



Nanomaterials: Compatibility Towards Biological Interactions

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Abstract

With the expansion of nanotechnology, health care sector has seen a boom in the field of diagnostics and therapeutics. Despite heaps of research, still the world of nanomaterials is illusive to us, especially its interaction with any biological system. This chapter is dedicated towards a basic understanding of the classifications and characteristics of nanomaterials used in biomedical applications, the nano-cellular system interaction mechanism, and the factors governing their interaction. Another vital topic discussed in this chapter is the compatibility of these nanomaterials in any in vivo biological application in connection with biodegradability, cytotoxicity, accumulation in tissues and organs, and possibility of excretion from the body.

Keywords

Biocompatibility · Cytotoxicity · Nanomaterial interaction · Organic nanomaterial · Inorganic nanomaterial

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1 Introduction

Nanomaterials are miraculous materials with unique properties that are distinctly different and enhanced to their own bulk counterparts. As of date, nanomaterial research in the fields of nanomedicine, nano-biology, and nano-toxicology as well as nano-diagnostics is growing rapidly and bringing in tremendous developments. As we talk about the role of nanomaterials in these biomedical applications, the interaction of nanomaterials with biological systems and their compatibility is extremely crucial to understanding the successful implementation of these nanomaterials. A clear insight on this interaction will facilitate to recognize the risk and the impact of nanomaterials on health and hence our life. There is a clear need to be concerned about the toxicity of nanoparticles; the potential long-term detriment nanomaterials could cause; because these are mostly newly explored, and many are still in the early stages of research with scary and positive evidence backed up by successful clinical trials. As nanomaterials interact with biological systems, there may be unpredicted biological effects that may be either adverse or favorable. There is a big question mark as to whether we should be concerned about the adverse effects or the potential compatibility of nanoparticles, or whether we should investigate and learn more to make the notion practically feasible.

The latest technologies have enabled the use of different characteristics properties at the nanoscale by the creation of nanomaterials in a controlled and refined manner. By controlling the size and shape of the nanostructures, nanotechnologies can also control the usage, design, arrangement, and their portrayal in biomedical fields. The use of nanomaterials has brought a revolution in the field of biomedicine by upgrading the prevention, detection, and treatment of diseases on cellular levels. The extent of functionalizing nanomaterials to alter their properties has made them an excellent resource for use in diagnosis and therapy of biological systems. Use of nanomaterials has given a significant breakthrough to solve critical problems relating to human health and to overcome age-related health issues (Azzawi et al. 2016). This is possible due to the compelling use of nanomaterials at cellular and subcellular levels. Various aspects of nanomaterials have been discussed in many research papers for a wide variety of biomedical application. This includes the study of drug expression in a particular population, use of nano-bots in medicine, their role in treating cancer, their use as bioimaging agents, skin-contacting sensors, and nano-devices to detect activities at cellular and subcellular levels and many more (Azzawi et al. 2016).

However, there are several gaps that still need attention to analyze the numerous constraints pertaining to the knowledge of nanomaterials, their structure, synthesis method, and characterization. Another major challenge is the mass production of these nano-moieties that can reduce the cost of these nano-based medical applications. There is also a need for quicker and more accurate methods to control the desired properties of nanomaterials, which can be a boon for their use in biomedical fields.

Nanomaterials can be ingested, inhaled, or they can penetrate the skin readily. Hence, there is a growing interest in evaluating their potential toxicity in the body. As of now, study of nanomaterials causing toxic effects to the human health is limited. However, several efforts have been made regarding the same, but they have

not been very much successful. Such efforts have led to the establishment of another subsidiary branch, known as “nano-toxicology.” Several published research articles are dedicated to keep a check on the toxic effects of nanomaterials.

In this chapter, we have addressed the current gap in knowledge as well as the developments made in this interdisciplinary field of research. Aimed at this goal, an extensive classification of various types of nanomaterials is presented, majorly classified into inorganic – metal and metal oxide nanoparticles; organic nanomaterials and natural nanomaterials such as lipids, micelles, dendrimers, etc. Further, the chapter encompasses understanding of different types of possible interaction of nanomaterials with biological systems and various factors affecting this interaction. Finally, an insight into the compatibility in terms of biodegradability, cytotoxicity, and stability is portrayed. The summary includes a future assessment of the topic of discussion and its possible fate in near future.

2 Classification and Characteristics of Nanomaterials

Nanomaterials undergo several significant changes in a tangible environment. It is therefore required to study the state of the nanomaterials and their characteristic features prior to the interaction probe. Many nanomaterial-related studies focus on issues that have an impact on the characteristics of the nanomaterial, eventually on the cellular attributes and bio-dispersal, which in turn depend upon the shape and size of the particle. Adding to it, the size and shape of the nanomaterial are analytical for regulating cellular spiking, intracellular location, and bio-disposal. As shown in Fig. 1, nanomaterials can be broadly distinguished under the categories of inorganic and organic.

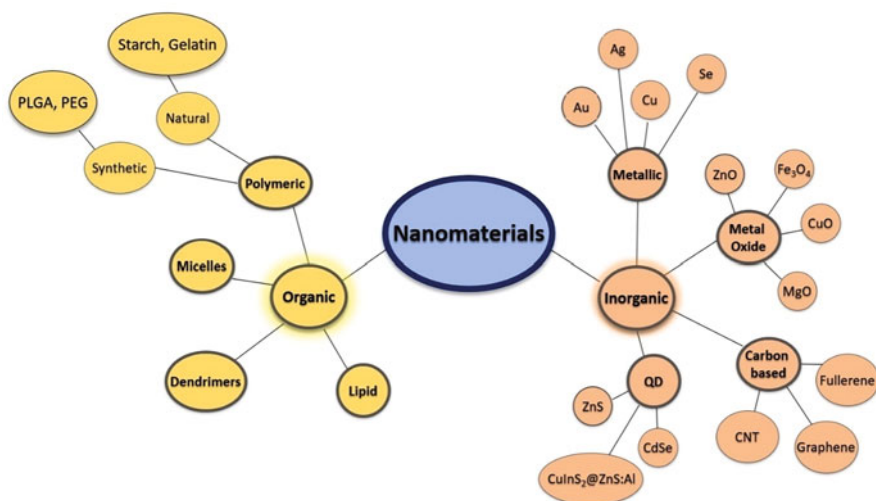


Fig. 1 Classification of nanomaterials

2.1 Inorganic Nanomaterials

A major classification of inorganic nanomaterials includes metallic nanoparticles, metal oxide nanoparticles, and semiconductor nanocrystals better known as semiconductor quantum dots. These nanomaterials with different shapes and dimensions have found loads of applications in almost every industrial sector including the health care and medical sector. Although these nanomaterials are still in their R&D stage of development, they hold a big promise of commercialization in various biomedical applications very soon.

2.1.1 Gold

The use of gold (Au) in biomedical applications is a part of ancient medicinal knowledge in several parts of the world. Typically, biomedical use of Au nanomaterials is a broad and popular field of research. Au nanomaterials are known for their unique properties like chemical inertness and high stability, which allow excellent biocompatibility in both in vivo and in vitro applications. Au nanomaterials of specific dimension of less than 50 nm also exhibit excellent optical properties like surface plasmon resonance with chemiluminescence and fluorescence property. These nanoparticles are easy to functionalize to enhance the interaction with biomolecules for a particular application. This is possible due to the charge present on its surface. Also, Au nanomaterials are less invasive and less toxic to human beings.

Applications

Au nanomaterials have been explored in a wide range of applications. For instance, in targeted drug-delivery systems, anticancer therapies, contrast agents in imaging, molecular imaging, antimicrobial treatments, biosensors for disease diagnostics including cancer and several chronic diseases, and intercellular studies, hyperthermia-based therapies to treat malignant tumors, biocatalysts (Giljohann et al. 2010), etc.

2.1.2 Silver

Silver (Ag) is the most widely used nanomaterial worldwide. These materials can be made into several shapes, but spherical particles are the most commonly used configuration. The large surface-to-volume ratio of Ag nanomaterials enhances binding with many ligands. Ag nanomaterials have very high electrical conductivity, high stability, and low frit temperature. The large area of plasmonic field and tunable structures paves the way for various applications.

Applications

Ag nanomaterials have found their applications in various biomedical fields, primarily due to their ease of handling and holding of biomolecules (Gnanadesigan et al. 2012). These are used in biosensors and are considered one of the best catalysts. Nano-silver has a high efficacy of delivering genes and drugs to target specific sites and is widely use in theragnostic applications. Ag nanomaterials are known to be

some of the best antibacterial, antiviral, and antifungal materials. Different nano-silver moieties have been used in wound healing and dressing applications. These materials are used in anticancer drugs and treatment. Ag nanomaterials are also used as implants for several body parts, for example, dental and bone implants. However, toxicity, potential efficiency, and costs remain a concern for their use in the human body.

