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Introduction to Nanotechnology

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Origin of Nanotechnology

The term nanotechnology comes from the combination of two words: the Greek numerical prefix nano referring to a billionth and the word technology (Logothetidis, 2012). As an outcome, nanoscience and nanotechnology are the study and application of extremely small things preferably less than 100 nanometers. Richard Feynman introduced the concept of nanotechnology in his pioneering lecture “There's plenty of room at the bottom” at the 1959 meeting of the American Physical Society (Feynman, 1959). Despite extensive research on nanotechnology in recent years, relatively a very little has been done to explore their biomedical application and potential toxicity.

Nanoscale science studies the phenomena, properties, and responses of materials at atomic, molecular, and macromolecular scales, and in general at sizes between 1 and 100 nm. In this scale, and especially below 5 nm, the properties of matter differ significantly from that at a coarse particle.

Inspired by Feynman's concepts, K. Eric Drexler in 1986 independently used the term "nanotechnology" in his book “Engines of Creation: The Coming Era of Nanotechnology”, which proposed the idea of a nanoscale "assembler" and other items of arbitrary complexity with atomic control.

Since 1980s, Drexler's had disseminated the concept of nanotechnology and draw attention on prospects of atomic control of matters. Further, invention of scanning tunnelling microscope (STM) in 1981 had excel the popularization of nanotechnology and provide better understanding about atoms and bonds. Theory of individual atom and their role was successfully conceptualized after the discovery of STM in 1989. Similarly, development of microscope was important discovery for unprecedented development of nanotechnological fields. In 1986, IBM Scientist Gerd Binning and Heinrich Rohrer have received Nobel Prize in Physics for their contribution in microscope development. Similarly, in 1996, Harry Kroto, Richard Smalley and Robert Curl had received Nobel Prize in chemistry for the discovery of fullerenes in 1985. By the mid-2000s new and serious scientific attention began to flourish. Nanoparticle research is currently an area of intense scientific interest due to a wide variety of potential applications in biomedical, optical and electronic fields (Kroto et al., 1985).

In the recent past, numerous developments have been made in nanotechnology. This timeline will helps to understand the evolution and future implication of nanotechnology Fig. 1.1.

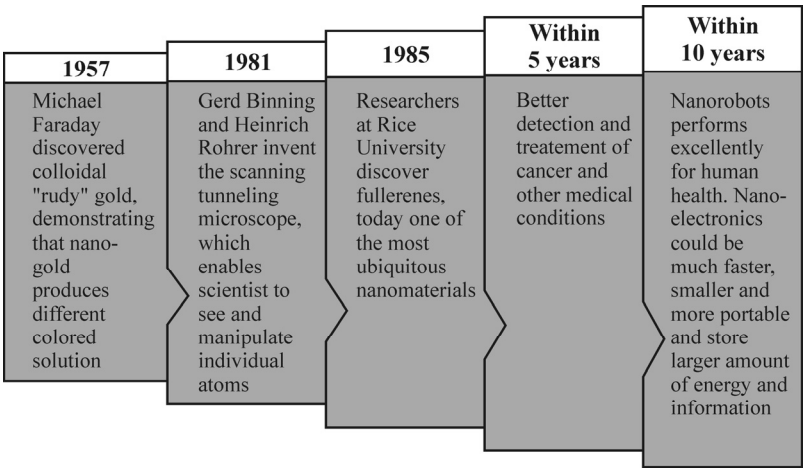


Fig. 1.1 Nanotechnology developed over the years.

Significance of the Nanoscale

Nanoparticles are of great scientific interest as they are, in effect, a bridge between bulk materials and atomic or molecular structures. A bulk material possesses constant physical properties regardless of its size, but at the nanoscale size-dependent properties are often observed. Nanoscale materials may differ from bulk materials by the following means.

- Electromagnetic forces play a dominate role over gravitational forces.
- Material at nanoscale often exhibit unexpected optical properties called Quantum effects. Nanomaterials due to their unique surface properties amplify light intensity.
- Greater surface area to volume ratios.
- Nanoparticles due to their high surface area to unit mass possess random molecular motion called Brownian movement.

Gravitational Forces Vs Electromagnetic Forces

Nanomaterials due to their small size and negligible mass, gravitational forces become negligible. Electromagnetic forces play central role in determining the behaviour of nanoparticles. Therefore nanoparticles provide a tremendous driving force for diffusion eventually result higher solubility, permeability and diffusivity (Juh et al., 2007).

Quantum Effects

Nanoparticles are so small that electrons are not free to move about as in case of bulk materials. As a result of this, nanoparticles react differently with light called quantum effect. For example gold nanoparticles usually appear red in solution. While gold appears as greenish yellow in bulk form (Hewkuruppu et al., 2013).

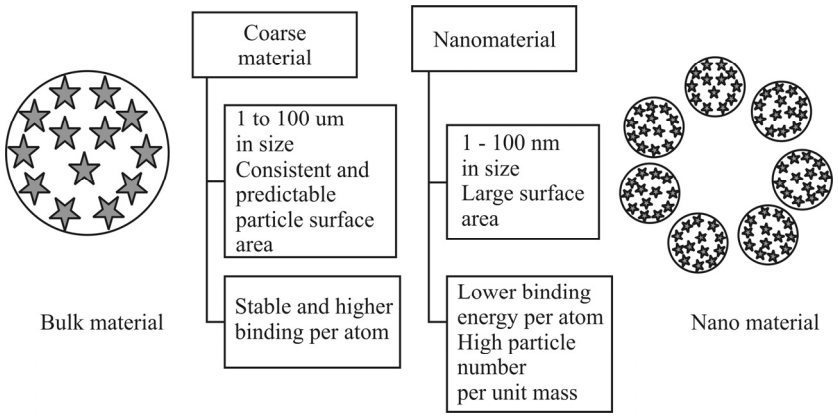
Surface Area to Volume Ratio

As surface area to volume ratio increases, nanoparticle shows strong interaction with surrounding material; eventually result higher wettability, solubility, permeability, bio-adhesion and diffusivity. This results in better catalysts, since a greater proportion of the material is exposed for potential reaction. Therefore nanoparticles can have deep access to the human body than bulk materials because of the particle size and control of surface properties. However bulk materials due to their large size show limited interaction with surrounding area (Gupta and Kompella 2006).

Random Molecular Motion

Nanoparticles because of their small size moves randomly. On the other hand larger particle easily sediment, since gravitational force overcomes the electromagnetic forces. At the nanoscale, the particle is moving wildly, called Brownian movement. The atoms situated at the surface have less neighbours than bulk atoms, resulting in lower binding energy per atom with decreasing particle size eventually result in the reduction of

melting point. The decrease in melting temperature can be on the order of tens to hundreds of degrees for metals with nanometer dimensions (Uma et al., 2012). Fig. 1.2 summarizes some of the differences between nanomaterials and bulk materials.



Bulk material properties

- **Physical form:** Particles form large groups of insoluble particles.
- **Surface area:** Particles can be seen with naked eye (larger than 100 nm).
- **Diffusion:** Gravitational force overcome electro-magnetic force, result settling of micro-particles.
- **Sedimentation:** Particles settle out with time.
- **Optical Properties:** Optical properties like refractive index remain unchanged.
- **Electrical Properties:** Electrical properties of bulk material are remaining unchanged.
- **Physical properties:** Micro-scale has a very small effect on the percentage of atoms on the surface.
- **Melting Point:** Has no or a very small effect.

Nanomaterial properties

- **Physical form:** Particles form groups of ions, atoms, or molecules.
- **Surface area:** Surface Area of nanoparticle = 10^7 times more than microparticles.
- **Diffusion:** The high surface area to volume ratio of nanoparticles provides a tremendous driving force for diffusion.
- **Sedimentation:** Particles do not settle out with time.
- **Optical Properties:** Nanoparticle exhibits unique optical properties because the particles are so small that electrons are not free to move about as in bulk gold.
- **Electrical Properties:** Electrical properties of nanoparticles change with diameter. They can be either conducting or semi-conducting in their electrical behaviour.
- **Physical properties:** Nano-scale has a big effect on the percentage of atoms on the surface.
- **Melting Point:** Melting point is lower for smaller particles.

Fig. 1.2 Difference between bulk materials and nanomaterials.

Nanomaterials and their Classification

Material whose size is less than 1μm in at least one dimension are defined as nanomaterials. Nanomaterials can be found in different forms and shapes. They are classified based on geometry, morphology, composition, and preparation approach or micrometric properties (Oberdorster et al., 2005).

- ***Nanomaterial classification based on geometry*** **(Fig. 1.3):**
  - 1D nanomaterials have one dimension in the nanometer scale. Structures which exhibit such properties include nanolayers, nanoclays or nanosheet. Common types of 1D nanomaterials include Graphite nano sheet and clay nanoplatelet.

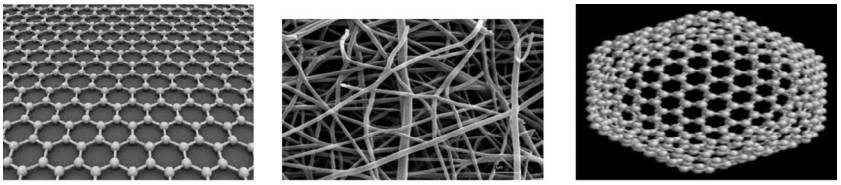


Fig. 1.3 Classification of nanomaterials based on geometry.

  - 2D nanomaterials have two dimensions in the nanometer scale. Two-dimensional nanomaterials includes carbon nanotubes, nanofibers etc.
  - 3D nanomaterials have nanoscale structure in all three dimensions. 3D nanomaterials structure includes nanoparticles, nanospheres, nanocapsules or nanoemulsion.
- ***Nanomaterial classification based on morphology*****:** Nanoparticle may be classified based on their morphological parameters such as aspect ratio (Ratio of particle long axis length to the particle diameter or thickness). Nanomaterials are generally classified as materials with high or low aspect ratio. High aspect ratio nanoparticles include nanotubes and nanowires, with various shapes, such as helices, belts, or perhaps nanowires with diameter that varies with length. Small-aspect ratio morphologies include spherical, oval, cubic, nanoparticles (Oberdorster et al., 2005).
- ***Nanomaterial classification based on composition*****:** Nanoparticles can be composed of a single constituent material or be a composite of several materials.

- ***Nanoparticle classification based on micrometric behaviour*:** Based on their physic-chemical and electro-magnetic properties, nanoparticles can exist as dispersed aerosols, as suspensions/colloids, or in an agglomerate state.

Impact of Nanoparticles in Pharmaceutical Science

Miniaturization of materials in nanoscale may reframe the physicochemical and biological properties of existing material. The decrease in the size of particles will not only increases the surface area of resultant particles but also provides increased number of molecules on the surface of particles. This may leads to changes the physicochemical properties of the material. The increased surface area of nanoparticles can significantly increase the dissolution of poorly water soluble drugs. Due to their small size, nanoparticles are less prone to gravitational settling and can be easily suspended in liquid formulation. Their size dependent optical properties are unique in their applications to the efficient labeling of biomolecules and tissues where the traditional fluorescent labels have been hardly accessible because of the size restrictions. Nanomaterials are being exploited in many different biological and medical fields due to their distinct optical, electrical, chemical and physical properties (Desai et al., 1996, Dressman et al., 1998). The Figs. 1.4, 1.5 &1.6 summarize key points illustrating the pharmaceutical significance of nanoparticles.

Nanomaterial in Biomedical Application

Nanomaterials due to their unique optical, electronic, magnetic, surface and chemical properties, they have a wide variety of applications, including medicine, drug delivery, biological imaging, and structural engineering. Nanomaterials due to their small size show distinct advantages over bulk materials. As the size of the material decreases, its surface-to-volume ratio increases. This presents considerable advantage to modify properties of nanomaterials through surface functionalization techniques (Emerich et al., 2003, Jain, 2003).

A class of nanoparticles known as “quantum dots”, which can emit different types of light depending on their size, have wide applications in diagnostics and bioimaging, including imaging in cancer studies. It is expected that the application of nanoparticles (gold, iron, quantum dots) could be extremely important for the development of contrast agents for almost all imaging techniques. Nanoparticles could also be used to treat cancer directly by targeting them to cancer sites and using light or magnetism to heat them, thereby destroying the cancer (Leoni et al., 2002).

