European Journal of Molecular & Clinical Medicine

ISSN 2515-8260 Volume 08, Issue 04, 2021

REVIEW ON CHARACTERIZATION OF NANOPARTICLES

Dr. N.SANTHI & Dr.ANTONY K.TERESA INFANTA S.

Assistant Professor, Department of Biotechnology,
New Prince Shri Bhavani Arts and Science college, Chennai-600100,
[1] n.santhisivakumar@gmail.com [2]teresainfanta@gmail.com

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. They have been used in vivo to protect the drug entity in the systemic circulation, restrict access of the drug to the chosen sites and to deliver the drug at a controlled and sustained rate to the site of action. 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.

Key words: nanoparticles, drug delivery, targeting, drug release

INTRODUCTION

Nanoparticles are well-defined as particulate dispersions with a dimension in the range of 10-1000nm. The drug is dissolved, deceived, encapsulated or attached to a nanoparticle matrix. Depending upon the method of preparation, nanoparticles, nanospheres or nano capsules can be obtained. Nano capsules are systems in which the drug is limited to a cavity surrounded by a unique polymer membrane, whereas nanospheres are matrix systems in which the drug is essentially 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 because of their ability to circulate for a prolonged period time target a particular organ, as carriers of DNA in gene therapy, and their ability to deliver proteins, peptides and genes 1-4. 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. Though liposomes have been used as potential carriers with unique advantages including protecting drugs from degradation, targeting to site of action and reduction toxicity or side effects, their applications are limited due to inherent problems such as low encapsulation efficiency, rapid leakage of water-soluble drug in the presence of blood components and poor storage stability. On the other hand, polymeric nanoparticles offer some specific advantages over liposomes. For instance, they help to increase the stability of drugs/proteins and possess useful controlled release properties 5, 6. The advantages of using nanoparticles as a drug delivery system include the following: 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 clearance of 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 numerous routes of administration including oral, nasal, parenteral, intra-ocular etc. In spite of these advantages, nanoparticles do have limitations. For example, their small size and large surface area can lead to particle aggregation, making physical handling of nanoparticles problematic in liquid and dry forms. In addition, small particles size and large surface area readily result in limited drug loading and burst 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.

Preparation of Nanoparticles

Nanoparticles can be prepared from a variety of materials such as proteins, polysaccharides and synthetic polymers. The selection of matrix materials is dependent on many factors including7 : (a) size of nanoparticles required; (b) inherent properties of the drug, e.g., aqueous solubility and stability; (c) surface characteristics such as charge and permeability; (d) degree of biodegradability, biocompatibility and toxicity; (e) Antigenicity of the final product. Nanoparticles have been prepared most frequency by three methods: (1) dispersion of preformed polymers; (2) polymerization of monomers; and (3) ionic gelation or coacervation of hydrophilic polymers. However, other methods such as supercritical fluid technology 8 and particle replication in non-wetting templates (PRINT) 9 have also been described in the literature for production of nanoparticles. The latter was claimed to have absolute control of particle size, shape and composition, which could set an example for the future mass production of nanoparticles in industry. Dispersion of preformed polymers: 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), 10-12. This technique can be used in various ways as described below. 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 an 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 and polymer concentration 13. In order to produce small particle size, often a high-speed homogenization or ultrasonication may be employed 14. Spontaneous emulsification or solvent diffusion method: This is a modified version of solvent evaporation method 15. 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. 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 and re-suspending the particles in an isotonic surfactant-free medium. This technique has been reported for making polybutylcyanoacrylate or poly (alkyl cyanoacrylate) nanoparticles16; 17. Nano capsule formation and their particle size depends on the concentration of the surfactants and stabilizers used 18. 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 19, 20. 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 polyanion sodium tripolyphosphate. In this method, positively charged amino group of chitosan interacts with negative charged tripolyphosphate 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 (1) due to ionic interaction conditions at room temperature. Production of nanoparticles using supercritical fluid technology Conventional methods such as solvent extraction-evaporation, solvent diffusion and organic phase 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 21. A supercritical fluid can be generally defined as a solvent at a temperature above its critical temperature, at which the fluid remains a single phase regardless of pressure 21. Supercritical CO2 (SC CO2) is the most widely used supercritical fluid because of its mild critical conditions 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, eg 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 8 . Thote and Gupta (2005) described the use of a modified SAS method for formation of hydrophilic drug dexamethasone phosphate drug nanoparticles for microencapsulation purpose 22. RESS differs from the SAS process in that its solute is dissolved in a supercritical fluid (such as supercritical methanol) and then the solution is rapidly expanded through a small nozzle into a region lower pressure 21, Thus the solvent power of supercritical

