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A REVIEW ON NANOPARTICULATE DRUG DELIVERY SYSTEM

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ABSTRACT

For the past few decades, there has been a considerable research interest in the area of drug delivery using particulate delivery systems as carriers for small and large molecules. Particulate systems like nanoparticles have been used as a physical approach to alter and improve the pharmacokinetic and pharmacodynamic properties of various types of drug molecules. Various polymers have been used in the formulation of nanoparticles for drug delivery research to increase therapeutic benefit, while minimizing side effects. Here, we review various aspects of nanoparticle formulation, characterization, effect of their characteristics and their applications in delivery of drug molecules and therapeutic genes. The ability to assemble and study materials with nanoscale precision leads to opportunities in both basic biology and development of new biological technologies.

INTRODUCTION

Nanoparticles are defined as particulate dispersions or solid particles with a size in the range of 10-1000nm. The drug is dissolved,entrapped, encapsulated or attached to ananoparticle. Depending upon the method of preparation, nanoparticles, nanospheres or nanocapsules can be obtained.Nanocapsules can be obtained. Nanocapsules are systems in which the drug is confined to a cavity surrounded by a unique polymer membrane, while nanospheres are matrixsystems in which the drug is physically and uniformly dispersed.

In recent years,biodegradable polymeric nanoparticles particularly those coated with hydrophilic polymer such as poly(ethylene glycol) (PEG)known as long-circulating particles, have been used as potential drug delivery devices becauseof their ability to circulate for a prolonged period time target a particular organ, as carriers of DNAin gene therapy, and their ability to deliver proteins, peptides and genes.

The major goals in designing nanoparticles as a delivery system are to control particle size surface properties and release of pharmacologically active agents in order to achieve the site-specific action of the drug at the therapeutically optimal rate and dose regimen.[3-12, 16, 19,24]

ADVANTAGES OF NANOPARTICLES:

1. Particle size and surface characteristics of nanoparticles can be easily manipulated to achieve both passive and active drug targeting after parenteral administration
2. They control and sustain release of the drug during the transportation and at the site of localization, altering organ distribution of the drug and subsequent clearanceof the drug so as to achieve increase in drug therapeutic efficacy and reduction in side effects.
3. 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.
4. Site-specific targeting can be achieved by attaching targeting ligands to surface of particles or use of magnetic guidance.
5. The system can be used for various routes of administration including oral, nasal, parentral, intraocular etc. [1-3, 5, 8]

LIMITATION OF NANOPARTICLES

Their small size and large surface area can lead to particle-particle aggregation, making physical handling of nanoparticles difficult in liquid and dry forms. In addition, small particles size and large surface area readily result in limited drug loading andburst release. These practical problems have to be overcome before nanoparticles can be used clinically or made commercially available. The present review details the latest development of nanoparticulate drug delivery systems, surface modification issues, drug loading strategies, release control and potential applications of nanoparticles.[2, 3, 22]

METHOD OF PREPARATION OF NANOPARTICLES:

Nanoparticles have been prepared most frequency by three methods: (1) dispersion of preformed polymers; (2) polymerization of monomers; and (3) ionic gelation or co-acervation of hydrophilic polymers. Nanoparticles can be prepared from a variety of materials such as a proteins,polysaccharides and synthetic polymers.The selection of matrix materials is dependent on many .factors including: (a) size of nanoparticles required; (b)inherent properties of the drug, e.g., aqueous solubility and stability; (c) surface characteristics such as chargeand permeability; (d) degree of biodegradability,biocompatibility and toxicity; (e) Drug release profile desired; and (f) Antigenicity of the final product. However, other methods such as supercritical fluid technology and particle replication in non-wetting templates (PRINT) 12 have also been described in the literature for production of nanoparticles. The latter was claimed to have absolute control of particle size, shapeand composition, which could set an example for the future mass production of nanoparticles in industry.Dispersion of preformed polymers is a common technique used to prepare biodegradable nanoparticles from poly (lactic acid) (PLA); poly (D,L-glycolide), PLG; poly (D, L-lactide-co-glycolide) (PLGA) and poly(cyanoacrylate) (PCA), This technique can be used in various ways as described below.

1) Solvent Evaporation Method:

In this method, the polymer is dissolved in an organic solvent such as dichloromethane, chloroform or ethyl acetate, which is also used as the solvent for dissolving the hydrophobic drug. The mixture of polymer and drug solution is then emulsified in an aqueous solution containing a surfactant or emulsifying agent to form oil in water (o/w) emulsion. After the formation of stable emulsion, the organic solvent is evaporated either by reducing the pressure or by continuous stirring.Particle size was found to be influenced by the type and concentrations of

stabilizer, homogenizer speed andpolymer concentration16. In order to produce small particle size, often a high-speed homogenization or ultrasonication may be employed.

