# Nanoparticle Synthesis, Characterization and Its Applications in Cancer Drug Delivery- A Review

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***Abstract*—Cancers are a large family of diseases that involve abnormal cell growth with the potential to invade or spread to other parts of the body. Variety of treatments have been developed and optimized for reducing their adverse effects and as a step for their cure. Major research is being carried out throughout the world for improvising existing treatments as well as developing new ones. Over the years, chemotherapy remains to be the most effective treatment available. As the painful aspect of chemotherapy is well known, researchers are now focusing on alleviating these side effects. Targeted drug delivery is considered more beneficial for this kind of treatment. In this context, Nanofibers offer numerous advantages, most notably high surface area, small pore size and high pore volume. These characteristics led to their exploitation in many innovative applications including drug delivery, wound healing, scaffold preparation, fuel cells etc. This review paper briefly deals with the nanoparticle preparation techniques, characterization, cytotoxicity assay and drug release kinetics and some of its applications.**

***Keywords*— *chemotherapy, nanoparticles, drug delivery, drug release kinetics***

## I. INTRODUCTION

Cancer is the leading cause of death in economically developed countries and the second leading cause of death in developing countries [1].

Normal cells in the body undergo an orderly path of growth, division, and death. In the Case of cancer, cells tend to grow uncontrollably and ultimately become immortal. Unlike regular cells, cancer cells do not experience programmed cell death. This leads to the formation of abnormal mass of cells called tumors. When a tumor spreads to the other body parts, it is said to have metastasized and results in serious conditions (invading and destroying other healthy tissues) and hence difficult to treat.

Cancer treatment depends on the type of cancer, the stage of the cancer, age, health status and additional personal characteristics. Major treatments for cancer include surgery, radiation, chemotherapy, immunotherapy, hormone therapy, or gene therapy. Sometimes even combinations of these treatments are used. Chemotherapy is generally used to treat cancer that has spread or metastasized, as the drugs travel throughout the body inhibiting proliferation of cells. It differs from surgery or radiation in that; it is always used as a systemic treatment. Nowadays more than 100 varieties of anticancer drugs are used.

Conventional chemotherapeutic agents are distributed non-specifically in the body where they affect both normal and tumour cells, thereby limiting the dose achievable within the tumour and thus resulting in suboptimal treatment. Thus, targeted therapy has emerged as one of the major approaches to overcome the lack of specificity of conventional chemotherapeutic agents [1].

A well designed controlled drug delivery system can overcome some of the problems of conventional therapy and enhance the therapeutic efficacy of a given drug. To obtain maximum therapeutic efficacy, it becomes necessary to deliver the agent to the target tissue in the optimal amount in the right period of time there by causing little toxicity and minimal side effects [3]. Microencapsulation provides the means of converting liquids to solids, of altering colloidal and surface properties, of providing environmental protection and of

controlling the release characteristics or availability of coated materials.

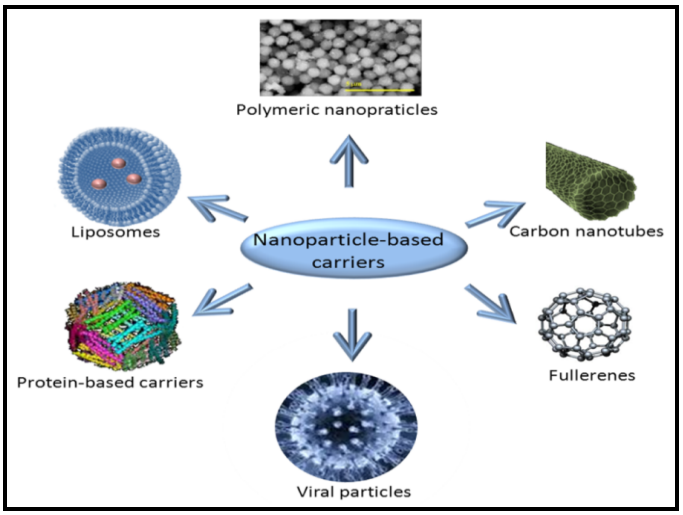


Fig. 1. Nanoparticle based carriers used for targeted drug / gene delivery [2]

The major goals of drug delivery include specific drug targeting, reduction in toxicity, safety, biocompatibility and faster development of medicine. Exploiting the use of drug delivery systems, targeted delivery can be achieved and some of the pharmacological properties of conventional drugs can also be improved. In general when a drug is associated with a carrier, the drug clearance decreases, half-life increases, volume of distribution decreases. In essence, the bio distribution and pharmacokinetics of the drug is improved [4].

