

Overview of Drug Delivery Systems : a weapon against disease [Review]

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Abstract

Nanoparticle Drug Delivery System (NDDS) is a common drug delivery system that incorporates nanoparticles into a drug administered as a living body. These systems have the advantage of being able to address several traditional drug-related problems, such as low water solubility, low biological availability, and non-specific distributions in the body, and additionally allow the drug to be used where it is intended. Important factors in these systems include drug storage, longevity, targeting, and drug releasing. In the case of drug storage, nanoparticles are mainly used, and Longevity prevents drugs from adhering to plasma proteins. Targeting includes passive targeting and active targeting. In the case of drug release, it can be classified as an exogenous drug that is inside NDD and is stimulated by stimulation, and an endogenous drug that is activated by stimulation. The use of biological pathways, biological proteins, or lipids in drug synthesis is called Green Nanoparticles, and they have the advantage of being less energy-consuming and economical than conventional synthesis methods. These drugs can be used to administer drugs to the blood-brain barrier.

Natural drug delivery is a drug obtained by extracting a specific compound from an organism and then concentrating, screening, and refining it, which can treat various diseases. Depending on the organism, it can be largely divided into animals, plants, and viruses, including Gelatin, Collagen, Milk Protein, Silk Fibrin, Elastin, Platelet, and plants such as Zein, Gliadin, Lectin, Soy Protein, and Sunflower pollen. To stabilize them when used, there is a cell membrane coding mechanism, also known as "trojan horse technology," which is a technology that packages drugs with bio membranes such as red blood cells, platelets, and cancer cells. In addition, technologies for having specific bonds using cell membranes are also being developed.

As the demand for field diagnosis and treatment increases, the demand for micro-robot and endoscopy is increasing. They have the advantage of being able to transport drugs by the autonomous peristaltic movement of the digestive system and injecting only the necessary amount at the correct target point. These drug release methods include manual and active dismantling, and the difference between them is the availability of voluntary monitoring through the camera. In the case of drugs used in the respiratory tract, drugs are administered in a simulation called Computational fluid dynamics.

The skin is considered a major obstacle to the delivery of drugs in vitro drugs. To break down these obstacles, various technologies have been developed to bypass the keratin layer of the epidermal layer and immune cells so that drugs can be absorbed into the dermis layer where many blood capillary tubes are located. Among them, Iontophoresis is the most widely used. This technique promotes skin penetration of hydrophilic and charged molecules by applying low voltage to non-invasive skin delivery technology to administer drugs. Another non-invasive

method is the transdermal patch, a medicinal adhesive patch that is attached to the skin to systematically deliver drugs over a period. Invasive methods include microneedles, which penetrate the stratum corneum of the skin and form microtubules to administer drugs. In addition, ultrasonic technology that interacts with ultrasonic reactants through ultrasonic waves to treat them, and nanofiber technology that uses physicochemical properties useful for treatment are also being developed.

Introduction

I. Nanoparticle

I-1. Nanoparticle drug delivery system

NDDSs (Nanoparticle drug delivery system) is a common drug delivery system used for treating diseases including cancer, cardiovascular diseases and infectious diseases by using in vivo drug holding nanoparticles. The general structure of NDDSs contains nanocarriers, polymer coats to increase longevity, targeting agent, stimuli-responsive moiety, imaging or contrast agent, and cell penetrating peptides.(Figure (1).) Depending on the components of the structure, which are directly related to the functions of NDDSs, NDDSs can be classified into 3 classes: (1) NDDS that combines at least two different functions such as longevity, targetability, stimuli-sensitivity or cell penetration. (2) more than one drug or gene therapy (3) so-called theranostic NDDSs, which have an additional diagnostic label for use with current clinical imaging modalities. The use of NDDSs can overcome several problems that are associated with traditional drugs, such as poor aqueous solubility, low bioavailability, the extent to which a substance or drug becomes completely available to its intended biological destination(s), and nonspecific distribution in the body. To be efficient NDDS, increased drug stability that usually means increased circulation time, two or more functions (either simultaneously or sequentially) to overcome multiple physiological barriers, great targeting, and the ability to bear a sufficient load are necessary conditions.

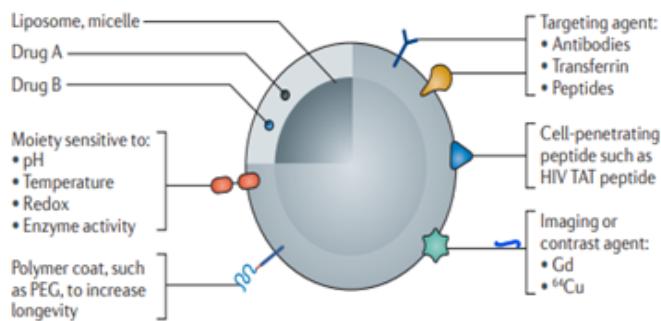


Figure (1) General structure of NDDS

In this chapter, general mechanism and various kinds of NDDSs are described based on the general process and main factors of NDDS: (1) Drug storage (2) Longevity (3) Targeting (4) Drug release (5) Penetrating (6) Degradation. Also in the end, newly developed NDDSs will be introduced.

1. Drug storage

Various nanoparticles, such as : liposomes; polymeric nanoparticles; polymeric micelles; silica, gold, silver and other metal nanoparticles; carbon nanotubes; solid lipid nanoparticles; niosomes; and dendrimers, are used as nanocarriers. (Figure (2).) For multifunctional NDDSs, more than one or two drugs are embedded in the different layers of the particle. They can be released spontaneously or time-separately.

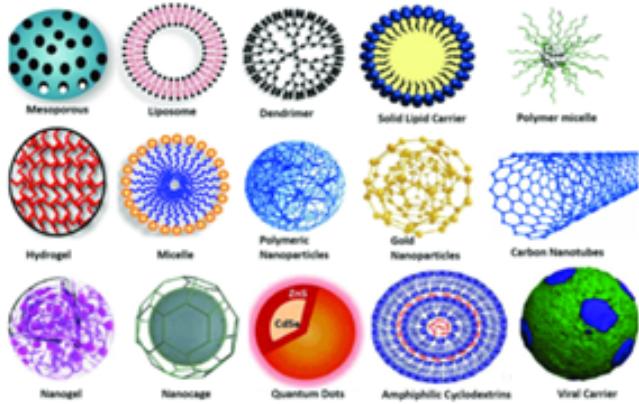


Figure (2) Drug storage nanoparticles

2. Longevity

To understand the mechanism of the longevity enhancement of NDDSs, must be to the biological process against the implantation of the nanocarrier complex device. Implantation causes local injury that provokes an immune response, initiating the Vroman effect in the blood vessels and promoting the inflammatory process. After that, different immune factors such as monocytes, macrophages, lymphocytes, and fibroblast act against the device. The inflammation could lead to a fibrotic encapsulation of the device, isolating it from the nearby tissue environment. Generally, the host response and the fibrotic encapsulation specifically blemish the device's functionality and longevity. Consequently, the adsorption of plasma proteins must be hindered. To do such, hydrophilic and flexible polymers are coated on the nanocarriers usually.

The most common used polymer to hinder the protein's adsorption is poly(ethylene glycol), PEG. PEG is non-toxic, colorless, inert, odorless, and non-volatile. Also, it is incredibly soluble in water, and organic solvents such as benzene. It was slightly negative to a neutral surface charge. The pegylation of NDDSs (1) prevents their interaction with opsonins and (2) impedes capture and clearance by the mononuclear phagocyte system. However, pegylation induces the production of antibodies that can accelerate the blood clearance. Also, particularly after repetitive administration, the mass of bound PEG was found important for determination of the time it remains in blood. Pegylation can be executed on various nanocarriers as part of NDDSs. Liposomal long-circulating NDDSs are the most frequently studied type using along with PEG. pegylated gold nanoparticles are used in photothermal tumour therapy. Pegylation reduced the toxicity of positive charge-bearing dendrimers in cell-culture experiments. Quantum dots can also be modified by pegylation and when pegylated, the

circulation times was been prolonged while the mononuclear phagocyte system was activated.

Not only PEG, anyway hydrophilic and soluble polymers include poly[N(2hydroxypropyl) methacrylamide], poly(acryloyl morpholine), polyNvinylpyrrolidones and polyvinyl alcohol) might be used as same as PEG in NDDSs. PEO, Poly(ethylene oxide) segments is one of them. PEO segments nicely fill out voids in the water structure and minimally perturb the structure of water itself, thereby minimizing the tendency for hydro-phobic interactions. PEO produces a surface that is in a liquid-like state with the polymer chains exhibiting considerable flexibility and mobility. The high mobility of PEO chains has been proposed to repel approaching proteins from the surface because the protein does not have sufficient contact time with the mobile chains to adsorb. Lower surface density of high molecular weight PEO is more effective in reducing protein adsorption than a higher surface density of the low molecular weight polymer.

3. Targeting

One of the coat polymers of NDDS is a targeting agent which targets the region for drug to work on. There are 2 types of targeting Passive targeting and active targeting. Passive targeting NDDSs tend to accumulate in tumours, probably through the EPR effect basis. EPR effect, also called enhanced permeability and retention effect, is the property through which macromolecules (such as nanoparticles) accumulate in areas of inflammation including tumors, owing to the increased vascular permeability or abnormal blood vessel architecture. By passive targeting, we cannot do the specific local targeting or therapy. In active targeting NDDSs, specific moieties attached to nanoparticulate pharmaceutical drug delivery systems force them to interact with a specific type of cell or tissue. This makes specific local targeting or therapy able. Next, properties of passive and active targeting is described.

There are some drawbacks of passive targeting. First, tumours — especially large, solid tumours — are pathophysiologically heterogeneous. Some parts of such tumours are not vascularized, do not exhibit the EPR effect, may have sizeable necrotic areas and have varied microvascular permeability. In addition, the increased interstitial pressure that exists within tumours may limit the EPR-mediated accumulation of NDDSs even if the vasculature is leaky. To use passive pathway, long circulation times to ensure that sufficient drug is delivered to the target tissue is needed.

To make use of active targeting, appropriate biomaterials as targeting ligands are essential and, monoclonal antibodies, transferrin, various peptides, folate, aptamers (single-stranded oligonucleotides) or certain sugar moieties are the things used a lot. To prevent steric hindrance between the targeting moiety and the protective polymer (such as PEG), these kinds of targeting ligand is usually attached to the chemically activated distal end of the NDDS-grafted polymeric chain. So in general form might be pNP-PEG-Ligand. Recently, self-assembling polyalkylcyanoacrylate-based nanoparticles and aptamers are

used a lot these days in active targeting. In the case of self-assembling polyalkylcyanoacrylate-based nanoparticles, it enables various ligands to be easily attached. (Figure(3)) In the case of aptamers, single or double-stranded fragments of DNA or RNA which binds to specific proteins, have been used as stable and efficient targeting moieties for NDDSs via both base pairing and affinity and specificity by electrostatic, hydrogen or hydrophobic bonding. (Figure(4))

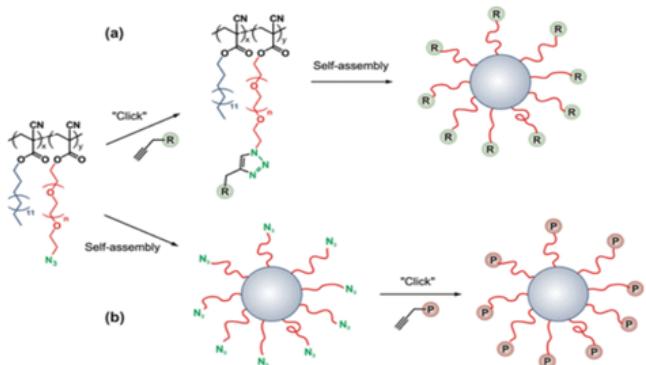


Figure (3) General approach to prepare functionalized poly(alkyl cyanoacrylate) nanoparticles: click reaction on the poly[(hexadecyl cyanoacrylate)-co-methoxypoly(ethylene glycol) cyanoacrylate] (P(HDCA-co-N₃PEGCA)) copolymer followed by self-assembly in aqueous solution (a) or click reaction at the surface of preformed P(HDCA-co-N₃PEGCA) nanoparticles (b).

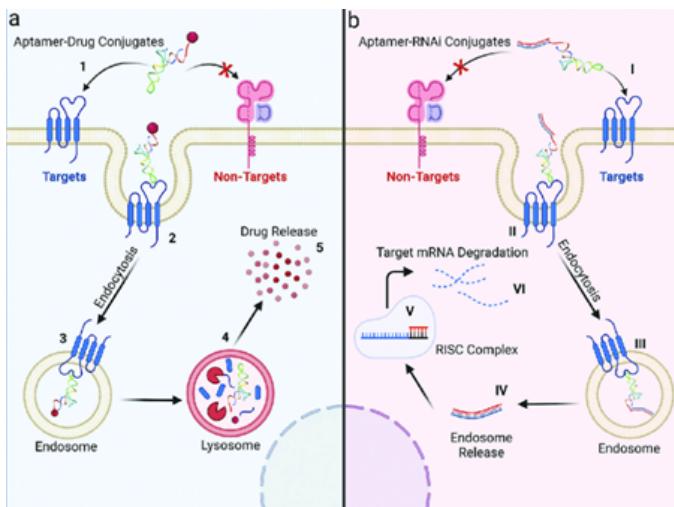


Figure (4) Electrostatic, hydrogen or hydrophobic bonding based aptamer NDDSs (a) and base paring based aptamer NDDSs (b).