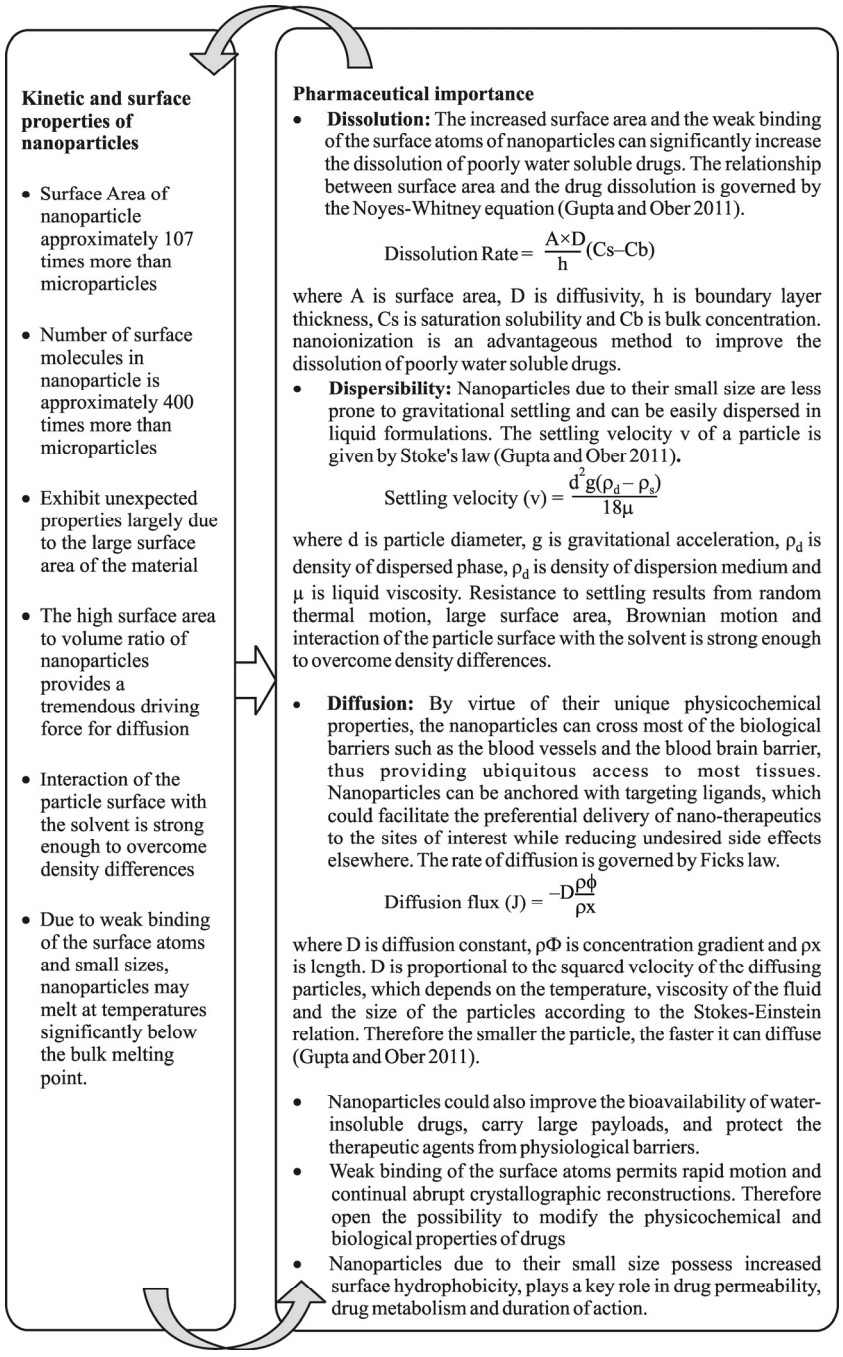


Fig. 1.4 Kinetic properties and their pharmaceutical importance.

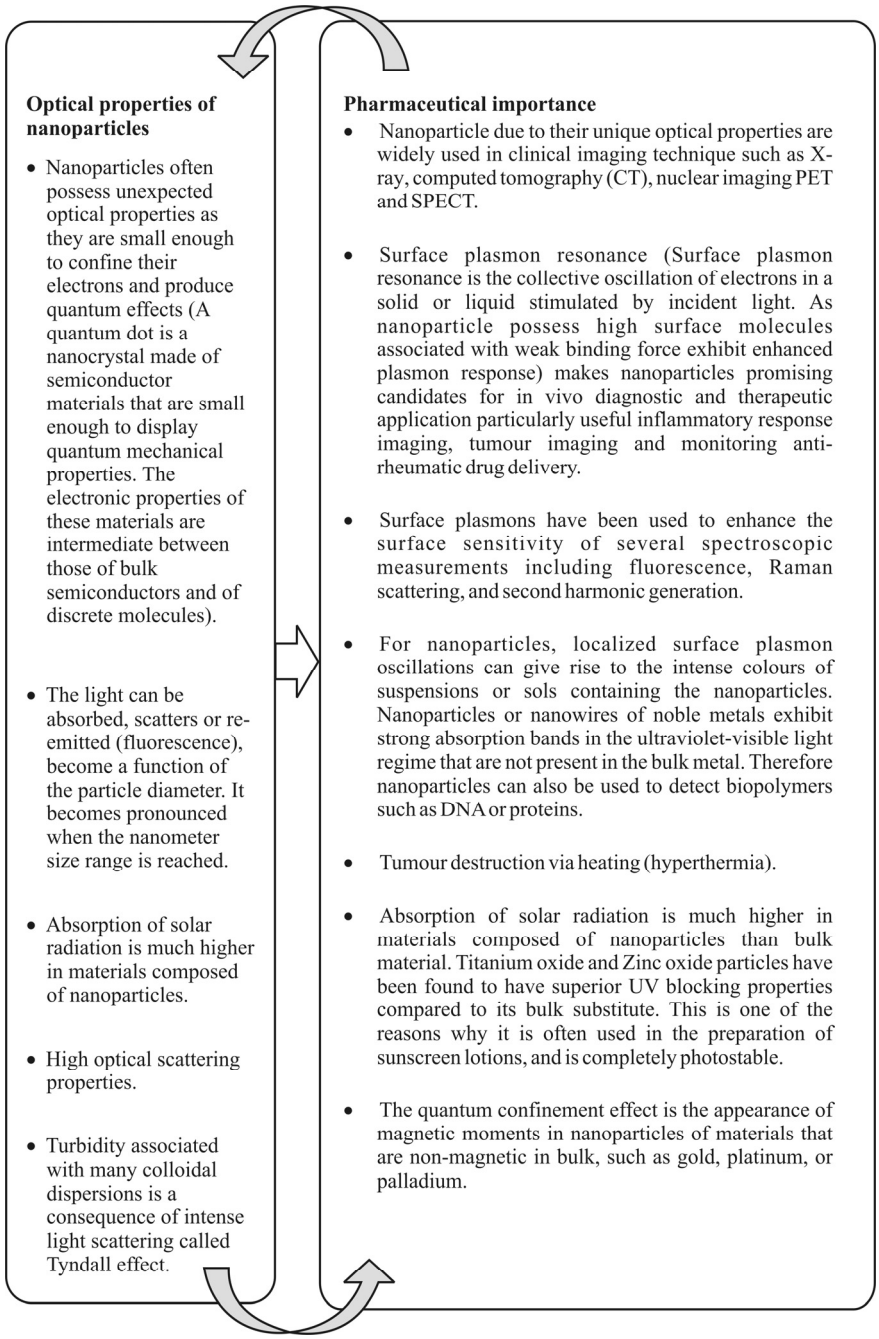


Fig. 1.5 Optical properties and their pharmaceutical importance.

Fig. 1.6 Electrical properties and their pharmaceutical importance.

The use of nanomaterials for drug delivery is an area with a great deal of activity that could have a major impact on the medical and pharmaceutical industry. For drug delivery, nanomaterials combine the advantages of high surface area, improved interfacial properties and size confinement to deliver drugs that have increased efficacy.

Nanoparticles may offer better solubility, leading to a better absorption. Also, drugs may be contained within a molecular carrier (Liposome, Solid lipid nanoparticle, polymeric nanoparticle), either to protect them from stomach acids or to control the release of the drug to a specific targeted area, reducing the likelihood of side effects (Mohanraj and Chen 2006).

Nanomaterials can potentially have roles in tissue engineering, in the production of artificial 'scaffolds' for the re-growing of tissues and organs specifically tailored to the patient, as well as implants and materials used in artificial joints (Chung et al., 2007). Fig. 1.7 summarizes emerging research of nanoparticles for bioimaging and medicine.

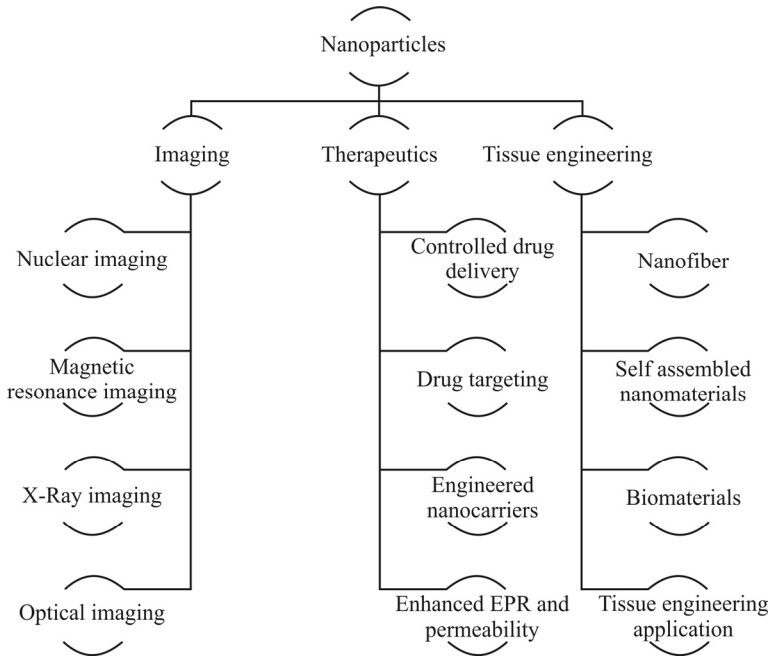


Fig. 1.7 Biomedical application of nanomaterials.

Nanoparticles for Imaging

Imaging techniques are widely used in medicine and biochemical research. A number of molecular imaging techniques, such as nuclear imaging (PET), optical imaging (OI), magnetic resonance imaging (MRI),

ultrasound imaging (USI), and others have been reported for imaging of biological specimens (Fass 2008, Debbage and Jaschke 2008, Thomas and Jeong 2013). Fig. 1.8 illustrates a schematic representation of different component of an engineered nanoparticle for *in-vivo* bio-imaging.

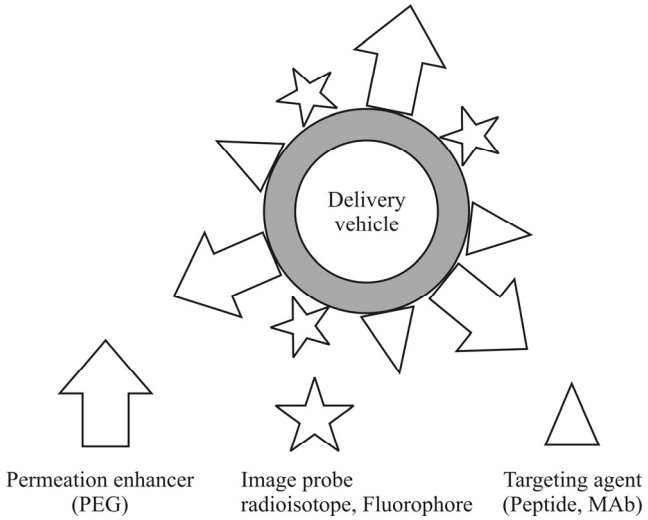


Fig. 1.8 Schematic representation of surface engineered nanoparticle for bio-imaging.

Nanoparticles due to their unique properties such as superior photo stability, narrow range of emission, broad excitation wavelength, multiple possibilities of modification, quantum dots makes these systems promising candidates for sensing applications. The most widely used nanoparticles in optical imaging are semiconductor nanocrystals, known as quantum dots. Their size dependent optical properties are unique in their applications to the efficient labeling of biomolecules and tissues where the traditional fluorescent labels have been hardly accessible to because of the size restrictions. In contrast, the size and shape of fluorescent nanoparticles can be rather easily controllable during their synthesis (Ocampo et al., 2011). In addition to beneficial properties of nanomaterials, there are other requirements need to be addressed for efficient diagnosis that includes:

- A delivery vehicle or core materials that can carry an optimal amount of radioisotopes and has favourable pharmacokinetics.
- Specific targeting ligands.
- The selection of imaging probe with a proper half-life; that are inexpensive to produce and can be readily conjugated to the delivery vehicles.