fluids dramatically decreases and the solute eventually precipitates. This technique is clean because the precipitate is basically solvent free. RESS and its modified process have been used for the product of polymeric nanoparticles 23. Supercritical fluid technology technique, although environmentally friendly and suitable for mass production, requires specially designed equipment and is more expensive. Effect of Characteristics of Nanoparticles on Drug Delivery 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 confirmed that nanoparticles of sub-micron size have a number of advantages over microparticles as a drug delivery system 24. Generally, nanoparticles have fairly higher intracellular uptake compared to microparticles and available to a wider range of biological targets due to their small size and relative mobility. Desai et al found that 100 nm nanoparticles had a 2.5-fold greater uptake than 1 μm microparticles, and 6-fold greater uptake than 10 μm microparticles in a Caco-2 cell line25. In a subsequent study 26, the nanoparticles penetrated throughout the submucosal layers in a rat in situ intestinal loop model, while microparticles were predominantly localized in the epithelial lining. 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 27. Tween 80 coated nanoparticles have been shown to cross the blood-brain barrier 28. In some cell lines, only submicron nanoparticles can be taken up efficiently but not the larger size microparticles 29. 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 (1) diffuse out 30. 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. For instance, the rate of PLGA polymer degradation was found to increase with increasing particle size in vitro 31. It was thought that in smaller particles, degradation products of PLGA formed can diffuse out of the particles easily while in large particles, degradation products are more likely remained within the polymer matrix for a longer period to cause autocatalytic degradation of the polymer material. Therefore, it was hypothesized that larger particles will contribute to faster polymer degradation as well as the drug release. However, Panyam et al prepared PLGA particles with different size ranges and found that the polymer degradation rates in vitro were not substantially different for different size particles 32. 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 33. The results obtained by photon-correlation spectroscopy are usually verified by scanning or transmission electron microscopy (SEM or TEM). 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 34. 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 34, 35. Binding of these opsonins onto the surface of nanoparticles called opsonization acts as a bridge between nanoparticles and phagocytes. 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 36. Generally, it is IgG, compliment C3 components that are used for recognition of foreign substances, especially foreign macromolecules. Hence, to increase the likelihood of the 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 with hydrophilic polymers/surfactants; (b) formulation of nanoparticles with biodegradable copolymers with hydrophilic segments such as polyethylene glycol (PEG), polyethylene oxide, polyoxamer, poloxamine and polysorbate 80 (Tween 80). PEG surfaces in brush-like and intermediate configurations reduced phagocytosis and complement activation whereas PEG surfaces in mushroom-like configuration were potent complement activators and favoured phagocytosis 2, 37. The zeta potential of a nanoparticle is commonly used to indicate the surface charge property of nanoparticles 38. It reflects the electrical potential of particles and is influenced by the composition of the particle and the medium in which it is dispersed. 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 centre of the nano capsule or adsorbed onto the surface (1) 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: • Incorporating at the time of nanoparticles production (incorporation method) • 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) 39- 41. The PEG moiety has no or little effect on drug loading 42. 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 19 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 43, 44. Drug release to develop a successful nanoparticulate system, both drug release and polymer biodegradation are important attention 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. The rapid initial release or ‘burst’ is mainly attributed to weakly bound or adsorbed drug to the large surface of nanoparticles 45. It is obvious that the method of incorporation has an effect on release profile. If the drug is loaded by combination method, the system has a relatively small burst effect and better sustained release characteristics 46. If the nanoparticle is coated by polymer, the release is then controlled by diffusion of the drug from the core across the polymeric membrane. Furthermore, release rate can also be affected by ionic