2.1.3 Copper

Copper (Cu) nanomaterials have gained interest due to their high melting point, immense conductivity, low electrochemical relocation behavior, and low cost. Cu nanomaterials are much more reactive as compared to their bulk counterparts. Cu nanomaterials are highly stable and are seen to possess antimicrobial properties.

Applications

Cu nanomaterials have been explored in several biomedical applications. Typically, Cu nanomaterials have demonstrated significant antibacterial and antifungal effects. Cu nanomaterials have also been utilized in molecular imaging and cancer therapy. Photothermal ablation of tumor cells has been performed with the help of Cu nanomaterials (Badawy et al. 2015). However, the cytotoxicity of Cu nanomaterials is a concern.

2.1.4 Selenium

Selenium (Se) nanomaterials have gained attention and are successfully used in the fields of biomedicine and nutritional additives. Se nanomaterials play a key role in the formation of antioxidants like deiodinase. Se nanomaterials show scavenging effects due to their free radicals that can work efficiently in both in vivo and in vitro conditions. Se nanomaterials possess antimicrobial effects against some microbial species. Se nano-moieties have also demonstrated some anticarcinogenic action. Se-based nanomaterials show much lower toxic effects as compared to their bulk state.

Applications

Se nanomaterials have been used for intercellular assays. These nanoparticles have also been used in targeted drug delivery systems as drugs and as enzyme-carrying vehicles. Its role in biosensors and antimicrobial activities is also significant. Se nano-moieties are used in anticancer treatment as they can induce tumor cell apoptosis. Se nanomaterials have also been used in antidiabetic therapies with their ROS-scavenging abilities. Se nanomaterials also aid in food feed for mainly farm animals (Iranifam et al. 2013). They are used in food supplements to enhance growth performance and to improve the body's immune function via antioxidant effect. These particles have also shown their anti-inflammatory reactions to improve the efficiency of vaccines.

2.1.5 Zinc Oxide

Zinc oxide (ZnO) is present naturally inside the earth's crust as zincite. ZnO nanomaterials have demonstrated versatile applications, including excellent use as antifungal, antibiotic, and antimicrobial materials. ZnO nanomaterials, being semi-conducting, can communicate with electrons at a very high rate, leading to great electrochemical properties. ZnO nanoparticles have been validated with a high surface-to-volume ratio, nontoxicity, and chemical stability.

Applications

ZnO nanomaterials have been extensively used for their antimicrobial properties by incorporating them into bandages, coatings, agrochemicals, alloys, and textiles. ZnO nanomaterials are used as contrast agents in MRI (magnetic resonance imaging), as catalysts, and in medical biosensors due to their magnetic properties (Venu Gopal and Kamila 2017). ZnO nanomaterials are also extensively studied for their use as drug/gene delivery vehicles in the treatment of diseases. ZnO-based nanomaterials are known to be used for highly target-specific tumor cell destruction. These materials are used in drug delivery, bioimaging, and in the detection of tumors and are used in biosensors and as biomarkers for detection. In addition, ZnO nanomaterials are widely used in cosmetic products due to their efficient UV blocking capabilities.

2.1.6 Iron Oxide

Iron oxide nanomaterials have found tremendous use in various fields due to their chemical stability, low toxic effects, biocompatible nature, and large surface area with small size. Iron oxide nanomaterials exhibit superparamagnetic behavior and ease of modification with an applied field that allows these materials to be recycled or reused. The ease of separation of iron oxide nanomaterials due to their excellent magnetic properties is also a boon which enables their use in various biomedical applications. They are popularly known as SPIONS – superparamagnetic iron oxide nanoparticles.

Applications

Iron oxide nanomaterials are commonly used as contrast agents in MRI – magnetic resonance imaging. Iron oxide nanomaterials have been used in drug and gene delivery with an externally applied magnetic field. Iron oxide-based nanomaterials have been tested as antimicrobial agents. These materials have also been explored in cancer diagnosis, treatment, and therapy using external magnetic fields (Espinosa et al. 2016). Iron oxide nanomaterials have also been explored in tissue engineering and intercellular analysis. SPIONS are excellent materials when it comes to magnetic bio-separation.

2.1.7 Copper Oxide

Copper oxide (CuO) nanomaterials have gained interest especially due to their strong antimicrobial and biocide effects. CuO nanomaterials also have a unique

blend of electrical, optical, and magnetic properties. These particles are highly stable with long shelf life and are cost efficient.

Applications

These materials are extensively used in wound healing applications and as microbial warfare agents. CuO nano-moieties are widely used as antimicrobial materials – antiviral, antifungal, and antibacterial. CuO nanomaterials are often used as catalysts and in cosmetic products. These materials are also used in different types of sensors – glucose sensors, immunosensors for detection of cancer, dopamine sensing, etc. Besides, the antioxidant and anti-inflammatory activities of the CuO nanomaterials are often utilized in various biomedical applications (Oliveri 2020).

2.1.8 Magnesium Oxide

Magnesium oxide (MgO) nanomaterials possess high solidity, high melting point, and high sterility. These are nontoxic and odorless materials. The high specific surface area and crystal structure are added advantages of these materials. The high surface reactivity, thermal stability, and chemical stability of MgO make it an encouraging material for various applications.

Applications

MgO nanomaterials have been investigated for bioimaging, coating of implants, wound healing and skin injuries, tissue regeneration, anticancer strategies (Sharma et al. 2017), and in drug and gene delivery applications. These materials have also been studied in nano-cryosurgery, as antimicrobial agents, and have also been applied as food additives and in food supplements.

2.1.9 Quantum Dots

Quantum dots (QDs) have acquired the captivating attention of researchers due to their extraordinary potential in biomedical and pharmaceutical sectors. QDs are semiconductor nanomaterials and show strong fluorescence under light sources like laser.

QDs can be fused via direct binding with biomolecules like nucleotides, proteins, and imaging agents, which are used for biosensing, bio-labelling, targeting, and imaging. Core shell quantum dots are used for a variety of applications in biomedical fields. The shell provides better bio-functionalization, dispersibility, and shows enhanced optical properties. CuInS₂@ZnS: Al, ZnS, and CdSe are some of the widely used QDs.

QDs have gained immense interest in bioimaging and bio-labelling applications because of significant benefits over the conventional organic fluorophores as listed below:

- QDs have high signal-to-noise ratio (S/N) compared to conventional organic dyes.
- QDs possess long fluorescence and notably high photoresistance.

- QDs are much better resistant to photobleaching, thus leading to an enduring photostability.
- These materials are roughly 10–15 times brighter than organic dye base fluorophore.
- QDs give sharp and narrow emission peak and broad excitation spectra.
- QDs can be easily sculptured into any shape, and a variety of biomolecules can be coated over these particles.
- By varying different aspects of QDs, like size of the core and its composition, shell composition, and exterior finish, their optical properties can be altered.

Applications

QDs find immense research interest in the field of life science. QDs are used in major imaging techniques like MRI (magnetic resonance imaging), nuclear and optical imaging. Different types of QDs can be used in various biomedical fields which differ in terms complexity, resolution, acquisition time, sensitivity, and cost. Direct imaging property of QDs can be used to overcome some disadvantages that conventional diagnostics methods possess. For instance, using QDs in cancer treatment for delivering the drug to the specific site and tracing its path with help of the imaging property of QDs. Functionalized QDs can be used in live cell operations as suitable probes. QDs are used in cells for biomolecular tracking, staining of tissues, tumor imaging, and vascular imaging (Schnee et al. 2012). QDs also have certain challenges that these materials are quite cytotoxic and the time of reaction is slow.

2.1.10 Fullerenes

Fullerenes are sp^2 -hybridized carbon cages formed with double bonds and single bonds. Fullerenes are the most symmetric members of the carbon family and have chemical and structural stability. These materials are an important part of medicinal research due to their promising biocompatible properties. The structure, electronic configuration, size, and hydrophobicity of fullerenes have made them extremely appealing in the biomedical field. Its caged structure gives much scope for modification and functionalization, which makes it an efficient candidate for various applications. Despite the low solubility of fullerenes in the physiological environment, they have attracted significant researchers.

Applications

Fullerenes can be used in radical scavenging and also as antioxidants. These materials can give a high quantum yield of singlet oxygen when exposed to light. With the use of this property, fullerenes can cleave DNA by directly transferring electrons from their excited state. These materials have been studied as carrier vehicles for drug and gene delivery. Fullerenes can inhibit the approach of substrates to the sites of enzymes. These can be used as excellent biomarker materials. Talking about the toxicity of fullerenes, data shows that pristine fullerenes are nontoxic. At the same time, many derivatives of fullerenes show toxic effects, although the degree of toxicity is moderate.