Anyways, both way of targeting are closely connected because NDDSs will accumulate in the target area via the EPR effect (passive targeting) before ligand-mediated interaction with target cells (active targeting) occurs. Another interesting issue is associated with the use of targeting ligands that are also naturally present (in their free form) in the circulation, such as folate, transferrin or certain peptides. Competition between the ligands attached to NDDSs and the native ligands for the binding sites is expected. However, it is experimentally overcome yet. This is probably achieved through multipoint interactions of ligand-modified NDDSs with the target and/or

because of the rapid recirculation of the cognate receptors, which provides sufficient opportunities for an interaction of the NDDSs with the receptor. NDDSs are used a lot as drug delivery on cancer, cardiovascular diseases, particularly atherosclerosis, and infectious diseases. Here are common used targets on each diseases.

In the case of cancer, folate and transferrin is common target of active targeting and, EPR effect is that of passive targeting. Folate easily conjugates to nanocarriers, and has high affinity for folate receptors. Also and the lower expression of folate receptors in normal cells than in cancer cells and activated macrophages that are typical of inflammatory diseases. Transferrin is an 80 kDa serum glycoprotein, binds to the transferrin receptor and is taken into cells via receptor-mediated endocytosis. Compared with normal cells, the expression of transferrin receptor in certain cancer cells is much higher because of their increased demand for iron. EPR effect, the tumour vasculature is the property through which macromolecules (such as nanoparticles) accumulate in areas of inflammation including tumours, owing to the increased vascular permeability or abnormal blood vessel architecture. Moreover, EGFRs are frequently overexpressed in solid tumours and are therefore popular targets for NDDSs. HER2, which is overexpressed in approximately 20% of breast cancers and some other cancers, is also a popular target for NDDSs.

In the therapy of cardiovascular disease, especially atherosclerosis, macrophage and clot proteins are commonly targeted during active targeting. Atherosclerosis is a common condition that develops when a sticky substance called plaque builds up inside your arteries so, the key of Atherosclerosis therapy is to make plaque smaller. And to do this, have to lower the LDL (low-density lipoproteins) uptake by macrophages because, increased uptake of LDLs by resident macrophages in plaques is associated with the progression of atherosclerosis. (Figure (5)) For instance, targeted polymeric NDDSs loaded with pravastatin specifically and dramatically inhibited the phagocytic activity of macrophages without affecting non-target cells. Multifunctional micellar NDDSs that combined a targeting pentapeptide, a fluorophore and a drug targeting atherosclerotic plaques by specifically binding to clotted plasma proteins. Or liposomes loaded with ATP or coenzyme Q accumulated well in the infarcted areas of the myocardium and improved cardiac parameters in rat and rabbit models of myocardial infarct.

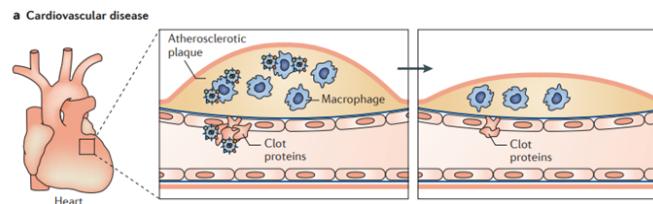


Figure (5) Active targeting on atherosclerosis.

Infectious diseases are cured by using active targeting which induces its apoptosis. Multicomponent NDDSs that contain metals, which can facilitate the formation of reactive oxygen species to induce membrane blebbing and DNA damage. NDDSs that combined iodinated chitosan and silver

nanoparticles bound to and killed bacteria better than either component alone. (Figure(6))

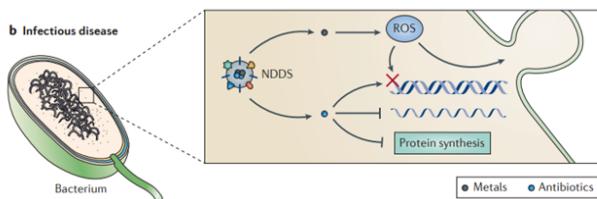


Figure (6) Therapy using NDDSs on infectious bacteria.

Lastly, there are two important considerations related with targeting, using NDDSs. First, target affinity and the density of the receptors that are present on the cell surface. In addition, with pegylated NDDSs, the surface-attached PEG could prevent or hinder the interaction between the targeting ligand and its receptor. Second, when to progress passive and active targeting. In general, if good accumulation of a drug in the pathological tissue is the primary goal, then EPR mediated accumulation may suffice. However, if the presence of the drug inside the cell is needed then ligands that can be internalized may be required.

4. Drug releasing

The most important part of NDDS is how to release the drug. Drugs in NDDS are released by externally applied stimuli physically, such as temperature changes, magnetic fields, and chemically, such as pH, redox, and enzyme. Drug releasing is executed in two ways: Exogenous, which drug is inside the NDDS and comes out due to the stimuli, and Endogenous, which drug is on outer layer of NDDS and activated by the stimuli. Below, each exogenous and endogenous drug release methods are described.

4-a. Exogenous stimuli-responsive drug release

1. Thermo-responsive system

Thermoresponsiveness is usually governed by a nonlinear sharp change in the properties of at least one component of the nanocarrier material with temperature. Such a sharp response triggers the release of the drug following a variation in the surrounding temperature. Ideally, thermosensitive nanocarriers should retain their load at body temperature ($\sim 37^{\circ}\text{C}$), and rapidly deliver the drug within a locally heated tumour ($\sim 40\text{--}42^{\circ}\text{C}$). There are two general ways of thermos-responsive NDDS: (1) Temperature-triggered unfolding of a leucine zipper peptide NDDS and, (2) Temperature-triggered generation of bubbles from the decomposition of encapsulated ammonium bicarbonate. In former, a leucine zipper peptide is installed in protection agents of NDDS that exhibit a lower critical solution temperature and when the temperature nearby gets 43°C , the zipper is unfolded and drug particles get released. In latter, NH_4HCO_3 is inside the NDDS with drugs. When the temperature nearby NDDS gets 43°C , by the chemical formula: $\text{NH}_4\text{HCO}_3 \text{ (aq)} \rightarrow \text{NH}_3 \text{ (aq)} + \text{H}_2\text{O} \text{ (l)} + \text{CO}_2 \text{ (g)}$, the bubbles are

formed which breaks the protection of NDDS such as liposomes and drug is released along them. (Figure (7)) Liposomes, or polymer micelles or nanoparticles (usually poly(N-isopropyl acrylamide), PNIPAM) that exhibit a lower critical solution temperature is commonly used as NDDS. And, *in vivo*, heat is generally applied by using temperature-controlled water sacks, radiofrequency oscillators or miniature annular-phased array microwave applicators.

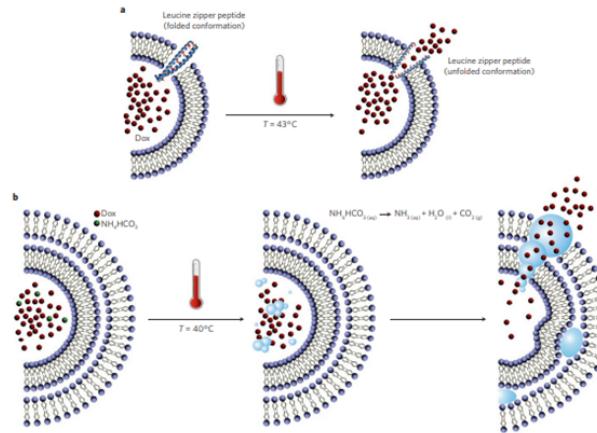


Figure (7) 2 ways of temperature-based actuation mechanisms for liposomal drug delivery.

2. Magnetically responsive system

Magnetic guidance is typically obtained by focusing an extracorporeal magnetic field on the biological target during the injection of a magnetically responsive nanocarrier. Candidate nanosystems for such a therapeutic approach are: core– shell nanoparticles (a magnetic core made of magnetite (Fe_3O_4) coated with silica or polymer), magnetoliposomes (Fe_3O_4 or maghemite (Fe_2O_3) nanocrystals encapsulated in liposomes), and porous metallic nanocapsules. Drugs are capped inside such nanosystem by nanovalve and when the alternating magnetic field (AMF) is applied, the nanovalve is uncapped so that the drugs can be released. To avoid limitations related to physical drug entrapment (for instance, uncontrolled burst release or poor drug loading), the drugs and the nanocarriers can be covalently linked. Also, the heat generated by an AMF can also trigger nanocarrier structural alteration, such as shell or bilayer porosity increase disintegration of the Fe_3O_4 core, or single-crystal nanoshell lattice deformation. So far, porous metallic nanocapsules is the most effective system. (Figure(8))

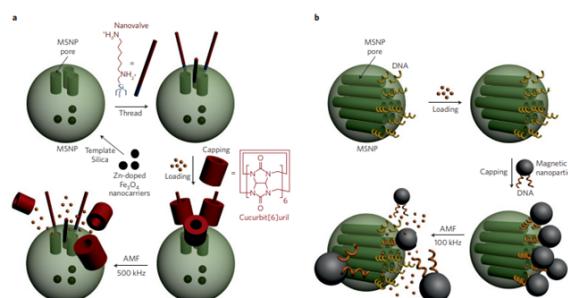


Figure (8) Actuation mechanisms based on the heat generated by an alternating magnetic field (AMF) leading to on-demand

pulsatile drug release from mesoporous silica nanoparticles (MSNPs)

3. Ultrasound-triggered drug delivery

Ultrasound waves can trigger the release of the drug from a variety of nanocarriers through the thermal and/or mechanical effects generated by cavitation phenomena or radiation forces. The cavitation threshold is easily achieved when low ultrasound frequencies (in the kHz range) are used. Physical forces associated with cavitation can induce nanocarrier destabilization, drug release and transient increase in vessel permeability, leading to the cellular uptake of therapeutic molecules. (Figure(9)) While using this method, short lifespan and absence of extravasation may limit the use of microbubbles for tissue targeting. This difficulty has been overcome by the development of perfluorocarbon (PFC) nanoemulsions that convert into microbubbles under the action of therapeutic ultrasounds. Furthermore, echogenic liposomes — also termed bubble liposomes — contain air pockets or nanoemulsions of liquid PFC44 and can integrate ultrasound responsiveness into a drug nanocarrier. The use of ultrasounds is also appealing because of their non-invasiveness, the absence of ionizing radiations, and the facile regulation of tissue penetration depth by tuning frequency, duty cycles and time of exposure.

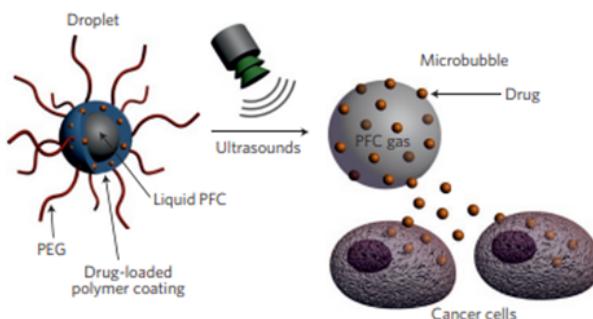


Figure (9) Drug delivery from echogenic perfluorocarbon (PFC)-containing nanoemulsions.

4. Light triggered drug delivery

A large variety of photoresponsive systems has been engineered in the past few years to achieve on-demand drug release in response to illumination of a specific wavelength (in the ultraviolet, visible or near-infrared (NIR) regions). Used materials should be light-absorbing, as the commonly used mechanism in light-responsive platforms are chemical bonds cleavage, conformation shift(usually from trans to cis), or a decrease in the hydrophobicity of the platform. A bioluminescence resonance energy transfer (BRET) system, based on luciferase-rose bengal conjugates for photodynamic therapy (PDT). The emission peak of the donor luciferase (535 nm) is correlated with the absorption peak of the acceptor rose bengal (550 nm). The reaction between the luciferase and its ligand CTZ generates single oxygen and drug is released with such change. Another common approach is using NIR radiation. In NIR-responsive platform for DOX release from

gold nanorods (AuNR) core, coated with MSN shell, Once NIR radiation was applied, the photothermal effect on the AuNR caused the detachment from the polynucleotides, and their denaturation. This provoke the reveal of the pores, leading to the drug release.

4-b. Endogenous stimuli-responsive drug release

1. pH-sensitive system

pH variations have been exploited to control the delivery of drugs especially in intracellular compartments (such as endosomes or lysosomes). Two main strategies exist: (1) the use of polymers (polyacids or polybases) with ionizable groups that undergo conformational and/or solubility changes in response to environmental pH variation; and (2) the design of polymeric systems with acid-sensitive bonds whose cleavage enables the release of molecules anchored at the polymer backbone, the modification of the charge of the polymer or the exposure of targeting ligands. As shown in Figure(10), Polyhistidine-based micelles could respond to acidic tumour microenvironments by efficient exposure of the transactivating regulatory protein (TAT) sequence(First way), and TAT-peptide decorated liposomes comprising an acidic hydrolyzable PEG shell allowed improved exposure of the TAT sequence at low pH(Second way). This system is usually used in cancer therapy, taking advantage of the slight difference of pH existing between healthy tissues (~7.4) and the extracellular environment of solid tumours (6.5–7.2). Also, because of the broad range of pH found throughout the gastrointestinal tract, pH-responsive systems for oral drug delivery have been designed to protect drugs from the harsh conditions found in the gastric cavity and to improve their absorption in the intestine. For instance, poly(methacrylic acid)-based copolymers have been used as pH-sensitive coatings at the surface of porous silica nanoparticles, as well as to prepare copolymer micelles able to disassemble at the intestinal pH.