Delivery Vehicle/Core Materials

Nanoparticles have an advantage for molecular imaging in that much functionality can be added to the surface and interior of the particle. The structure of nanoparticle allows organic and inorganic nanoparticles that have been functionalized so that radio nuclides, targeting ligands, and polyethylene glycol can be attached to provide the imaging signal, target the particle, and alter the pharmacokinetics of the particle (Hong et al., 2009). There are several advantages of using nanoparticles as a core shell material to deliver diagnostic agents:

- Increasing the *in-vivo* circulation half-life of encapsulated substances.
- Provide better permeability, retention and uptake of diagnostic agents into target site.
- It may be utilized to deliver multiple diagnostic agents with different markers in single carrier.
- This technology is an important tool for better understanding to chemistry of cell, cell surface, cell receptor interaction, and other biological signals and their pathways
- Multipurpose delivery systems may be developed that not only diagnosed the site but also treat the abnormality of causes.

Due to small size and versatile surface chemistry, nanoparticle platforms may offer appropriate pharmacokinetics for optimal delivery of radioisotopes or imaging probe into the target cell, thereby improve the efficacy and sensitivity of diagnostic techniques. Several nanocarrier devices as a core shell materials have been applied in imaging (Amir and Faraji 2009). Table 1.1 enlists specific nanoscale devices used in bio-imaging technique.

Table 1.1 Nanoscale devices as delivery vehicle used in biomedical imaging.

Nano core scale device	Comments
Liposome	Liposome composed of one or more phospholipid bilayer membranes; smallest liposomes are in nanometre size range. Liposomes have gained increasing interest as delivery vehicle due to their low toxicity and biodegradability. Liposomes with modified surfaces have also been developed using specific ligands to improve the efficacy of imaging.
Dendrimer	Dendrimers are repetitively branched molecules with monodispersed structures of 2–20 nm. With the advantage of tunable surface modification, dendrimers can be easily functionalized to exhibit desirable characteristics, ideal for the molecular imaging of organs and other target-specific locations.

Table 1.1 ***Contd…***

Nano core scale device	Comments
Polymeric nanoparticle	Various polymers are used to produce biocompatible, biodegradable nanoparticles of 50-2000 nm. Surface properties of these nanoparticles can be modified to target specific cell types and thereby improve the sensitivity of imaging technique.
Nanotube	Carbon nanotubes (CNTs) are building blocks of carbon with a cylindrical nanostructure, The unique properties of CNTs makes them promising biological imaging probe and have been intensively explored for biological and biomedical applications in the past few years.
Solid lipid nanoparticles	Solid lipid nanoparticles are lipid-based submicron colloidal carriers. SLNs due to their controllable pharmacokinetic parameters and surface hydrophobicity hold promise for use as targeted agents and multimodal imaging agents.
Nanocrystals	Nanocrystals are aggregates of thousands of molecules surrounded by a thin coating of surfactant. Quantum dot nanocrystals are fluorophores where emitted light is of longer wavelength than the incident; they absorb photons of light and then re-emit longer-wavelength photons nearly instantaneously. They have extensive uses in materials research, chemical engineering, and as quantum dots for biological imaging.
Calcium phosphates nanoparticle	Calcium phosphates, also known as hydroxyapatite, represent the majority of the inorganic matter of human hard tissue such as bone and teeth. Calcium phosphates due to their strong affinity towards bone tissue, these particles are used for bone imaging.
Quantum dots	Quantum Dots (QDs) are inorganic semiconductor nanocrystals of a few nanometers in diameter with unique optical and chemical properties. Quantum dots have been increasingly used in fluorescence applications in biological research due to their ability to increase the intensity of emission spectra. Further quantum dots due to their long term photostability, enable researcher to investigate basic cellular and molecular processes such as cell migration, differentiation, and metastasis.
Magnetic nanoparticles	Magnetic nanoparticles are a class of nanoparticle which can be manipulated using magnetic field. Such particles commonly consist of magnetic elements such as iron, nickel and cobalt. The size and surface of these particles can be specifically functionalized to target cells (tumor, transplanted cells).

Targeting Ligands

The selection of ligands that needs to be attached to the nanomaterial surface plays an importance role in order to achieve desired results. A variety of different ligands may be attached to a nanomaterial depending on what effect is to be achieved. Several factors that could be considered include ligand biocompatibility, cell specificity, binding affinity, and purity of the ligand. Other important factors that have to be taken into account are the size and charge of the ligand molecule, and

their ease of modification and conjugation to the nanoparticles. The smaller the nanomaterial, the larger is the impact of the ligand on it. There are six different classes of targeting ligands, commonly used including antibodies or their fragments, aptamers, protein, peptides, sugars, and small molecules. Table 1.2 enlists the commonly used ligands in bio-medical imaging.

Table 1.2 Ligands used in biomedical imaging.

Types of ligand	Comments	Limitation	Application
Antibodies and antibody fragments	Antibodies and antibody fragments with a size range from 10-15 nm form an important class of targeting ligands with a high degree of specificity for cellular receptors and a wide range of binding affinities and have been extensively investigated in biomedical imaging	Large MW (150 KD) limits its application in cellular targeting. May be Immunogenic (Need special process conditions during conjugation process)	mAb against PSMA, was conjugated to PAMAM dendrimers and showed enhanced binding affinity for LNCaP cells
Aptamers	Nucleic acid aptamers are single-stranded DNA or RNA oligonucleotides with well defined, three-dimensional structures. Aptamers can recognize a wide variety of molecules (e.g., proteins, phospholipids, sugars, and nucleic acids) with high affinity and specificity	Poor serum stability and limited cell uptake	aptamer against VEGF known as Pegaptanib, used for the treatment of age-related macular degeneration
Protein	Endogenous proteins that selectively bind to specific membrane-bound receptors on cells can be used.	Immunogenic and poor affinity for target. High molecular weight restricts the passage of protein into cell. Protein susceptible to proteolytic enzyme.	Transferrin (Tf), epidermal growth Factor, Nerve Growth Factor, etc. The receptors of Tf and EGF are overexpressed on cancer cells.

Table 1.2 ***Contd…***

Types of ligand	Comments	Limitation	Application
Peptide ligands	Peptide ligands have shown significant targeting potential because of their small size, high stability, and relative ease of large-scale synthesis with excellent quality control.	Difficult to mapping and susceptible to proteolytic enzymes under physiological conditions	Peptide SP5-52 can recognize tumor neovasculature. Arg–Gly–Asp-based (RGD) peptide extensively investigated tumor angiogenesis
Sugars	Specific sugar molecules (e.g. , lactose, galactose, and mannose) can recognize lectins that are overexpressed on the surface of numerous cancer cells	Less binding energy and the affinity depends on the density of sugar molecules.	Galactose could recognize the asialoglycoprotein receptor which is expressed on hepatocytes.
Small molecules	Small molecules have potential targeting ligands due to their low molecular weights, low production costs, and easy conjugation with nanoparticles e.g., folic acid	Also expressed in normal cells leads uptake of encapsulated markers to non-target cells.	Folic acid have specificity in recognizing folate receptors that are over expressed in many types of tumor cells

Imaging Probe

Nanotechnology is one of important tool for unprecedented growth in the field of diagnosis and imaging. Nanotechnology provides nanosized imaging probes that can be functionalized to improve selectivity towards specific sites. Further, nanoionization of lots of material has shown improved optical, thermal, and magnetic properties (Quantum dots, carbon nanotubes, fullerene etc.) that has been proven their potential for diagnosis and imaging for the diseased or disordered site within human body. Nanotechnology have quested many hidden diseased site, organs, organelles, cells and receptors. Different diagnostic tool in association with imaging agents have been used for diagnostic purpose Table 1.3 such as x-rays, sonography, magnetic resonance imaging (MRI), computed tomography (CT), positron emission tomography (PET), single-photon emission computed tomography (SPECT), magnetic and fluorescence imaging techniques (Thomas and Jeong 2013).

Table 1.3 Advantages and limitations of imaging modalities.

Imaging modalities	Advantages	Limitations
PET	• Provides biochemical information • High sensitivity • Three-dimensional imaging • Can monitor changes in tumour pathology and drug metabolism	• Limited anatomical information • Requires specialized and sophisticated equipment • Requires special care and handling due to the use of radio-nucleotide Expensive
MRI	• High resolution • Good soft tissue contrast • Provides both anatomical and physiological information	• Low sensitivity • Relatively long acquisition time Expensive
CT	• High-sensitivity anatomical imaging • Provides three-dimensional image	• Limited functional information • Poor soft tissue contrast • Expensive
Optical imaging	• Wide applicability and simple handling • Simultaneously monitor several molecular events • Economical and easy to operate	• Requires genetic manipulation of investigated cells • Provides limited anatomical • Reduced sensitivity with increased imaging depth

***Nuclear imaging*:** Nuclear imaging techniques require radioactive substance (Thallium 201, Technetium-99m, iodine 123, cobalt 60, Gallium 67), which emits gamma rays. The radioactive gamma rays emitted are captured by detectors that surround the body. A special method of nuclear imaging is based on positron emission tomography (PET) and SPECT (single-photon emission computed tomography). Under these techniques radioactive agents such as Technetium-99m is attach to the nanoparticles, which provide the contrast that allows for imaging. In addition, the nanoparticles are labeled with a specific ligand that targets the target site. When injected into the body, the targeted nanoparticles will find and illuminate these vessels (Thomas and Jeong 2013).

***Magnetic resonance imaging*:** Magnetic nanoparticles can be used for imaging applications such as in magnetic resonance imaging (MRI) as the magnetic nanoparticles can be easily guided through the body with the application of an external magnetic field. MRI is a useful problem-solving diagnostic tool in the clinical field because it has higher spatial resolution and contrast in soft tissue than other imaging modalities. Various contrast agents that are used in MRI includes gadolinium, iron,

manganese etc. Among these, magnetic nanocrystals provide the excellent probe for the magnetic resonance imaging (MRI), which is widely used imaging modality to present a high spatial resolution and great anatomical detail. Recently, superparamagnetic iron oxide (SPIO) nanoparticle has become the gold standard for MRI cell tracking. Gadolinium (Gd) complex based contrast agents can be good alternative MRI contrasts to generate the unambiguous positive contrast (hyper-intensity) and developed. Even if they produce positive contrast and increase the visibility of cells in low signal tissue, they have short residence time and can't pass through the cell membrane easily. Therefore, there have been developed some of Gd ion based nanopaticulate contrast agents to overcome these disadvantages of the complex agents. Other nanoparticles that are being investigated for use as a carrier for MRI contrast agent are fullerenes, liposomes, polymeric nanoparticles and dendrimer (Debbage and Jaschke 2008).

***Computed tomography*:** Computed tomography (CT) uses x-rays to obtain images of specific area treated with specific contrast agents. The major advantage of the CT imaging technique is that it produces images with high spatial resolution. Most commonly used CT contrast agents are iodine-based compounds. These agents work by blocking X-rays, thereby providing contrast and enhancing a part of the body. Among non-ionic and ionic contrast agents, ionic agents prove to be more harmful, especially for patients with renal problems. Therefore, to overcome the limitations of conventional contrast agents, recent research has focused on developing a gold nanoparticle based contrast agent for CT. Gold nanoparticles are biocompatible and are capable of targeting the tumor by the EPR effect. This material has a very high X-ray absorption coefficient, which makes it a suitable agent for replacing iodine in CT imaging. Gold is a metal with a high atomic number and is therefore considered a strong candidate for CT imaging because it provides better x-ray attenuation and contrast (Thomas and Jeong 2013).

***Optical imaging*:** Optical imaging involves an optical contrast agent that can emit fluorescence at various excitation wavelengths. Previously optical imaging was used in cancer diagnosis based on the variation in endogenous fluorescence of neoplastic tissue. However, due to the difficulty in distinguishing the diagnostic signal component from background fluorescence, exogenous contrast agents were developed. Nanoparticle based optical contrast agents, such as "quantum dots," were thus developed to have superior imaging properties compared to organic counterparts. The major issues encountered by optical imaging with conventional contrast agents are the inability to quantify the image and

the auto fluorescence of normal tissue against the fluorescence of the contrast agent, which can severely impair the image quality. To overcome the limitations of conventional contrast agents, the semiconductor particles with unique optical properties are extensively studied for biological imaging are generally made of a cadmium selenide core surrounded by a shell of zinc sulphide. They are of the nanometer scale and when illuminated, the quantum dot emits a particular colour based on its size. Since the colour of quantum dots is very specific to the size and composition, allowing the simultaneous fluorescence of many different, specific colours and therefore allowing the easy discrimination between tagged targets, even in a single cell. Further conventional fluorophores suffer from short life span. In contrast, quantum dots remain stable for days to months (Thomas and Jeong 2013).