The basic functional subunits of cells and tissues are defined at the nano scale; hence application of Nanobiology represents a new frontier in research [5]. Nanoparticles are solids and spherical structures ranging around 100 nm in size and prepared from natural or synthetic polymers [6]. Nanoparticle research is currently an area of intense scientific interest due to a wide variety of potential applications in biomedical, optical and electronic fields.

Nanoparticles used in drug delivery must gather some requirements like drug compatibility, biocompatibility, proper degradation kinetics and ease of processing. Their unique features such as high surface to volume ratio, their quantum properties and their ability to absorb and carry any other compound makes them attractive for drug delivery. The advantages of nanoparticles include ease of manipulation of particle size and surface characteristics, better drug utilization, site specific targeting and controlled release. Inspite of these advantages, the nanoparticles do possess some limitations. Due to their particle size, physical handling becomes difficult and particle-particle aggregation takes place resulting in limited drug loading and burst releases.

The engineered nanoparticles may be of biological origin like dextran, chitosan or may be chemical based like polymers, carbon and silica. Polymer-based nanoparticles are submicron-sized polymeric colloidal particles in which a therapeutic agent of interest can be fixed or encapsulated inside their polymeric matrix or adsorbed or conjugated on to the surface.

From a broader perspective in medicine, nanoparticles have been used in specific applications such as tissue engineered scaffolds and devices, site specific drug delivery systems, cancer therapy and clinical bio analytical diagnostics and therapeutics [6,7].

For targeted delivery, the persistence of nanoparticles in systemic circulation for a long period of time is important [8]. However, nanoparticles can be rapidly eliminated by macrophages in the Reticulo-Endothelial system located primarily in the liver and spleen, resulting in a short half-life in the circulation after intravenous administration [9].

The binding of opsonin proteins present in the blood serum to the injected nanoparticles, causes attachment of opsonised particles to macrophages and consequently leads to their internalization by phagocytosis [10]. Therefore coating materials such as poloxamines, poloxamers, and poly ethylene glycol have been used which protect the nanoparticles from being phagocytosed and eliminated [11].

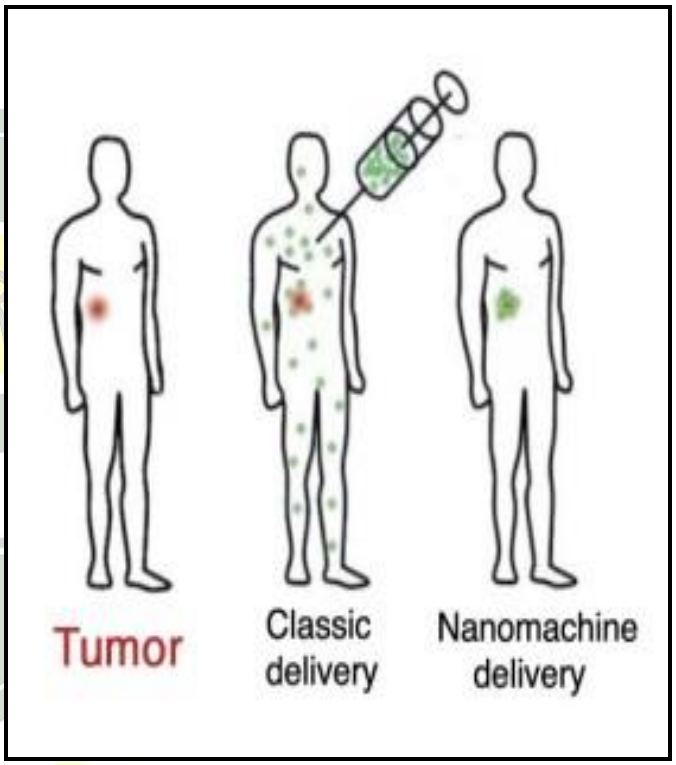


Fig. 2. Drug Delivery Mechanism [12]