interaction between the drug and addition of auxillary ingredients. When the drug is elaborate 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 43; 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 ultracentrifugation/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. Applications of Nanoparticulate Delivery Systems Tumor targeting using nanoparticulate delivery systems The rationale of using nanoparticles for tumor targeting is based on 1) nanoparticles will be able to deliver a concentrate dose of drug in the (1) 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 health tissues by limiting drug distribution to target organ. Verdun et al confirmed in mice treated with doxorubicin combined into poly (isohexylcyanoacrylate) nanospheres that higher concentrations of doxorubicin manifested in the liver, spleen and lungs than in mice treated with free doxorubicin 47. 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 MPS and endocytosis/phagocytosis process 48. Recently Bibby et al reported the biodistribution and pharmacokinetics (PK) of a cyclic RGD doxorubicin-nanoparticle formulation in tumor bearing mice 49. Their biodistribution studies revealed decreasing drug concentrations over time in the heart, lung, kidney and plasma and accumulating drug concentrations in the liver, spleen and tumor. The majority injected dose appeared in the liver (56%) and only 1.6% in the tumour at 48 hrs post injection, confirming that nanoparticles have a great tendency to be captured by liver. This indicates the greatest challenge of using nanoparticles for tumour targeting is to avoid particle uptake by mononuclear phagocytic system (MPS) in liver and spleen. 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 hepatocarcinoma, hepatic metastasis arising from digestive tract or gynaecological cancers, bronchopulmonary tumors, primitive tumors and metastasis, small cell tumors, myeloma and leukemia. Histological examination showed a considerable accumulation of nanoparticles in the lysosomal vesicles of Kupffer cells, whereas nanoparticles could not be clearly identified in tumoral cells. Thus, Kupffer cells, after a massive uptake of nanoparticles by phagocytosis, were able to induce the release of doxorubicin, leading to a gradient of drug concentration, favorable for a prolonged diffusion of the free and still active drug towards the neighboring metastatic cells 50. When conventional nanoparticles are used as carriers in chemotherapy, some cytotoxicity against the Kupffer cells can be expected, which would result in deficiency of Kupffer cells and naturally lead to reduced liver uptake and decreased therapeutic effect with intervals of less than 2 weeks administration 51. Therefore, the ability of conventional nanoparticles to enhance anticancer drugs efficacy is limited to targeting tumors at the level of MPS-rich organs. Also, directing anticancer drug-loaded nanoparticles to other tumoral sites is not feasible if a rapid clearance of nanoparticles

occurs shortly after intravenous administration. Long circulating nanoparticles to be successful as a drug delivery system, nanoparticles must be able to target tumors which are localized outside MPS-rich organs. In the past decade, a great deal of work has been devoted to developing so-called "stealth (1) particles or PEGylated nanoparticles, which are invisible to macrophages or phagocytes 52. A major 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 52, 53. These coatings provide a dynamic "cloud" of hydrophilic and neutral chains at the particle surface which repel plasma proteins 54 55. 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. Considering that fact that folate receptors are over stated on the surface of some human malignant cells and the cell adhesion molecules such as selectins and integrins are involved in metastatic events, nanoparticles bearing specific ligands such as folate may be used to target ovarian carcinoma while specific peptides or carbohydrates may be used to target integrins and selectins 56. Oyewumi et al demonstrated that the benefits of folate ligand coating were to facilitate tumor cell internalization and retention of Gd-nanoparticles in the tumor tissue 57. MDR occurs mainly due to the over expression of the plasma membrane glycoprotein (Pgp), which is capable of extruding various positively charged xenobiotics, including some anticancer drugs, out of cells 58. The rationale behind the association of drugs with colloidal carriers, such as nanoparticles, against drug resistance derives from the fact that Pgp probably recognizes the drug to be effluxed out of the tumoral cells only when this drug is present in the plasma membrane, and not when it is located in the cytoplasm or lysosomes after endocytosis 59 60. Nanoparticles for oral delivery of peptides and proteins Significant advances in biotechnology and biochemistry have led to the discovery of a large number of bioactive molecules and vaccines based on peptides and proteins. (1) 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 61. The surface area of human mucosa extends to 200 times that of skin 62. 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) mucus layer and epithelial cell lining itself 63. The histological architecture of the mucosa is designed to efficiently prevent uptake of particulate matter from the environment. Certain glycoproteins and lectins bind selectively to this type of surface structure by specific receptor-mediated mechanism. Different lectins, such as bean lectin and tomato lectin, have been studied to enhance oral peptide adsorption 64 65. The ability to increase oral bioavailability of various peptides (e.g., granulocyte colony stimulating factor, erythropoietin) and particles by covalent coupling to vitamin B-12 has been studied 66, 67. For this intrinsic process, mucoprotein is required, which is prepared by the mucus membrane in the stomach and binds specifically to cobalamin. The mucoprotein completely reaches the ileum where resorption is mediated by