Nanoparticles are believed to be of high significance for the progress of diagnostics in the future. Research is focused on the development of better contrast agents for nearly all imaging techniques (Thomas and Jeong 2013). Table 1.4 illustrates the potential application of important classes of nanomaterials and their intrinsic properties that contribute to their unique biomedical imaging (Huang et al., 2009).

Table 1.4 Nanoparticles in bio imaging technique.

Category of nanomaterials	Examples	Intrinsic properties	Application
Gold nanoparticle	Gold nanoparticles with ligand gastrin peptide receptor and Technetium-99m radioisotope	Extensive surface plasmon resonance with specific affinity for gastrin over expressed cell	SPECT/CT imaging of gastrin releasing peptide-receptor in breast and prostate cancer detection , and multimodal probe for possible thermotherapy
Silver nanoparticles	Silver nanoparticles with permeation enhancer Poly(N-vinyl-2- pyrroli-done) and radioisotope^{125}	Extensive surface plasmon Resonance with higher permeability for vascular and inflamed tissue	*In vivo* imaging and biodistribution of radiolabeled NPs
Supermagnetic iron oxide nanoparticle	Iron oxide nanoparticle with alendronate/ osteoclastic surface and radioisotope Technetium-99m radioisotope	Magnetic attraction, commonly recommended for targeting peripheral tissue	Platform for SPECT/MRI images

Table 1.4 ***Contd…***

Category of nanomaterials	Examples	Intrinsic properties	Application
Gold nanoparticles	Gold nanoparticles dispersion with PEG	Contrast agents for X-rays. This material has a very high X-ray absorption coefficient	Gold nanoparticles are biocompatible and are capable of targeting the tumor by the EPR effect.
Perfluorocarbon nanoparticle	Gadolinium chelates concentrated in perfluorocarbon nanoparticle emulsion	Excellent magnetic properties	Diagnosis of arteriosclerosis plaques.
Fullerenes	Gadolinium entrapped in fullerenes	Excellent magnetic properties	These nanoparticles can be used to detect early stage blood clot formation
Quantum dots	Conjugation of biotinylated fibrinogen to QDs	Unique optical properties	QDs allow for the non-invasive visualization of blood vessel development over time

Nanotechnology in Drug Delivery

Drug delivery describes a process whereby a therapeutic agent is administered to the body in a controlled manner so that an optimum amount reaches the target site (Amir and Faraji 2009).

Nanomaterials due to their unique properties show promise in targeted and controlled delivery of biopharmaceuticals (Jaspreet et al., 2005 and Jain et al., 2010). The advantages of using nanoparticles as a drug delivery system include the following:

- Particle size and surface characteristics of nanoparticles offers numerous opportunity for drug targeting including both passive and active drug targeting.
- Nanoparticle due to their small size exhibit higher intracellular uptake and can penetrate the sub mucosal layers while the microcarriers are predominantly localized on the epithelial lining.
- Nanoparticles due to their functional properties control and sustain release of the drug during the transportation and at the site of localization, altering organ distribution of the drug and subsequent clearance of the drug so as to achieve increase the drug therapeutic efficacy of drug and reduction in side effects.

- Controlled release and particle degradation characteristics can be readily modulated by the choice of matrix constituents.
- Drug loading is relatively high and drugs can be incorporated into the systems without any chemical reaction; this is an important factor for preserving the drug activity.
- Site-specific targeting can be achieved by attaching targeting ligands to surface of particles or use of magnetic guidance.
- The system can be used for various routes of administration including oral, nasal, parenteral, intra-ocular etc.
- Protect drugs from hepatic inactivation, enzymatic degradation and rapid clearance *in-vivo*. Fig. 1.9 illustrate the properties of nanoparticle useful in drug delivery.

Above advantages of nanomaterials is attributed due to their unique properties particularly associated to their particle size, surface area, hydrophobicity, surface charge and crystallinity Fig. 1.9.

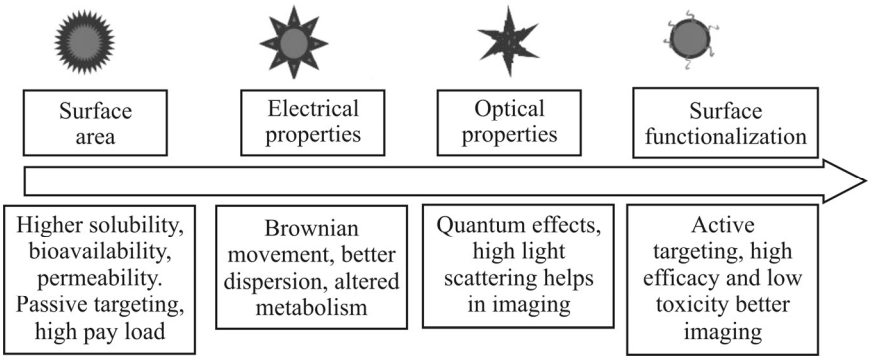


Fig. 1.9 Nanomaterial properties useful in drug delivery.

Particle Size

The sub-micron size of nanoparticles offers a number of distinct advantages over microparticles particularly in drug delivery (Panyam and Labhasetwar 2003). It has been reported that, nanostructures have the ability to protect drugs from the degradation in the gastrointestinal tract; the technology can allow target delivery of drugs to various areas of the body Fig. 1.10.

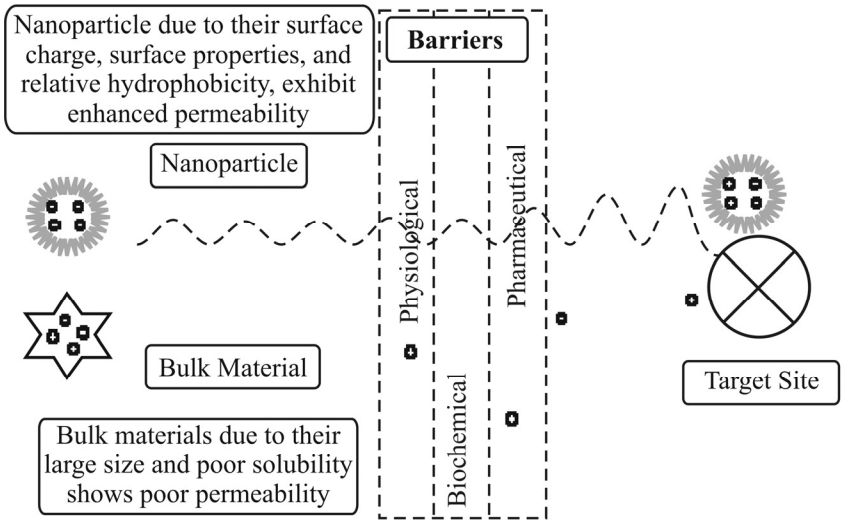


Fig. 1.10 Schematic presentation describing barriers to drug targeting and the role of nanosystems in overcoming these barriers.

Nanotechnology have potential to improve the uptake of poorly water soluble drugs from gastrointestinal tract and minimize first pass metabolism. Further, nanotechnological approaches may also be utilized for sustained and controlled release of drugs (Mohanraj and Chen 2006). Nanosized particles not only reaches to specific organs and tissues, but also taken up by specific cell and intracellular compartments. Uptakes of these nanomaterials is 100-200 or even more times better than its counter parts that is in micron range. Surface decorative nanoparticles may be also designed for organ or site specific delivery of therapeutic entities. Thus, Nanotechnology may be utilized for both active and passive targeting of the nanomaterials (McNeil 2005 and Bae and Park 2011).

***Passive targeting*:** Passive targeting refers to the accumulation of drug or drug-carrier system at a particular site due to physico-chemical or pharmacological factors. Passive targeting, refers to the preparation of a drug carrier complex that avoids removal through body mechanisms like metabolism, excretion, opsonisation, and phagocytosis, so that the complex remains circulating in the blood stream permitting its transmission to the target receptor by properties like pH, temperature, molecular size, or shape. Spontaneous drug accumulation in areas with leaky vasculature is a form of passive targeting. The physiology of diseased tissues, altered in different pathological conditions, is exploited for passively targeting drugs (Bae and Park 2011). Nanoparticles used in

a drug delivery system should be large enough to thwart their speedy outflow into blood capillaries. But they should be small enough to escape capture by fixed macrophages stuck in the reticuloendothelial system (RES), such as the liver and spleen (Jaspreet et al., 2005).

Particularly particles less than 100 nm in size allows these particles to reach virtually all tissues in the body (Jaspreet et al., 2005, Mcnail 2005 and Koziara et al., 2003). Nanoparticles due to their submicron size have relatively higher intracellular uptake compared to microparticles and available to a wider range of biological targets. It was found that the uptake as well as the saturation concentration varied with the different sized nanoparticles particularly with 50-nm-size particles being the most efficient in their uptake, indicating that there might be an optimal size for efficient nanomaterial uptake into cells (Bae and Park 2011). Fig. 1.11 illustrates the process of uptake of nanoparticles into cells (Verma and Stellacci 2009).

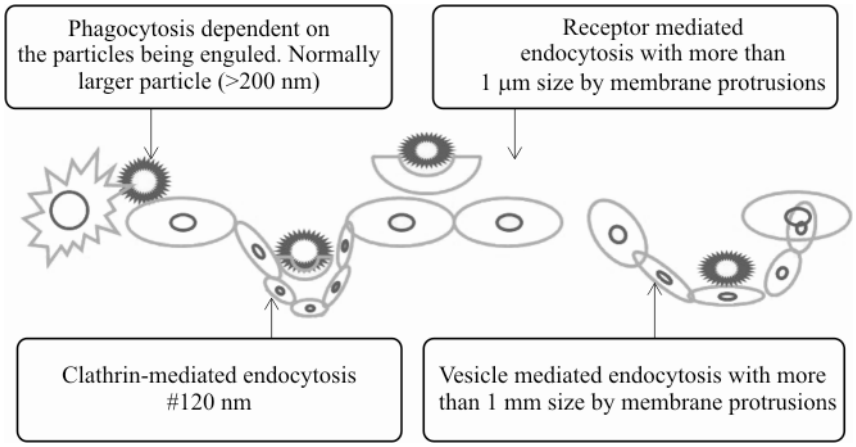


Fig. 1.11 Cellular uptake of nanoparticles.

Besides rapid uptake by RES, another significant barrier for injectable particulate systems is the endothelial lining between the vascular space and extravascular target tissue. In most tissues the vascular system is lined with a continuous layer of endothelial cells often supported by a basement membrane. This barrier virtually excludes extravasation of nanoparticles except for a few selected sites where the endothelial lining is discontinuous. Fortunately, it has been found that regions of increased capillary permeability include pathological sites such as tumors and sites of infection and inflammation. Nanoparticles have been shown to extravagate into these pathological areas (Jaspreet et al., 2005). The

mechanism of extravasation is often referred to as 'enhanced permeability and retention (EPR) effect.

An additional factor participating in targeting is the unique microenvironment flanking tumor cells, which is distinctly dissimilar from that of normal cells.

Taking together, passive targeting of nanomaterials is based on selective but non-specific extravasation into pathological tissues accessible from the circulation due to a locally increased vascular permeability. However, passive targeting with nanoparticles encounters many obstacles on the way to their target; these include mucosal barriers, non-specific uptake of the particles and nonspecific delivery of the drug (Schroeder et al., 2012).

Further, when target cells are not localized in the extravascular space but for example in the blood circulation, the localization process requires more sophisticated strategies such as specific carrier target cell recognition. In these situations surface conjugated targeting ligands and membrane translocating functionalities for intracellular delivery have to be included in the nanomaterial system.

Therefore, appropriate size and functionalization with specific ligands can provide means of enhanced delivery of drugs and reduced non-specific toxicity. Fig. 1.12 explores the biological interaction of nanoparticles.

	Cell Vs Nanoparticle • Nanoparticle penetrate cell through passive uptake or adhesive mechanism. • The maximum size of nanoparticle allowing penetration through cell membrane is known to be 500 nm. • Nanoparticle internalization depends on nanoparticle size. Nanoparticles (< 100 nm) localize in organelles, such as mitochondria (Bay and park 2011). • Nanoparticle with a diameter from 1-20 nm, are able to penetrate cells through ion channels or via pores in the cell membrane celle. The process of nanoparticle uptake by cells is clinically used today in targeted drug delivery and cell imaging. Nanoparticle uptake by red blood cells exclusively depends on their size. • Nanoparticle charge plays an essential role in their uptake by platelets and WBC.