2.1.11 Graphene and its Derivatives

Graphene is a single layer of carbon with densely packed sp^2 -hybridized atoms, forming a honeycomb-like lattice structure. It is often referred to as “wonder material.” Graphene and its derivatives have gained significant research interest due to their excellent physiochemical properties. These materials have high mechanical strength, stretchability, flexibility, biocompatibility, and high impermeability. Graphene and its derivatives are chemically inert. The exceptionally thin structure and high surface area paves its way to be applied in several applications, where its bulk counterparts cannot be used. Graphene and its derivatives can be processed in aqueous conditions and are amphiphilic in nature. It presents ease of functionalization and can suppress fluorescence.

Applications

Graphene and its derivatives have been used as biosensing and imaging materials due to their selectivity, solubility, and outstanding biocompatibility. Gene and drug delivery with graphene and graphene-based materials has been successfully demonstrated due to their large surface area, high loading capacity, ease of membrane penetration, and high purity (Chung et al. 2013). These materials have found intriguing applications in cell growth, cell culture, and tissue engineering. These materials can also be used for bio-functionalization of proteins and in teeth and bone implantations. It is to be noted that graphene and its derivatives are toxic and can contain impurities due to methods of synthesis. Mass production of graphene-based products is a tedious work and is cost-consuming. Moreover, it is difficult to control the thickness and size of graphene materials.

2.1.12 Carbon Nanotubes

Carbon nanotubes (CNT) are hollow and ordered carbon-based nanostructures arranged in the form of cylinders. These carbon atoms have sp^2 hybridization. It is classified into two basic types based on the structural formation: single-walled and multi-walled CNT. Single-walled CNTs are a single layer of carbon atoms rolled into a cylindrical shape, whereas multi-walled CNTs contain many sheets of carbon rolled into a cylindrical shape in a concentric manner. CNTs are highly elastic and very flexible. CNTs have magnificent mechanical strength and high thermal and electrical conductivity. The high aspect ratio and high surface area of CNTs are utilized in various applications. CNTs can be functionalized easily by covalent and non-covalent methods. Modification of CNTs can be done on their surface and tip ends. By modifying the structure, various functional groups can be added to the CNTs, which makes them useful in almost every field.

Applications

CNTs have been employed in targeted gene and drug delivery systems as they can penetrate easily through the cells and keep the cargo intact. CNTs have often found their use in electrochemical biosensors for detecting various biomolecules and in tissue engineering. These materials have also been broadly investigated for diagnostic purposes. CNTs have immense potential in bio-medicinal fields. Being small and

light in weight, CNTs can go well in the air and can be inhaled, causing toxic effects. CNTs can also cause dermal toxicity and lead to skin diseases. However, their toxicity depends upon the shape, structure, and the functional groups attached.

2.2 Organic Nanomaterials

Although inorganic nanomaterials are being extensively explored in biomedical applications, a major concern of cytotoxicity limits their uses in *in vivo* applications. With substantial interest in biocompatible and nontoxic nanomaterials, many organic nanomaterials, particularly polymeric nanomaterials, are being broadly explored.

2.2.1 Micelles

Micelles are aggregates of amphiphilic block copolymers or lipid molecules in a spherical shape. These structures are formed by the self-assembling of polymeric molecules upon the surface saturation of the solution at a particular polymer concentration. This concentration, above which polymeric entities begin assembling as micelles, is known as the critical micelle concentration (CMC). The hydrophobic chain of the amphiphilic polymer starts to aggregate, decreasing its water contact, forming a central core and hydrophilic part pointing outwards to the aqueous medium, forming the corona/shell of the micellar structure. Various copolymers in the form of di-block and tri-block, including polyethylene glycol (PEG), poly(N-isopropyl acrylamide) p(NIPAAm), Poly(ϵ -caprolactone) (PCL), poly(N-vinyl pyrrolidone) (PVP), and poly(lactic-co-glycolic acid) (PLGA), lead to the formation of micellar structures. The corona, being the outer part of the micelle, is responsible for determining its biocompatibility, circulation half-life, and stability in the environment. Subsequently, it also helps in determining the efficacy of micelle by interacting with the cells and other biological components of the blood for use in biomedical applications. On the other hand, the central core of the micelle is used for the loading of drugs. The core being hydrophobic in nature allows only the hydrophobic drugs to be loaded inside it. This helps in increasing the water solubility and decreasing the toxicity of the hydrophobic drug. Interestingly, the shell/corona of micelles helps in keeping the micelles stable for a longer duration in plasma because of their hydrophilic nature and prevents their opsonization, resulting in their escape from the reticuloendothelial system (RES), subsequently avoiding the pre-degradation of drug and increasing its efficiency. Along with this, the nano-size of micelles allows easy penetration and accumulation into the tissues, which results in a high concentration of drugs at the target site by the enhanced permeation and retention effect (EPR), mainly observed around the tumor regions. Additionally, a higher drug loading capacity is seen in the case of micelles. Despite several benefits, use of micelles for drug delivery is obstructed by the fact that upon dilution, micelles tend to lose their shape, which leads to undesired release of the drug. To overcome this, amphiphilic copolymers having a low CMC value are used to prepare micelles or they are stabilized by post-polymerization after the self-assembly of

monomers, which would help in maintaining a concentration equilibrium between the solution and the micelle.

Applications

Drug delivery – In today's era, it is estimated that more than 70% of all the entities that have been researched upon for use as drugs are hydrophobic in nature. Thus, it becomes necessary to develop methods to increase their water solubility for human use. Polymeric micelles are one such solution to overcome this problem by acting as carriers for hydrophobic drugs. Hydrophobic drugs are loaded inside the core of micelles, and their outer shell helps in increasing the water solubility and decreasing the toxicity of the drug. Many experimental studies have shown the increase in drug water solubility of 10–8400 times. For example, paclitaxel loaded in poly(D, L-lactide)-methoxy PEG micelle showed 5000 fold times increase in the water solubility of paclitaxel drug (Burt et al. 1999). With the increase in water solubility and the nano-size of micelles, the absorption of drug inside the cells becomes more effective. It is also possible to modify the outer shell of the micelles using different kinds of ligands such as antibodies, antigens, genes, aptamers, or nucleic acids to enhance their biological properties. With the help of ligand modification, micelles can cross the blood-brain barrier by helping in transcytosis (transport of molecules from one side of a cell to other) to cross epithelial cells or by using Pluronic copolymers which inhibit P-glycoprotein for penetration of drug molecules into bovine mammary epithelial cells. These ligands also make target-specific delivery possible. One of the active areas for the use of polymeric micelles as drug delivery carriers is in tumor treatment. Micelles increase the solubility of hydrophobic anticancer drugs and their permeation inside the tumor cells by enhancing permeation and retention effects. Genexol – a polymer micelle US FDA approved drug – is used for breast cancer treatment.

Diagnostics: Contrast agents play a crucial role in imaging techniques such as magnetic resonance imaging (MRI), computed tomography (CT), and nuclear imaging. Contrast agents in micellar forms have been shown to be more advantageous than conventionally used contrast agent molecules because of their high biocompatibility and ability to be tuned according to our area of use. MRI contrast agents use pH-sensitive micelles to detect cancer. These exhibit great contrast enhancement in low pH biological compartments and simplify the detection of very small size tumors as well in vivo. Micelles can simultaneously carry anticancer drugs as well as imaging probes for the detection of cancer, showing theragnostic (diagnostics and therapeutics) properties. Similarly, a CT scan applies contrast agents such as iodine, which are able to absorb X-rays for imaging in high resolution. To overcome the side effects of such heavy elements, polymeric micelles can be used. For example, PLL-PEG polymer micelle containing iodine was developed (Movassaghian et al. 2015) that helped in improving the circulation of iodine and decreased the amount of iodine used in maintaining the same contrast.

2.2.2 Dendrimers

Dendrimers are architectural motifs with well-defined hyperbranched symmetrical structures of polymeric molecules. It contains a small polymeric core surrounded by branching units representing a “branching tree” shape. The three main parts of the dendrimer structure are the central core, repeated internal branching units, and the terminal functional groups. These are self-assembled structures formed by the combination of different types of interaction such as electrostatic, drug-polymer interaction or complexes with nucleic acids. Additionally, unlike micelles, their self-assembly is independent of the concentration, which makes them more structurally stable in *in vivo* conditions. The structure of dendrimers highly resembles with many proteins, which makes dendrimers a promising material for use in medicine. The third generation of PAMAM dendrimers, for example, resemble insulin in size and shape, which makes them potential synthesizers of protein scaffolds. The size and shape of dendrimers can be altered with successive generations (conjugation of further monomer molecules) during their formation. Alongside, dendrimers are also being used as delivery vehicles, contrast agents, and as biomimicking agents in the sensors. Drugs are loaded within the core and polymer branches can be functionalized with various antibodies, molecules, and genes resulting in multi-functional dendritic structures. In some cases, dendrimers act as “*prodrug*” by forming covalent linkages with biomolecules and perform their activity only at the time of interaction with a cell. Typically, dendrimers are much more preferred over liposomes and polymeric micelles because of their *in vivo* uniform dispersion and stability.

