
REVIEW

POROUS NANOPARTICLES IN DRUG DELIVERY SYSTEMS

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ABSTRACT

This article concentrates mainly on fabrication of porous nanoparticles, its characterisation and its use for controlled release of drug. It also encompasses the strategies that have been used to translate and fabricate a wide range of particulate carriers e.g., nanospheres, liposomes, micelles, oil-in-water emulsions, with prolonged circulation and/or target specificity. Sol-gel technique is one of the most widely used techniques to fabricate porous nanoparticles within the polymer. Such nanoparticles have also applications in vascular drug delivery and release, site-specific targeting, as well as transfusion medicine.

With regard to the targeting issues, attention is particularly focused on the importance of physiological barriers. We have also critically reviewed and assessed the fate and activity of biodegradable polymeric drug delivery vehicles because the uniformity in degradation of these polymers is questionable.

This article will highlight rational approaches in design and surface engineering of nanoscale vehicles and entities for site-specific drug delivery. Potential pitfalls or side effects associated with nanoparticles are also discussed.

Keywords: Nanotechnology; nanoparticles; nanofibers; controlled-release; nanofabrication; biopharmaceuticals; porous nanoparticles; nanosized drug delivery systems; macrophage; endothelium; intracellular delivery; extravasation; toxicity; antituberculosis drugs; nanoparticles tuberculosis therapy. intracellular internalization; endocytosis; bone marrow differentiation.

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INTRODUCTION

Nanotechnology is a science of atomic scale phenomenon and mostly deals with particles ranging from 100 nm – 0.1 nm. It has now become possible to handle individual atoms; pick them up or place them from one place to another. Nanoparticles, now has endless uses and applications like manufacture of fogless car mirrors, fabric which does not absorb ink, mirror of TiO_2, nanosensors, carbon nanotubes etc. This multidisciplinary scientific field involves creation and utilization of materials, devices or systems which has enabled the development of an amazing variety of methods for fabricating nanoparticles in recent years. This technology is equally innovative and has a critical role in controlled release of drug delivery.

Research into the rational delivery and targeting of pharmaceutical, therapeutic, and diagnostic agents is at the forefront in nanomedicine. These involve the identification of precise targets (cells and receptors) related to specific clinical conditions and choice of the appropriate nanocarriers to achieve the required responses while minimizing the side effects. Mononuclear phagocytes, dendritic cells, endothelial cells, and cancers (tumor cells, as well as tumor neovasculature) are key targets. Today, approaches to particle design and formulation are expanding the market for many drugs and are forming the basis for a highly profitable niche within the industry, but some predicted benefits are hyped.

The development of a wide spectrum of nanoscale technologies is beginning to change the foundations of disease diagnosis, treatment, and prevention. These technological innovations, referred to as nanomedicines by the National Institutes of Health (Bethesda, MD, USA), have the potential to turn molecular discoveries arising from genomics and proteomics into widespread benefit for patients. Nanomedicine is a large subject area and includes nanoparticles that act as biological mimetics (e.g., functionalized carbon nanotubes), "nanomachines" (e.g., those made from interchangeable DNA parts and DNA scaffolds such as octahedron and stick cube), nanofibers and polymeric nanoconstructs as biomaterials (e.g., molecular self-assembly and nanofibers of peptides and peptide-amphiphiles for tissue engineering, shape-memory polymers as molecular switches, nanoporous membranes), and nanoscale microfabrication-based devices (e.g., silicon microchips for drug release and micromachined hollow needles and two-dimensional needle arrays from single crystal silicon), sensors and laboratory diagnostics (Moein *et al*, 2005).

Physicochemical characteristics

The clearance behaviour and tissue distribution of intravenously injected particulate drug carriers are greatly influenced by their size and surface characteristics (Poznansky and Juliano, 1984; Patel, 1992; Moghimi and Davis, 1994). These physicochemical parameters can control the degree of particle self-association (Ahl *et al*., 1997) in the blood as well as particle opsonization in biological fluids. The size of a particle may change substantially upon introduction into a protein-containing medium (e.g., plasma). Therefore, in the blood, particles and their aggregates should be small enough so that they are not removed from the circulation by simple filtration in the first capillary bed encountered (e.g., rat or mouse lung following tail vein injection). The opsonization process is the adsorption of protein entities capable of interacting with specific plasma membrane receptors on monocytes and various subsets of tissue macrophages, thus promoting particle recognition by these cells (Chonn *et al*., 1992; Moghimi and Davis, 1994; Gref *et al*., 1995; Moghimi and Patel, 1998; Moghimi and Hunter, 2000). Classical examples of opsonic molecules include various subclasses of immunoglobulins, complement proteins like C1q and generated C3 fragments (C3b, iC3b), apolipoproteins, von Willebrand factor, thrombospondin, fibronectin, and mannose-binding protein (Absolom, 1986; Patel, 1992; Serra *et al*., 1992; Chonn *et al*., 1995; Moghimi and Patel, 1996; Szebeni, 1998). On exposure to blood, particles of differing surface characteristics, size, and morphology attract different arrays of opsonins as well as other plasma proteins, the content and conformation of which may account for the different pattern in the rate and site of particle clearance from the vasculature (Moghimi and Patel, 1998). Since opsonization plays a major role in particle clearance from the blood, then interindividual variations in blood opsonic activity and concentration must also be considered. Undoubtedly, a clear understanding of such events is the first rational step for the design of colloidal carriers that target not only a relevant macrophage population but also for the engineering of long-circulating or macrophage-evading particles. It should also be emphasized that the interaction of particles with blood protein may have effects beyond opsonization. These may include interference with the blood-clotting cascade, a process that may lead to fibrin formation, and anaphylaxis because of complement activation.