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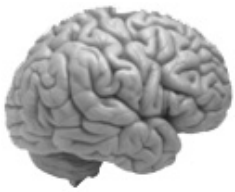
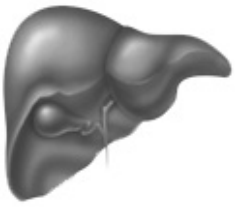
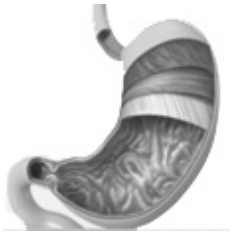
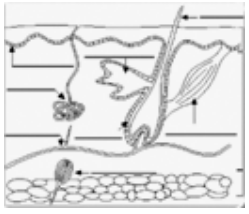
	Blood Brain Barrier Vs Nanoparticle • Neuronal uptake of inhaled nanoparticles may take place via the olfactory nerves or/and blood-brain-barrier. • Translocation of nanoparticles into deeper brain structures may be possible. • Blood-brain-barrier permeability is dependent upon the charge of nanoparticles. • It allows cationic nanoparticles to pass compared to neutral or anionic particles, due to the disruption of its integrity. • The blood brain barrier including CNS has exhibited a vascular pore cut off size of 10 nm (Schroeder et al., 2012).
	Liver Vs Nanoparticle • Nanoparticles with size range less than 2 nm penetrate the epithelial's tight junction present in liver and kidney. • In liver, the endothelium is fenestrated with pores of up to 100 nm, allowing easier passage of larger particles. • In the presence of inflammation the permeability of the endothelium is increased, allowing a larger passage of particle. • The proposed cut off size for particle for liver extravasation in 200 nm (Schroeder et al., 2012). • Liver has the blood vessels with fenestration of 200 nm.
	GIT Vs Nanoparticle • A small fraction of inhaled nanoparticles was found to pass into the gastrointestinal tract. • The extent of particles absorption in the gastro-intestinal tract is affected by size, surface chemistry and charge, length of administration, and dose. • Particles with size between 50 nm and 3 μm possess higher uptake. • Diseases, such as diabetes, may lead to higher absorption of nanoparticles in the gastrointestinal tract. • Nanoparticles can translocate to blood, spleen, liver, bone marrow, lymph nodes, kidneys, lungs and brain, stomach and small intestine. • Particle below 1 μm were taken up by peyer patches.
	Skin Vs Nanoparticle • Nanoparticle penetration through the skin typically occurs at hair follicles, sweat gland, sebaceous gland and broken size. • Nanoparticles with diameter between 750 nm and 2 microns selectively penetrate the skin at hair follicles. • Translocation of nanoparticles through skin into the lymphatic system. • The proposed cut off size for particle for skin penetration is 1000 nm.

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Injectables Vs Nanoparticle

- The translocation of nanoparticles following injection depends on the site of injection: Intravenously injected nanoparticles quickly spread throughout the circulatory system.
- Nanoparticles injected intravenously are retained longer in the body than ingested ones.
- Intravenously injected nanoparticles size ranging 10-240 nm show localization in different organs, such as liver, spleen, small intestine, brain, lungs.

Fig. 1.12 Nanoparticles Vs biological system.

Active targeting: Active targeting employs specific modification of nanocarrier with active agents having selective affinity for recognizing and interacting with a specific cell, tissue or organ in the body. Active targeting offers more selectivity and specificity in drug targeting than passive targeting Table 1.5.

Table 1.5 Comparison between passive and active targeting (Khanna 2012).

Parameters	Passive targeting	Active targeting
Requirement to accessed the target	Utilizes the special deviated conditions prevailing in the diseased portion of the body	Depends on the species that is over expressed during disease
Selectivity and specificity	Less selective	High selective
Application	Restricted in use	Very versatile
Performance	More likely to produce side effects	Less likely to induce side effects

Coupling of drug carrier nanosystems to ligands allows drug molecules by means of receptor targeted ligands. This modification is usually on the surface of the particle, introducing ligands, which facilitates the homing, binding and internalization of the formulation to the targeted cells. Active targeting aims at improving the therapeutic availability of drugs to target cells within the pathological site and to minimize undesired side effects to non-target cells within the pathological tissue (Jaspreet et al., 2005). Target cells located in the circulation can be expected to be readily accessible. Target cells outside the vasculature are more difficult to reach and nanomaterials need to extravasate before being able to bind. Most research has focused on the specific targeting of cells expressing disease-associated biomarkers, as in the case of cancer. Various moieties have been examined as targeting agents, including monoclonal antibody, vitamins, carbohydrates, aptamers, peptides

(e.g., Arg-Gly-Asp) and proteins (e.g., lectins, and transferrin) Fig. 1.13 (Svenson and Prud'homme 2012). However, active agents, such as ligands for the receptors and antibodies to the surface proteins have been used extensively to target specific cells, the majority of research today has focused on antibodies.

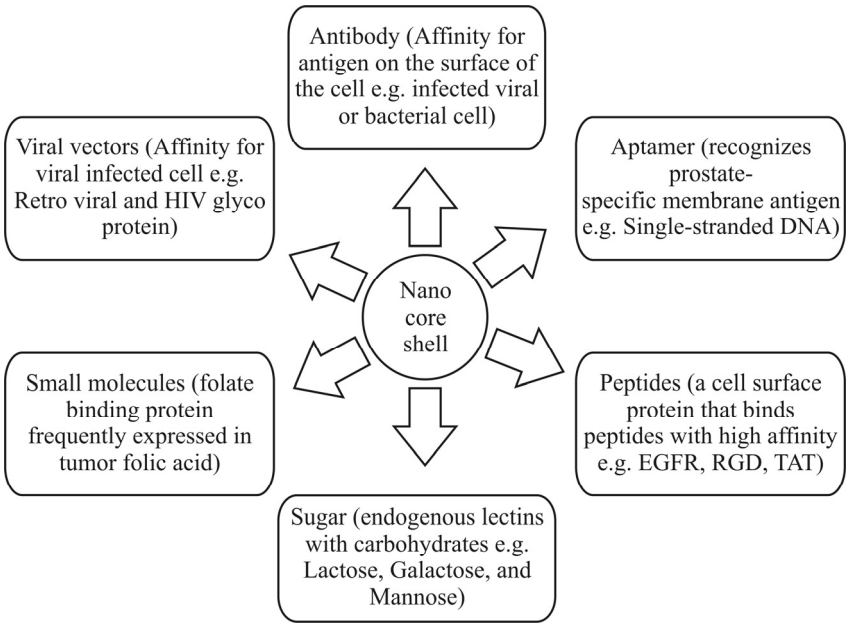

Fig. 1.13 Different ligands used in active targeting.

- ***Antibodies:*** An immunoglobulin generally found in the blood that detects and destroys invaders, like bacteria. Antibodies against specific antigen can be conjugated either directly to the drugs (immuno therapeutics) or to the nano-sized delivery systems (e.g., immuno liposomes, immuno micelles, etc.) to achieve drug targeting. Some of the receptor likes growth factor, epidermal growth factor etc., is over expressed in cancer, mAbs against this receptor is a viable technique used for drug targeting. However antibodies due to their large molecular weight exhibit poor permeability thereby reduces the targeting efficacy of the nanostructure carrier. Antibody fragments F_{ab} due to its small molecular weight shows excellent permeability increases the targeting efficacy of the carrier system (Jaspreet et al., 2005). Compared to mAbs, antibody fragments have demonstrated higher potential for targeting as they are smaller in size and lack the

complement activation region of mAbs, while retaining the antigen binding specificity (Svenson and Prud'homme 2012).

- ***Aptamer:*** Aptamers are oligonucleotides or peptide molecules that bind to a specific target molecule. Nucleic acid aptamer due to their low immunogenicity and small size offer enough flexibility to be used as ligand. Aptamers can recognize a wide variety of molecules likes proteins, phospholipids, sugars, and nucleic acids with high affinity and specificity. Most notably, the FDA approved an aptamer against VEGF 165, known as Pegaptanib, for the treatment of age-related macular degeneration (Svenson and Prud'homme 2012).
- ***Peptides:*** Peptides are natural or synthetic, short polymer chain compound, containing two or more amino acids linked by the carboxyl group of one amino acid to the amino group of another. Peptide ligands have shown significant targeting potential because of their small size, high stability, and relative ease of large-scale synthesis with excellent quality control. Peptide-conjugated nanoparticles have been widely used for targeting cancer cells and tumor vasculature e.g., the peptide SP5-52 can recognize tumor neovasculature (Svenson and Prud'homme 2012).
- ***Sugars:*** Chemically sugars are polyhydroxyl aldehyde or ketones. Monosaccharide and oligosaccharides due to their low molecular weight and simple chemical configuration finds useful in drug targeting. Presence of endogenous lectins on the epithelial cells of different gut regions further potentiates the utility of sugar as targeting moiety (Ohannesian et al., 1995). Specific sugar molecules likes lactose, galactose, and mannose can recognize lectins that are over expressed on the surface of numerous cancer cells (Rodríguez et al., 2006). Galactose shows greater interaction in proximal regions of gut while fucose binds to the distal positions (Jaspreet et al., 2005).
- ***Small molecules:*** Small molecules like folic acid, intrinsic factor etc., show considerable attention as potential targeting ligands due to their low molecular weights, low production costs, and easy conjugation with nanoparticles. Folic acid can be useful as a targeting moiety for the folate receptor-α, over expressed in several human tumors, including ovarian, lung, brain, head and neck, and breast tumors (Ross et al., 1994).
- ***Viral vectors:*** Viral vectors are protein, peptide, glycoprotein or lipoprotein commonly used by molecular biologists to deliver

genetic material into cells. Viral vectors are commonly used to target viral infected cells particularly in case of HIV, HSV etc. (Jaspreet et al., 2005).

Hydrophobicity

The size of nanoparticles is not the only factor influencing cellular uptake; their surface hydrophobicity determines the amount of adsorbed blood components, mainly proteins (opsonins). This in turn influences the *in-vivo* fate of nanoparticles. Binding of these opsonins onto the surface of nanoparticles called opsonisation acts as a bridge between nanoparticles and phagocytes (Mohanraj and Chen 2006). Hence, to increase the efficiency of drug targeting by nanoparticles, it is necessary to minimize the opsonisation and to prolong the circulation of nanoparticles *in-vivo*. This can be achieved by surface coating of nanoparticles with hydrophilic polymers/surfactants or making a formulation of nanoparticles with biodegradable copolymers with hydrophilic segments such as polyethylene glycol (PEG), polyacrylamide (PA), Poly vinyl alcohol (PVA) and hydroxyl propyl methyl cellulose (HPMC) (Muller and Wallis 1993).

Drug Loading

Ideal nanoparticulate system should have a high drug pay load thereby reduce the quantity of matrix materials for administration. Drug loading can be done by two methods: incorporating at the time of nanoparticles production (passive method) or absorbing the drug after formation of nanoparticles (Active technique) (Govender et al., 1999 and Mohanraj and Chen 2006).

Crystallinity

Crystallinity in nanoparticle formulation is an important consideration during the development process that greatly affects the solubility and dissolution characteristics of the drug. In general, amorphous forms of the particles are more soluble than crystalline form (Izumikawa et al., 1991).

Nanocarriers in Drug Delivery Systems

Nanocarriers are nanoscale delivery vehicle range from 1-200 nm with optimised physiochemical and biological properties. Nanoparticles due to their unique surface properties are selectively taken up by cells than the macro molecules, so they can be successfully used as delivery vehicle for drug delivery. They consist of nanostructured materials from natural or

synthetic source and can be used as drug carriers in which the active ingredient is dissolved, entrapped, encapsulated, adsorbed or chemically attached. Nanocarriers due to their submicron size shows a promising alternative for non-invasive routes of administration such as pulmonary, vaginal, oral, nasal and ocular routes.

Various nanocarriers commonly used in the biomedical application include liposome, solid lipid nanoparticles, dendrimer, polymeric nanoparticles etc. (Jaspreet et al., 2005). Table 1.6 summarize various Nanocarriers describing their advantages and disadvantages in drug delivery

Table 1.6 Nanocarriers used in drug delivery.