2) Spontaneous Emulsification or Solvent Diffusion Method:

This is a modified version of solvent evaporation method. In this method, the water miscible solvent along with a small amount of the water immiscible organic solvent is used as an oil phase. Due to the spontaneous diffusion of solvents an interfacial turbulence is created between the two phases leading to the formation of small particles. As the concentration of water miscible solvent increases, a decrease in the size of particle can be achieved. Both solvent evaporation and solvent diffusion methods can be used for hydrophobic or hydrophilic drugs. In the case of hydrophilic drug, a multiple w/o/w emulsion needs to be formed with the drug dissolved in the internal aqueous phase.

3) Polymerization Method:

In this method, monomers are polymerized to form nanoparticles in an aqueous solution. Drug is incorporated either by being dissolved in the polymerization medium or by adsorption onto the nanoparticles after polymerization completed. The nanoparticle suspension is then purified to remove various stabilizers and surfactants employed for polymerization by ultracentrifugation andre-suspending the particles in an isotonic surfactant-free medium. This technique has been reported for making polybutylcyanoacrylate or poly (alkylcyanoacrylate) nanoparticles Nanocapsules formation and their particle size depend on the concentration of the surfactants and stabilizers used.

4) Coacervation or Ionic Gelation Method:

Much research has been focused on the preparation of nanoparticles using biodegradable hydrophilic polymers such as chitosan, gelatin and sodium alginate. Calvo and co-workers developed a method for preparing hydrophilic chitosan nanoparticles by ionic gelation. The method involves a mixture of two aqueous phases, of which one is the polymer chitosan, a di-block co-polymer ethylene oxide or propylene oxide (PEO-PPO) and the other is a poly-anion sodium tri-polyphosphate. In this method, positively charged amino group of chitosan interacts with negative charged tri-polyphosphate to form coacervates with a size in the range of nanometer. Coacervates are formed as a result of electrostatic interaction between two aqueous phases, whereas, ionic gelation involves the material undergoing transition from liquid to gel release. These practical problems have to be overcome before nanoparticles can be used

clinically or made commercially available. The present review details the latest development of nanoparticulate drug delivery systems, surface modification issues, drug loading strategies, release control and potential applications of nanoparticles.

5) Production of Nanoparticles Using Supercritical Fluid Technology:

Conventional methods such as solventextraction-evaporation, solvent diffusion and organicphase separation methods require the use of organic solvents which are hazardous to the environment as well as to physiological systems. Therefore, the supercritical fluid technology has been investigated as an alternative to prepare biodegradable micro- and nanoparticles because supercritical fluids are environmentally safe.A supercritical fluid can be generally defined asa solvent at a temperature above its critical temperature, at which the fluid remains a single phase regardless of pressure. Supercritical CO2 (SC CO2) is the most widely used supercritical fluid because of its mild critical conditions (Tc = 31.1 °C, Pc = 73.8 bars), nontoxicity,non-flammability, and low price. The most common processing techniques involving supercritical fluids are supercritical anti-solvent (SAS) and rapid expansion of critical solution (RESS). The process of SAS employs a liquid solvent, e.g. methanol, which is completely miscible with the supercritical fluid (SC CO2), to dissolve the solute to be micronized; at the process conditions, because the solute is insoluble in the supercritical fluid, the extract of the liquid solvent by supercritical fluid leads to the instantaneous precipitation of the solute, resulting the formation of nanoparticles.Supercritical fluid technology technique, although environmentally friendly and suitable for mass production, requires specially designed equipment and is more expensive.[2, 3, 10-17, 22-24]

EFFECT OF CHARACTERISTICS OF NANOPARTICLES ON DRUG DELIVERY:

1) Particle Size:

Particle size and size distribution are the most important characteristics of nanoparticle systems. They determine the in vivo distribution, biological fate, toxicity and the targeting ability of nanoparticle systems. In addition, they can also influence the drug loading, drug release and stability of nanoparticles. Many studies have demonstrated that nanoparticles of sub-micron size have a number of advantages over microparticles as a drug delivery system. Generally nanoparticles have relatively higher intracellular uptake compared to microparticles and available to a wider range of biological targets due to their small size and relative mobility. It was also reported that nanoparticles can across the blood-brain barrier following the opening of

tight junctions by hyper osmotic mannitol, which may provide sustained delivery of therapeutic agents for difficult-to-treat diseases like brain tumors. In some cell lines, only submicron nanoparticles can be taken up efficiently but not the larger size microparticles. Drug release is affected by particle size. Smaller particles have larger surface area; therefore, most of the drug associated would be at or near the particle surface, leading to fast drug release. Whereas, larger particles have large cores, which allow more drug to be encapsulated and slowly diffuses out. Smaller particles also have greater risk of aggregation of particles during storage and transportation of nanoparticle dispersion. It is always a challenge to formulate nanoparticles with the smallest size possible but maximum stability. Polymer degradation can also be affected by the particle size. The rate of PLGA polymer degradation was found to increase with increasing particle size in vitro. Currently, the fastest and most routine method of determining particle size is by photon correlation spectroscopy or dynamic light scattering. Photon-correlation spectroscopy requires the viscosity of the medium to be known and determines the diameter of the particle by Brownian motion and light scattering properties. The results obtained by photon-correlation spectroscopy are usually verified by scanning or transmission electron microscopy.

2) Surface Properties of Nanoparticles:

When nanoparticles are administered intravenously, they are easily recognized by the body immune systems, and are then cleared by phagocytes from the circulation. Apart from the size of nanoparticles, 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 opsonization acts as a bridge between nanoparticles and phagocytes. The association of a drug to conventional carriers leads to modification of the drug biodistribution profile, as it is mainly delivered to the mononuclear phagocytes system (MPS) such as liver, spleen, lungs and bone marrow. Indeed, once in the blood stream, surface non-modified nanoparticles (conventional nanoparticles) are rapidly opsonized and massively cleared by the macrophages of MPS rich organs. Hence, to increase the likehood ofthe success in drug targeting by nanoparticles, it is necessary to minimize the opsonization and to prolong the circulation of nanoparticles in vivo. This can be achieved by (a) surface coating of nanoparticles withhydrophilic polymers/surfactants; (b) formulation ofnanoparticles with biodegradable copolymers with hydrophilic segments such as polyethylene glycol (PEG),polyethylene oxide, polyoxamer, poloxamine andm-polysorbate 80 (Tween 80).

The zeta potential of a nanoparticle is commonly used to characterize the surface charge property of nanoparticles35. Nanoparticles with a zeta potential above (+/-) 30 mV have been shown to be stable in suspension, as the surface charge prevents aggregation of the particles. The zeta potential can also be used to determine whether a charged active material is encapsulated within the center of the nanocapsule or adsorbed onto the surface.

3) Drug Loading:

Ideally, a successful nanoparticulate system should have a high drug-loading capacity thereby reduce the quantity of matrix materials for administration. Drug loading can be done by two methods:

a) Incorporating at the time of nanoparticles production (Incorporation Method) b) Absorbing the drug after formation of nanoparticles by incubating the carrier with a concentrated drug solution (Adsorption /Absorption Technique). Drug loading and entrapment efficiency very much depend on the solid state drug solubility in matrix material or polymer (solid dissolution or dispersion), which is related to the polymer composition, the molecular weight, the drug polymer interaction and the presence of end functional groups (ester or carboxyl). The PEG moiety has no or little effect on drug loading. The macromolecule or protein shows greatest loading efficiency when it is loaded at or near its isoelectric point when it has minimum solubility and maximum adsorption For small molecules, studies show the use of ionic interaction between the drug and matrix materials can be a very effective way to increase the drug loading.

4) Drug Release:

To develop a successful nanoparticulate system, both drug release and polymer biodegradation are important consideration factors. In general, drug release rate depends on: (1) solubility of drug; (2) desorption of the surface bound/ adsorbed drug; (3) drug diffusion through the nanoparticle matrix; (4) nanoparticle matrix erosion/degradation; and (5) combination of erosion/diffusion process. Thus solubility, diffusion and biodegradation of the matrix materials govern the release process. It is evident that the method of incorporation has an effect on release profile. If the drug is loaded by incorporation method, the system has a relatively small burst effect and better-sustained release characteristics. If the nanoparticle is coated by polymer, the release is then controlled by diffusion of the drug from the core across the polymeric membrane. The membrane coating acts as a barrier to release, therefore, the solubility and diffusivity of drug in polymer membrane becomes determining factor in drug release. Furthermore release rate can

also be affected by ionic interaction between the drug and addition of auxillary ingredients. When the drug is involved in interaction with auxillary ingredients to form a less water-soluble complex, then the drug release can be very slow with almost no burst release effect. Various methods which can be used to study the in vitro release of the drug are: (1) side-by-side diffusion cells with artificial or biological membranes. (2) dialysis-bag diffusion technique.(3) reverse-dialysis bag technique.(4) agitation followed by ultra centrifugation/centrifugation.(5) Ultra-filtration or centrifugal ultra-filtration techniques. Usually the release study is carried out by controlled agitation followed by centrifugation. Due to the time-consuming nature and technical difficulties encountered in the separation of nanoparticles from release media, the dialysis technique is generally preferred.[6, 9, 12, 14, 22-28]