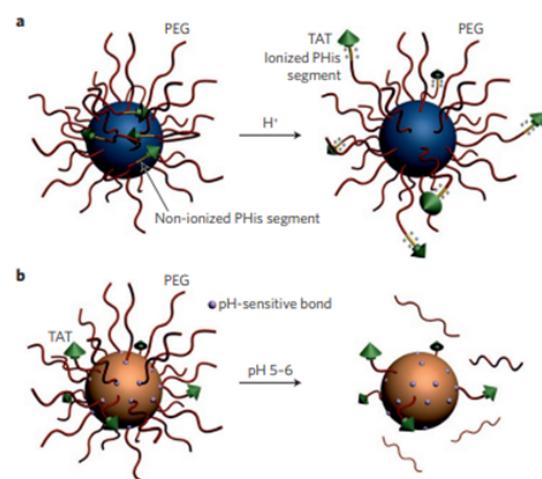


Figure (10) pH-sensitive nanocarriers for efficient TAT-peptide exposure.

2. Redox-sensitive system

Disulphide bonds, prone to rapid cleavage by glutathione (GSH), can be used to attain redox sensitivity. The cytosolic release of drugs can then be triggered by the different concentrations of GSH found in extracellular (~ 2 – 10 μM) and intracellular (~ 2 – 10 mM) compartments, and in tumour tissues compared with healthy ones. This has been achieved by reductively degradable micelles from self-assembled amphiphilic copolymers containing disulphide links within the hydrophobic backbone or bearing a single disulphide bond at the connection of the two polymer blocks. Other routes used GSH-sensitive crosslinking agents incorporated either in the shell or in the core of the micelles, leading to rapid micelle disassembly followed by specific intracellular release of hydrophobic drugs. Oxidation responsiveness was also explored for triggered drug release in inflammatory tissues, which are characterized by an accumulation of reactive oxygen species.

3. Enzyme-sensitive system

The altered expression profile of specific enzymes (such as proteases, phospholipases or glycosidases) observed in pathological conditions, such as cancer or inflammation, can be exploited to achieve enzyme-mediated drug release with accumulation of drugs at the desired biological target. Most of the systems devoted to enzyme-mediated drug delivery exploited the presence of enzymes in the extracellular environment. There are two big ways : (1) Using enzyme-sensitive property on penetrating and (2) Using that on releasing or drug releasing relying on degradation. First way is: short peptide sequences, cleavable by enzymes, as linkers between surface PEG chains and either TAT-functionalized liposomes so that when the NDDS gets into the region, the linker gets cleaved. After cleavage of the PEG shell in the tumour microenvironment, surface bioactive ligands became exposed, and this enhanced intracellular penetration compared with nanocarriers without cleavable linkers. (Figure(11)-a) In the case of second way, as you can see in the Figure(11)-b, The enzyme-mediated disintegration of the polymer-DNA electrostatic interaction promoted gene release and transcription.

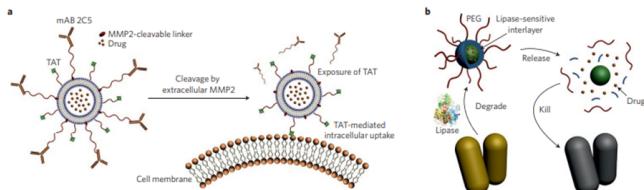


Figure (11) Enzyme-sensitive drug delivery.

To enhance the effectiveness of NDDSs, sometimes various drug releasing strategies are multiply combined. Such combined use of two different stimulus-sensitive approaches could be beneficial, as was shown for copolymeric micelles that were sensitive to both pH and redox potential. Because of the coexistence of a pH gradient and an oxidative environment in certain pathological conditions, in certain cases pH and redox responsiveness can be used in combination. For example, doxorubicin delivery to HepG2 tumour cells by these

dual-sensitive NDDSs was more effective than with single-stimulus-sensitive control NDDSs. Also, thermosensitive and magnetically sensitive liposomes loaded with methotrexate had more favourable drug pharmacokinetic properties than single-stimulus-sensitive delivery systems. They can be used multiply due to a temperature increase when an alternating magnetic field is applied. Of all the stimuli considered above, pH and temperature are the easiest to use among the intrinsic stimuli, as well as magnetic field and ultrasound.

5. Penetrating- intracellular delivery and organelle targeting

After reaching the target, NDDSs may still need to cross the barrier of the cell membrane to deliver their drug load into specific organelles within the cytoplasm. To make cellular internalization, targeting and cell-penetrating moieties are included in NDDS with agents that can destabilize the lysosomal membrane, such as fusogenic lipids and pore-forming proteins. These agents will be activated by the internal stimuli such as pH, enzyme, or glutathione variation and consequently make selective internalization of NDDS. Complexes of HIV TAT peptide (TATp)-liposomes and a plasmid encoding green fluorescence protein (pGFP) were used for successful in vitro transfection of several tumour and normal cells, as well as for the in vivo transfection of Lewis lung carcinoma tumour cells in mice. Octa-arginine-modified, bleomycin- or doxorubicin loaded liposomes demonstrated good intracellular penetration and strong inhibition of tumour growth in murine model. Also to provide intracellular drug delivery, the cell-penetrating function should be shielded until the NDDS is inside the target cell. In this case, PEG coating is used such as targeted long-circulating pegylated liposomes and PEG-phosphatidylethanolamine-based micelles that possess several functionalities.

6. Removal

In sum, inflammation could lead to a fibrotic encapsulation of the device which will carry out the removal. This fibrous capsule is mainly created by myofibroblast and fibrocytes, forming collagen and extracellular matrix (ECM) layer around the device. This FBR will last as long as the device is in the body, usually leading to fibrosis: isolation of the devices from the nearby tissues by creating a buffer multi-component layer on top of the device. The FBR is composed of different immune factors such as macrophages, fibroblasts, foreign body giant cells (FBGCs), etc., depending on the device's surface characteristics. Since the phagocytosis of nondegradable element will fail, the immune response will eventually lead to the inflammation and device encapsulation, as part of the foreign body response (FBR). As the body tries to heal the injury or to remove the foreign object, it will try to clear it out. Immune system removes the NDDS devices.

Green Nanoparticles refers to nanoparticles or nanomaterials obtained by combining biotechnology and nanotechnology using biological pathways or products such as proteins and lipids of living organisms (plants, microorganisms, viruses, etc.). They have the advantage of reducing the use of expensive chemicals, consuming less energy during production, and producing eco-friendly products than conventional physical and chemical technologies. The disadvantages of this technology include difficulty in obtaining raw materials, low yield, and long production time. The organisms used in GNP are mainly plants, and their extracts are used as reducing agents to reduce Cobalt, Copper, Silver, Gold, Palladium, Platinum, Zinc Oxide and Magnetite. Previous studies have shown that their biological systems in algae, diatoms, bacteria, yeast, fungi and human cells, as well as plants, can convert inorganic metal ions into metal nanoparticles by their ability to reduce proteins and metabolites, and synthesized metal nanoparticles exhibit unique optical and chemical properties. Compared to the conventional physical and chemical nanoparticle synthesis methods, green synthesis through living things has the following advantages: first, the process step is short. Second, energy consumption during synthesis is low. Third, it is economical. (Use less expensive chemicals and do not require complex equipment and synthesis steps). Fourth, it is not toxic and has high stability. On the other hand, the disadvantages can be classified as those that are common to nanoparticles (e.g., when NP enters the respiratory tract, it can cause heavy metal poisoning and nano-pollution), and the disadvantages of having only GNP are as follows: first, there is a difficulty in extracting raw materials (Seasonal and Regional Availability Issues). Second, the synthesis and reaction time are long. Third, there may be problems with the quality of the final product. (The particle size of the product is very heterogeneous). Fourth, low yield.

GNP technology mainly uses plant extracts, and the general process is to use dry biomass and metal salts of plants as bio-reducing agents and precursors, respectively. If you look at this process in detail, there are three main steps, and the steps are as follows.

1. Determine the solvent to be used.
2. Decide on eco-friendly and environmentally friendly reducing agents.
3. A non-toxic substance as a capping agent is determined.

These major steps increase the stability of the synthesized nanoparticles. When GNP is used for drug delivery, it is absorbed into target cells through capillaries, and then efficient drug accumulation is possible there, and continuous drug release is possible. In addition, there is no biological toxicity of the carrier while transporting the drug, so it is highly stable and advantageous for large-scale production and sterilization, so the outlook in the future medical industry is bright.

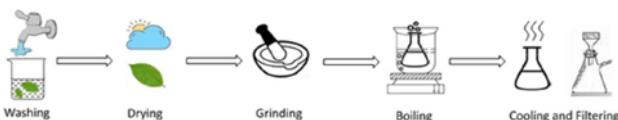


Figure (12) The basic process of obtaining plant extracts that are the basis of GNP

I-3. Blood-Brain Barrier

The blood-brain barrier (BBB) is the term that refers to the continuous nonfenestrated vessels of the central nervous system (CNS) that regulates the movement of molecules, ions, and cells between the blood and the CNS. The tight capillary in the BBB is made from endothelial cells (ECs), pericytes, astrocytes, tight junctions (TJs), neurons, and basal membrane. Additionally, the interendothelial junctions including adherens junctions, TJs, and gap junctions control the permeability of the BBB. There are two pathways in which molecules take to cross the BBB: paracellular pathway and transcellular pathway. The paracellular pathway indicates passive diffusion in which the molecules use the concentration gradient to pass the BBB. The transcellular pathway includes several mechanisms including passive diffusion, receptor-mediated transport, and transcytosis. The BBB can be disrupted due to various physiological conditions of diseases, including stroke, diabetes, seizures, hypertensive encephalopathy, acquired immunodeficiency syndrome, traumatic brain injuries, multiple sclerosis, Parkinson's disease (PD) and Alzheimer's disease (AD), and the disrupted BBB often becomes highly permeable.

As a restricting barrier, it maintains the CNS homeostasis and prevents harmful substances such as pathogens and toxins from entering the CNS, as well as protects the CNS from diseases and inflammation. Thus, while it is an obstacle for the nanoparticles that aim to enter the CNS, a healthy brain's blood-brain barrier serves as a critical tool for proper neuronal function. According to the previous research, it has been found that the nanoparticles themselves don't cross the BBB even with their small sizes. Instead, it was observed that the nanoparticles increase the drug concentration in the cells that are present in the BBB, increase the half-life of the drug, and increase the circulation time of the drugs in the blood, therefore providing more opportunity for drug uptake. Additionally, the nanoparticles themselves likely enter the damaged brain from stroke or sclerosis through the disrupted BBB. Making the nanoparticle themselves cross the BBB of the healthy brain, however, requires further research.

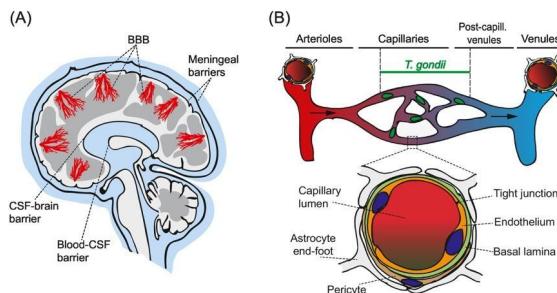


Figure (13) Schematic representation of blood-brain barrier

For the drug delivery to the CNS to occur, the nanoparticles must overcome the blood-brain barrier, and to see if the nanoparticles went through the BBB, they must be labeled or "barcoded" for detection and tracking. Medina et al. attached the amine-functionalized PEG-coated quantum dots (QDs) to the poly(lactic-co-glycolic acid) (PLGA), which was then conjugated to the nanoparticles. The barcoded QD-PLGA nanoparticles were simultaneously tracked through fluorescent signaling, and they observed that the nanoparticles entered the CNS through circumventricular organs or leaky vasculature of tumors. Thus,

this means that the increased vascularity of the tumor facilitates the nanoparticle entrapment within endothelial cells in the tumor. They concluded that this is the reason why using fluorescent dyes for tracking nanoparticles can give misleading information about the localization of individual nanoparticles.

There have been several attempts to make nanoparticles cross the BBB, which often involves incorporating various kinds of biopolymers and peptides into the nanoparticles. The research done by Monge-Fuentes et al. demonstrated the poly (D, L-lactic-co-glycolic acid) (PLGA) polymeric nanoparticles deliver dopamine to the brain tissue by circumventing the BBB structure. Albumin was selected for the experimental nanosystems for its ability to pass the BBB through the receptor-mediated pathways. As a result, they observed red fluorescent dots scattered throughout the brain tissue, meaning that the albumin/PLGA nanosystem agglomerates were successful in passing through the BBB.

