that convert locally supplied fuels to force and movement or externally powered motors that mostly use magnetic and ultrasound energies to drive their motion. Chemically powered motors can propel themselves through aqueous solution by using surface reactions to generate local gradients of concentration, electrical potential, and gas bubbles. Magnetic swimmers successfully use magnetic actuation to reproduce the motions of natural swimming microorganisms with helical or flexible flagella.
Tremendous efforts from the nanorobotic community have greatly improved the power, motion control, functionality versatility, and capabilities of the various micro/nanorobotic prototypes. Many studies have demonstrated that these micro/nanorobots can navigate through complex biological media or narrow capillaries to perform localized diagnosis, remove biopsy samples, take images, and autonomously release their payloads at predetermined destinations.
Many of the micro/nanorobots are made of biocompatible materials that can degrade and even disappear upon the completion of their mission. These preliminary in vivo micro/nanorobot operations have demonstrated their enhanced tissue penetration and payload retention capabilities. Such untethered micro/nanorobots represent an attractive alternative to invasive medical robots and passive drug carriers and are expected to have a major impact on various aspects of medicine.

Microbiology

- Although microrobots and nanorobots can be constructed and have function, their use within the vascular system is limited by challenges with transportation and propulsion.
- An effective strategy for enabling propulsion of microrobots and nanorobots is coupling them to magnetotactic bacteria such as Magnetococcus, Magnetospirillum magnetotacticum or Magnetospirillum magneticum.
- The smallest known species of magnetotactic bacteria is the marine magnetotactic spirillum, which is 0.5 μm (500 nanometers).
- magnetotactic cocci are more useful for intravascular function.
- The components of the magnetotactic bacteria that are responsive to the magnetic field are called magnetosomes.

- Magnetosomes are prokaryotic pseudo-organelles with about 15-20 magnetite crystals, each about 50 nm in diameter, contained within an invagination of the prokaryotic cell membrane.
- Magnetotactic cocci have been found to travel in consistent and predictable patterns following established geomagnetic lines.

Hematology

- From uses ranging to emergency transfusions of non-blood oxygen carrying compounds to restoring primary hemostasis, there is a wide array of applications under study for nanorobotics in hematology.
- One of these devices currently under design is a nanorobot dubbed a respirocyte (artificial red blood cells).
- First, collecting oxygen as it passes through the respiratory system for distribution throughout the bloodstream.
- Second, collecting carbon dioxide from tissues for release into the lungs. And finally, metabolizing circulating glucose to power its own functions.
- **The total size of the robot would be about one micron, or 1,000 nanometers**. However, the contained components would be constructed on the nanoscale.
- These include an onboard computer of 58 nm diameter, and oxygen and carbon dioxide loading rotors with a maximum 14 nm diameter in any one dimension.
- The **respirocyte is designed to carry 236 times more oxygen per unit of volume compared to red blood cells**.

Micro/nanorobots for precision surgery

robot-assisted surgery is a rapidly evolving field that allows doctors to perform a variety of minimally invasive procedures with high precision, flexibility, and control

Unlike their large robotic counterparts, tiny robots can potentially navigate throughout human body, operate in many hard-to-reach tissue locations, and hence target many specific health problems.

Untethered nanorobotic tools, ranging from nanodrillers to microgrippers and microbullets, offer unique capabilities for minimally invasive surgery.

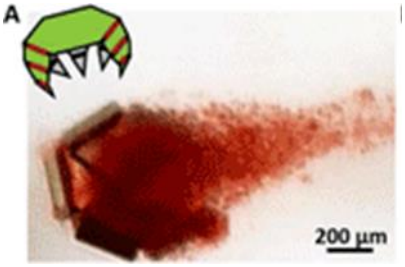
Powered by diverse energy sources, the moving micro/nanorobots with nanoscale surgical components are able to directly penetrate or retrieve cellular tissues for precision surgery.

Unlike their large robotic counterparts, these tiny robots can navigate through the body's narrowest capillaries and perform procedures down to the cellular level. Tetherless microgrippers represent an important step toward the construction of autonomous robotic tools for microsurgery. These mobile microgrippers can capture and retrieve tissues and cells from hard-to-reach places. Similar to their large tethered counterparts, the gripping operation of untethered microgrippers commonly involves an opening/closing of the device.

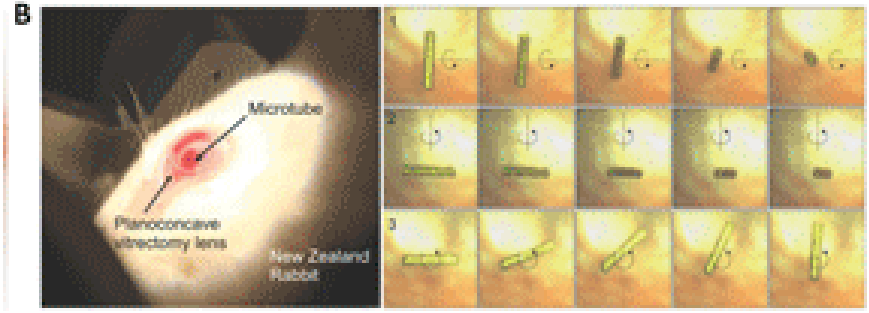
Different responsive mechanisms, based on temperature, pH, or enzyme stimuli, have been explored for actuating self-folding microgrippers autonomously in specific environments.

For example, Fig. illustrates the ability of a tetherless thermobiochemically actuated microgripper to capture a cluster of live fibroblast cells from a dense cell mass in a capillary

tube. The microgripper could subsequently move out of the capillary tube with the captured cells in its grasp, demonstrating its strength for performing an in vitro tissue biopsy.



Magnetically actuated microrobots have also shown considerable promise for minimally invasive in vivo surgical operations because magnetic fields are capable of penetrating thick biological tissues. An implantable magnetic tubular microrobot was able to perform such surgery at the posterior segment of the eye. The electrochemically prepared microrobot was injected with a 23-gauge needle into the central vitreous humor of the eye and monitored with an ophthalmoscope and integrated camera. Wireless control was used to rotate the intraocular magnetic microrobot around three axes in the vitreous humor of a living rabbit eye. Similar magnetic microtubes can be developed and applied as implantable devices for targeting other diseases in different confined spaces of the human body.



Recent proof-of-concept studies have demonstrated that the untethered micro/nanorobots can perform surgical operation on a single-cell level. Solovev et al described nanoscale tools in the form of autonomous and remotely guided catalytic InGaAs/GaAs/(Cr)Pt microjets.
With diameters of 280 to 600 nm, these self-propelled rolled-up tubes can reach a speed of up to 180 µm/s in hydrogen peroxide solutions. The effective transfer of chemical energy to a translational corkscrew-like motion has allowed these tubes to drill and embed themselves into biological samples such as a single cell.