Applications

Drug and nucleic acid delivery – The high surface area of dendrimers offers the possibility of delivering both hydrophilic and hydrophobic drugs using them. The hydrophobic drugs are loaded in the core of the dendrimer, and hydrophilic drugs are attached to its branches. Dendrimers can easily deliver drugs through oral as well as nasal pathways due to their nano-dimension and high solubility. PAMAM dendrimers show potential in this type of drug delivery. Additionally, target specific delivery is carried out using dendrimers as their polymeric branches are highly acceptable to modifications using specific antibodies, ligands, and other biomolecules as well. These modifications also allow a slow and regulated delivery of drugs. For example, it was seen that when cisplatin was encapsulated in PAMAM dendrimers, a long-term slow release of cisplatin was achieved (Gupta and Nayak 2015). Similarly, studies are going on to treat genetic disorders by transferring genes using dendrimers (Lyu et al. 2020). siRNA widely helps in many therapeutic applications, but its use has become limited because of its unstable nature and easy degradation.

Artificial proteins – Because of their close resemblance with some of the protein structures, like hemoglobin and insulin, dendrimers are referred to as “artificial proteins.” The high molecular weight and the hyperbranched structure of dendrimers are two contributing factors for their biomimicking properties. Dendrimers with structures like proteins inhibiting angiogenesis are being developed for their use in

cancer treatment. These dendrimers act as proteins and inhibit the growth and differentiation of tumor cells.

Bioimaging – Dendrimers are used in contrast agents for MRI and CT techniques to improve the performance of contrast agent elements. In MRI, signals are generated by the relaxation rate of water protons in vivo. To enhance these signals and obtain a better result, contrast agents are incorporated inside the human body. One of the mainly used contrast agents in MRI is gadolinium (Gd) chelates. However, toxicity and biocompatible concerns are related to their use in in vivo conditions, and thus Gd is incorporated into the dendrimers to improve its circulation time and higher signal. Gd chelates are attached to the dendritic branches for their easy interaction with the protons that result in the MRI images. Similarly, for optical imaging purposes, fluorescent dyes can be attached to the dendrimers which would produce light on interaction or reaction at the specific sites resulting in efficient diagnosis technique.

2.2.3 Lipid Nanoparticles

Liposomes are phospholipid bilayer structures that are being extensively exploited as the drug delivery and gene delivery carriers. A simple liposome typically comprises of a hollow core of diameter ~ 50 nm to $1 \mu\text{m}$ surrounded by phospholipids bilayers. The hollow core acts as an accumulation site for therapeutic molecules. The number of phospholipid bilayers further classifies liposomes into different configurations such as multi-lamellar, small unilamellar, and large unilamellar. As the name implies, multi-lamellar vesicles are made up of several lipid bilayers that are separated from each other via aqueous spaces, whereas unilamellar vesicles have a single bilayer with entrapped aqueous regions. This unique biphasic nature of liposomes allows delivery of both hydrophobic as well as hydrophilic drugs congruently. Drug delivery system using lipid nanoparticles conventionally adopt the methodology of enhanced permeability and retention (EPR) effect. The EPR effect was first introduced by *Morgan* et al., where he first reported in vivo liposome imaging of deep-sealed infections and solid tumors using Indium 111 radiolabels (*Morgan et al. 1985*). Further, the discrete structural configuration, flexibility, and size of liposomes allow them to easily pass through the cell membrane followed by releasing the carried content, and thus, they can be recognized as smart carriers for targeted drug delivery. It is seen that liposomes have a short circulation half-life; to overcome this drawback, liposomes are conjugated with polyethylene glycol (PEG) polymer, which helps in increasing the overall stability of liposomes.

Applications

Cancer therapy – Most of all the available anticancer drugs till date are highly toxic and hydrophobic in nature. These drugs not only affect tumor cells but also the nearby healthy cells. Liposome-based delivery system has great potential in cancer therapies. The encapsulation of anticancer drugs in liposomes helps in increasing their solubility and reduces the risk of toxicity by preventing leakage of drugs at undesired locations. Several anticancer-based liposomal formulations have already been approved by the FDA for use in cancer therapies. Doxorubicin encapsulated in

PEGylated liposomes was the first liposomal delivery system approved by the FDA in 1995. Many other ligand-modified liposomal formulations are also under clinical tests.

Microbial infections – Fungal infections, though rare, have a high mortality rate. Amphotericin B is a standard drug used to treat a wide spectrum of fungus. Regrettably, toxicity risks are involved with its use, for which its encapsulation in liposomes is carried out. A liposomal formulation of Amphotericin B, AmBisome, was prepared which showed a high decrease in toxicity level while maintaining the same effectiveness of the drug (Faustino and Pinheiro 2020). Researchers are investigating to prepare oral formulations of AmBisome, as the presently available is costly and requires medical administration during its use (Faustino and Pinheiro 2020). Another serious problem related to microbial infections is the increase in resistant bacteria against the antibacterial drugs. With the frequent use of antibiotics, bacteria have started developing resistance, which leads to a decrease in drug potency. Liposomal formulations are seen to be a promising solution to maintain the drug efficacy. Arikace, an FDA approved Amikacin liposome-encapsulated formulation, is used against *Pseudomonas aeruginosa* and other lung diseases.

Gene delivery – Small-sized liposomes containing cationic lipids are found to be effective delivery carriers for DNA and RNA due to their low surface charge and long circulation time. Cationic lipid-based liposomes can easily encapsulate nucleic acids with a negative charge at low pH. Lipofectin™ is an effective, commercially available liposome prepared using dioleoyloxypropyl-trimethylammonium bromide (DOTMA) for DNA transfer. The positive headgroup of DOTMA forms a complex with the negative phosphate group of DNA and helps in transfection in cells.

2.2.4 Starch

Starch is one of the most common polysaccharides, produced by plants for energy storage. Nowadays, starch is being used as a biodegradable, nontoxic polymer for varied applications in the form of starch nanoparticles. Typically, starch nanoparticles are built from amylose and amylopectin in crystalline and semicrystalline structure. The physiochemical properties of starch nanoparticles depend on the source from which starch is obtained such as potato, wheat, and maize, because starch in its native form possesses some limitations which are overcome by using modified starch nanoparticles. Some of these are oxidized starch, cadexomer iodine starch, hydroxyethyl starch having improved solubility, stability, and properties to pass through biological barriers. Cadexomer iodine is physically entrapped in the starch matrix which is hydrophilic in nature. This is used for the slow release of iodine onto the wound with the help of its pores. On the other hand, oxidized starch is prepared under specific environmental conditions using an oxidizing agent. It forms a highly stable film which can be used as a binder in the paper industry. Additionally, hydroxyethyl starch is produced by a reaction between starch and ethylene oxide to be used as plasma expanders for the treatment of articular perfusion. Additionally, since starch is derived from plants, it is inherently biocompatible, which favors its use as nanofillers for scaffolds in tissue engineering along with drug delivery. Studies have also shown that the coating of starch on inorganic

nanoparticles such as PbSe nanoparticles renders it nontoxic and biocompatible (Torres and Arce 2015).

Applications

Scaffolds – Various studies show that starch nanoparticles when used as nanofillers enhance the structural properties. These are used as bone cements to provide temporary supports. Scaffolds are prepared using starch-based polymers which support the growth of epithelial cells on their surface for new bone formation and are later degraded into nontoxic residues. A nanocomposite of multi-walled CNTs and starch nanoparticles was developed as tissue scaffolds for regenerating bones (Famá et al. 2011).

Drug delivery – Starch nanoparticles have been explored as nanocarriers for drugs, increasing their absorption and bioavailability in the human body. Curcumin when delivered using starch maleate nanoparticles showed 300 times increase in curcumin solubility as compared to free curcumin (Pang et al. 2014). Similarly, indomethacin loaded in starch nanoparticles showed an improved pharmacokinetic behavior (Simi and Emilia Abraham 2007). It is also seen that starch nanoparticles permit controlled drug release in both acidic and basic mediums.