II. Natural Drug Delivery

NDD refers to transporting a therapeutic agent to a specific location and releasing it at a specific speed using a delivery tool or carrier. It can be used to promote the transfer of small compounds as well as large molecules such as peptides, nucleic acids, polymers, and poorly soluble treatments. They can be obtained by extracting them from plants, marine life, fungi, microorganisms, and invertebrates, and then concentrating, sorting, and refining them. The NDD obtained in this way can be used to treat diabetes, cancer, neurodegenerative diseases and infections.

II-1. Animal Origin Natural drug delivery

1. Gelatin Nanoparticles

Gelatin is a denatured protein derived from the acidic hydrolysis or collagen bases in animals and stimulates the immune system through the denaturation of gelatin. They are used as stabilizers and plasma volume enhancers in gelatin sponges such as protein formulations, vaccines, and gel foam. It can play an important role in cell binding and cell transfer by binding to beta subunits of integrin receptors on the cell surface. Gelatin's activators are used to produce target drug delivery carriers and to attach large amounts of drugs to the carrier by allowing various chemical changes to the gelatin, either directly or using other linkers. Gelatin can be used to deliver both hydrophilic and hydrophobic drugs to Polyampolyte. Gelatin nanoparticles are synthesized through precipitation, phase separation, emulsion solvent evaporation, self-assembly by binding chemical functional groups of gelatin molecules, self-assembly between drugs and gelatin molecules, and micro-emulsion. In most cases, crosslinking agents are combined to stabilize nanoparticles in the final phase of synthesis.

2. Collagen Nanoparticles

It is widely used in medicine because it is biocompatible, has small immune system stimuli, and is biodegradable. Collagen NP is removed by the action of the macrophage system, allowing certain compounds such as anti-AIDS drugs to be absorbed into macrophages. It is used as a drug carrier for long-term administration of antibacterial and steroid drugs in dermatology by forming a stable and transparent colloidal solution dispersed by water, and due to its small size and wide contact surface.

3. Milk Proteins

Depending on the structure of the natural carrier of biologically active substances, there are two types of linear proteins, including casein, and spherical proteins, including whey proteins. Linear proteins are inexpensive, easily accessible, and are used in drug-carrying nanoparticles with high stability. In addition, unlike spherical proteins, it has the advantage of not being sensitive to temperature. The casein film has high tensile strength and is used as a purification coating to protect sensitive compounds. On the other hand, the disadvantages of casein are that direct intravenous injection can cause immunosuppression and allergies. Spherical proteins are characterized by high resistance to enzymes that break down proteins.

4. Silk Fibroin

It is composed of fibrous linear proteins and adhesive proteins such as serein surrounding them, and these are mainly studied in drug delivery and tissue engineering. The low inflammatory response at the decomposition site and the formation of nanoparticles through them can protect peptides with proteins such as conjugated insulin and vascular endothelial growth factors in an environment where serum and digestive enzymes are present, resulting in an increased active release period of drugs.

5. Elastin

It is similar to Elastin fibers in tissues (arterial, lung, skin, etc.) in the body, and has the advantage that the immune system does not respond. The possibility of designing and manufacturing similar Elastin proteins provides the advantages of protein nanoparticles, including the ability to achieve pharmacokinetic properties, accurately control molecular weight, and the production of single-size polymers.

II-2. Plant Origin Natural drug delivery

1. Zein

Seventy-five percent of the amino acids, which consist of water-soluble and alcohol-soluble proteins found in grains, are hydrophobic. With this characteristic, it can be used as a carrier for hydrophobic drugs, and a hydrophilic compound can be trapped to enable transport and release. It has excellent biodegradability, low absorption, and high-temperature

resistance, and can be used as a decomposable coating agent for pharmaceuticals.

2. Gliadin

It is similar to creatine and the protein is rich in proline, so it can interact with the skin creatine epidermis. Through these properties, skin formulations can be produced. It shows its ability as an emission control system for hydrophobic compounds such as vitamins A and E and amphiphilic compounds. And it has a high binding force with the cell membrane through many hydrogen bonds and hydrophobic interactions in the body's mucous membrane. Through this, it is useful for the manufacture of oral formulations for the treatment of gastric diseases. It is effective in removing *Helicobacter pylori* by increasing bioavailability due to interaction with gastric mucus and increasing drug release time.

3. Lectin

Wheat Germ Agglutinin (WGA) gained great interest due to its high stability in lectin, low toxicity and immunogenicity, resistance to proteolysis, and specific identification and binding site of glycosylated in the intestinal mucosa and found that the absorption of oral drug agents could be improved. It is being studied to improve the absorption of existing drugs with low bioavailability and manufacture target drug formulations for cancer treatment, and to induce apoptosis in cancer cells, resulting in high anti-tumor effects. There are many proteins and phospholipids in the cell membrane attached to the roots of oligosaccharides, and they have the property of being able to bind specifically. Target drug action can be performed through these characteristics.

4. Soy proteins

Soy protein extracts are a balanced combination of polarity, non-polarity, and pregnant amino acids that can be used in various drugs. It has a spherical structure consisting of a hydrophilic shell and a hydrophobic nucleus.

5. Sunflower Pollen

Pollen has the ability to protect plant genetic material against long-term dryness, high temperatures, ultraviolet rays, and damage caused by microorganisms. Using these properties, pollen grains have a high potential as a transport medium for encapsulation of various substances, including medicines, vaccines, formulants, and oils, in solid exine capsules separated from plant spores.

on the type of virus. It typically has excellent stability in a variety of chemical environments and can be used to design protein carriers that perform multiple tasks simultaneously, such as drug loading, imaging agents, and cage targeting agents for specific cells or tissues. It also has a uniform size, making it possible to load a relatively specific amount of drugs into these nanoparticles, which is an important pharmacokinetic feature of the drug formulation. The produced protein cage is naturally stable in many physiological environments and protects drugs and treatments from chemical and enzymatic degradation.

2. Non-Viral Protein Cages

In addition to viruses, ferritin/apophytin protein cages and small heat shock protein are among the protein cages. In the case of the ferritin cage, there are 14 channels for material exchange between external environments in the form of a hollow structure, and about 4,500 iron ions can be stored in the inner space. Numerous cancers, such as neuroendocrine, pancreatic, prostate, lung, bone marrow, and leukemia tumors, have been shown to be able to load large amounts of this radioactive substance into the body and increase its stability in the body. The higher dose of radiation to the tumor tissue will be during radiotherapy

II-4. Characteristics of plant-derived drug carriers and animal-derived drug carriers

In the case of plant origin, it is hydrophobic and has a longer drug release period than animal origin. It can also be stabilized without the use of fewer physicochemical treatments and chemical linker molecules than animal proteins, and the product is widely available. And the cost of synthesis is relatively low. It is highly stable because it is very unlikely to transmit diseases from plant to human.

Among animal-derived carriers, formulations based on albumin nanoparticles have several advantages. First of all, albumin is one of the important proteins in plasma and plays many physiological roles. If a high concentration of albumin is present in the body, a significant amount of albumin without side effects or with fewer side effects can be injected into the body. In addition, albumin has the advantage of being easy to access and low cost in mass production. And albumin has many functional groups and can bind to various drugs. In addition, it has the advantage of being easily deformed according to the environmental conditions where albumin is located and changing the binding of ligands to return to its original state with the help of disulfide binding. This characteristic imparts denaturation of the drug and increases stability.

II-3. Protein Cages

1. Viral Protein Cages

Virus cage is a structural shell of a virus that does not contain nucleic acid, and its shape, size, and stability vary depending

II-5. Platelets

Platelets, also known as thrombocytes, are cells that circulate within the blood that function in thrombosis and hemostasis in which they repair damaged blood vessels. They are either concave, oval, or disc-shaped, and they are formed in the

cytoplasm of mature megakaryocytes in the bone marrow. There have been several studies done on the relationship between the activated platelets and cancer cells, and the research identified receptor-ligand interactions including P-selectin and its ligand, α IIb β 3 integrins, the fibrinogen, and more. Platelets are known to play a major role in tumor cell proliferation by protecting the circulating tumor cells from physical obstacles including intravascular shear stress and helping them evade the host defense system. They also promote tumor extravasation and metastasis by allowing the tumor cells to migrate to secondary sites on the vascular wall, as well as facilitating tumor-related angiogenesis and growth by forming new blood vessels. Additionally, platelets aggregate with tumor cells in circulation, which facilitates tumor cells' adhesion to the vascular endothelium.

Platelets can be used in the drug delivery system using their ability to secrete biologically active molecules in a soluble form or packaged into extracellular vesicles (EVs) upon activation, which can be delivered to different cells including cancer cells. Since the presence of the platelets in the microenvironment of different kinds of tumors has been confirmed, there has been considerable research done on the ability of the platelets loaded with anticancer drugs and their therapeutic effects. Sarkar et al. demonstrated a platelet-based natural drug delivery system based on the concept that the activated platelets would release the loaded anticancer drug with the granules to the target site. They loaded the fluorescent-labeled doxorubicin (DOX) into the platelets and observed successful drug release by platelet. Loading drugs into the platelets was only possible since the entity engulfed by platelets does not get metabolized via conventional phagocytosis, but instead, remains intact inside the platelet. Moreover, Sarkar et al. observed that the release of drugs by platelets required a surprisingly low volume of drugs, as well as that there was no cross-reactivity observed since the platelets do not have a nucleus and DNA and don't express Rh antigen.

The research done by Zhang et al. is about engineering MK progenitor cells that are capable of creating platelets that express PD-1 on their surface and are loaded with cyclophosphamide, which is a regulatory T cell (Treg) inhibitor. Cyclophosphamide functions by causing the depletion of Tregs within the tumor microenvironment, which in turn improves the response rate of PD-1/PD-L1 blockade. By developing PD-1-expressing platelets, they demonstrated a cellular drug delivery system that can block PD-L1 on residual tumor cells and revert CD8+ T cells to eliminate the tumor cells. These platelets can also act as drug carriers, and they demonstrated this by loading cyclophosphamide.

II-6. Biomimetic

The efforts to improve the nanoparticle drug delivery system are mainly focused on synthesizing polymers for the nanoparticles, which can be manufactured reliably. Coating polyethylene glycol (PEG) to increase systematic circulation by stabilizing nanoparticles as well as protecting them from opsonization is the most well-understood method to create long-circulating nanoparticles. However, there have been several

attempts to incorporate natural biomaterials into synthetic nanoparticles to make nature-inspired biomimetic delivery systems. This is the cell-membrane coating mechanism, also known as the "Trojan horse technology." The mechanism is about covering the nanoparticles that contain loaded drugs with the membranes of biological cells, including erythrocytes, mesenchymal stem cells, WBC, RBC, platelets, and cancer cells. The cell membrane-camouflaged nanoparticle achieves longer circulation, which allows sustained systemic delivery and improved targeting.

Improving the cell-specific targeting is one of the most critical features of the nanoparticles that work as carriers of therapeutic drugs. It is a desired trait since this ability can potentially reduce off-target side effects as well as increase drug delivery. To improve cell-specific targeting of the cell membrane-camouflaged nanoparticles, there have been studies on inserting lipids into the cell membranes, which is possible due to the fluid and dynamic nature of the membrane bilayers. In addition to this method, the cell membrane used to coat the nanoparticles can be utilized to have cell-specific binding.

Hu et al. demonstrated the natural RBC membrane-coated nanoparticles and functionalizing the nanoparticles with native immunomodulatory proteins. The "marker-of-self" proteins, including native CD47, to the particle surfaces in the right-side-out orientation. CD47 is an integral membrane protein of RBCs that has an IgV-like extracellular domain that is responsible for keeping the RBCs alive in circulation. They yielded nanoparticles that have the same CD47 surface density as the natural RBCs that are capable of undergoing molecular interactions. This RBC-NPS evaded the macrophage uptake, which verifies that the CD47 present on the surface is a functional immunomodulatory protein.

Jing et al. demonstrated the platelet-camouflaged nanococktail as a possible treatment for multidrug resistance (MDR) cancer. They encapsulated melanin nanoparticles (MNPs) and doxorubicin (DOX) inside the RGD peptide-modified nanoscale platelet vesicle (RGD-NPVs). Using the RGD-NPVs as the drug carrier allowed the therapeutic agents to evade immune surveillance and facilitate the targeting of α β 3 integrins that are overexpressed on the tumor vasculature. They observed no toxicity in the test cells as a response to RGD-NPVs, as well as that the RGD-NPVs successfully inhibited the growth and metastasis of the MDR breast cancer.

III. Robotics

III-1. Microrobots for drug delivery

To minimize the discomfort of invasive gastrointestinal (GI) examination, small bowl endoscopy has been developed. Nowadays, not only images but also on-the-spot diagnosis and therapy are required, and various microrobots containing drugs and sometimes endoscopy are meeting such requirement. They are generally between 3 and 4.5 g in weight and approximately 26 × 11 mm in size and have a GI tract from the mouth to the anus by autonomic peristaltic movements (passive locomotion) as a pathway. Along with such a pathway, microrobots should find the desired area and release the desired amount of drug

accurately, and differently depending on the type of therapy. Microrobots for drug delivery generally have two important factors: Releasing and Anchoring.