APPLICATIONS OF NANOPARTICULATE DELIVERY SYSTEMS:

1. Tumor Targeting Using Nanoparticulate Delivery Systems:

The rationale of using nanoparticles for tumor targeting is based on following characteristics

1) Nanoparticles will be able to deliver a concentrate dose of drug in the vicinity of the tumor targets via the enhanced permeability and retention effect or active targeting by ligands on the surface of nanoparticles.

2) Nanoparticles will reduce the drug exposure of healthy tissues by limiting drug distribution to target organ. Studies show that the polymeric composition of nanoparticles such as type, hydrophobicitiy and biodegradation profile of the polymer along with the associated drug's molecular weight, its localization in the nanospheres and mode of incorporation technique, adsorption or incorporation, have a great influence on the drug distribution pattern in vivo. The exact underlying mechanism is not fully understood but the biodistribution of nanoparticles is rapid, within ½ hour to 3 hours, and it likely involves mononuclear phagocytic system (MPS) and endocytosis/phagocytosis process. Such propensity of MPS for endocytosis/phagocytosis of nanoparticles provides an opportunity to effectively deliver therapeutic agents to these cells. This biodistribution can be of benefit for the chemotherapeutic treatment of MPS- rich organs/tissues localized tumors like hepato-carcinoma, hepatic metastasis arising from digestive tract or gynacological cancers, bronchopulmonary tumors, primitive tumors and metastasis, small cell tumors, myeloma and leukemia.

2. Ligand Attached Nanoparticles:

To be successful as a drug delivery system, nanoparticles must be able to target tumors, which are localized outside MPS-rich organs41. In the past decade, a great deal of work has been devoted to developing so called "stealth" particles or PEGylated nanoparticles, which are invisible to macrophages or phagocytes. Amajor breakthrough in the field came when the use of hydrophilic polymers (such as polyethylene glycol, poloxamines, poloxamers, and polysaccharides) to efficiently coat conventional nanoparticle surface produced an opposing effect to the uptake by the MPS, These coatings provide a dynamic "cloud" of hydrophilic and neutral chains at the particle surface, which repel plasma proteins 44,45. As a result, those coated nanoparticles become invisible to MPS, therefore, remained in the circulation for a longer period of time and hence called as long circulating nanoparticles. Hydrophilic polymers can be introduced at the surface intwo ways, either by adsorption of surfactants or by use of block or branched copolymers for production of nanoparticles. Studies show nanoparticles containing a coat of PEG not only have a prolonged half-life in the blood compartment but also be able to selectively extravasate in pathological sites such as tumors or inflamed regions with a leaky vasculature. As a result, such long-circulating nanoparticles have increased the potential to directly target tumors located outside MPSrich region. The sizes of the colloidal carriers as wellas their surface characteristics are the critical to the biological fate of nanoparticles. A size less than 100 nm and a hydrophilic surface are essential in achieving the reduction of opsonisation reactions and subsequent clearance by macrophages. Coating conventional nanoparticles with surfactants or PEG to obtain a long circulating carrier has now been used as a standard strategy for drug targeting in vivo. Extensive efforts have been devoted to achieving "active targeting" ofnanoparticles in order to deliver drugs to the right targets, based on molecular recognition processes such as ligand receptor or antigen-antibody interaction. Considering that fact that folate receptors are over expressed on the surface of some human malignant cells and the cell adhesion molecules such as selectins and integrins areinvolved in metastatic events, nanoparticles bearingspecific ligands such as folate may be used to target ovarian carcinoma while specific peptides or carbohydrates may be used to target integrins and selections. Targeting with small ligands appears more likely to succeed since they are easier to handle and manufacture. Furthermore, it could be advantageous when the active targeting ligands

are used in combination with the long-circulating nanoparticles to maximize the likelihood of the success in active targeting of nanoparticles.