Advantages	Disadvantages	Application
Liposome		
• Versatility in surface modification • Non-immunogenic • Controlled drug delivery • Biodegradable and non-toxic • Carry both water and oil soluble drugs • Improve the stability of proteins	• Very limited loading capacity • Low carrier stability & expensive • Rapid clearance from circulation by RES • Leakage and fusion of encapsulated drug / molecules. • Phospholipid undergoes oxidation and hydrolysis like reaction	• Controlled and targeted drug delivery • Improve drug solubility, permeability and bioavailability • Surface modified liposome permit target drug delivery • Bio-sensing
Dendrimer		
• Utilise to improve drug solubility • Dendrimers have nanoscopic particle size (1-100 nm) makes them less susceptible for RES uptake • Outer surface of dendrimers has multiple functional groups permit surface modification • Low immunogenicity	• Toxic • Expensive • Difficulty in synthesis • Poor EPR not suitable for extended or controlled drug delivery	• Delivery of nucleic acids, vaccine and drug • Controlled drug release by film forming agent • Diagnostic reagents • Biosensor systems (systems containing dyes, iron oxide magnetic particle or imaging probe) • Dendrimers can also be used as a solubilizing agent

Table 1.6 ***Contd…***

Advantages	Disadvantages	Application
Polymeric nanoparticle		
• Suitable for parenteral drug delivery • Ease for surface modification • Surface properties helps to improve solubility • Controlled drug delivery • Biodegradable and biocompatible • Allow both passive and active targeting	• Difficulty to scale-up • Difficulty to correlate *in-vitro in-vivo* correlation • Difficulty to develop stable oral formulation • Increased cellular and molecular toxicity	• Chemical modification of drug • Targeted drug delivery • Enhanced solubility, permeability and bioavailability • Improve the sensitivity of bio-sensing technique
Solid lipid nanoparticle		
• Site specific delivery of drugs, enhanced drug penetration into the skin via dermal application • Possibility of scaling up • Protection of chemically labile agents from degradation in the gut and sensitive molecules from outer environment • SLNs have better stability compared to liposomes • Enhance the bioavailability of entrapped bioactive and chemical production of labile incorporated compound.	• Poor drug loading capacity, • Drug expulsion after polymeric transition during storage • Relatively high water content of the dispersions	• Controlled and targeted drug delivery • Enhanced solubility, permeability and bioavailability • Improve permeability against through rigid anatomical barrier
Carbon nanotubes		
• Unique mechanical properties offer *in vivo* stability • Extremely large aspect ratio, offers template for development of multimodal devices	• Non-biodegradable • Large available surface area for protein opsonisation • As-produced material insoluble in most solvents; need to surface treatment to confer aqueous solubility	• Intracellular targeting of protein, gene and DNA delivery • Targeting of anti cancer drugs • Treatment modality for intracellular pathogen

Table 1.6 ***Contd…***

Advantages	Disadvantages	Application
• Capacity to readily cross biological barriers • Unique electrical and semiconducting properties; constitute advanced components for *in vivo* devices • Hollow, fibrous, light structure with different flow dynamics properties; advantageous *in vivo* transport kinetics • Mass production – low cost; attractive for drug development	• Bundling; large structures with less than optimum biological behaviour • Healthy tissue tolerance and accumulation; unknown parameters that require toxicological profiling of material.	• Carbon nanotubes (CNTs) emerged as novel electronic and optical bio-sensing materials • immunodiagnostic to investigate the intracellular pathway of protein and peptide
Quantum dots		
• They have greater photostability than traditional dyes improving the signal to noise ratio • Very narrow emissions enable multiplexing assays • They are highly photo-resistant with significantly longer fluorescence • Fluorescence yield of QDs is higher than the unconjugated QDs	• The size of QD complexes limits tissue penetration • QD complexes, including their capping materials may be immunogenic • The heavy metals contained in the core, and the materials used for capping may be toxic • QD complexes precludes renal excretion	• *In-vivo* imaging • Imaging probe for both fluorescence microscopy and MRI • QD allow long-term imaging in the cellular environment with high photo stability • As a result of their superior optical properties QD can be adopted for screening candidate drug as alternative to organic fluorophore.
Inorganic nanoparticles		
• Due to their small size allows for efficient drug accumulation at the target sites in the body. • Retention of drug at the active site and longer clearance time • Increased therapeutic efficacy • Magnetic and quantum properties allows its uses in bio-imaging	• Discontinuation of therapy is not possible • Cytotoxicity, Pulmonary inflammation, pulmonary carcinogenicity and alveolar inflammation. • The disturbance of autonomic imbalance by nanoparticles having direct effect on heart and vascular function	• Bio-compatible inorganic materials serve as a base in tissue engineering • Carriers of antigens & vaccines • Controlled & targeted drug delivery • Carriers of diagnostic agent • Carriers of MRI contrast

Table 1.6 *Contd…*

Advantages	Disadvantages	Application
Nanofiber		
• Easy to scale-up • Low density, large surface area to mass, high pore volume makes it suitable floating drug delivery system • Surface topography provide better interaction with biological tissue thus emerged as an excellent approach for tissue engineering • Simple to fabricate • High surface to volume ratio allows easy functionalization	• The problem with nanofiber is that they are not easily obtainable • Their structure cannot be controlled, and results cannot be repeated • There are currently few efficient and economic processes for making high quality nanofibers at a commercial scale	• Tissue engineering • By virtue of their high surface area and porosity, they have the potential to provide enhanced cell adhesion • Nanofibrous is extensively explored as biological scaffold materials for bone, cartilage, ligament, and skeletal muscle control drug delivery • Improve the stability of encapsulated biological • Nanofibers can improve performance of fibrous filter media
Lipoplexes		
• Suitable as Injectable • Versatility in monomer species • Easy for surface modification • Applicable to protein and gene delivery • Enhancing oral adsorption • Relatively easy preparation methods	• Frequently low drug solubility in lipid • Difficulty in lipid selection • Susceptible to oxidation • Poor pay load for hydrophilic drugs	• Least toxic and more stable Colloidal carrier systems as alternative materials to polymers

Nanocarriers are useful in the drug delivery process because they can deliver drugs to site-specific targets, allowing drugs to be delivered in certain organs or cells but not in others. Site-specificity is a major therapeutic benefit since it prevents drugs from being delivered to the wrong places. One potential problem with nanocarriers is unwanted toxicity from the type of nanomaterial being used. Therefore the selection of nanocarrier is utmost important to achieve the desired therapeutic outcomes. Nanocarriers used for medical applications should have the following properties (Bagul et al., 2012).

- Prolonged circulation in the blood and ability to accumulate at the target site via EPR effect
- Biodegradable, biocompatible, non-immunogenic, and nontoxic

- High drug pay load and preferably particle size is < 100 nm
- The ability of specifically recognize and bind target tissues or cells via the surface attached specific ligand (specific antibody, sugar, aptamer, peptide)
- The ability to respond to local stimuli characteristic of the pathological site by for example releasing an entrapped drug or specifically acting on cellular membranes under the abnormal pH or temperature in disease sites
- The ability to penetrate inside cell for efficient targeting of intracellular targets
- They must have a shelf life long enough to allow storage and distribution.

Liposomes: Liposomes are spherical vesicles composed of amphiphilic phospholipids and cholesterol, which self-associate into bilayers to encapsulate an aqueous interior. The amphiphilic character of the liposomes, with the hydrophobic bilayer and the hydrophilic inner core, enables solubilisation or encapsulation of both hydrophobic and hydrophilic drugs. Along with their good solubilisation power, a relatively easy preparation and a rich selection of physicochemical properties have made liposomes attractive drug carrier systems. Liposome can be prepared by different techniques such as detergent depletion, ethanol injection, reverse-phase evaporation and emulsification (Mathiowitz 1999). Liposomes vary greatly in size, most are 500 nm or less. Depending upon their size and number of bilayers, liposomes can be classified into three categories: multilamellar vesicles, large unilamellar vesicles, and small unilamellar vesicles. Liposomes can be classified in terms of composition and mechanism of intracellular delivery into five types: conventional liposomes, pH-sensitive liposomes, cationic liposomes, immunoliposomes, and long-circulating liposomes Fig. 1.14. Liposomes because of the following properties represent ideal drug delivery systems

- Liposomes are biodegradable and biocompatible.
- Liposomes can entrap both hydrophilic and hydrophobic drugs.
- Liposome can protect the drug from the adverse external conditions.
- Liposomes provide a unique opportunity to deliver pharmaceuticals into cells or even inside individual cellular compartments.

- Liposome due to their unique surface properties provides ample opportunities for surface functionalization.

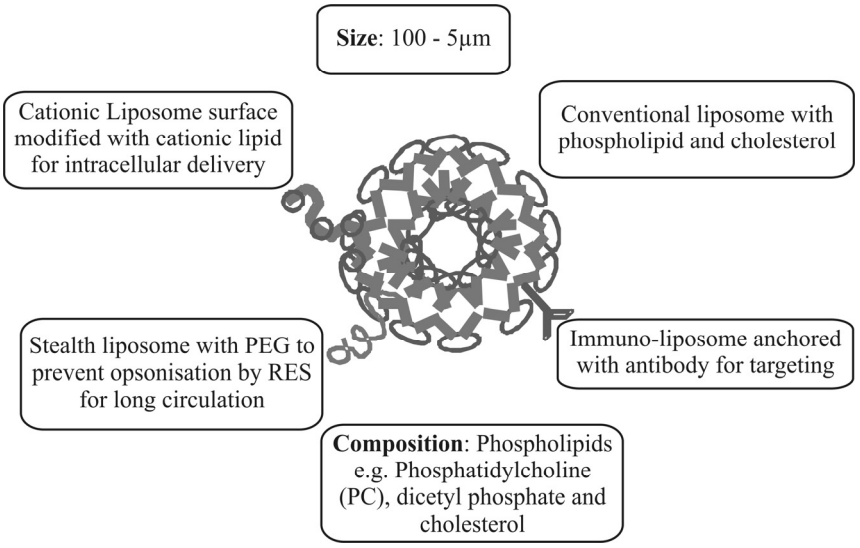

Fig. 1.14 Composition, size and types of liposome.

Dendrimers: Dendrimers, a unique class of polymers, are highly branched macromolecules whose size and shape can be precisely controlled Fig. 1.15. Dendrimers are fabricated from monomers using either convergent or divergent step growth polymerization (Amir and Faraji 2009). The void area within a dendrimers, the extent of its branching and its unique surface properties offer great potential for drug delivery. Dendrimers used in drug delivery and imaging are usually 10 to 100 nm in diameter with multiple functional groups on their surface,

Drug molecules can be incorporated into dendrimers via either complexation or encapsulation. Dendrimers used in drug delivery typically incorporate one or more of the following polymers: polyamidoamine (PAMAM), melamine, poly (L-glutamic acid) (PG), polyethyleneimine (PEI), poly (propyleneimine), and poly (ethylene glycol) (PEG), Chitin. Polycationic dendrimers have demonstrated great potential in the delivery of anticancer therapeutic agents. The polycationic surface of dendrimers is however limited its application in drug delivery, due to their toxic effect on cell membranes.

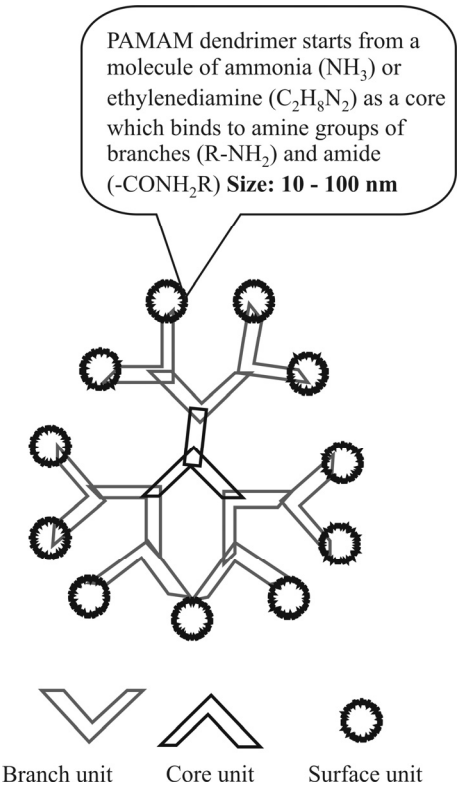

Fig. 1.15 Different structural unit of dendrimers.