1. Releasing

First, microrobot's releasing mechanism can be divided into two types: (1) passive releasing, and (2) active releasing. Their main difference is the availability of spontaneous monitoring through a camera, can be related to diagnosis, and remote of activation based on it. In the passive-releasing microrobots, the drug is exposed to the GI environment whenever an external trigger (e.g., pH or temperature). In the active-releasing microrobots, the active expulsion of the drug is driven by the remote activation of the release mechanism. However, their trigger energy source and mechanism of drug release are almost the same (e.g., radio frequency, magnetic field). Under, each passive and active-releasing microrobots are explained.

1-a. Passive releasing microrobots

Passive-releasing microrobots lack monitoring and communication with computer device, so they usually use an external trigger energy source. The drug release might be triggered by an external radiofrequency generator that heats and melts a thread, thus piercing a latex balloon containing the chemical with a released needle. When the carrier capsule consists of two magnetic parts bonded together and containing a reservoir, the drug release is performed by an external magnetic field that separates the capsule's two components. However, the activation may fail due to interposed tissues weakening the electromagnetic signal easily in passive-releasing methods.

1-b. Active releasing microrobots

Active-releasing microrobots can be simply explained as a combination of a drug releasing mechanism of passive release and capsule endoscopy or a sensor that can communicate with a computer. The active-releasing microrobot consists of a cap (containing 0.3 mL of liquid drug), microprocessor, battery, pH and temperature sensors, RF transceiver and pump. Computer devices resident times throughout the GI tract and local temperatures and pH with wireless data transmission allowing automated drug release with pH/temperature-based positioning. Also, because the active-releasing platform has a communicating device or sensor inside, the trigger energy source is usually positioned internally. For example, in Figure (14), an internal heating element triggers the capsule's opening and rapidly ejects the drug with a piston. In Figure (15), gas pressure is produced to empty the drug reservoir using a microigniter. Figure (16) is the form of a typical active-release microrobot platform. It has four main components necessary for ultrasound-mediated targeted DD: a focused US transducer, a DD channel, a video camera and a light source.

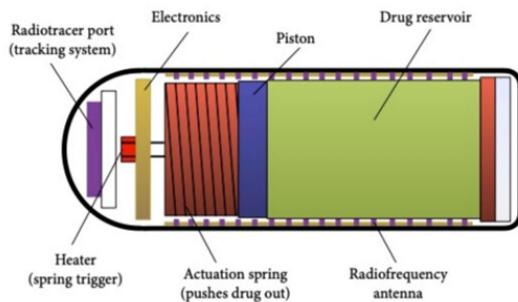


Figure (14). Schematic drawing of the Enterion™ capsule.

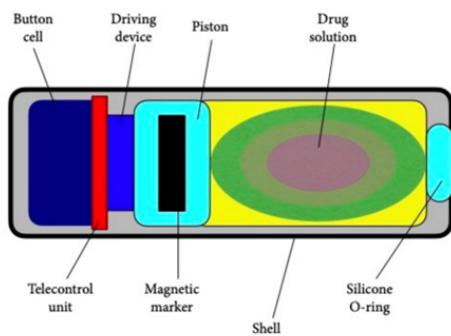


Figure (15). Schematic drawing of the remotely controlled capsule (RCC).

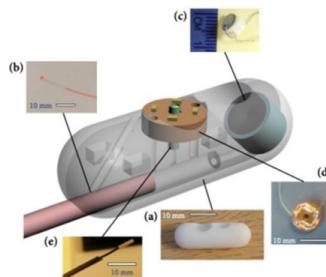


Figure (16). (a) the capsule body, (b) the tether with the electrical cables and the drug delivery channel, (c) the ultrasound source, (d) the light source and (e) the miniature camera

2. Anchoring

They should be able to withstand the forces of peristalsis. String attachments or “leg”-based designs are the most used. To target an area of interest within the intestine, prevent rapid transit and allowing the capsule to be retrieved, and release drugs or implement the diagnosis, anchoring the microrobot to the area is necessary.

1. CFD

Respiratory drug delivery is a surprisingly complex process with a number of physical and biological challenges. Computational fluid dynamics (CFD) is a scientific simulation technique that is capable of providing spatially and temporally resolved predictions of many aspects related to respiratory drug delivery from initial aerosol formation through respiratory cellular drug absorption. The dynamic model and quantitative drug delivery model of the targeted drug delivery microrobot driven by the spiral jet structure are established, and the motion characteristics of the targeted drug delivery microrobot are simulated and analyzed by the method of Computational Fluid Dynamics (CFD)

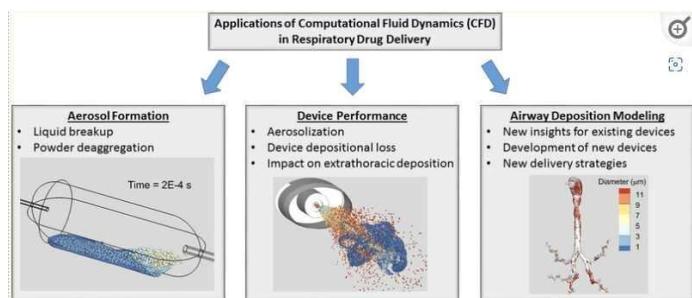


Figure (17)

2. Advantages

CFD models of respiratory drug delivery have a number of advantages compared with semiempirical and 1D whole-lung approaches. CFD simulations are based on solution of the underlying transport equations, which can directly account for factors such as transient flow, turbulence and turbulent particle dispersion, hygroscopic particle size change, and fluid-wall interactions in complex geometries. With CFD simulations, realistic airway geometries are employed, which are necessary to account for deposition in complex structures like the larynx, airway bifurcations, and constricted airways. Highly realistic models of the alveolar region including wall motion are also possible. CFD simulations can directly predict the effects of jet and spray momentum on an aerosol as it is emitted from an inhaler into the mouth-throat and upper tracheobronchial airways.

3. Limitations

Limitations of CFD models include complexity in capturing the physics associated with pharmaceutical aerosol generation and delivery, difficulty in resolving flow dynamics in the vast expanse of the bifurcating airways, and computational expense. As a result, CFD models are typically limited to sections of the respiratory tract, such as from the oral cavity to as far as approximately the sixth airway generation.

IV. In vitro device

IV-1. Iontophoresis

Biological polymeric drugs are considered superior to low molecular drugs due to their high specificity and favorable safety profiles. However, these polymer drugs have limitations in that the oral bioavailability is low and half. These drugs are administered as non-invasive transdermal delivery, and a major problem with this method is the presence of the outermost keratin layer of the skin. Iontophoresis relies on a low level of electrical action for transdermal drug delivery to promote skin permeation of hydrophilic and charged molecules. Iontophoresis is a method of administering drugs by applying a low voltage to non-invasive skin transmission technology.

In 2020, the FDA approved a total of 53 new treatments across several treatment areas, and these macromolecular drugs have fewer side effects, high specificity, and high endogenous target binding affinity. However, these drugs do not pass through the epithelium well due to their large molecular size and high polarity and are very likely to be inactivated in the digestive tract by various digestive enzymes. Existing methods (such as parenteral administration/intra-vacuum or subcutaneous injection) had side effects, and non-invasive pathways are being studied to solve them.

The mammalian skin consists of a double layer of the epidermis and dermis. The follicles, sweat glands, and sebaceous glands are known as skin appendages, which originate from depressed epidermal tissue and are often rooted in the dermis. The outermost layer of the epidermis acts as a barrier to the external environment and consists of dead cells called keratinocytes. In the process of damage and recovery, they mechanically push the absorbed drug out of the body. In addition, the active cell transport process does not exist in non-living keratinocytes, so it is difficult to deliver drugs to keratinocytes. In addition, they are composed of hydrophobic substances, making it challenging to spread hydrophilic drugs.

Solving this problem was approached by a chemical and physical method. Chemically, it is a passive technology that modifies the lipid structure, increases drug penetration to the stratum corneum, or increases the permeability of the stratum corneum by combining the two. A recent study revealed effective ion water-based skin delivery of siRNA (used by organic solvents, fatty acids, and surfactants). In addition, encapsulations or particulate formulations are using lipid-based nanoparticles (liposomes, ethosomes, transferosomes, and niosomes), dendrimers, and polymer nanoparticles. Physically, there are ultrasound, fine needle, electrical perforation, pyrojet injectors and thermal resection, and Iontophoresis.

In the case of Iontophoresis (IP), weak current (0.5 mA/cm^2) is the driving force, and the advantage is that it can be transferred regardless of molecular size, and it can be performed simply without damaging cells. The disadvantages are that skin irritation may occur, and there is a risk of burns if the current is selected incorrectly.

The IP consists of an anode and a cathode, a drug store, an electronic controller, and a power source. They place active electrodes including drug stores on the skin surface and attach return electrodes including opposite ions adjacent to the active electrodes to complete the circuit. Thereafter, the drug is injected

by inducing the flow of current from the electrode to the skin. The current used here provides a driving force for the drug to penetrate and penetrate the stratum corneum. The penetration efficiency of the drug is determined by the intensity and duration of the current and the area of the skin surface in contact with the active electrode.

Researchers such as Hama also studied the biological effects of IP and the penetration of skin barriers. The researchers found that applying IP to skin surfaces activates an intracellular signaling pathway leading to the opening of intercellular spatial devices that facilitate liposome movement through skin barriers. In addition to these studies, when low current is applied to cultured cells, the induced cell absorption path is found to be intracellular migration, but it is known that macromolecules of more than 70,000 Da (dalton) are emitted. In addition, specific signaling molecules that contribute to low current medium intracellular migration were identified.

In conclusion, we found that low-current IP activates intracellular signaling pathways that collaboratively promote propulsion and penetration of skin barriers in drug delivery. In the case of IP, it is known that aqueous pores are not generated in the stratum corneum. Recently, it has been reported that this method opens intercellular junctions to produce fast transport pathways, and therefore polymer drugs delivered to IP can follow paths around cells that cross skin barriers. (drug delivery through hair follicles, sweat glands, and glands) That is, during IP, electricity preferentially passes through hair follicles and sweat glands and induces solvent flow through these accessory pathways. Drugs can be absorbed through this process.

A recent development in IP-mediated transdermal delivery of biological polymer drugs has shown that

1. IP-mediated intradermal delivery of siRNA in the skin with atopic dermatitis
2. IP-mediated transdermal delivery of biological macromolecules (CpG oligodeoxyribonucleotides) for cancer immunotherapy
3. Psoriasis Targeting by IP-Mediated Transdermal Transfer
4. IP-mediated percutaneous transdermal transport of Cetuximab (chimera monoclonal antibody that binds to human epidermal growth factor receptors) (SCC) is a non-melanoma skin cancer originating from viable epidermal keratin cells
5. IP-mediated transdermal transfer of biologically active human primary fibroblast growth factor
6. IP application for internal organs

There are, and they combine IP and other penetration technologies to apply them. These limitations include that drug delivery is limited when the molecular weight is 15 kDa or more, and the transfer efficiency may vary depending on the physicochemical characteristics (solubility and stability) of each polymer. In addition, the electrical amplitude used is physiologically acceptable but can have several effects on the skin. (skin irritation, numbness, itching, and erythema) There is also a risk of burns due to electric currents.

The development of biological polymeric drugs has continued to expand in recent years as a non-invasive route of administration

is preferred for this type of drug. IP has gained significant interest in non-invasive skin delivery of biological giant molecular medications due to a simple application procedure. In this review, we discussed the potential application of IP and its underlying mechanisms to overcome problems related to non-invasive transdermal delivery of biological macromolecules. Various studies have demonstrated successful and effective IP-mediated transdermal delivery of biological macromolecular drugs. However, in recent studies, IP was applied to internal organs such as the liver and pancreas, but the therapeutic effect was mainly limited to skin diseases.

IV-2. Invasive & non invasive drug delivery through skin

The most general methods of in-vitro drug delivery is done through the skin, whether it is invasive or non-invasive. In invasive way, there are hypodermic needle and microneedle(MN). In non-invasive way, there are topical cream and transdermal patch methods.(Figure(18)) Each latters are the new, potentiated technologies in the industry. Invasive ways have advantages on high bioavailability, the quickness of achieving the plasma level, accurate dose, but having disadvantages on pain, needles disposal. Non-invasive ways have advantages on less pain, ease on application, availability of self-administration, reducing frequency if administration, easy application, but disadvantaged on possibility of irritation at the applied site, limited suitable drugs and slowness or reaching the plasma level.

	Topical cream	Transdermal patch	Hypodermic needle	Microneedle
Description	Emulsion/ emulgel/ cream/ ointments	Adhesive patch to be placed on the skin	Fine, hollow tube having a sharp tip with small opening at the end	Micron size needles are aligned on the surface of a small patch
Onset of action	Slow	Painless	Painful	Painless
Pain	Painless	Painless	Sufficient	Sufficient
Bioavailability	Poor	In sufficient	Less	Better
Patient compliance	Less	More	Not possible	More
Side effects/contraindications	Possible	Drug has to cross stratum corneum barrier, thus poor diffusion of large molecules	Drug placed directly in the dermis	Bypass stratum corneum and drug placed directly into epidermis or dermis hence enhanced permeability
Mechanism of drug delivery	Permeation through skin pores.			