specific receptors. The paracellular route of absorption of nanoparticles utilises less than 1% of mucosal surface area. Using polymers such as chitosan 68, starch 69 or poly(acrylate) 70 can increase the paracellular permeability of macromolecules. This process is initiated by an unspecific physical adsorption of material to the cell surface by electrostatic forces such as hydrogen bonding or hydrophobic interactions 71. Adsorptive endocytosis depends primarily on the size and surface properties of the material. If the surface charge of the nanoparticles is positive or uncharged, it will provide an affinity to adsorptive enterocytes though hydrophobic, whereas if it is negatively charged and hydrophilic, it shows greater affinity to adsorptive enterocytes and M cells. This shows that a combination of size, surface charge and hydrophilicity play a major role in affinity. This is demonstrated with poly (styrene) nanoparticles and when it is carboxylated 72. 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. (1) humoral and cell-mediated immunity because intracellular production of protein, as opposed to extracellular deposition, stimulates both arms of the immune system 73. Nanoparticles loaded with plasmid DNA could also serve as an efficient sustained release gene delivery system due to their rapid escape from the degradative endo-lysosomal compartment to the cytoplasmic compartment 74. Hedley et al. 75 reported that following their intracellular uptake and endolysosomal escape, nanoparticles could release DNA at a sustained rate resulting in sustained gene expression. It effectively prevents the passage of water-soluble molecules from the blood circulation into the CNS, and can also reduce the brain concentration of lipid-soluble molecules by the function of enzymes or efflux pumps 76. For example, polysorbate 80/LDL, transferrin receptor binding antibody (such as OX26), lactoferrin, cell penetrating peptides and melanotransferrin have been shown capable of delivery of a self non transportable drug into the brain via the chimeric construct that can undergo receptor-mediated transcytosis 77-81. It has been reported poly(butyl cyanoacrylate) nanoparticles was able to deliver hexapeptide dalargin, doxorubicin and other agents into the brain which is significant because of the great difficulty for drugs to cross the BBB 77. Despite some reported success with polysorbate 80 coated NPs, this system does have many shortcomings including desorption of polysorbate coating, rapid NP degradation and toxicity caused by presence of high concentration of polysorbate 80.37. OX26 MAbs (anti-transferrin receptor MAbs), the most studied BBB targeting antibody, have been used to enhance the BBB penetration of lipsosomes 82. However, recently, Ji et al. demonstrated that brain uptake of lactoferrin, an iron-binding glycoprotein belonging to the transferrin (Tf) family, is twice that of OX26 and transferrrin in vivo 79. It is possible soon we will see these BBB specific molecules used for targeting nanoparticles to the brain.

CONCLUSION

The foregoing show 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.

Further advances are needed in order to turn the concept of nanoparticle technology into a realistic practical application as the next generation of drug delivery system.

References

1. Langer R. Biomaterials in drug delivery and tissue engineering: one laboratory's experience. Acc Chem Res 2000; 33: 94-101.

2. Bhadra D, Bhadra S, Jain P, Jain NK. Pegnology: a review of PEG-ylated systems. Pharmazie 2002; 57: 5-29.

3. Kommareddy S, Tiwari SB, Amiji MM. Long-circulating polymeric nanovectors for tumor-selective gene delivery. Technol Cancer Res Treat 2005; 4: 615- 25.

4. Lee M, Kim SW. Polyethylene glycol-conjugated copolymers for plasmid DNA delivery. Pharm Res 2005; 22: 1-10.

5. Vila A, Sanchez A, Tobio M, Calvo P, Alonso MJ. Design of biodegradable particles for protein delivery. J Control Release 2002; 78: 15-24.

6. Mu L, Feng SS. A novel controlled release formulation for the anticancer drug paclitaxel (Taxol(R)): PLGA nanoparticles containing vitamin E TPGS. J Control Release 2003; 86: 33-48.