Polymeric nanoparticles: Polymeric nanoparticles (PNPs) are structures with a diameter ranging from 10 to 100 nm Fig. 1.16. Polymeric nanoparticles provide significant flexibility in design because polymers can be biodegradable or non biodegradable, and can be made from synthetic or natural sources (Amir and Faraji 2009). Polymers which are commonly used for nanoparticle formation include poly lactic acid (PLA), dextran, and chitosan. Degradation and drug release kinetics can be precisely controlled by the physicochemical

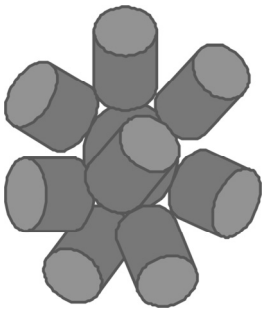

Fig. 1.16 Polymeric nanoparticles.

Size: 10-100 nm, Composition: Natural (Albumin, Gelatin, Chitosan) Synthetic (Polyester, Polyiminocarbonate, Poly-phosphazine)

properties of the polymer, such as molecular weight, dispersity index, hydrophobicity, and crystallinity. The nanoparticle surface is usually sterically stabilized by grafting, conjugating, or adsorbing hydrophilic polymers such as PEG to its surface, which can also reduce hepatic uptake and improve circulation half-life.

Polymeric micelles: Polymeric micelles are nanostructure, spherical colloidal particles with a hydrophobic core and a hydrophilic exterior Fig. 1.17. Their main utility is in the preparation of pharmaceutical formulations, notably agents that are regularly soluble in water (Aliabadi et al., 2008). Drugs or contrast agents may be entrapped within the hydrophobic core or linked covalently to the surface of micelles. The small size (<100 nm) allows micelles for efficient accumulation in pathological tissues with permeabilized vasculature via the enhanced permeability and retention (EPR) effect. Polymeric micelles may circulate for prolonged periods in the blood, evading host defences. With their property of continued stability in the blood, polymeric micelles can be used to gradually release drugs and facilitate *in vivo* imaging. To support prolonged systemic circulation, shells of polymeric micelles are designed to be thermodynamically stable and biocompatible.

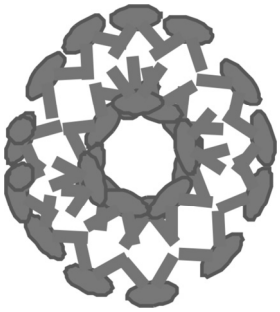

Fig. 1.17 Polymeric micelle: Organized auto-assembly formed in a liquid.

Composition: Amphiphilic macromolecules, and hydrophobic blocks. Size: 10-100 nm

Lipid nanoparticles: Solid lipid nanoparticles are lipid-based submicron colloidal carriers. In general, they are more stable than liposomes in biological systems due to their relatively rigid core consisting of hydrophobic lipids that are solid at room and body temperatures, surrounded by a monolayer of phospholipids (Amir and Faraji 2009). These aggregates are further stabilized by the inclusion of high levels of surfactants Fig. 1.18. Because of their ease of biodegradation, they are less toxic than polymer or ceramic nanoparticles. They have controllable pharmaco-kinetic parameters and can be engineered with three types of hydrophobic core designs: a homogenous matrix, a drug-enriched shell, or a drug-enriched core. NLC (Nanostructured lipid carrier) and LDC (Lipid drug conjugates) are modifications of lipid based nanoparticles

that have been developed to overcome the limitations of conventional SLN (Jain et al., 2010 and Amir et al., 2009). NLC are produced by mixing solid lipids with liquid lipids, which leads to special nanostructure with increased drug payload and prevented drug expulsion. LDC was developed in order to expand applicability of liquid based carriers to hydrophilic drug molecules. These insoluble drug-lipid conjugates can be prepared by salt formation (Amir et al., 2009).

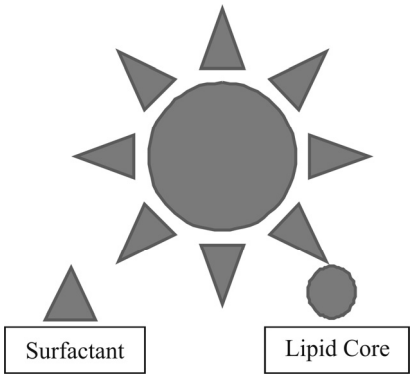

Fig. 1.18 Solid lipid nanoparticles.

Size: 100 nm-5 µm, Composition: Compritol, Carnauba wax, Beeswax, Cetyl alcohol, Emulsifying wax, cholesterol and Non-ionic surfactant include Glyceryl Monostearate Witepsol Egg lecithin8 Tricaprin

Nanocrystals: Nanocrystals are aggregates comprising several thousands of molecules that combine into specific lattice conformation to form a nanocluster (Amir and Faraji 2009) Fig. 1.19. Typical sizes of these aggregates are between 10-100 nm and they exhibit physicochemical properties linked between atoms and bulk matter.

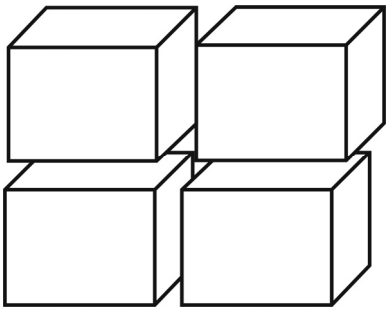

Fig. 1.19 Nanocrystal.

Size: < 100 nm, Composition: Nanocrystals is a crystalline nanoparticle consist of molecular precursors, like manganese oxide, Cadmium telluride and surfactants (Tween)

By controlling the size and surface area of nanocluster, properties such as surface charge, crystalline structure and melting temperature can be altered (Amir and Faraji 2009). The nanocrystals must be stabilised to prevent the formation of larger aggregates. Nanocrystals are produced by ultrasonication, nanosuspension, high speed stirring, wet milling, high pressure homogenisation, nanocrystallisation and spray drying technique. Nanocrystallisation approaches being used to improve the solubility and bioavailability of poorly soluble drugs.

Inorganic nanoparticles: Inorganic nanoparticles are typically composed of inorganic compounds such as silica or alumina Fig. 1.20. One of the advantages of these particles is that their preparation is very simple (Amir and Faraji 2009). They are unaffected by changes in pH or temperature. It is possible to manipulate many features of these nanoparticles, including size, shape, porosity, inertness etc., and they can easily be modified to attach different biomolecules. Their typical size is around 50 nm. Ceramic nanoparticles have been used to encapsulate hydrophobic drug molecules, acid labile enzyme and increase the transfection efficiency of DNA. Inorganic materials have been extensively studied for imaging using magnetic resonance and high-resolution superconducting quantum interference devices. However inorganic particles may not provide added advantages over other nanocarriers for systemic targeting of cancer cells because they are not biodegradable, have low payloads, and have no controlled release properties.

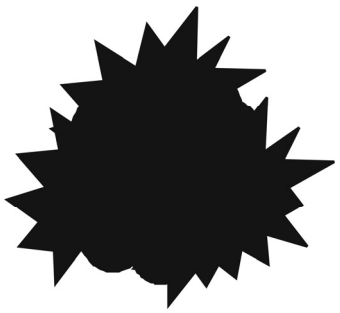

Fig. 1.20 Inorganic nanoparticle.

Inorganic nanoparticles often exhibit novel physical properties as their size approaches nanometer scale dimensions, Composition: Li_2, MoO_4, NaCl, $NaBiO_3$, $MgCO_3$, $MgTiO_3$, $AlPO_4$ and TIO_2, Size: 10-100 nm

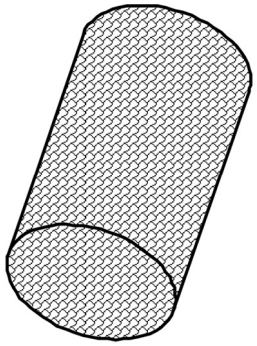

Fig. 1.21 Carbon nanotubes (CNTs).

Carbon nanotubes are allotropes of carbon with a cylindrical nanostructure. Types: Single-wall Nanotubes (SWNT), Multi-wall Nanotubes (MWNT) and Double-wall Nanotubes (DWNT). Size: 1-50 nm

Carbon nanotubes: Carbon nanotubes (CNTs) are allotropes of carbon with a cylindrical nanostructure Fig. 1.21. Nanotubes have been constructed with diameter of 1-50 nm. These carbon molecules have unusual properties (Amir and Faraji 2009). Carbon nanotube technology has shown to have the potential to alter drug delivery and biosensing methods, and thus, carbon nanotubes have recently garnered

interest in the field of medicine. Functionalization of single-walled nanotubes (SWNTs) has proven to enhance solubility of poorly aqueous soluble drugs and allow for efficient tumor targeting/drug delivery. It prevents SWNTs from being cytotoxic and altering the function of immune cells. In concern to the pharmaceutical need CNT dispersion in aqueous medium should also be stable and uniform. Many approaches have been applied to overcome the dispersion problems, including use of solvent dispersion technique, functionalization of sidewalls, application of surfactant and use of biomolecular dispersion in complete dispersion. CNTs show no sign of aggregation and phase separation for several months.

***Quantum dots*:** Quantum dots (QDs) are colloidal semiconductor nanocrystals ranging from 2 to 10 nm in diameter Fig. 1.22. QDs can be synthesized from various types of semiconductor materials via colloidal synthesis or electro-chemistry (Amir and Faraji 2009). The most commonly used QDs are cadmium selenide (CdSe), cadmium telluride (CdTe), indium phosphide (InP), and indium arsenide (InAs). In bio-imaging these particles serve as contrast agents, providing much greater resolution than existing fluores-cent dyes. These particles can absorb white light and re-emit it within nanoseconds with different bulk band gap energies corresponding to different combinations of particles. QDs also provide enough surface area to attach therapeutic agents for simultaneous drug delivery and *in vivo* imaging.

Fig. 1.22 Quantum dots.

Quantum dot is a nanocrystal made of semiconductor materials that are small enough to display quantum mechanical properties Composition: Cadmium selenide, Lead selenide, Lead sulphide and Lead telluride
Size: 1-10 nm

Nanofibers: Nanofibers are defined as fibers with diameters less than 100 nanometers Fig. 1.23. Nanofibers are prepared through electro-spinning process. Electro-spinning has gained widespread interest as a potential polymer processing technique to produce ultrafine polymer fibers for drug delivery applications. It has been proven to be a relatively simple and versatile method for producing polymeric fibers with diameters ranging from tens of nanometers to microns. Electrospun fibers have a high surface to volume ratio which makes them promising

candidate in adsorption of less-soluble drugs. As a fibrous scaffold, nanofibers are able to entrap drugs with a high loading capacity and high encapsulation efficiency because of their low weight and inherent high surface to volume ratio (Chew et al., 2008). They have been designed as promising carriers for delivering anticancer drugs, especially in postoperative local chemotherapy via surgical implantation of the scaffold.

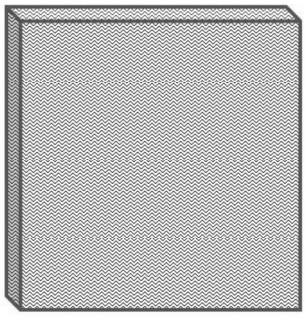

Fig. 1.23 Nanofiber.

Nanofibers are defined as fibres with diameters less than 100 nanometers Composition: Synthetic polymer (PCL, PVA, PEO) and Natural (Gelatine, Collagen, etc) Size: 2D Nanoscale products with at least one dimension less than <100 nm

***Lipopolyplexes*:** These are nano structured assemblies, which form spontaneously between nucleic acids and polycations polymers or cationic liposomes Fig. 1.24. Lipopolyplexes are used in transfection of drugs and DNA (Pelisek et al., 2006). Because of its cellular transfection capability, are useful in the delivery of anti-viral drugs and vaccine. The shape, size distribution, and transfection capability of these complexes depends on their composition and charge ratio of nucleic acid to that of cationic lipid/polymer. Commonly used polycations polymers used in gene transfer/therapy protocols include poly-L-lysine, linear- and branched-poly (ethylene-eimine), poly-amino esters, and cationic cyclodextrin. Table 1.7 enlists some examples of nano-scale carrier system used for the delivery of pharmaceuticals (Willie et al., 2008).

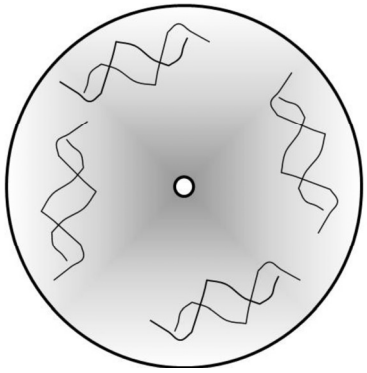

Fig. 1.24 Lipopolyplexes.