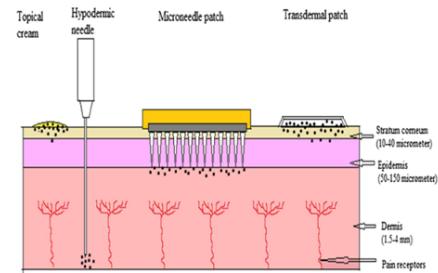


Figure (18). 4 methods of drug delivery through skin

To understand both invasive and non-invasive way, understanding the basic mechanism of drug delivery through skin is essential. Skin is composed of 3 layers: Epidermis, dermis, and hypodermis. (Figure(19)) The most important part of most drug delivery through skin is to bypass the stratum corneum (SC) and immune cells of epidermis layer (especially the stratum spinosum, an epidermal layer), and be absorbed to dermis layer which many blood capillaries are located. (Figure(20)) There are 2 big pathways to absorb drug via SC: transepidermal and transappendageal. The first way, transepidermal is the method that the large surface area of the SC allows drug from transdermal patch to spread onto the skin surface and permeate into the cells (transcellular) or interspaces between the cells (intercellular). The transepidermal route can be further subdivided into two pathways, namely transcellular and

intercellular. In the transcellular route, drugs diffuse through SC cells during the absorption process. Therefore, drugs have to pass the membranes, which are composed of lipid bilayers, which means that this route is mostly taken by hydrophobic drugs. The second route is the intercellular, in which the drugs have to diffuse through the lipid matrix of the intercellular space of residing keratinocytes in the SC. Hydrophilic compounds or small molecules are transported via this route. The intercellular route is the dominant pathway for drug absorption yet.(Figure(21)) The second pathway is transappendageal which is defined as drug delivery via hair follicles or sweat glands in the skin. This route is necessary for the transport of polar or ionisable compounds and is useful for transport of large macromolecules which have problems passing through the epidermal cells due to the molecular size and different partition properties. Nevertheless, the usage of this pathway is somewhat limited due to the smaller absorption area ($\sim 0.1\%$ of total skin area), compared to that available for the transepidermal route. Thus, researchers have developed methods to enhance drug absorption across the skin by modifying the structure of the SC, either chemically, physically or using combinations of these methods.(Figure(21))

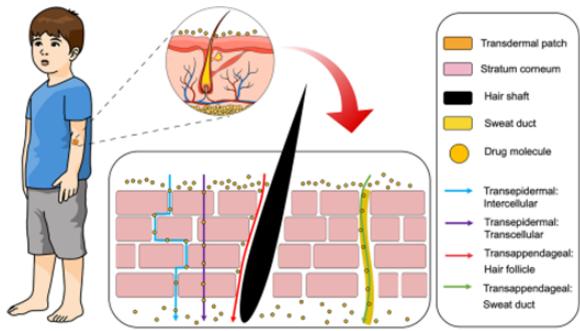


Figure (21). 2 big pathways of drug delivery through skin.

Below, each invasive methods: hypodermic needle, microneedle and non-invasive methods: topical cream, transdermal patch drug delivery is described.

1. Invasive in-vitro drug delivery

1-a. Hypodermic needle

A hypodermic (hypo – under, dermic – the skin) needle is a hollow needle commonly used with a syringe to inject substances into the body or extract fluids from it. They may also be used to take liquid samples from the body, for example taking blood from a vein in venipuncture. Unlike most of other in-vitro drug delivery system in this paper, the drug is injected directly to the dermis layer. So, it provides rapid delivery of liquids. Also it can provide sterile conditions. It significantly reduces contamination during inoculation of a sterile substrate in two ways. First, its surface is extremely smooth, preventing airborne pathogens from becoming trapped between irregularities on the needle's surface, which could subsequently be transferred into the media as contaminants. Second, the needle's point is extremely sharp, significantly reducing the diameter of the hole remaining after puncturing the membrane, which consequently prevents microbes larger than the hole from contaminating the substrate. However the second advantage is more improved in microneedle technology.

1-b. Microneedle(MN)

Microneedles (MNs) are micron-sized needles, on a solid support, with needle heights ranging between 25 and 2000 μm . These needles can pierce the SC and create microconduits, following insertion into the skin. MNs are able to penetrate the SC of the skin and reach the dermis without breaching nerve endings and blood vessels in the dermal layer. Consequently, upon administration, MNs are less painful and more comfortable compared to hypodermic injection. MNs are generally manufactured using biocompatible substances or biodegradable polymers, such as silicon, metal, ceramic, silica glass, carbohydrate, polymers including poly (methyl methacrylate) (PMMA), polylactic acid (PLA), poly (lactic-co-glycolic acid) (PLGA), polyglycolic acid (PGA), poly (carbonate), cyclic-olefin copolymer and etc. Polymer and metal is the material used most and ceramic and glass is the least used. It is important to ensure that MN materials are safe and do not induce inflammation response after the insertion. Also it must be sharp and slender

Figure (19). 3 layers of human skin

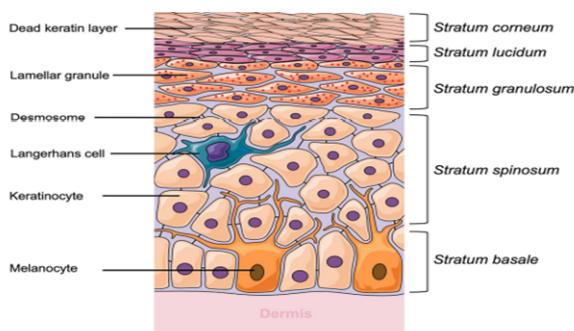


Figure (20). 3 Schematic representation of epidermis layer of human skin.

enough so that it can easily penetrate into the skin and also be strong enough so that it does not break when inside the skin. Two important factors for a safe and efficient design of microneedles are the force at which the microneedle loses its structural integrity and the insertion force. The ratio of these two forces is called as the ‘safety factor’ which is used to determine its compatibility. The ratio is preferred to be as high as possible. MNs can be cylindrical, triangular, pointed, pentagonal, octagonal and are available in many more shape. MN can be sorted in 6 types: solid, coated, hollow, dissolving, hydrogel-forming(a.k.a swellable), and porous. (Figure(22)) Below, each type is described. Also, sometimes MNs can be used in biosensing, and this is described briefly too.

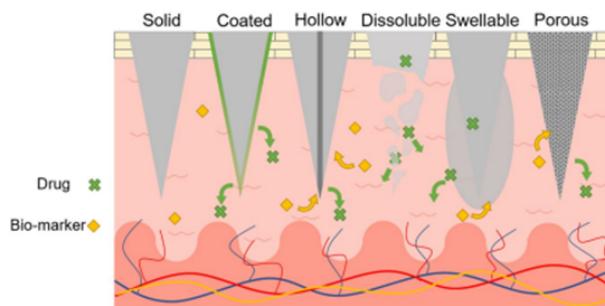


Figure (22) 6 type of microneedle used in drug delivery and biosensing.

(1) Solid MN

Solid MN is usually just for penetrate and create the pathway for drug to move. Following insertion, the MNs are removed and a transdermal patch is applied onto the microconduits (Figure(25)-a). Then, the drugs are released via passive diffusion from the drug formulation and permeate through the microconduits into the skin. Solid MNs might be fabricated using a variety of different materials such as silicon, tungsten, calcium sulfate dihydrate and polylactic acid.

(2) Coated MN

Coated MNs are fabricated by coating the needle tips of the MN with drug formulation, either a drug solution or dispersion layer. To prepare the coating, drugs are mixed with thickening agents and surfactants in order to ensure the drugs adhere onto the MN tips prior to insertion into the skin. (Figure(25)-b) In terms of materials, the MN can be prepared from metals or polymer, since the basic technology requires the production of a solid MN. In Figure(6), needle tips of coated MNs are shown.

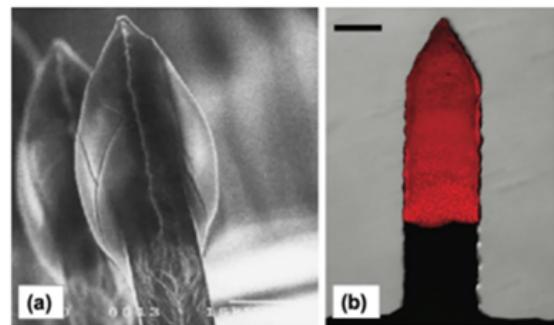


Figure (23) : (a) Desmopressin (reproduced with permission from Copyright 2004, Elsevier). (b) DNA (reprinted with permission from Copyright © 2010, American Chemical Society)

(3) Hollow MN

In hollow MN, the drug formulation (solution or dispersion) is loaded in the interior of the MN, then the drug is injected and transferred into the skin after insertion of the MN. (Figure(25)-c) Hollow MNs allow greater amounts of drug loading, when compared to solid and coated MN. Similar to solid MN, hollow MN can be manufactured using metals, silicon, glass or polymer. However, the major disadvantage of using hollow MN is the possibility of blockage after the needles are inserted due to the open needle bores. In Figure(24), needle tips of coated MNs are shown.

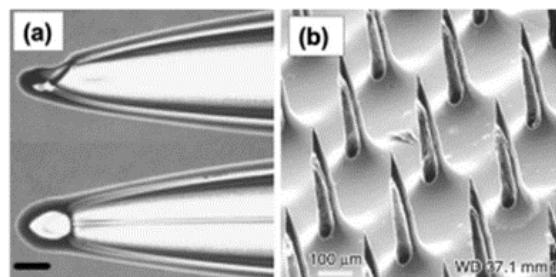


Figure (24): Hollow MNs made of (a) glass (reproduced with permission from Copyright 2006, Elsevier) and (b) silicon (this is an open access article)

(4) Dissolving MN

In the case of dissolving MN, following insertion, the needles dissolve in the skin and the drugs are released gradually from the MN matrix. For fabricating dissolving MN, the drugs are mixed with soluble and biocompatible polymers. Thus, drug release rate is mainly influenced by the polymer composition and MN matrices. (Figure(25)-d) Dissolving MNs have been manufactured using numerous different polymers, such as poly(lactide-co-glycolide), carboxy methyl cellulose, poly(vinyl alcohol), poly(vinyl pyrrolidone), hyaluronic acid and copolymers of methyl vinyl ether and maleic acid. However, dissolving MN also have some inherent drawbacks, such as the deposition of polymers in the skin, limited amounts of drug that can be formulated in the needles and subsequently delivered into the skin.

(5) Swellable MN

Instead of mixing the drugs with polymers, swellable MN utilises a drug-containing reservoir which is then integrated with blank MN upon application. The difference here is that, after insertion into the skin, the MN can absorb a considerable amount of interstitial fluid and swell in the skin. Then, the drug-containing reservoir dissolves and drug molecules diffuse through the swollen MN conduits into the skin. (Figure(25)-e) Swellable MNs are generally fabricated using physical crosslinking techniques such as exposure to ultraviolet (UV) light with photoinitiators, so the drug release can be controlled. For instance, Water-soluble swellable MNs with encapsulated drugs could achieve prolonged and uniform drug release with adjustable delivery rates by altering the crosslinking degree. There are two big advantages of such MN. Firstly, as the drugs are not a component part of the MN matrix, it is possible to load a greater amount of drug into the associated drug-loaded reservoir than could be loaded into the MN arrays themselves. Consequently, this can lead to increased drug concentrations delivered into the skin. Secondly, following application, the swollen MN are removed intact from the skin; hence, there is no polymer deposition in the skin.

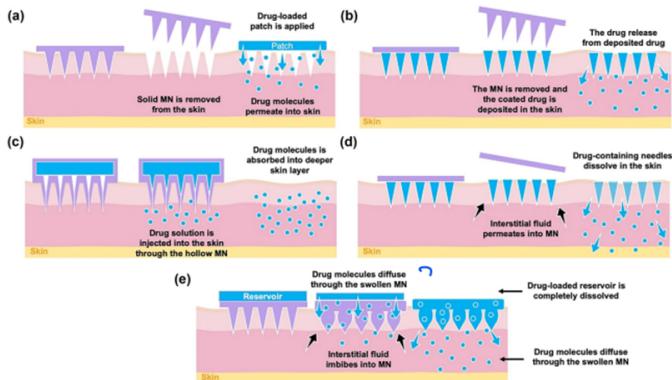


Figure (25). Various MNs: (a) solid, (b)coated, (c)hollow, (d)dissolving, (e)swellable

(6) Porous MN

Porous MNs, which have interconnected micro-sized pores throughout the entire structure of MN, have attracted significant attention. Porous MNs are generally created from biocompatible metals, ceramics, or polymers with small randomly distributed and interconnected pores. Figure(26) schematically illustrates one example of porous MN using pH change. The key component of this system is NaHCO_3 , which can be effortlessly incorporated into the pores of porous polymer coatings together with a drug, using an emulsion method. NaHCO_3 is a gas-generating agent under acidic conditions. Reaction of NaHCO_3 and an acid yields a salt and carbonic acid, which easily decomposes to carbon dioxide (CO_2) and water. This effect generates pressure inside the pores of the porous polymer coating and ruptures the thin polymer membrane, thereby releasing the encapsulated drug. By taking advantage of continuous pores, the drug solution can be absorbed spontaneously by capillary action with an active pharmaceutical ingredient (API) being stored or maintained in a dried form for additional minimally invasive

drug delivery. However, a trade-off between the porosity and the mechanical strength is necessary owing to the large volume of voids inside the MN when compared to solid MNs, which results in an increase in fragility.