3. Nanoparticles for Oral Delivery of Peptides and Proteins:

Significant advances in biotechnology and biochemistry have led to the discovery of a large numberof bioactive molecules and vaccines based on peptides and proteins. Development of suitable carriers remains a challenge due to the fact that bioavailability of these molecules is limited by the epithelial barriers of the gastrointestinal tract and their susceptibility to gastrointestinal degradation by digestive enzymes. Polymeric nanoparticles allow encapsulation of bioactive molecules and protect them against enzymatic and hydrolytic degradation. For instance, it has been found that insulin-loaded nanoparticles have preserved insulin activity and produced blood glucose reduction in diabetic rats for up to 14 days following the oral administration. The surface area of human mucosa extends to 200 times that of skin. The gastrointestinal tract provides a variety of physiological and morphological barriers against protein or peptide delivery, e.g., (a) proteolytic enzymes in the gut lumen like pepsin, trypsin and chymotrypsin;(b) proteolytic enzymes at the brush border membrane(endopeptidases); (c) bacterial gut flora; and (d) mucuslayer and epithelial cell lining itself. 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 epithelial cells in the GI tract.

4. Nanoparticles for Gene Delivery:

Polynucleotide vaccines work by delivering genes encoding relevant antigens to host cells where they are expressed, producing the antigenic protein within the vicinity of professional antigen presenting cells to initiate immune response. Such vaccines produce both humoral and cell-mediated immunity because intracellular production of protein, as opposed to extracellular deposition, stimulates both arms of the immune system. The key ingredient of polynucleotide vaccines, DNA, can be produced cheaply and has much better storage and handling properties than the ingredients of the majority of protein-based vaccines. Hence, polynucleotide vaccines are set to supersede many conventional vaccines particularly for immunotherapy. However, there are several issues related to the delivery of polynucleotides, which limit their application. These issues include efficient delivery of the polynucleotide to the target cell population and its

localization to the nucleus of these cells, and ensuring that the integrity of the polynucleotideis maintained during delivery to the target site.Nanoparticles loaded with plasmid DNA could also serveas an efficient sustained release gene delivery system dueto their rapid escape from the degradativeendolysosomalcompartment to the cytoplasmic compartment. Hedley et al reported that following theirintracellular uptake and endolysosomal escape,nanoparticles could release DNA at a sustained rateresulting in sustained gene expression. This gene deliverystrategy could be applied to facilitate bone healing byusing PLGA nanoparticles containing therapeutic genessuch as bone morphogenic protein.

a) Gene Therapy Using Nano-Delivery Systems:

Gene therapy involves the delivery of one ormore genes and the sequences controlling theirexpression into the target cell or tissue. These newlydelivered genes can then replace a defective gene or addgenes, which "rewrite" certain aspects of the cell'sfunctions, thus producing new proteins. The delivery ofgenes to the cell or tissue needs to be carried out using avehicle, approved for clinical applications, whichfacilitates the gene's entrance into the cell. We havedeveloped two new vehicles for gene delivery:Nanoparticles and ultrasound waves. The nanoparticlescontaining the new gene are injected into the site ofinterest where they are taken up by the cells and releasetheir gene contents in the cells. The ultrasound energy,which is given from outside the body, forces the entranceof genes into the organ without the need of invasivesurgery. Both technologies are used to deliver genes,which encode for the anticancer drugs.

5. Nanoparticles for Drug Delivery into the Brain:

The blood-brain barrier (BBB) is the mostimportant factor limiting the development of new drugsfor the central nervous system. The BBB is characterizedby relatively impermeable endothelial cells with tightjunctions, enzymatic activity and active efflux transportsystems. It effectively prevents the passage of watersolublemolecules from the blood circulation into theCNS, and canalso reduce the brain concentration oflipid-soluble molecules by the function of enzymes orefflux pumps. Consequently, the BBB only permitsselective transport of molecules that are essential forbrain function. Strategies for nanoparticle targeting to thebrain rely on the presence of and nanoparticle interactionwith specific receptor-mediated transport systems in theBBB. For example polysorbate 80/LDL, transferrinreceptor binding antibody (such as OX26), lactoferrin,cell penetrating peptides and melanotransferrin.[2-8, 12, 17, 22-28]

CONCLUSION

The above review concludes that nanoparticulate systems have great potentials, being able to convert poorly soluble, poorly absorbed and labile biologically active substance into promising deliverable drugs. The core of this system can enclose a variety of drugs, enzymes, genes and is characterized by a long circulation time due to the hydrophilic shell which prevents recognition by the reticular-endothelial system. To optimize this drug delivery system, greater understanding of the different mechanisms of biological interactions, and particle engineering, is still required.

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