## II. BRIEF REVIEW

### A. *Tumor Microenvironment*

Different strategies are used to target specific disease phenotypes so as to reduce side effect of the drugs. Therefore understanding the tumor environment can be potentially useful to design these strategies.

Tumor microenvironment consists of non-neoplastic cells, extracellular matrix, signalling molecules and vascular networks (for nutrient and exchange of waste) that support tumor growth and invasion by establishing a favourable niche for the growth of complex tissues and also foster drug resistance. Tumor blood vessels are generally characterized by abnormalities such as high proportion of proliferating endothelial cells, pericyte deficiency and abnormal basement membrane formation leading to enhanced permeability [13].

Nanoparticles/Nanocarriers can extravasate and accumulate inside the interstitial spaces and also lymphatic vessels are absent or non-functional which constitutes incomplete drainage, thus the nanoparticles are not removed and are preserved in the tumor. This phenomenon is termed as Enhanced Permeability and Retention Effect and has shown ten times increase in the retention of drugs packed in Nano carriers at the tumor site. The development of Multiple Drug Resistance is a main barrier to effective cancer chemotherapy, which can decrease the therapeutic efficiency leading to treatment failure. Tumor cells have highly ordered internal resistance which is typically caused by complex mechanisms. Mostly the cancer cells turn out to less efficient method of glycolysis for energy production, releasing large amount of lactic acid, causes acidification of the surrounding microenvironment, which promotes tumor invasion and suppresses immune surveillance. This phenomenon is known as Warburg Effect. This is utilized to design pH-sensitive nanoparticles that are stable at physiological pH but are degraded to release active drug in a pH less than physiological pH. Recently, it has also been shown that nanoparticles composed of weak polybases when exposed to a pH gradient tend to accumulate preferentially and increase in size (swell) when in low pH regions by a phenomenon termed as pH phoresis. This has also been exploited in facilitating enhanced delivery of drugs at the tumor site [13].

### *B. Methods of Preparation of Nanoparticles*

Depending on the preparation process, nanoparticles can vary structurally. The drug is either entrapped inside the core of a nanocapsule or entrapped in or adsorbed on the surface of the matrix nanosphere. There are various methods available for the synthesis of nanoparticles.

Electrospinning is a spinning technique, which uses electrostatic forces to produce fine fibers from polymer solutions or melts and the fibers thus produced have a thinner diameter and a larger surface area than those obtained from conventional spinning processes. Electrospun, nanofiber scaffolds have highly controllable three-dimensional structures, high porosity and high surface area-to-volume ratios, providing excellent surfaces for vascularisation, cell migration, attachment and proliferation. These fibers have enormous applications in nano-catalysis, tissue scaffolds, protective clothing, filtration, and optical Electronics [14]

*1) Experimental Method:* Electrospinning is conducted at room temperature with atmospheric conditions. The set up consists of three major components: a high voltage power supply, a spinneret (e.g., a pipette tip) and a grounded collecting plate (usually a metal screen or a plate). The polymer solution held by its surface tension at the end of a capillary tube is subjected to an electric field and an electric charge is induced on the liquid surface due to this electric field. These charged ions move in response to the applied electric field towards the electrode of opposite polarity, thereby transferring tensile forces to the polymer liquid. When the electric field applied reaches a critical value, the repulsive electrical forces overcome the surface tension [16]. After the initiation from the cone, the jet undergoes a chaotic motion or bending instability and is field directed towards the oppositively charged collector, which collects the charged fibers [17]. As the jet travels through the atmosphere, the solvent evaporates, leaving behind a dry fiber on the collecting device.