2.2.5 Chitosan

Chitosan, a linear polysaccharide, is derived from the chitin of seashells. Chitosan nanoparticles are polymeric self-assembled nanoparticles that are highly biocompatible and biodegradable in nature. However, chitosan is water insoluble and poses significant hurdles regarding various applications, but its modification using functional groups helps in overcoming this limitation and widens the area of application. The amine groups of chitosan are responsible for its various physical and chemical properties. Literature shows that chitosan nanoparticles help in achieving antitumor activities by improving the body's immune functioning (Nanoparticle et al. 2019). Besides, chitosan is a cationic polymer, which helps it adhere to bacterial cell walls. This adherence prohibits further bacterial growth, showing great inherent antibacterial properties. According to studies, chitosan with a low molecular weight is more effective against gram-negative bacteria (Younes et al. 2014). Chitosan nanoparticles also show antioxidant properties by scavenging reactive oxygen species, preventing the damage of proteins and other lipid membranes. Enzymes present in biological organisms can easily depolymerize chitosan, which prevents its accumulation inside the body, making them highly nontoxic in nature.

Applications

Antimicrobial agent – Chitosan nanoparticles, because of their cationic nature, show antibacterial activity against a wide range of bacteria. These can enter the cell wall of bacteria and inhibit DNA transcription and mRNA synthesis. Studies show that the type of bacteria affected by chitosan nanoparticles depends on its molecular weight, surrounding pH, and then its acetylation (Zeng et al. 2014). The low pH and molecular weight of chitosan favor antimicrobial activity against gram-negative bacteria because of more negative charge on cell wall leading to strong interaction

between chitosan and bacteria. Chitosan nanoparticles also showed antifungal activity against *Fusarium oxysporum*, which increases by increasing the molecular weight and deacetylation of chitosan.

Wound healing – ChiGel, Tegasorb™, and ChitoFlex are some of the commercially available forms of chitosan for use in the wound healing process. Chitosan nanoparticles, being charged particles, attract the red platelets to speed up the coagulation at the site of the wound. These are also seen to show macrophage movement and enact cell expansion. Along with speeding up the healing process, chitosan nanoparticles prevent any bacterial infection around the wound. Chitosan-based nanofibers are used as cement-based chitosan for dressing material. Chitosan, in the form of scaffolds and sponges, is also used in wound healing applications.

Drug and gene delivery – Chitosan nanoparticles are able to carry drugs as well as nucleic acids for delivery. Chitosan is viscous in nature, which helps in the slow release of drugs, improving their therapeutic efficiency. Chitosan nanoparticles have proven to be good candidates for oral and nasal delivery of drugs, as in the microsphere form, these particles are easily absorbed by epithelial cells.

2.2.6 Gelatin

Gelatin is one of the important natural polymers used in various applications. The wide scope of using gelatin is possible because of its biocompatibility, biodegradability, readily accessible, and economical properties. Gelatin is formed by either acidic or basic hydrolysis of collagen in two forms: Type A and Type B gelatin nanoparticles. The isoelectric point of Type A gelatin is around seven, whereas for Type B gelatin it is around four, and among these, Type B gelatin nanoparticles exhibit better efficiency of drug delivery. The US FDA has approved gelatin as Generally Recognized as Safe material for its use in in vivo applications such as tissue engineering and drug delivery systems. Gelatin nanoparticles can effectively carry drugs and help in their enhanced delivery.

Applications

Drug and gene delivery – Gelatin nanoparticles are promising nanocarriers for both drugs as well as genes. These assist in reducing the toxic effects of drugs and also in increasing their biocompatibility. Drugs travel easily across the plasma without getting pre-degraded. By enhanced permeation and retention effects, gelatin nanoparticles accumulate a high concentration of drug on the target sites. It was seen that the toxicity level of noscipine decreased considerably by loading it into gelatin nanoparticles (Madan et al. 2011).

Tissue engineering – Gelatin, being a denatured polymer, offers an advantage over other biopolymers in making scaffolds for tissue engineering purposes. Gelatin nanocomposites are formed with greater mechanical strength for bone scaffolds. A study showed that bone can easily penetrate inside the scaffold made using gelatin and hydroxyapatite composite because of its high elastic nature (Venugopal et al. 2008). Studies prove that composites with gelatin conjugation are one of the best materials for bone regeneration application (Peter et al. 2010).

2.2.7 Synthetic Polymeric Nanomaterials

Poly(Lactic-Co-Glycolic Acid)

Poly(lactic-co-glycolic acid) (PLGA) is one of the most successful synthetic polymers to be used in the biological applications approved by the US FDA. The main reason is its biodegradability. PLGA nanoparticles are hydrolyzed into lactic acid and glycolic acid which are then metabolized into carbon dioxide and water via Krebs cycle. Additionally, the surface of PLGA can be easily decorated using various ligands such as antibodies, genes, and other biomolecules. PLGA is a copolymer of polyglycolic acid (PGA) and polylactic acid (PLA) linked by ester linkages. The ratio of these monomers determines the molecular weight and degradation time of PLGA nanoparticles. Alongside, by varying the monomer ratio, PLGA can be made into any configuration for carrying a wide variety of biomolecules.

Applications

Typically, polymeric nanoparticles are used for drug delivery and therapeutic purposes, exhibiting stimuli-responsive activity and stability in the extreme pH and temperature conditions of the human body.

Drug delivery for cancer therapy – PLGA nanoparticles can encapsulate hydrophobic as well as hydrophilic drugs for their efficient delivery. In case of cancer treatment, PLGA nanoparticles are used to increase the solubility and efficiency of hydrophobic drugs such as doxorubicin. For instance, to treat breast cancer, salinomycin and paclitaxel were encapsulated in PLGA nanoparticles and further anti-CD133 antibodies were grafted on their surface for targeting breast cancer cells and on-site release of the drugs (Swaminathan et al. 2013). PLGA nanoparticles have also been explored for pH and heat-responsive drug release.

Imaging and diagnosis of cancer – Imaging plays a vital role in keeping a check on the recurrence of tumors and also monitoring any therapeutic response. Alongside, it is also important to diagnose cancer at an early stage for efficient treatment. For this purpose, PLGA nanoparticles containing superparamagnetic iron oxide were prepared to be used as contrast agents in MRI (Wang et al. 2008). In another study, radiotracer technetium-99 m was encapsulated in PLGA nanoparticles for the detection of sentinel lymph nodes (Acharya and Sahoo 2011).

Polyethylene Glycol

Polyethylene glycol (PEG), also called as stealth polymer, is one of the widely used polymers in drug delivery applications. It is highly biocompatible, water soluble, biodegradable, nontoxic, and nonimmunogenic in nature. For use in various biomedical applications, PEG with different molecular weights and shapes are considered, depending upon the field of application. Typically, three shapes of PEG are used, i.e., linear, branched, and Y-shaped polyethylene glycol. Linear PEG contains one functional group which undergoes conjugation with protein on one side of the chain and a methoxy group on the other side. Two or more linear PEG together makes branched PEG structure which shows better immunological and

pharmacokinetic properties. On the other hand, Y-shaped PEG shows more stability against pH, temperature, and other physiological environment (Mohapatra et al. 2019). The chain of PEG molecule does not contain any bulky group which makes its structure flexible. It also allows binding of water molecules leading to more hydrodynamic volume. The properties of PEG nanoparticles not only make it a versatile nanocarrier but also a good coating material for other nanoparticles termed as PEGylation. As a coating, PEG makes the nanoparticles hydrophilic due to the presence of repeating units of ethylene glycol. Additionally, PEG enhances the stability by reducing the charge interaction between protein and nanoparticles. It also acts as a barrier against immune system increasing the circulation time of nanocarriers.

Applications

PEG nanoparticles are majorly used as drug delivery carriers for both hydrophobic as well as hydrophilic drugs. Drug molecules are conjugated to PEG nanoparticles using linkers which on reaching the target site initiate their release. In one of the studies, camptothecin (CPT) was conjugated with PEG nanoparticles to form a CPT-PEG prodrug complex (Yu et al. 2005). CPT is an enzyme inhibitor which on binding with topoisomerase and DNA inhibits relegation of DNA and tumor cells are destroyed. But its use is limited because of low water solubility and toxicity to normal cells. To overcome this issue, CPT-PEG complex was formed in which PEG nanoparticles helped in making CPT water soluble and less toxic. Studies involving loading of doxorubicin (DOX) drug in PEG nanoparticles have shown to enhance its antitumor activity and prevent pre-degradation during its use (Veronese et al. 2005). Another area of application for polyethylene glycol is to coat metallic nanoparticles such as gold and silica with a layer of PEG. The coating of PEG improves the biocompatibility of such nanoparticles and protects them against various biological barriers during in vivo applications.