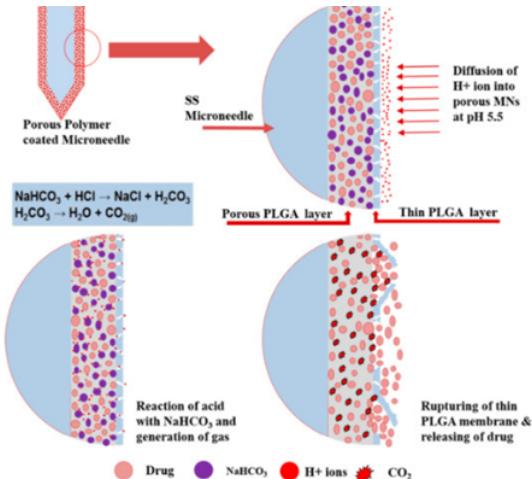


Figure (26). pH sensitive Porous MN

Here, general MN technologies are reviewed. Besides the delivery function, MNs have also been investigated for therapeutic drug monitoring and biosensing too. This technology was considered as a minimally invasive monitoring method because it utilises the micronized needles for taking the biological samples, such as skin interstitial fluid (ISF) or blood. Solidtype MNs were used to collect dermal ISF by piercing the skin followed by absorption using a paper strip. Hollow MNs were employed for rapid ISF extraction by capillary action (attraction between MN and the interested solution) owing to their structure. They were also integrated with biosensors or microfluidic channels to achieve the transportation of collected fluids and the subsequent analysis. Swellable MNs, generally created from crosslinked hydrogels, enable the continuous absorption of body fluids until saturated. In addition, the rapid absorption and collection of ISF can be achieved using porous MNs through capillary action. Thus, ISF extraction and the subsequent analysis of biomarkers in fluids can be achieved using porous MNs integrated with lab-on-chip biosensing devices.

2. Non-invasive in-vitro drug delivery

2-a. Topical drug administration

Topical drug administration is a localized drug delivery system anywhere in the body through ophthalmic, rectal, vaginal and mainly skin as topical routes. Topical drug administration must need following requirements. First, Container Selection and Product Stability. Container Selection and Product Stability Depending on the properties of the combined ingredients, a dispensing container will be chosen (i.e., tube, jar, can, etc.) to provide a stable physicochemical environment that protects the active compound(s) from chemical degradation. The formulation can be a liquid or semi-solid, monophasic or multiphasic (e.g.,

oil-in-water or water-in-oil). Second, Skin penetration. Once the product is applied on the skin, a complex interaction occurs between the formulation, the active compounds, and the skin itself. The penetration of the active compound(s) into the skin follows Fick's first law of diffusion (Formula (1)), which can be only applied in slow releasing (usually -0.5 release exponent over time), none-drug concentration changing condition. Also, most of the topical drug administration is done by transappendageal pathway which is defined as drug delivery via hair follicles or sweat glands in the skin. Third, it should be easy to applied. For example, the application of the drug on large, hairy surfaces like the chest and the back might be well-done. Generally, topical preparations meant for systemic or local effect are classified as 3 types: solid, semi-solids, liquids. Semi-solid form is the most used and creams, gel, ointments, paste, which are possessing drugs in a one type among various bases matrix such as hydrocarbon, water-removable, water-soluble bases matrix, are the examples.

$$J_B = -D_B^* \frac{dC_B}{dx}$$

Formula(1) Fick's first law / J is Flux, which represents the number of atoms passing through the unit area per unit time, D is the diffusion coefficient of atoms, C is the concentration, and x is the direction.

2-b. Transdermal patch

A transdermal patch is a medicated adhesive patch that is placed on the skin to deliver a time released dose of medication systemically for treating illnesses. It is a form of developed topical drug administration in various physico-chemical ways. Transdermal patch also utilize the fickian diffusion of drug as topical cream, which means it follows the Fick's law. Basic components of transdermal drug delivery systems are: Polymer matrix, the drug, permeation enhancers, adhesives, and backing membrane. First, the polymer controls the release of drug from the device. The polymer used should be stable, non-reactive with the drug, should allow the drug to diffuse properly and release through it. Natural polymers such as cellulose, synthetic elastomers, synthetic polymers are mainly used. Second, the drug should have molecular weight less than 1000 Daltons, and have affinity for both lipophilic and hydrophilic phases to be delivered through transdermal patch. Third, permeation enhancers promote skin permeability by altering skin as a barrier to the flux of a desired penetrant. Solvents, which increase penetration possible by swelling the polar pathway and/or fluidizing lipids, and surfactants, which the polar head group and the hydrocarbon chain length alters the penetration to enhance polar pathway transport, especially of hydrophilic drugs are the example. Forth, transdermal devices to the skin is done by using a pressure sensitive adhesive, which can be positioned on the face of the device or to the back of the device and extending peripherally. It should be physically and chemically compatible with the drug. Lastly, flexible and impermeable backing membrane provide a good bond to the drug reservoir, prevent drug from leaving the dosage form through the top, and accept printing. These essential components present differently depends on the patch's basic

mechanism of delivery – which is affected by how the drug is reserved. (Figure(10)) According to this, there are 4 big types of transdermal patch described below.

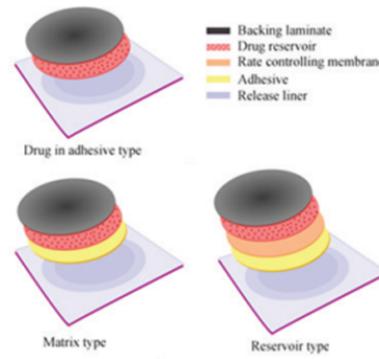


Figure (27) 4 types of transdermal patch. Adhesive type has drug inside the adhesive layer. Matrix type has drug inside the polymer matrix. Reservoir type has the drug inside the rate controlling membrane. Not shown in the figure, but lastly there is micro reservoir type which has both properties of reservoir and matrix type.

(1) Adhesive Dispersion-Type Systems (a.k.a Adhesive type)

This is the most simplified form as shown in Figure(10). The drug reservoir is formulated by directly dispersing the drug in an adhesive polymer e.g., Poly (isobutylene) or Poly (acrylate) adhesive and then spreading the medicated adhesive on to a flat sheet of drug impermeable metallic plastic backing to form a thin drug reservoir layer. (Figure(30)) Rate controlling adhesive polymer of a specific permeability and constant thickness are applied to produce an adhesive diffusion-controlled delivery system. This also follows the Fick's law considering adhesives' physical properties too.

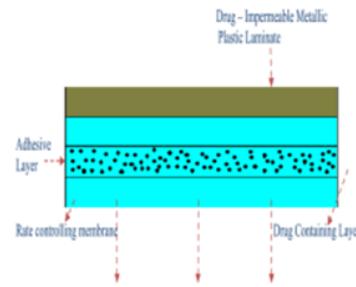


Figure (28) Adhesive type transdermal patch

(2) Matrix Diffusion-Controlled Systems (a.k.a Matrix type)

The drug reservoir is prepared by homogenously dispersing drug particles in a hydrophilic or lipophilic polymer matrix. The drug reservoir can be formed by dissolving drug and polymer in a common solvent followed by solvent evaporation. The drug reservoir containing polymer disc is then pasted onto an

occlusive base plate of a drug impermeable plastic backing. The adhesive polymer is then spread along the circumference to form a strip of adhesive rim around the medicated disc. (Figure(29)) The drug dispersion in the polymer matrix is accomplished mainly by blending therapeutic dose of drug with polymer or highly viscous base polymer, followed by cross linking of polymer. Because the drug reservoir is not directly attached to the backing and polymer can not rupture, this type of system has the absence of dose dumping. The rate of drug release from this type of system is controlled by initial drug loading dose, diffusivity of drug in polymer matrix and drug solubility in polymer, following the Formula (3), which is induced from the Fick's law. (Formula (1)).

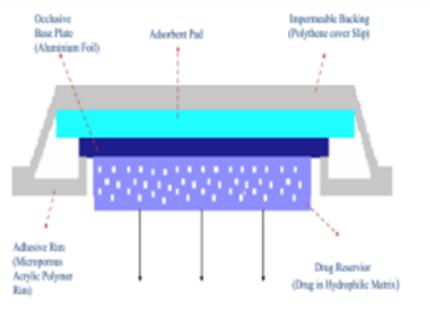


Figure (29) Matrix type transdermal patch

$$\frac{Q}{\sqrt{t}} = \sqrt{2AC_R D_s}$$

Formula (2) Matrix type drug release rate/ A is initial drug loading dose in polymer matrix, D is diffusivity of drug in polymer matrix, C is drug solubility, the actual initial concentration in polymer.

(3) Membrane Permeation – Controlled Systems (a.k.a Reservoir type)

In this type of system, the drug reservoir is totally encapsulated in a shallow compartment moulded from a drug-impermeable metallic laminate and a rate controlling membrane. (Figure(13)) The dosage rate per patch area is controlled by altering the composition and thickness of the inert membrane which has a constant thickness x, drug diffusivity D, drug solubility K, thus a constant permeability DK. The rate of drug release from this type of system is controlled by the polymer composition, permeability coefficient and thickness of the rate limiting membrane and adhesive, following the Formula (3), which is induced from the Fick's law. (Formula (1)). Breakage of the rate controlling membrane can result in dose dumping or a rapid release of the entire drug in this form.

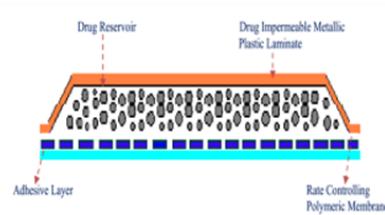


Figure (30) Reservoir type transdermal patch

$$J = \frac{(K_m K_c D_d D_m) C_R}{(K_m D_m h_d) + (K_c D_d h_m)}$$

Formula (3) Reservoir type drug release rate/ r is reservoir, m is membrane a is aq/ K is partition coefficient of drug, D is diffusion coefficient, h is thickness of layer, C is drug concentration.

(4) Micro reservoir Type or Micro sealed Dissolution Controlled Systems

The drug reservoir is formed by first suspending the drug solids in an aqueous solution of water-soluble liquid polymer e to form several discrete, unleachable microscopic spheres of drug reservoirs. After, by immediately cross-linking the polymer chains in situ, which produces a medicated polymer disc with a constant surface area and fixed thickness, the unstable drug dispersion is quickly stabilized. The medicated disc is positioned at the center and surrounded with an adhesive produce. (Figure(31)). The rate of the drug delivery is controlled by partition coefficient, diffusivity, and solubility of drug, similarly formed as Formula(2).

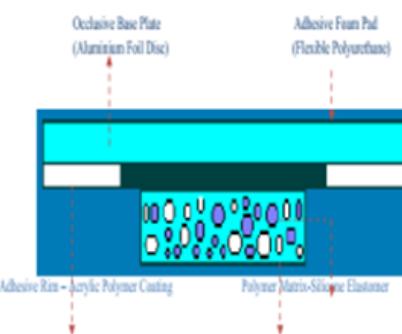


Figure (31) Micro reservoir type transdermal patch.

Transdermal patch is developed upon these basis so far. Also these days it is combined with electrical devices to used in therapy and diagnosis spontaneously. It is essential for transdermal patch to be statically stable, especially against shear force too. It should be both adhesion and cohesion not to failure.(Figure(32)) The higher the adhesive thickness, the higher the number of shearing layers and, therefore, the lower the

matrix cohesion, so have to find the right material making moderate ratio between them.

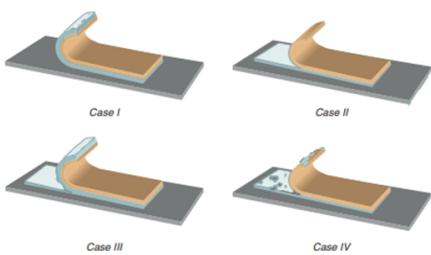


Figure (32) Patch modes of failure. When a patch is peeled away from an adherend, four types of failure can occur. Case I (adhesive failure) is the only acceptable form of patches. When the pressure-sensitive adhesive does not strictly adhere to the backing layer, it may transfer to the adherend, leaving no matrix on the backing layer (case II). Case III refers to what happens when the matrix has good adhesive strength but poor cohesive strength. Case IV is a combination of adhesive and cohesive failure at the same time. The shift from one to another type of failure is affected not only by additives but also by peel rate

IV-3. Ultrasound

Ultrasound waves are well-known for diagnostic imaging applications, including baby monitoring, tumor imaging, or blood flow evaluation. However, the application of ultrasound waves in the field of drug delivery has also been a prominent topic for research. The ultrasonic drug delivery is based on the enhancement of the therapeutic effect via the interaction between ultrasound and ultrasound-responsive materials, such as liposomes or microbubbles. The first research on ultrasound to treat disease was done in 1954 when the treatment of digital polyarthritis was successful by combining hydrocortisone with ultrasound as the treatment method. This method was named sonophoresis or phonophoresis, and this has been a topic of research for facilitating transdermal drug delivery and developing needle-free drug administration.

As for designing the ultrasound-responsive materials, it is important to aim for a high therapeutic index (TI). TI is calculated as the drug dose that produces toxicity in 50% of the population divided by the minimum dose that is effective for 50% of the population.

$$TI = \frac{TD_{50}}{ED_{50}} \quad (1)$$

Thus, the drug's effectiveness in therapy increases proportionally with the dose required for toxicity and disproportionately with the dose required to take effect for half of the population. There are three conditions to be met to achieve a significant increase in TI, which include:

1. stable encapsulation of the drug compound with an ultrasound-responsive material
2. release of the drug by ultrasound

3. imaging of the drug carrier and monitoring of the drug delivery.