Currently, there are two standard electrospinning setups, vertical and horizontal. With the expansion of this technology, several research groups have developed more sophisticated systems that can fabricate more complex nanofibrous structures in a more controlled and efficient manner [18].

The preparation of nanoparticles based on the ionotropic gelation method is extremely mild and involves the mixing of two aqueous phases at room temperature. The polysaccharides are dissolved in water and this solution is added drop wise to solutions containing their counter ions. Due to the complexation between oppositively charged species, polysaccharides undergo ionic gelation and precipitate to form spherical particles [19]

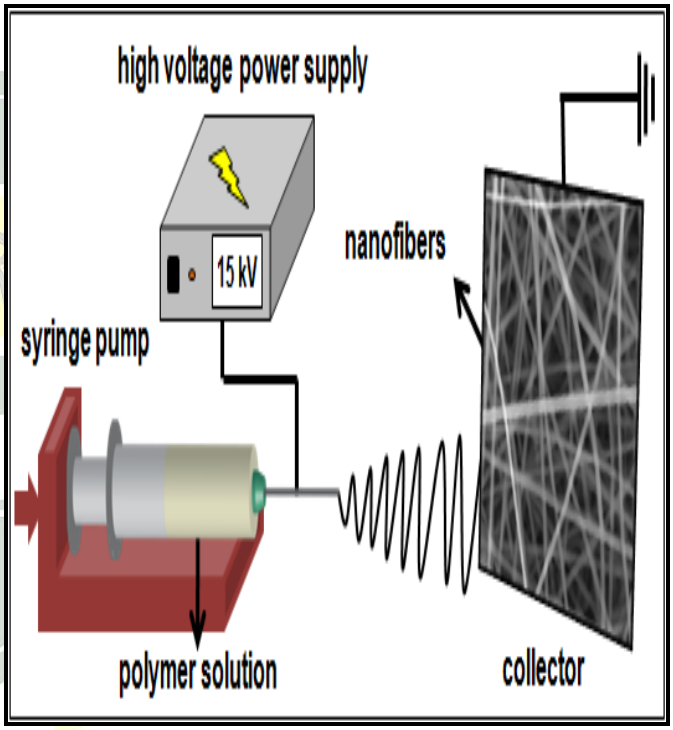


Fig. 3. Electrospinning experimental set up [15]

*a) Solvent Evaporation:* In Solvent Evaporation Method the polymer is dissolved in an organic solvent. The mixture of the solvent and drug is then emulsified in an aqueous solution containing a surfactant or emulsifying agent to form oil in water emulsion. After formation of a stable emulsion the organic solvent is evaporated either by reducing pressure or by continuous stirring [20].

The most generally used method for Poly lactic- co-glycolic acid (PLGA) nanoparticle formation is the Single Or Double Emulsion Solvent Evaporation, where single emulsion process involves oil-in-water emulsion perfect for water insoluble drugs (steroids) and double emulsion process involves water-in-oil-in-water which is best suited for water soluble drugs (peptides, proteins, vaccines) [21].

*b) Nano precipitation:* The Nano precipitation technique also known as solvent displacement method is usually used for

hydrophobic drug entrapment but also has been suited for hydrophilic drugs.