7. Kreuter J. Nanoparticles. In Colloidal drug delivery systems, J, K., Ed. Marcel Dekker: New York, 1994; pp 219-342.

8. Reverchon E, Adami R. Nanomaterials and supercritical fluids. The Journal of Supercritical Fluids 2006; 37: 1-22.

9. Rolland JP, Maynor BW, Euliss LE, Exner AE, Denison GM, DeSimone JM. Direct fabrication and harvesting of monodisperse, shape-specific nanobiomaterials. J. Am. Chem. Soc. 2005; 127: 10096-10100

10. Kompella UB, Bandi N, Ayalasomayajula SP. Poly (lactic acid) nanoparticles for sustained release of budesonide. Drug Deliv. Technol. 2001; 1: 1-7.

11. Ravi MN, Bakowsky U, Lehr CM. Preparation and characterization of cationic PLGA nanospheres as DNA carriers. Biomaterials 2004; 25: 1771-1777.

12. Li YP, Pei YY, Zhou ZH, Zhang XY, Gu ZH, Ding J, Zhou JJ, Gao, XJ, PEGylated polycyanoacrylate nanoparticles as tumor necrosis factor-[alpha] carriers. J Control Release 2001; 71: 287-296.

13. Kwon, HY, Lee JY, Choi SW, Jang Y, Kim JH. Preparation of PLGA nanoparticles containing estrogen by emulsification-diffusion method. Colloids Surf. A: Physicochem. Eng. Aspects 2001; 182: 123-130.

14. Zambaux M, Bonneaux F, Gref R, Maincent P, Dellacherie E, Alonso M, Labrude P, Vigneron C. Influence of experimental parameters on the characteristics of poly(lactic acid) nanoparticles prepared by double emulsion method. J. Control. Release 1998; 50: 31-40.

15. Niwa T, Takeuchi H, Hino T, Kunou N, Kawashima Y. Preparation of biodegradable nanoparticles of water-soluble and insoluble drugs with D,Llactide/glycolide copolymer by a novel spontaneous emulsification solvent diffusion method, and the drug release behavior. J. Control. Release 1993; 25: 89-98.

16. Zhang Q, Shen Z, Nagai T. Prolonged hypoglycemic effect of insulin-loaded polybutylcyanoacrylate nanoparticles after pulmonary administration to normal rats. Int. J. Pharm. 2001; 218: 75-80.

17. Boudad H, Legrand P, Lebas G, Cheron M, Duchene D, Ponchel G. Combined hydroxypropyl-[beta]- cyclodextrin and poly(alkylcyanoacrylate) nanoparticles intended for oral administration of saquinavir. Int J. Pharm. 2001; 218: 113-124.
18. Puglisi G, Fresta M, Giammona G, Ventura CA. Influence of the preparation conditions on poly(ethylcyanoacrylate) nanocapsule formation. Int. J. Pharm. 1995; 125: 283-287.
19. Calvo P, Remunan-Lopez C, Vila-Jato JL, Alonso MJ. Novel hydrophilic chitosan-polyethylene oxide nanoprticles as protein carriers. J. Appl. Polymer Sci. 1997; 63: 125-132.
20. Calvo P, Remunan-Lopez C, Vila-Jato JL, Alonso MJ. Chitosan and chitosan/ethylene oxide-propylene oxide block copolymer nanoparticles as novel carriers for proteins and vaccines. Pharm Res. 1997; 14: 1431-1436.
21. Jung J, Perrut M. Particle design using supercritical fluids: Literature and patent survey. J. Supercritical Fluids 2001; 20: 179-219.
22. Thote AJ, Gupta RB. Formation of nanoparticles of a hydrophilic drug using supercritical carbon dioxide and microencapsulation for sustained release. Nanomedicine: Nanotech. Biology Medicine 2005; 1: 85-90.
23. Sun Y, Mezian M, Pathak P, Qu L. Polymeric nanoparticles from rapid expansion of supercritical fluid solution. Chemistry 2005; 11: 1366-73.
24. Panyam J, Labhasetwar V. Biodegradable nanoparticles for drug and gene delivery to cells and tissue. Adv Drug Deliv Rev 2003; 55: 329-47.
25. Desai MP, Labhasetwar V, Walter E, Levy RJ, Amidon G L, The mechanism of uptake of biodegradable microparticles in Caco-2 cells is size dependent. Pharm Res 1997; 14: 1568-73.
26. Desai MP, Labhasetwar V, Amidon GL, Levy RJ. Gastrointestinal uptake of biodegradable microparticles: effect of particle size. Pharm Res 1996; 13: 1838-45.
27. Kroll RA, Pagel MA, Muldoon LL, Roman-Goldstein S, Fiamengo SA, Neuwelt EA. Improving drug delivery to intracerebral tumor and surrounding brain in a rodent model: a comparison of osmotic versus bradykinin modification of the blood-brain and/or blood-tumor barriers. Neurosurgery 1998; 43: 879-86; discussion 886-9.
28. Kreuter J, Ramge P, Petrov V, Hamm S, Gelperina SE, Engelhardt B, Alyautdin R, von Briesen H, Begley DJ. Direct evidence that polysorbate-80-coated poly(butylcyanoacrylate) nanoparticles deliver drugs to the CNS via specific mechanisms requiring prior binding of drug to the nanoparticles. Pharm Res 2003; 20: 409-16.
29. Zauner W, Farrow NA, Haines AM. In vitro uptake of polystyrene microspheres: effect of particle size, cell line and cell density. J Control Release 2001; 71: 39-51.
30. Redhead HM, Davis SS, Illum L. Drug delivery in poly(lactide-co-glycolide) nanoparticles surface modified with poloxamer 407 and poloxamine 908: Mohanraj & Chen 572 Trop J Pharm Res, June 2006; 5 (1) in vitro characterisation and in vivo evaluation. J Control Release 2001; 70: 353-363.
31. Dunne M, Corrigan OI, Ramtoola Z. Influence of particle size and dissolution conditions on the degradation properties of polylactide-co-glycolide particles. Biomaterials 2000; 21: 1659-1668.
32. Panyam J, Dali MM, Sahoo S K, Ma W, Chakravarthi SS, Amidon GL, Levy RJ, Labhasetwar V. Polymer degradation and in vitro release of a model protein from poly(,-lactide-co-glycolide) nano- and microparticles. J Control Release 2003; 92: 173- 187.