Poly- ϵ -Caprolactone

Poly- ϵ -caprolactone (PCL), a biodegradable polymer, is exploited for its use in biological fields such as tissue engineering, wound dressing, and drug delivery. In addition to drug delivery, PCL is also used to deliver other proteins, vaccines, and biomolecules for the treatment of various diseases. Despite these advantages, PCL is not used as much as compared to other polymeric nanoparticles such as PLGA and PGA because of its slow degradation rate. PCL is highly stable as compared to other biodegradable polymers because of a smaller number of free ester bonds which lowers its degradation rate. Although this property of slow degradation helps PCL nanoparticles to be used effectively in the making of implants and bone tissue that need the material with very slow or no degradation (Woodruff and Hutmacher 2010). Studies on the toxicity of PCL nanoparticles showed that they are non-mutagenic and safe for use.

Applications

Scaffolds in tissue engineering – PCL is the most favored nanomaterial for the use as scaffolds in tissue engineering because of its slow degradation rate which matches with time required for bone ingrowth and repair. Also, the pore size and interconnection are important during the development of scaffolds as they should not permit cell movement across them but also at the same time should allow the blood vessel growth inside them, and this can be easily achieved using PCL which allows easy tenability in this structure during preparation. Along with PCL, ceramics are used as fillers in them to enhance the mechanical stiffness of scaffold. Additionally, PCL nanoparticles are also being explored for the wound repair in soft tissues of the human body. It is seen that nanofibers of PCL help in improving the mechanical strength and cell interaction when used as fillers in natural polymers like chitosan.

Drug delivery – PCL nanoparticles are preferred for a long-term slow release of drug in the system. PCL, due to its hydrophobic nature, easily encapsulates hydrophobic drugs, whereas hydrophilic drugs are adsorbed on their surface. PCL nanoparticles help these drugs to pass through blood-brain barriers and the immune system, further allowing easy interaction with cell membranes to deliver drugs inside the cells. For example, carboplatin drug was loaded inside PCL nanoparticles using solvent-emulsion evaporation method for the brain delivery (Karanam et al. 2015). Studies showed that PCL nanoparticles loaded with curcumin drug exhibited slow- and long-term release of the curcumin drug (Espinoza et al. 2020).

Polylactic Acid

Poly(lactic acid) (PLA) is an aliphatic, biodegradable polymer made up of lactic acid monomers. PLA can be easily derived from carbon dioxide, rice, wheat, which makes it very environmentally friendly. Along with this, PLA nanoparticles are very biocompatible, recyclable, and easy to produce, which makes it an excellent candidate to be used for biological applications. According to the area of use, it is easy to tune the mechanical properties of PLA. It is seen that sometimes the hydrophobic nature of PLA is responsible for the inflammatory response of the tissue.

Applications

Tissue engineering – PLA is used to form porous scaffolds due to their good mechanical strength and biocompatibility. These scaffolds are used to initiate the regrowth of tissues by acting as supports for them. A composite of PLA along with hydroxyapatite has been used to initiate osteogenesis and be used in dental applications (Kim et al. 2020). PLA scaffolds are also used for the growth of epithelial cells. PLA along with PGA showed good results for this purpose (Kim et al. 2020).

Drug delivery – PLA nanoparticles are used as nanocarriers for delivery of drugs used in the treatment of various diseases. A major benefit of using PLA in drug delivery systems is that it easily dissolves in extracellular matrix and allows the delivery of drugs in a slow and controlled manner for prolonged effect. Studies showed a greater amount of drug accumulation at the target site when docetaxel was

encapsulated in PLA nanoparticles as compared to when it was used as a free drug (Hrkach et al. 2012).

3 Interaction of Nanomaterials with Biological Systems

3.1 Governing Factors

There are various factors that can modify the conformation of nanomaterials and can be a potential cause for deteriorating their biological properties. The biophysical properties of nanomaterials display their recognition and effect on artificial moieties contained within, among all comprising morphology, chemical framework, solubility, shape and structure, size, and their aggregation status. These features also play a key role in studying specific mechanisms like cell biocompatibility. Some of the vital factors that strongly affect the interaction of nanomaterials on any biomolecules are: (a) interaction of the nanomaterial with its own surroundings, that is with other neighboring nanomaterials; and (b) physicochemical property and design architecture of the nanomaterial, for instance, size, shape, surface modification, protein corona effect, etc., as shown in Fig. 2.

3.1.1 Size

The size of nanomaterials plays a pivotal role in biological interactions with external systems and other biological mechanisms in the nano-regime, like cellular uptake and the processing effectiveness of the particle. The smaller the size of the nanoparticle, the faster will be its ionic release rate and interactivity with cell structures. The toxicity of nanomaterials is said to be directly dependent upon their size. A decrease in the particle size leads to an increase in the surface area to volume ratio, implying that the area of contact for the nanomaterial increases. Thus, its probability of invading into a cellular system gets higher and thereby increases the toxicity level.

It is clear that the size of the nanomaterials is extremely crucial in any *in vivo* administration or medicinal behavior. Nanomaterials greater than 1 μm cannot penetrate the cell membrane easily, but they can be linked to proteins absorbed in the cell. It has been reported that nanomaterials more than around 6 nm in dimension have a high chance of getting accumulated in different organs such as the kidneys where they cannot be further filtered. There are other studies reporting hepatotoxicity caused by CdSe quantum dots (Sanford et al. 2004). Other studies have investigated bioaccumulation and bio-dispersibility of different sizes of gold nanoparticles in the bloodstream and indicated that smaller gold nanoparticles stayed for longer durations in the blood and were also accumulated to a greater extent in the organs (Sonavane et al. 2008).

3.1.2 Shape

The toxicity of nanomaterials is also determined by their shape. Nanomaterials can be synthesized in different morphologies such as spheres, clusters, fibers, planes, tubes, and many other irregular shapes (Lee et al. 2019). Processes like

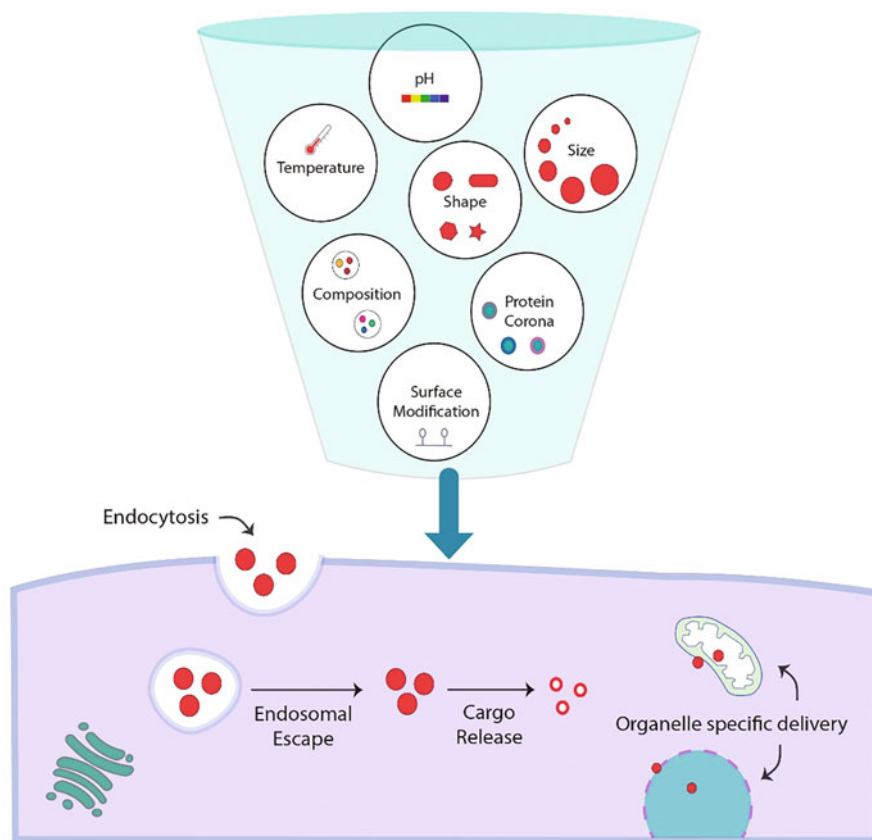


Fig. 2 Factors governing interaction of nanomaterials with biological systems

phagocytosis, absorption, bio-dispersal, and elimination are highly influenced by the shape of the nanomaterial. For instance, the spherical nanomaterials have been found to be comparatively less toxic as compared to other shapes. Moreover, spherical particles with an even size distribution are absorbed faster as compared to rod-shaped materials.

3.1.3 Surface Modification

The nature of a nanomaterial's surface is strongly responsible for its solubility and cell-to-nanomaterial interaction. A surface coating or modification can immensely affect the pharmacology, dispersal, aggregation, and toxicity of any nanomaterial (Favi et al. 2015).