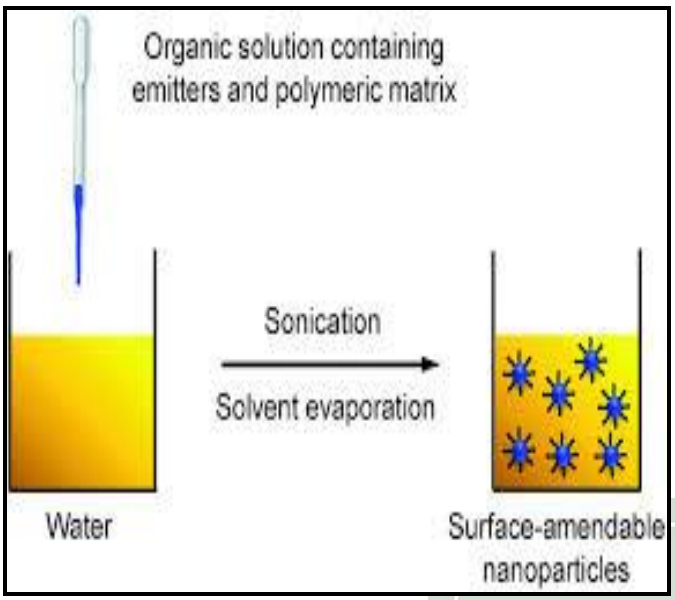


Fig. 4. Schematic illustration of the preparation of polymer-encapsulated organic nanoparticles from nanoprecipitation [22]

The polymers and drugs are dissolved in a polar solvent and the solution is poured in controlled manner into an aqueous solution with surfactant. Nanoparticles are formed immediately by rapid solvent diffusion [23]

*c) Reverse Salting Out:*The emulsification Reverse Salting Out method involves the addition of polymer and drug solution to a water miscible solvent and to an aqueous solution containing the salting out agent (magnesium chloride, calcium chloride etc.) under forceful mechanical stirring. As this oil-in-water emulsion is diluted with a plenty of water it induces the creation of Nanoparticles [24].

*d) Solvent Diffusion Method:* The emulsification Solvent Diffusion Method involves the solvent and water saturated at room temperature, the solvent containing the polymer and drug is emulsified in an aqueous surfactant solution and subsequently added under stirring to the emulsion system. The nanoparticles are formed due to the phase transformation and outward diffusion of the solvent leading to Nano precipitation [25].

*e) Spray Drying Method:* Spray Drying Method is one of the methods for preparation of nanoparticles, in which the drug is dissolved in chitosan-acetic acid solution and then a suitable cross linking agent is added. This solution is then atomized in a stream of hot air, which leads to formation of small droplets, from which the solvent evaporates leading to formation of free flowing powders [20].

### *C. Characterization of Nanoparticles*

Characterization of nanoparticles is necessary for a thorough understanding of their properties before developing them further for pharmaceutical functions [6].

Nanoparticles are generally characterized by their size, morphology and surface charge, using such advanced microscopic techniques as scanning electron microscopy (SEM), transmission electron microscopy (TEM) and atomic force microscopy (AFM).

The particle size of the Nanoparticle is significant, not only in determining the release profile and degradation patterns but also in determining the efficacy of the therapeutic agent in terms of tissue penetration and cellular uptake [26]. The particle size and size distribution is measured by Dynamic Light Scattering [9], Photon Correlation Spectroscopy [27], scanning electron microscopy [28], transmission electron microscopy [29] and atomic force microscopy [30].

The zeta potential can influence nanoparticle constancy and mucoadhesion as well as intracellular trafficking of particles as a function of pH. The values may be positive or negative based on the nature of polymer or material used for surface modification [6]. The zeta potential of the nanoparticles is determined by laser Doppler anemometry. The recent literature has reported that a higher absolute value of zeta potential indicates a more stable suspension and a lower value indicates colloid instability which could lead to nanoparticles aggregation.

The surface morphology is investigated by Field Emission Scanning Electron Microscopy. The molecular weight of the polymer influences the nanoparticle size, encapsulation efficiency and degradation rate of the polymer [31]. It is determined by Size Exclusion Chromatography [32].

The drug content and encapsulation efficiency is determined by high pressure liquid chromatography (HPLC). The drug encapsulation efficiency was defined as the amount of drug encapsulated in the nanoparticles to that added in the process. To investigate the uptake of polymeric nanoparticles, confocal laser scanning microscopy was used.