33. Swarbrick J, Boylan J. Encyclopedia of pharmaceutical technology. 2nd ed.; Marcel Dekker: New York, 2002.
34. Muller RH, Wallis KH. Surface modification of i.v. injectable biodegradable nanoparticles with poloxamer polymers and poloxamine 908. Int. J. Pharm. 1993; 89: 25-31.
35. Brigger I, Dubernet C, Couvreur P. Nanoparticles in cancer therapy and diagnosis. Adv. Drug Deliv. Rev. 2002; 54: 631-651.
36. Grislain L, Couvreur P, Lenaerts V, Roland M, DeprezDecampeneere D, Speiser P. Pharmacokinetics and distribution of a biodegradable drug-carrier. Int. J. Pharm. 1983; 15: 335-345.
37. Olivier JC. Drug transport to brain with targeted nanoparticles. NeuroRx 2005; 2: 108-119.
38. Couvreur P, Barratt G, Fattal E, Legrand P, Vauthier C. Nanocapsule technology: a review. Crit Rev Ther Drug Carrier Syst 2002; 19: 99-134.
39. Govender T, Stolnik S, Garnett MC, Illum L, Davis SS. PLGA nanoparticles prepared by nanoprecipitation: drug loading and release studies of a water soluble drug. J. Control. Rel. 1999; 57: 171-185.
40. Govender T, Riley T, Ehtezazi T, Garnett MC, Stolnik S, Illum L, Davis SS. Defining the drug incorporation properties of PLA-PEG nanoparticles. Int J Pharm 2000; 199: 95-110.
41. Panyam J, Williams D, Dash A, Leslie-Pelecky D, Labhasetwar V. Solid-state solubility influences encapsulation and release of hydrophobic drugs from PLGA/PLA nanoparticles. J Pharm Sci 2004; 93: 1804-14.
42. Peracchia M, Gref R, Minamitake Y, Domb A, Lotan N, Langer R. PEG-coated nanospheres from amphiphilic diblock and multiblock copolymers: investigation of their drug encapsulation and release characteristics. J Control Release 1997; 46: 223–231
43. Chen Y, McCulloch, RK, Gray BN. Synthesis of albumin-dextran sulfate microspheres possessing favourable loading and release characteristics for the anti-cancer drug doxorubicin. J Control Release 1994; 31: 49-54.
44. Chen Y, Mohanraj VJ, Parkin JE. Chitosan-dextran sulfate nanoparticles for delivery of an antiangiogenesis peptide. Letters in Peptide Science 2003; 10: 621-627.
45. Magenheim B, Levy MY, Benita S. A new in vitro technique for the evaluation of drug release profile from colloidal carriers - ultrafiltration technique at low pressure. Int. J. Pharm. 1993; 94: 115-123.
46. Fresta M, Puglisi G, Giammona G, Cavallaro G, Micali N, Furneri PM. Pefloxacin mesilate-and ofloxacinloaded polyethylcyanoacrylate nanoparticles; characterization of the colloidal drug carrier formulation. J. Pharm. Sci. 1995; 84: 895-902.
47. Verdun C, Brasseur F, Vranckx H, Couvreur P, Roland M. Tissue distribution of doxorubicin associated with polyhexylcyanoacrylate nanoparticles. Cancer Chemother. Pharmacol 1990; 26: 13-18.
48. Couvreur P, Kante B, Lenaerts V, Scailteur V, Roland M, Speiser P. Tissue distribution of antitumor drugs associated with polyalkylcyanoacrylate nanoparticles. J Pharm Sci 1980; 69: 199-202.
49. Bibby DC, Talmadge JE, Dalal MK, Kurz SG, Chytil KM, Barry SE, Shand DG, Steiert M. Pharmacokinetics and biodistribution of RGD-targeted doxorubicinloaded nanoparticles in tumor-bearing mice. Int. J. Pharm. 2005; 293: 281-290.