Phenomenon like *colloidal conduct*, *protein-plasma binding*, *absorption*, and *crossing of blood-to-brain barrier* are known to be influenced by the surface chemistry of a nanomaterial. For example, acetylation of starch nanoparticles leads to cross-linking, which in turn decreases the solubility and swelling property of

starch. An increase in the charge at the surface of a nanomaterial leads to its increased cytotoxicity. This indicates that greater cell interactions occur due to high charge accumulation and, subsequently, more endocytic metabolism. It is proven that higher toxicity is caused by uptake of positively charged nanomaterials as compared to negatively charged ones. Other studies have verified this fact where positively charged nanomaterials were observed to aggregate more in tumor cells as compared to the negatively charged nanomaterials (Hoshyar et al. 2016). Another study has shown surface charge-mediated negative effect on the adsorption rate of chitosan nanoparticles on different biological uses during drug delivery and other uses. Precise surface modification of nanomaterials may also lead to increased stability, a decrease in toxicity, and controlled and modulated cellular incorporation. Organic moieties like amine, hydroxyl groups, PEG are often used in surface modification.

3.1.4 Protein Corona

A corona is formed around the nanomaterials when they encounter different biomolecules in the bloodstream. The corona formed by proteins mainly consists of proteins with a varied nature of interactions: *clusterin*, *fibrinogen*, *immunoglobulin*, *albumin*, and *apolipoproteins*. Adsorption affinity of the nanomaterials on the protein surface plays a vital role in the corona formation.

The physicochemical properties and the biological specifications of nanomaterials change once the protein corona is formed around them. The characterization of the protein–nanomaterial interaction is challenging. But an in-depth understanding could bring awareness about the negative impacts that corona formation can have on the activity of nanomaterials.

According to the Vroman effect (Hirsh et al. 2013), corona formation is initially restricted to surface proteins with a higher congregation and lower binding affinity and then with high affinity proteins in a lower congregation. Protein corona is of two types: hard and soft. Hard corona has more binding affinity and a longer time of exchange. It is also closer to the nanomaterial surface. The soft corona layer has lower binding affinity and a fast time of exchange. Various techniques used in the characterization of protein–corona interactions include SDS-PAGE, UV-vis spectroscopy, centrifugation, special chromatography techniques, and calorimetry.

3.1.5 pH Response

pH plays an important role during the synthesis of various nanomaterials as well as affects some of their physicochemical properties. Gold nanomaterials are better synthesized in a low pH medium. pH value also affects gold nanoparticles' bonding with various organic functional groups and biomolecules, changing their biological activities. Similarly, iron nanoparticles are observed to be suitable for bioimaging purposes in an environment with a high pH as they exhibit superparamagnetic behavior in alkaline medium. In vitro studies with PLGA nanoparticles have shown favorable activity in both strongly acidic as well as strongly alkaline medium. The surrounding pH of the chitosan nanoparticles also causes changes in its outer structure and modifies its surface. Studies show that the therapeutic effect of chitosan

nanoparticles is changed by the acidic pH present around the tumor cells (Nguyen and Lee 2017). Additionally, the antimicrobial and antioxidant behavior of chitosan nanoparticles also depends on the pH of their surrounding medium. Studies also demonstrate that pH is important during dendrimer-mediated drug delivery where both the interior and exterior surfaces of dendrimers can be influenced by the surrounding pH, which ultimately affects the loading of various drugs (Maiti et al. 2005).

3.1.6 Temperature

The stable synthesis of nanomaterials is strongly dependent on the temperature of synthesis. Typically, gold nanomaterials are synthesized at high temperatures. Not only that, the plasmonic behavior of gold nanomaterials is well utilized in photothermal therapy where local heat is generated by light-induced excitation of these nanomaterials. As the gold nanomaterials start resonating, and upon an increase in temperature, gold nanomaterials burst out, releasing the drug on site (Vines et al. 2019). The formation of polymeric micelles and their shape and size are also highly dependent on temperature. A slight change in temperature can change the CMC value during the micelle formation, leading to a change in their size or shape. Likewise, at high temperatures, gelatin nanoparticles tend to loosen its structure by becoming flexible. This thermosensitive behavior of gelatin nanoparticles assists in realizing thermo-responsive drug delivery. Temperature is also an important factor in determining the fate of PLA nanoparticles. High temperatures favor the degradation process of PLA nanoparticles.

3.2 Interaction Mechanism

Several classifications of nanomaterials have been discussed in the above sections. The interaction of nanomaterials with biomolecules occurs through a wide-angle point-of-view. Nanomaterials interact with biomolecules in several biomedical applications, such as diagnostic tools like biosensors, bioimaging tools, nanocarriers, targeted drug delivery, etc.

The most pertinent biomolecules that interact with the surface of nanomaterials are nucleic acids and proteins. Binding with proteins can be via specific and non-specific adhesion as proteins have many contrasting binding sites due to post-translational changes and key amino acid structures. Adding to that, immune response to the biocompatibility of nanomaterials is critical in proteins. The highly specific base pairing, good physicochemical and mechanical stability, and ease of accessibility of nucleic acids result in an appropriate receptor for biomolecular nano-assembly.

Typically, during the nanomaterial interaction with physiological biomolecules: (a) the structure and intended purpose is compromised once the chosen nanomaterial is surrounded by other dormant biomolecules. Hence, custom-made nanomaterials play a vital role here. They are highly specific to their target biomolecules; (b) nanomaterials entering different pathways in human anatomy are significantly

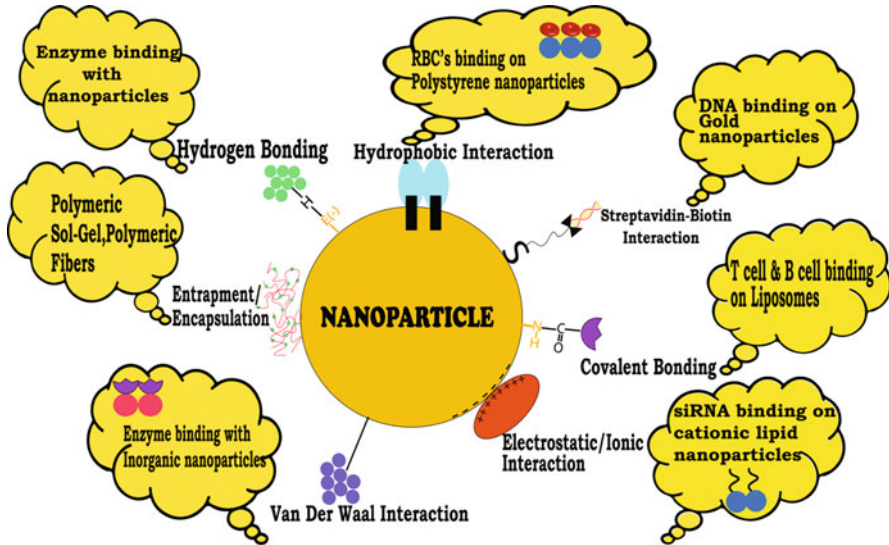


Fig. 3 Types of interaction between nanoparticles and biomolecules

influenced by their force of interaction. For instance, inhaled nanomaterials will directly interact with and affect the pulmonary system of the body. Based on these interactions, two major methods of immobilization are studied through interactions with various kinds of biomolecules, namely, physical and chemical (Fig. 3).

3.2.1 Physical Immobilization

Adsorption

This method involves the physical binding of the biomolecule to the surface of the nanomaterial. The nano-moiety can be either organic or inorganic. Binding occurs with the help of hydrogen bonds or Van der Waal forces. This method is widely used for the attachment of nucleic acids and binding proteins, avidin-biotin, hormones, and receptors as it is a simple and economic method of immobilization with minimal loss of activity. However, it provides a lower surface area for the binding to occur, and the yield is also low due to desorption and inactivation of the material. For example, polystyrene nanoparticles get attached on red blood cells (RBCs) by hydrophobic interactions, and various enzymes bind with inorganic nanoparticles through Van Der Waal as well as hydrogen bonding.

Similarly, adsorption of oppositely charged biomolecules on nanoparticles such as negatively charged siRNA on cationic lipid nanoparticles is also observed by electrostatic/ionic interactions. Binding of β -lactoglobulin on silicon nanoparticles with negative charge also occurs through ionic interaction.