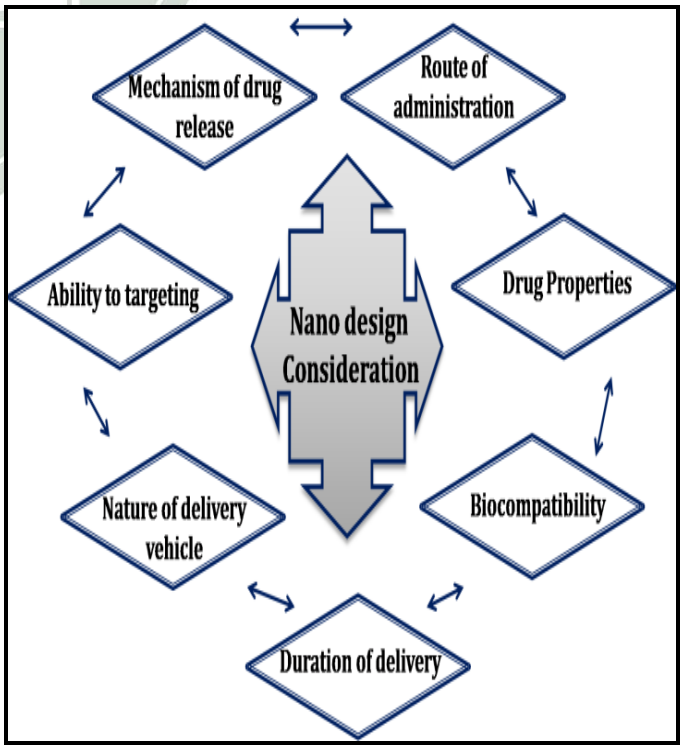


Fig. 5. Nanoparticle Design Consideration [33]

*D. Cytotoxicity assay*

Cancer cell viability was assayed using MTT toxic assay which is based on the capacity of living cells to metabolize the yellow tetrazolium salt MTT to a chromophore formazan product, the absorbance of which can be determined by spectrophotometric measurement [6]. Cell viability was assessed microscopically using tryphan blue penetration as an indication of cell death. Coulter counting was used to assess the cell numbers [34].

*E. Drug Release Kinetics*

Drug release depends on the physiochemical properties of the drug as well as the pH, crosslinking, morphology, size and density of the particulate system. The in-vitro release of the drug depends on factors like polarity and presence of enzyme in the medium.

Drug release depends on three different mechanisms:

*(1) Release from surface of the particle*

*(2) Diffusion through swollen rubbery matrix*

*(3) Release due to errosion*

*(4) The methods used in study of the in-vitro drug release are:*

- Dialysis by diffusion technique
- Reverse dialysis bag technique
- Side by side diffusion with artificial or biological membrane
- Ultracentrifugation
- Centrifugal ultrafiltration

The amount of drug release was monitored by the UV spectrophotometer as a function of time. The fibre mat was immersed in phosphate buffer saline at 7.4 pH, 37°C and constant stirring. Samples were periodically removed for analysis [35].

The drug loaded nanoparticle was dispersed in the release medium and then the suspension was introduced into a regenerated cellulose dialysis membrane and then the closed bag was put into a centrifuge tube and immersed in the release medium. The tube was put into an orbitol water bath shaking for 120 rpm at 37°C. Samples were periodically removed for analysis and replaced with fresh medium. Drug release was determined by high pressure liquid chromatography [9].

*F. Antimicrobial Activity*

Disk diffusion methods: the test samples are placed on solid nutritive media (agar plate) which contain a suspension of microbes swabbed or sprayed evenly on its surface. In the case of antibiotic tests, the samples are filter disks impregnated with the dilution of antibiotic. In the case of plastics, the sample is a plastic disk with the same contact surface area as at the antibiotic test. The incubation condition depends on the culture used, and the time of incubation is usually overnight, 18-24 hours. After the incubation period, the inhibitory zones have to be determined around the samples on the agar plate. The solid medium is generally Mueller-Hinton agar (containing beef extract, peptone and starch) or bloody agar, but BHI (brain-heart infusion) agar is also used. Both of these media are suitable for raising the most of aerobic and facultative anaerobic pathogenic microorganisms. (ASTM E2180 – 07 Standard test method for determining the activity of incorporated antimicrobial agent(s) in polymeric or hydrophobic materials) [36].