50. Chiannilkulchai N, Ammoury N, Caillou B, Devissaguet JP, Couvreur P. Hepatic tissue distribution of doxorubicin-loaded nanoparticles after i.v. administration in reticulosarcoma M 5076 metastasis-bearing mice. Cancer Chemother Pharmacol 1990; 26: 122-6.
51. Moghimi SM, Hunter AC, Murray JC. Long-circulating and target-specific nanoparticles: theory to practice. Pharmacol Rev 2001; 53: 283-318.
52. Storm G, Belliot S, Daemen T, Lasic D. Surface modification of nanoparticles to oppose uptake by the mononuclear phagocyte system. Adv. Drug Deliv. Rev. 1995; 17: 31-48.
53. Torchilin V, Trubetskoy V. Which polymer can make nanoparticulate drug carriers long circulating? Adv. Drug Deliv. Rev. 1995; 16: 141-155.
54. Jeon SI, Lee JH, Andrade JD, De Gennes PG. Protein-- surface interactions in the presence of polyethylene oxide : I. Simplified theory. J. Colloid Interface Sci. 1991; 142: 149-158.
55. Jeon SI, Andrade JD. Protein--surface interactions in the presence of polyethylene oxide : II. Effect of protein size. J. Colloid Interface Sci. 1991; 142: 159-166.
56. Stella B, Arpicco S, Peracchia M, Desmaele D, Hoebeke J, Renoir M, d'Angelo J, Cattel L, Couvreur P. Design of folic acid-conjugated nanoparticles for drug targeting. J. Pharm. Sci 2000; 89: 1452-1464.
57. Oyewumi MO, Yokel RA, Jay M, Coakley T, Mumper RJ. Comparison of cell uptake, biodistribution and tumor retention of folate-coated and PEG-coated gadolinium nanoparticles in tumor-bearing mice. J Control Release 2004; 95: 613-626.
58. Krishna R, Mayer L. Multidrug resistance (MDR) in cancer-mechanisms, reversal using modulators of MDR and the role of MDR modulators in influencing the pharmacokinetics of anticancer drugs. Eur. J. Cancer Sci 2000; 11: 265-283.
59. Larsen AK, Escargueil AE, Skladanowski A. Resistance mechanisms associated with altered intracellular distribution of anticancer agents. Pharmacol Ther 2000; 85: 217-29.
60. Bennis S, Chapey C, Couvreur P, Robert J. Enhanced cytotoxicity of doxorubicin encapsulated in polyisohexylcyanoacrylate nanospheres against multidrug-resistant tumour cells in culture. Eur J Cancer 1994; 30A: 89-93.
61. Damge C, Michel C, Aprahamian M, Couvreur P, Devissaguet JP. Nanocapsules as carriers for oral peptide delivery. J. Control. Release 1990; 13: 233-239.
62. Brandtzaeg P, Berstad A, Farstad I, Haraldsen G, Helgeland L, Jahnsen F, Johansen F, Natvig I, Nilsen E, Rugtveit J. Mucosal immunity – a major adaptive defense mechanism. Behring Inst. Mitt 1997; 98: 1-23.
63. Lee V, Yamamoto A. Penetration and enzymatic barriers to peptide and protein absorption. Adv. Drug Deliv. Rev. 1990; 4: 171-207.
64. Haltner E, Easson J, Lehr C. Lectins and bacterial invasion factors for controlling endo- and transcytosis of bioadhesive drug carrier systems. Eur. J. Pharm. Biopharm 1997; 44: 3-13.
65. Hussain N, Jani PU, Florence AT. Enhanced oral uptake of tomato lectin-conjugated nanoparticles in the rat. Pharm Res 1997; 14: 613-8.
66. Russell-Jones GJ, Arthur L, Walker H. Vitamin B12- mediated transport of nanoparticles across Caco-2 cells. Int. J. Pharm. 1999; 179: 247-255.
67. Russell-Jones GJ. The potential use of receptormediated endocytosis for oral drug delivery. Adv. Drug Deliv. Rev. 2001; 46: 59-73.