Lipopolyplexes is a complex of plasmids mixed with lipids that spontaneously forms a vesicle containing therapeutic genes of interest Composition: Cationic lipids and Nucleic acid Size: 100-1 µm

Table 1.7 Some nanoscale based carrier system used to deliver various pharmaceuticals.

Formulation	Drug	Route of Administration	Application
Liposome	Amphotericin B	IV infusion	Serious Fungal infections
Liposome	Cytosine arabinoside	IV infusion	Lymphomatous meningitis
Liposome	doxorubicin	IV infusion	Head and neck cancer
Dendrimers	Nadifloxacin	topical	Anti-bacterial
Dendrimer	Ketoprofen	Oral	Analgesic
Albumin bound nanoparticles	Paclitaxel	IV injection	Metastatic breast cancer
Gelatin nanoparticles	Rifampicin	IV injection	Tuberculosis
Solid lipid nanoparticle	Paclitaxel	IV Injection	Metastatic breast cancer
Solid lipid nanoparticle	Insulin	IV Injection	Diabetic
Nanocrystals	Sirolimus	IV injection	Immunosuppressant
Nanocrystals	Megaestrol acetate	Oral	Anorexia
Hydroxy apatite	Estradiol	Transdermal	Moderated to severe Vasomotors dysfunction

Nanotechnology in Tissue Engineering

Tissue and organ failure are serious and common medical conditions for which treatment options include organ transplantation, surgical repair, artificial prostheses, and drug therapy. Transplantation is frequently hindered by the lack of tissue donors. To address this challenge, tissue engineering approaches are being developed to generate functional three-dimensional (3D) tissues (Mohamed and Xing 2012).

Primary objectives of tissue engineering's are to recreate an appropriate simulated biological environment that supports the control and regulation of cell functions (Langer and Vacanti 1993). Therefore the structural scaffold should be biocompatible. The scaffold should also be porous with a high surface-volume ratio to allow for cell attachment and in-growth, as well as exchange of nutrients. Furthermore, the porous

nature of the scaffold will allow for angiogenesis upon implantation in a defect site (for vascularized tissues). Also, because the scaffold acts as a temporary support for the cells to adhere and proliferate, it should mimic native extra cellular matrix (ECM) both architecturally and functionally. To be able to mimic natural environment it is essential to build scaffold with nanoscale materials. In addition most of the scaffold that is used in tissue engineering lacks the function of active agent storage and release (Shrivastava 2008).

Nanoscale structure due to their surface properties dramatically increased surface area, surface roughness and surface area to volume ratios lead to superior physiochemical properties (i.e., mechanical, electrical properties, etc.). Therefore, nanomaterials with such excellent properties have been extensively investigated in a wide range of biomedical applications, in particular regenerative medicine and tissue engineering. Nanoscale products particularly nanofiber and nano-scaffolds are useful for mimicking native tissues because many biological structures, such as ECM fibres are in the range of tens of nanometers. Further nanotechnology can be used to modify the surface topography to regulate cell adhesion, morphology and migration.

In constructing an engineered tissue, the cells are initially isolated from the donor tissue and cultured under *in-vitro* conditions Fig. 1.25. A polymeric scaffold is designed by means of various processing methods such as solvent casting, phase separation, self-assembly, and electrospinning. The cells are then seeded and cultured on this scaffold (or cell carrier). In order to imitate the natural environment of cells, the above steps are performed in either static culture conditions or dynamic bioreactor systems.

In general, the synthesis of nano-structured materials can be generated by using one of two approaches. In one approach, nanomaterials are synthesized by miniaturizing existing materials with nanoscale resolution. These techniques include nanopatterning and electrospinning. In the other approach, molecular build-up, such as self-assembly and layer-by-layer deposition can be used to generate nanomaterials.

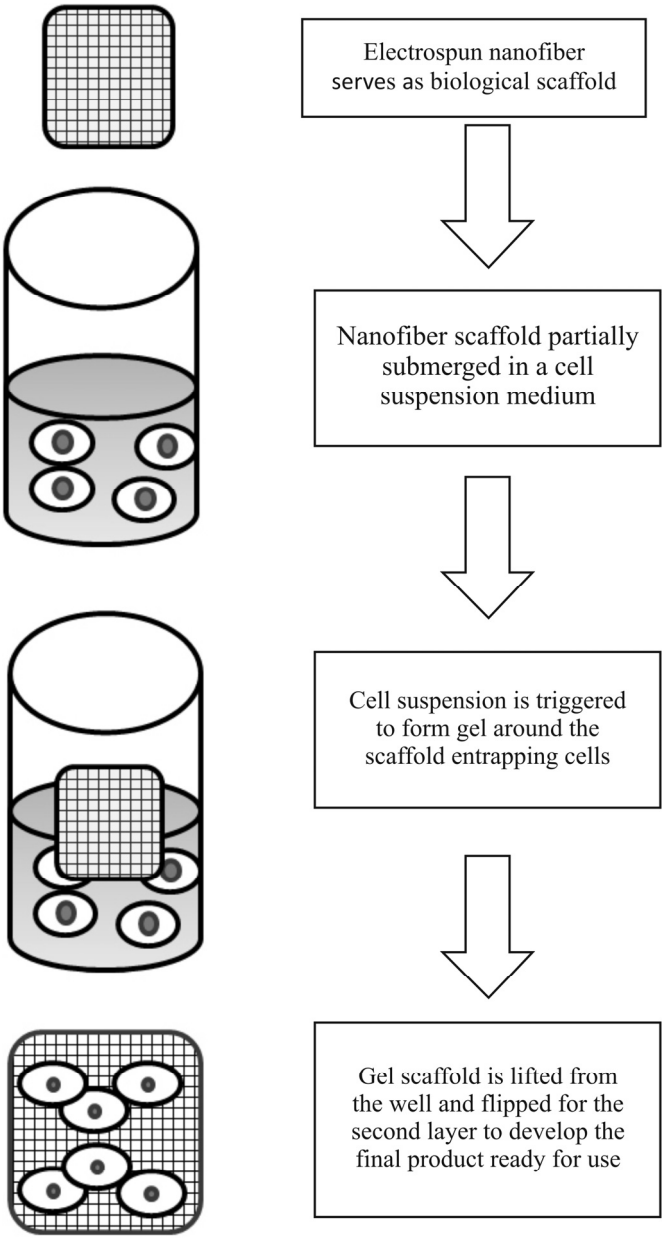

Fig. 1.25 Process illustrating the step involves in the preparation of scaffold.

Electrospun Nanofibers

Electrospun nanofibers are versatile tools to fabricate tissue engineering scaffolds with biomimetic mechanical, chemical and biological properties. One attractive feature of electrospinning is the simplicity and inexpensive nature of the setup; the typical electrospinning setup consists of a syringe pump, a high voltage source, and a collector Fig. 1.26. During the electro spinning process, a polymer solution is held at a needle tip by surface tension. The application of an electric field using the high-voltage source causes charge to be induced within the polymer, resulting in charge repulsion within the solution. This electrostatic force opposes the surface tension; eventually, the charge repulsion overcomes the surface tension, causing the initiation of a jet. As this jet travels, the solvent evaporates and an appropriate collector can be used to capture the polymer fiber. Typically, electrospun scaffolds are highly porous and can be engineered with controlled sizes, shapes, and fiber alignments. Electrospinning has been widely used for the fabrication of a variety of tissues (e.g., bone, cardiac muscle) using a number of synthetic and natural polymer such as PLGA, PLLA, collagen, and alginate (Yoshimoto et al., 2003).

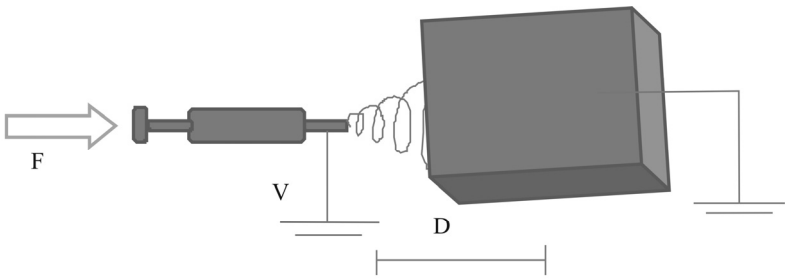

Fig. 1.26 Typical electrospinning setup., flow rate; F, distance between plate and needle; D, applied voltage; V.

Self-assembled Nanomaterials

Self-assembled nanostructures can be generated from different materials, such as peptide amphiphile (PA), hyaluronan, chitosan, and apatite/ amelogenin. Several methods, such as pH induction, layer-by-layer deposition, electrolytic deposition (ELD) and biomimetic coating, can be used to induce self-assembly. Molecular self-assembly of peptides and proteins can be used to make hydrogels for tissue engineering applications. Self-assembled peptides typically contain hydrophobic and hydrophilic regions that assemble into sheets or fibers, which can be

further assembled into hydrogels. Table 1.8 enlists the application of nanostructured biomaterials in tissue engineering (Panyam and Labhasetwer, 2003).

Table 1.8 Application of nanostructure based biomaterials in tissue engineering.

Biomaterials	Materials used	Application
Poly sialic acid	Hydrogel modified with adsorbed poly-L-Lysine or laminin or collagen	Cell adhesion properties
Silicon	Silicon wafer consisting of collagen and chondroitin sulphate	Deep and partial burn
Porcine collagen	Porcine collagen with growth factor	Partial and full thickness burn and venous and diabetic ulcer
Nylon	Nylon seeded with human dermal fibroblast	It serve as a temporary wound cover silicone membrane, used in partial and full thickness burn
Poly (β-hydroxyl butyrate)	Sheets impregnated with extracellular matrix molecules	Cell adhesion and proliferation
Poly caprolactone-nanofiber	Electrospinning and thermal fiber bonding	Mechanical strength
Poly caprolactone nanofiber	Composite nanofibers	Biological properties
Collagen	From calf skin, hydrogel cross-linked with YIGSR peptide based dendrimer from human placenta	Biological function (promote the growth of corneal epithelial cells)
Collagen	Composed of bovine collagen, fibroblast and keratinocyte	Provide living cell to the wound with potential for temporary stimulation.
Gelatin	hydrogel cross-linked with YIGSR peptide modified dendrimers	Mechanical strength
Alginate	Polysaccharide that have been extensively used for extracellular matrix	Extracellular matrix with good mechanical strength
Hyaluronic acid	From linear polysaccharide that is abundant in the synovial fluid and plays important role in wound healing, cell differentiation and cell mobility	Non-antigenic, non-inflammatory and generally non-tissue reactive.

Potential Risk of Nanotechnology in Biomedical Application

Nanomaterials of 1 to 100 nanometers size are being increasingly used for a variety of clinical and commercial purposes due to their large surface-to-volume ratio and unique physico-chemical, mechanical and electronic properties. While utilizing them for their beneficial functions it has some serious limitations

- Nanoparticles due to their small size penetrate various biological structures, disrupting their normal function
- Presently, nanotechnology is very expensive, difficult to scale-up and reproducible.
- There are no specific FDA directives to regulate pharmaceutical nanotechnology based products and related issues.
- Nanomaterials are developed for their unique properties in comparison to bulk materials. When nanoparticles are used for their unique reactive characteristics it may be expected some major health risk includes cytotoxicity, translocation to undesired cells, acute and chronic toxicity; some unknown, unpredictable and undefined safety issues, environmental impacts of nanomaterials and non-biocompatibility.
- Another is the solubility and persistence of nanomaterials. If they can't be broken down and digested or degraded, there is a danger of accumulation and damage organs.

Future Prospect

Nanomaterials promise to revolutionize medicine and are increasingly used in bio-imaging, drug delivery or tissue engineering applications. In near future, increasing use of nanobiotechnology by the pharmaceutical and biotechnology industries is anticipated. Nanotechnology will be applied at all stages of drug development - from formulations for optimal delivery to diagnostic applications in clinical trials. The most important pharmaceutical applications are in drug delivery. Apart from offering a solution to solubility problems, nanobiotechnology provides and intracellular delivery possibilities. A particularly effective application is as miniature devices such as nanorobots could carry out integrated diagnosis and therapy by refined and minimally invasive procedures, nanosurgery, as an alternative to crude surgery. The future of nanotechnology could improve the outlook for medical patients with serious illnesses or injuries. There is no doubt to presume that in next ten

years market will be flooded with nano-enabled delivery devices and materials regulate nanobiotechnology but full attention is needed to safety and toxicological issues.

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