*G. Applications of Nanoparticles*

Nanomaterials have been attracting the attention of global materials research these days primarily due to their enhanced properties required for application in specific areas like catalysis, filtration, NEMS, nanocomposites, nanofibrous structures, tissue scaffolds, drug delivery systems, protective textiles, storage cells for hydrogen fuel cells.

Based on the ability to deliver the requisite dose load of drug in the vicinity of the tumor due to the enhanced permeability and retention effect or active targeting by ligands on the surface of nanoparticles and also to reduce the drug exposure

to healthy tissues by limiting drug distribution to the target organ nanoparticles are used in tumor targeting. The histological architecture of the mucosa is designed to efficiently prevent uptake of particulate matter from the environment.

One important strategy to overcome the gastrointestinal barrier is to deliver the drug in a colloidal carrier system, such as nanoparticles which is capable of enhancing the interaction mechanisms of the drug delivery system and the epithelia cells in the Gastro Intestinal tract. The blood brain barrier effectively prevents the passage of water soluble molecules from the blood circulation into the Central Nervous System, and consequently only permits selective transport of molecules that are essential for brain function [20].

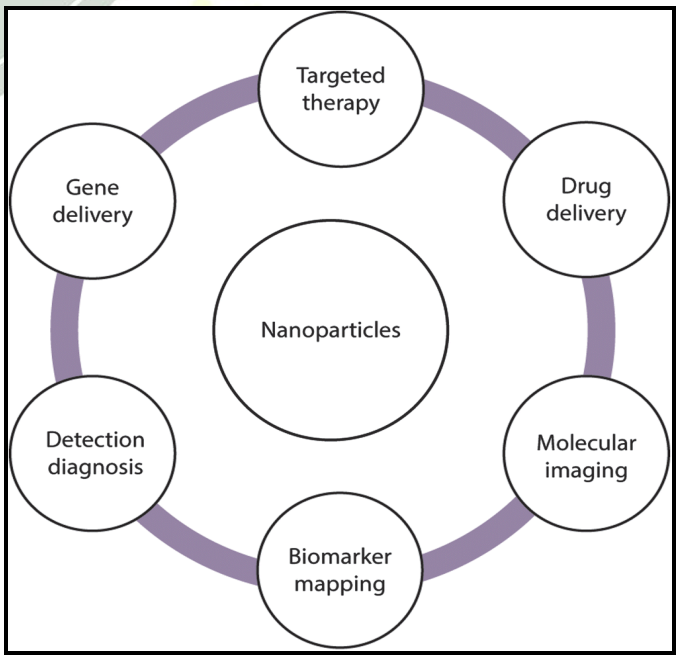


Fig. 6. Biomedical applications of nanoparticles[37]

Polymeric nanofibers have been used in air filtration applications for more than a decade. For filtration, the channels and structural elements of a filter must be matched to the scale of the particles or droplets that are to be captured in the filter. Thus, we can take advantage of the unique properties of electrospun membranes consisting of very-small diameter fibers.

A variety of polymeric nanofibers have been considered for use as scaffolds for engineering tissues such as cartilages, blood vessels, dermal tissue etc. [38]. Use of polymeric nanoparticles and lipid carrier systems, has limitations such as drug leakage and high water content of dispersions. Thus, lipid polymer hybrid nanoparticles (LPNs) have been explored by the researchers to provide a better effect using properties of both polymers and lipids [39].

Ribavirin, an antiviral drug used for the treatment of chronic hepatitis C induces severe side effects such as hemolytic anaemia. Therefore biodegradable nanoparticles as ribavirin carriers were prepared to modulate the pharmacokinetics of the drug [40]. Hydrophobic moiety-conjugated glycol chitosan forms amphiphilic self-assembled glycol chitosan nanoparticles and the hydrophobic drug molecules is encapsulated inside their hydrophobic core. This tumor-targeting drug delivery results in improved therapeutic efficiency

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