68. Schipper N, Olsson S, Hoogstrate J, de Boer A, Varum K, Artursson P. Chitosans as absorption enhancers for poorly absorbable drugs. 3: Influence of mucus on absorption enhancement. Eur J Pharm Sci 1999; 8: 335-43.
69. Lehr C, Bowstra J, Tukker J, Junginer H. Intestinal transit of bioadhesive microspheres in an in situ loop in the rat J Control Release 1990; 13: 51-62.
70. Bjork E, Isakkson U, Edman P, Artursson P. Starch microspheres induce pulsatile delivery of drugs and peptides across the epithelial barrier by reversible separation of the tight junctions. J Drug Target 1995; 6: 501-507.
71. Florence AT, Hussain, N. Transcytosis of nanoparticle and dendrimer delivery systems: evolving vistas. Adv Drug Deliv Rev 2001; 50 Suppl 1: S69-89.
72. Jani P, Halbert GW, Langridge J, Florence AT. The uptake and translocation of latex nanospheres and microspheres after oral administration to rats. J Pharm Pharmacol 1989; 41: 809-12.
73. Gurunathan S, Wu C, Freidag B. DNA vaccines: a key for inducing long-term cellular immunity. Curr. Opin. Immunol, 2000; 12: 442-447.
74. Panyam J, Zhou WZ, Prabha S, Sahoo SK, Labhasetwar V. Rapid endo-lysosomal escape of poly(DL-lactide-co-glycolide) nanoparticles: implications for drug and gene delivery. Faseb J 2002; 16: 1217-26.
75. Hedley M, Curley J, Urban R. Microspheres containing plasmid-encoded antigens elicit cytotoxic T-cell responses. Nat Med 1998; 4: 365-368.
76. Chen Y, Dalwadi G, Benson H. Drug delivery across the blood-brain barrier. Current Drug Delivery 2004; 1: 361-376.
77. Kreuter J. Influence of the surface properties on nanoparticle-mediated transport of drugs to the brain. J Nanosci Nanotechnol 2004; 4: 484-8.
78. Pardridge WM. Drug and gene targeting to the brain with molecular Trojan horses. Nat Rev Drug Discov 2002; 1: 131-9.
79. Ji B, Maeda J, Higuchi M, Inoue K, Akita H, Harashima H, Suhara T. Pharmacokinetics and brain uptake of lactoferrin in rats. Life Sciences 2006; 78: 851- 855.
80. Scherrmann JM, Temsamani J, The use of Pep: Trans vectors for the delivery of drugs into the central nervous system. International Congress Series 2005; 1277: 199-211.
81. Gabathuler R, Arthur G, Kennard M, Chen Q, Tsai S, Yang J, Schoorl W, Vitalis TZ, Jefferies WA. Development of a potential protein vector (NeuroTrans) to deliver drugs across the bloodbrain barrier. International Congress Series 2005; 1277: 171-184.
82. Pardridge WM. Drug and gene targeting to the brain via blood-brain barrier receptor-mediated transport systems. International Congress Series 2005; 1277: 49-62.