Entrapment/Encapsulation

In this method of immobilization, biomolecules are not directly attached to the nanomaterial; they are attached with the help of a polymer matrix. They are attached with the help of gels or fibers, which are in turn attached to the nanomaterials. A variety of materials can be used for this purpose, like sol-gels, polymers or polymer sol-gel conjugates, and many other inorganic materials. Generally, chemical modification of the molecule is not required during this process, leading to the formation of more stable structures. However, the biomolecule may leak from the matrix formed. Another widely used physical interaction is the streptavidin-biotin complex. Interaction using streptavidin-biotin is considered as the strongest non-covalent interaction. It helps in binding DNA onto gold nanoparticles for DNA delivery applications. Additionally, it is also seen that human embryonic kidney cells form a streptavidin-biotin interaction with PLA-PEG nanoparticles.

3.2.2 Chemical Immobilization

Covalent Binding

In this method of immobilization, the biomolecules are bound to the surfaces of nanomaterials with the help of functional groups that form covalent bonds between them. Different types of organic and inorganic groups like phenolic groups, carboxyl groups, etc., are used for this purpose. Covalent binding is very strong and leaves no scope for the leakage of the biomolecule. For example, T cells and B cells containing free thiol groups on their surfaces bind covalently onto nanoparticles like liposomes. However, these bonds can alter the activity of the biomolecules.

Cross-Linking

Cross-linking involves direct binding of the biomolecules to the surface of the nanomaterials with or without the use of support structures. This method is generally used for water-insoluble molecules. The most commonly used reagent for this purpose is glutaraldehyde. Cross-linking is highly stable and there is little chance of the structure getting distorted. However, this method is costly and can cause changes in the active site of the nanomaterial.

3.2.3 Generation of Reactive Oxygen Species on Nanoparticle-Cell Interaction

Nanoparticles, besides self-oxidation, also produce oxidant effects by initiating the generation of reactive oxygen species (ROS). Highly reactive surface, redox surface reaction in metallic nanoparticles, and nanoparticle interaction with cells are the main factors responsible for the generation of ROS during the use of nanoparticles in biological applications. Besides, physiochemical properties of nanomaterials initiate the production of ROS on interaction with phagocytic cells. Typically, the smaller sized nanoparticles are responsible for more ROS production due to their highly reactive surface. The amount of ROS generated by nanoparticles directly affects their toxicity extent. Many nanoparticles on internalization in the cells reach the mitochondria and depolarize its membrane, which leads to abnormal electron transport,

stimulating the production of ROS. The depolarization of the mitochondrial membrane is caused due to damage of phospholipids by the ROS present in the mitochondria. Many nanoparticles such as zinc and copper show this mechanism on interaction with human cells. One such study showed that when nanoparticles accumulated in the lungs, inflammatory cells were activated, which led to the generation of ROS, thus damaging the lung cells (Manke et al. 2013).

4 Compatibility of Nanomaterials in Biological Systems

It is inevitable that the design of nanomaterials, including its shape, size, surface modification, plays a crucial role in optimizing their favorable interactions in each biological system. Studies have demonstrated that small nanoparticles of size less than 10 nm can enter the human cellular system. Therefore, it needs a critical understanding to control the concentration of these nanomaterials that keeps the cytotoxicity level under non-adverse limits. Several studies are being conducted to evaluate the possible biocompatibility and toxicity of these nanomaterials in any biological microenvironments and to engineer biologically safe nanomaterials which may be of potential use in biomedical applications.

4.1 Inorganic Nanomaterials

With the rapidly increasing use of nanomaterials in biological applications, utilization of metallic nanoparticles is also increasing at a very fast rate. Due to their unique innate physiochemical properties, metallic nanoparticles are being widely used in *in vitro* applications such as bioimaging and biosensing. Despite several prospective applications, concern about the toxicity of inorganic nanomaterials is still a major concern. The toxicity of inorganic nanomaterials refers to the side effects caused by the nanoparticles to its surrounding medium during their activity. Because the cause and extent of toxicity exhibited by nanoparticles vary with shape, size, and concentration, toxicological studies for each nanomaterial are needed to be carried out before their actual use in *in vivo* conditions. Au nanoparticles are currently the most explored nanomaterial for use in the medical field, though it is seen that gold nanoparticles with a size below 4 nm show great toxicity by penetrating inside the cell and binding with DNA (Kamiar et al. 2013). A study showed that accumulation of Au nanoparticles in the liver and spleen regions of rat models ultimately causes alteration in their gene expression (Kamiar et al. 2013). It has been reported that Cu nanoparticles when used in a high concentration of more than 100 μM remain accumulated in human cells for a long time, which may damage the cell membrane and induce oxidative stress, which ultimately leads to cell death. Toxicity caused by Cu nanoparticles is primarily due to the generation of reactive oxygen species. Moreover, their tendency to oxidize limits their storage in a vacuum-sealed, dry, and cool environment. On the other hand, Se nanoparticles are less toxic in nature and the toxicity is dose dependent. Iron oxide nanoparticles, which are frequently

used in imaging and drug delivery, show different biochemical reactions depending on their surface chemistry. Their toxicity effect is shown by the generation of a high amount of reactive oxygen species, which results in cell injury. Due to their nontoxic nature, ZnO nanoparticles are widely used in cosmetics; moreover, cosmetics containing ZnO nanoparticles do not penetrate deeply into the skin. Among the various inorganic nanomaterials, the highest level of toxicity is shown by quantum dots. Quantum dots can easily get excited and cause harm to human body, and their oxidative nature directly causes damage to DNA; thus, quantum dots are most preferred in *in vitro* applications like biosensing and bioimaging. Despite the toxicity issues, inorganic nanomaterials possess overwhelming advantages for use in the medical field. For this purpose, many surface modifications are carried out to improve the biocompatibility of the inorganic nanomaterials. Often, biopolymers are coated on these nanoparticles, which prevent unnecessary interaction between cells and inorganic nanoparticles, and green synthesis methods are adopted for these nanoparticles, which can inherently make them biocompatible in nature and decrease their toxic effect.

4.2 Organic Nanomaterials

Organic nanomaterials, either natural or synthetic, are highly biocompatible and biodegradable in nature. These are the least toxic nanomaterials reported till date. However, in some situations, organic nanomaterials can also show a low amount of toxicity which is caused as a result of changes in the molecular structure of the material or high surface energy which makes them highly active to interact with the cells and other biomolecules inside the human body. For instance, cellulose nanofibers were seen to cause inhalation toxicity in rats. Similarly, when chitosan nanoparticles are taken through the nasal pathway, pro-inflammatory responses are triggered. Different pathways of taking organic nanoparticles can cause different effects. A study showed that chitosan nanoparticles can enter the brain and their accumulation may lead to apoptosis of neurons and inflammatory response (Huang et al. 2005). Typically, by taking care of organic nanoparticles during their formation and preventing accumulation in any part of the body, the current low amount of toxicity exhibited by these nanomaterials can also be prevented, making organic nanomaterials promising materials for biomedical applications.

5 Conclusions

Nanotechnology has a remarkable influence in the field of biomedical field. The nanomaterials used for the application in this field have a direct impact on our lives. Thus, to get a clear insight, in this chapter, we have classified the nanomaterials and extensively discussed their characteristic features and applications. Further, we focused on the interaction mechanism of nanomaterials with various biomolecules and factors affecting this interaction, which is a key point to be taken care of while

using nanomaterials in medicine. Finally, the compatibility in terms of stability and cytotoxicity is described in detail.

The various inorganic and organic nanomaterials used for different biomedical applications have their own unique features as well as limitations. Inorganic nanomaterials such as gold, silver, copper, etc., are easy to synthesize with the possibility of alterations in properties according to one's use of interest. However, organic nanomaterials such as liposomes, micelles have a definite set of properties which cannot be altered. On the other hand, polymeric (organic) nanoparticles find an upper hand in biocompatibility as compared to metal and metal oxide nanomaterials. Polymeric nanomaterials such as liposomes, starch, dendrimers, micelles, etc., show very less cytotoxicity because of their easy biodegradability to simpler non-toxic molecules. Typically, polymeric nanoparticles are more widely used in *in vivo* applications of drug and gene delivery, preparing scaffolds for implants as they can easily interact with cellular components, whereas inorganic nanoparticles are applied more in *in vitro* techniques such as bioimaging techniques as contrast agents. Many nanomaterials approved by the US FDA are already being used in clinical practices. Some of these include Lipofectin – a liposomal formulation being used for DNA transfer; Genoxal – a polymeric micelle used in the treatment of breast cancer; ZnO nanoparticles incorporated in various cosmetic products; silver nanoparticles used in bone implants; gold nanoparticles utilized in the treatment of various types of cancer using photothermal technology; etc.

Despite so many accomplishments till date, it is evident that we are still waiting to cross the tunnel and fully utilize the promising potential of nanomaterials. A deeper understanding of toxicity related to nanomaterials and practices to overcome this issue need to be worked upon. Alongside, further studies related to the interaction of nanomaterials inside biological systems will result in more efficient and safer biomedical applications.

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