The release of the drug cargo occurs with the ultrasound triggers, and the primary effect is pressure variation. The interaction of ultrasound with cells and tissues causes periodic pressure oscillations, and this simultaneous contracting and expanding from compression and refraction cycles from the ultrasound waves is the factor that facilitates drug release. The amplitude and frequency of the passing acoustic waves, as well as the size and material used to make the carrier determines the type of cavitation. The intensity of the ultrasound waves is understood via its mechanical index (MI), which is calculated by dividing the in situ peak negative pressure (PnP) by the center frequency (F_c).

$$MI = \frac{PnP}{\sqrt{F_c}} \quad (2)$$

Higher MI indicates stronger oscillation and therefore can result in the violent collapse of gas bubbles, which is known as “inertial cavitation.” Generally, the therapeutic ultrasound uses high MI values to disrupt the drug carrier and enhance drug uptake due to the extreme, localized pressures and temperatures.

There are four different types of mechanisms of cavitation-based ultrasound therapies: (a) acoustic streaming, (b) sonochemistry, (c) shock waves, (d) liquid microjets. Acoustic streaming indicates localized particle displacements and fluid currents caused by radiation forces experienced by reflectors and scatterers. During this mechanism, the bubbles induce stress on cells and tissues by oscillating around their resonant size, which generates velocities. Sonochemistry indicates when momentary high temperatures in the bubble core get generated due to the sudden collapse of the bubble. The hot bubble created from this mechanism influences the surrounding medium chemically. For (c), the shock waves that can disrupt the tissues thereby increasing drug uptake are formed by the sudden collapse of cavitation bubbles. Liquid microjets refer to high-velocity microjets

that can penetrate into the tissue or cause secondary shock waves. This is formed as the bubbles collapse near a surface where it experiences non-uniformities in their surroundings.

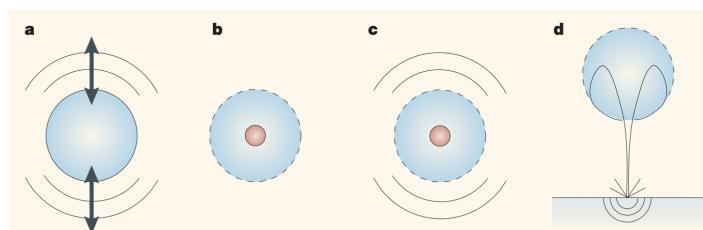


Figure (33) Mechanisms of cavitation-based ultrasound therapies

Micelles are formed as amphiphilic molecules such as lipids self-assemble via hydrophobic forces when exposed to an aqueous solution to form a hydrophobic core and a hydrophilic surface. They are known to release their drug cargo when exposed to ultrasound by ultrasound-induced cavitation. Husseini et al. loaded the anti-cancer agent doxorubicin (DOX) into the

micelles and exposed it to the continuous wave and pulsed ultrasound at the frequency range of 20 to 90 kHz (20 kHz, 47 kHz, 67 kHz, and 90 kHz). They observed the re-encapsulation of the released drug when the ultrasound went off. This suggests that the drugs would stay encapsulated upon leaving the sonicated volume, thus indicating that they wouldn't interact with normal tissues. They demonstrated the most efficient drug delivery at the lowest ultrasound frequency 20 kHz and that the efficiency decreases with the increasing ultrasound.

Liposomes are drug carriers that are most commonly used for their biocompatibility and versatility. Cavitation, thermal effects, and acoustic streaming are the potential mechanisms that can be used for drug release from liposomes. As for cavitation, the low-frequency ultrasound destabilizes liposomes to release their drug cargo. Schroeder et al. demonstrated the release of encapsulated drugs from the liposomes using low-frequency ultrasound (LFUS). The therapeutic agents (doxorubicin, cisplatin, and MPS) were loaded to sterically stabilized liposomes (SSLs). When the liposomes were ultrasonically irradiated, it was observed that the liposomal MPS release was biphasic. Furthermore, they demonstrated that the drug release occurs only during the LFUS exposure time and reasoned this by explaining that LFUS is capable of increasing the permeability of the liposomal membranes by forming pore-like defects that are transient depending on the LFUS irradiation.

Microbubbles refer to the vascular probes that have a gas-filled core and a shell of either lipid, polymer, and/or protein. They are relatively larger than the other commonly used drug carriers such as micelles or liposomes, so they cannot passively extravasate. They instead circulate in the system until they dissolve or are cleared by the mononuclear phagocyte system (MPS) as most of them get taken up by the target tissue. Microbubbles are capable of facilitating cavitation-related phenomena, and their volumetric oscillations can enhance the generation of acoustic streaming and shear forces. These responses result in the physiological changes in target cells and tissues that promotes the extravasation of circulating drugs.

Snipstad et al. demonstrated a multifunctional drug delivery system using the microbubbles stabilized by polymeric nanoparticles (NPMBs), in which the drug delivery is activated via ultrasound. They used poly(2-ethyl-butyl cyanoacrylate) (PEBCA) nanoparticles in a triple-negative breast cancer cell line. They observed the increased drug uptake by 2.3 times when the microbubbles were exposed to higher acoustic pressures, and assumed that higher pressure destroys the NPMB, indicating enhanced permeability. They concluded that ultrasound sonication and destruction of NPMBs improve the accumulation and distribution of the nanoparticles in tumors without harming normal tissues.

IV-4. Electrospinning

Among all the alternatives, nanofibers produced with biodegradable and biocompatible polymers gained increasing interest due to their broad flexibility, effectiveness, and unique physicochemical properties such as a large surface area, small diameter, and high aspect ratio. Also, targeted *in situ* application

of nanofibrous scaffolds could minimize the disadvantages of systemic perfusion with the free drug or other drug delivery systems, and on the other hand, maximize drug action pharmaceutical by a controlled and sustained release directly at the site of action. For instance, a nanofibrous scaffold can reduce the threat of antibiotic-resistant bacteria and multi-drug resistance in cancer therapy by site-specific, dose-specific, and timed release of different types of drugs.

Another great advantage is given by the similarity of the fibers with the natural fibrillary extracellular matrix (ECM), which facilitates cell attachment and proliferation for biomedical applications. Over the years, electrospinning proved to be one of the most cost-effective, simple, and flexible fabrication techniques for the production of polymer nanofibers.

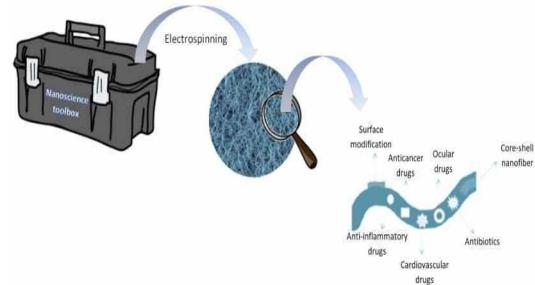


Figure (34)

Electrospinning is performed by applying a high voltage electrostatic field to a suitable polymer solution flowing through a needle. A specific feature of the final electrospun fiber is that structural design parameters such as porosity, morphology, and surface area could be tuned easily by modification of the environmental and processing conditions, according to the specific requirements for the delivery conditions

Drugs can be incorporated into the fiber by different approaches: by direct blending between the drug and the polymer solution, by surface immobilization after the spinning process, and by using an emulsion. Each method provides a different profile of drug release. A large variety of molecules has been successfully incorporated into electrospun fibers, from small molecules to proteins and nucleic acids. More sophisticated devices can also deliver multiple drugs with synergistic effects or selectively tune the release of the incorporated drug in response to external stimuli.

The overall process is carried out by using a polymer solution or a melted polymer. The polymer must be pumped through a spinneret (usually a syringe needle), to which a high voltage is applied. The applied voltage induces a charge movement in the polymer liquid, able to stretch the shape of the pendant drop, normally a sphere formed by the surface tension. Once the electrostatic repulsion of the charged polymer liquid becomes higher than the surface tension, a conical shape known as Taylor's cone is formed and the jet initiation starts from the cone tip. Remarkably, the two forces that induce the formation of Taylor's cone are controlled indirectly by flow rate and applied voltage. Therefore a good balance between the two parameters favors the formation of a stable jet. If enough

cohesive force exists in the polymer liquid, a stable jet is ejected from Taylor's cone, allowing the polymer chains to stretch each other and form a uniform filament.

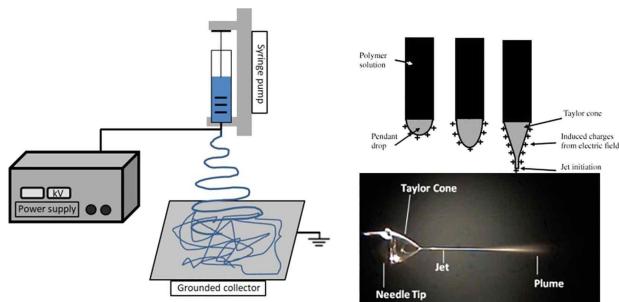
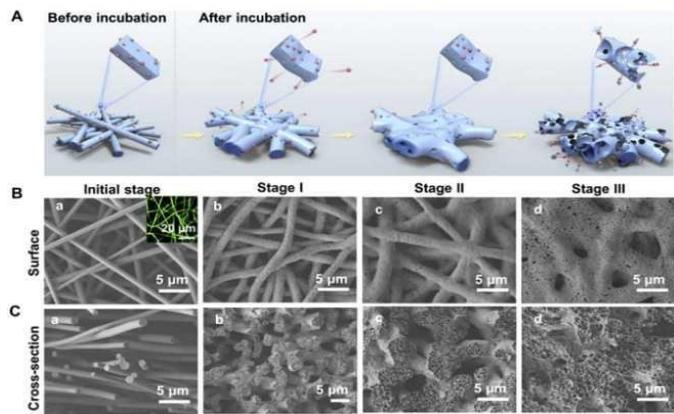


Figure (35)

Release kinetics drove the identification of three different stages: during the first few hours, the release occurred through stage one, described by a first-order equation in which the diffusion of the embedded molecules is controlled by the swelling of the fibers. The second stage, whose duration is around a few days, had a more flattened and sustained release, described by the zero-order equation of the Higuchi model. In this stage, the rate-determining step is the movement of the drug to the surface of the fiber. The release became proportional to time but independent of the concentration of the molecule. The third and final stage was mainly characterized by the hydrolysis of small oligomers from the scaffold, their diffusion with the entrapped molecules controls the rate of release. This stage has a square-root time dependence.



Fibers production by electrospinning takes place by application of high voltage to a liquid polymer flowing through a spinneret. A classification of different electrospinning methods is possible by analyzing the liquid source and the type of spinneret.

Two different techniques are employed for the generation of liquid polymers: The first involves the dissolution of polymer in a suitable solvent; the second makes use of the melted polymer. While, in solution electrospinning, the liquid is a solution of the polymer in a suitable solvent or mixture of different solvents, Melt electrospinning, uses heat to liquefy the polymer.

Spinneret or nozzle geometry governs the production of fibers

with different morphologies. In practice, the nozzle is the component in which the polymer flows and the voltage is applied. Single-axial represents the easiest setup for electrospinning. In this technique, a single capillary or a single syringe is used as the nozzle. A more sophisticated setup involves multi-channel or multi-axial technologies. In particular, the side-by-side and coaxial spinneret is composed of two or more capillaries, placed one adjacent to the other and one inside the other, respectively. Both techniques allow the use of several polymers. However, conversely to blending, by using those techniques the polymers coexist in the final fiber without physical mixing.

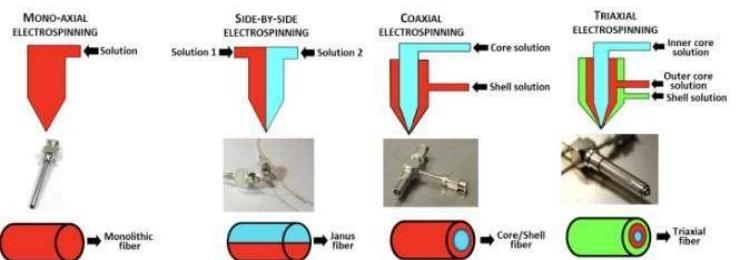


Figure (36)

Conclusion

Drug delivery system can be sorted in 4 big types: Nanoparticle drug delivery, Natural drug delivery, Robotics, and in-vitro drug delivery. Each system have advantages beyond each other on protection against degradation, accuracy of targeting, longevity, and accurate and broad range mobility. These days drug delivery industry is developing in the direction of theranostic, which enables therapy and diagnosis simultaneously. Theranostic NDDS and microrobotics are the proof of these enhancements. Also, developers are trying to approach increased circulation time, containing two or more functions or drugs, enhanced targeting. With the development of material science, pharmaceutical sciences and biomedical science, various controlled releasing nanomaterials will be used for smart DDSs in the future too. It will be an enormous challenge for researchers to improve preclinical research of advanced DDSs to reproducible and translatable production to clinical-trial success. Future work about smart DDSs for controlled drug delivery should be focused on the study of clinical translation to ensure more stimulus- sensitive nanomedicine to be clinically utilized.

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