It appears that evasion of particulate binding to, or uptake by, macrophages could be achieved to a certain extent by interference with protein adsorption and classical mechanisms of opsonization (e.g., prevention of complement activation in relevant species). In support of this statement, an early study with liposomes (Senior and Gregoriadis, 1982) demonstrated that small neutral unilamellar vesicles (100 nm or below), made from equimolar amounts of saturated phospholipids and cholesterol, have a longer circulation time in rats (half-lives up to 20 h) than their anionic counterparts (half-lives less than 1 h). These observations are in agreement with the process of complement opsonization of liposomes; neutral vesicles are poor activators of the complement system when compared with anionic liposomes (Volanakis and Wirtz, 1979; Chonn *et al*., 1991; Devine and Bradley, 1998). Therefore, the small-sized neutral vesicles are not efficiently coated with the opsonizing complement proteins and as a result are poorly recognized by Kupffer cells. However, in the case of larger neutral or anionic liposomes, clearance rates increased progressively with increasing size (Senior *et al*., 1985). This indicates that surface curvature changes may affect the extent and/or type of protein or opsonin adsorption. Indeed, vesicle size has been shown to play a critical role in complement activation (Devine *et al*., 1994; Harashima *et al*., 1994). From static in vitro studies, it appears that at a fixed lipid concentration larger liposomes are more efficient at activating complement than smaller vesicles (Devine *et al*., 1994). This probably suggests the importance of geometric factors and surface dynamics on the initial assembly of proteins involved in complement activation. Therefore, for vesicles larger than 100 nm other strategies must be sought to prevent surface opsonization processes. Indeed, the simplicity of the above approaches have led to the development of DaunoXome. This is a regulatory approved (U.S. Federal Drug Administration) formulation of daunorubicin citrate entrapped in small neutral unilamellar liposomes, made of high melting point phospholipid DSPC, for the treatment of Kaposi's sarcoma lesions.

Controlled drug delivery systems

Conventional dosage forms such as oral delivery and injections are predominant routes for drug delivery. However these types of dosage are not easily able to control the rate or drug delivery or the target area of the drug and are often associated with an intermediate or rapid drug release. Consequently the initial concentration of the drug in the body peaks above the level of toxicity and then gradually diminishes over time to an ineffective level. The duration of therapeutic efficacy then becomes dependent on the frequency of administration, and half life of the drug. High dosage of non targeted drugs are often administered to achieve an effective blood concentration (Tao and Desai, 2003).

Controlled drug delivery occurs when a polymer, whether natural or synthetic, is judiciously combined with a drug or other active agent in such a way that the active agent is released from the material in a pre-designed manner. The release of active agent may be constant over a long period of time, it may be cyclic over long period, or the environment or other external events may trigger it. In any case the purpose behind controlling the drug delivery is to achieve more effective therapies while eliminating the potential for both under and overdosing. Other advantages of using controlled release drug delivery are; maintenance of drug levels within desired range, need of fewer administration, optimal use of drug in question and patient compliance (Pillai & Panchagnula, 2001; Kwon & Kanob, 1996).

Where there are some advantages of controlled release of drugs, there are certain disadvantages, e.g., possible toxicity, non-compatibility of the material used, undesired by-products obtained by degradation of material and the higher cost of controlled release system as compared to traditional pharmaceutical formulations (Discher & Eisenberg, 2002).

An immense amount of interest has been increasingly placed on controlled release of drug delivery systems to maintain the therapeutic efficacy of these drugs. There are a number of mechanisms that can provide such controlled release of drugs, including transdermal patches, implants, bio-adhesive systems, and micro encapsulation (Tao & Desai, 2003). Controlled release of drugs using degradable polymers is well known and research is being carried out in designing newer class of materials including those whose release rate can be changed in vivo (Pillai & Panchagnula, 2001).

Nanovehicles as drug carriers

There are numerous engineered constructs, assemblies, architectures, and particulate systems, whose unifying feature is the nanometer scale size range (from a few to 250 nm). These include polymeric micelles, dendrimers, polymeric and ceramic nanoparticles, protein cage architectures, viral-derived capsid nanoparticles, polyplexes, and liposomes (Allen & Cullis, 2004; Moghimi *et al*., 2001; Sahoo & Labhasetwar, 2003; Panyam & Labhasetwar, 2004; Oh *et al*., 2004; Adams *et al*., 2003; Gao *et al*., 2002; Morgan *et al*., 2003; Krämer *et al*., 2004; Haag, 2004; Kossovsky 1996; Schmidt-Wolf & Schmidt-Wolf 2003; Raja 2003; Fenske *et al*. 2001; Verderone *et al*. 2000). First, therapeutic and diagnostic agents can be encapsulated, covalently attached, or adsorbed on to such nanocarriers. These approaches can easily overcome drug solubility issues, particularly with the view that large proportions of new drug candidates emerging from high-throughput drug screening initiatives are water insoluble. But some carriers have a poor capacity to incorporate active compounds (e.g., dendrimers, whose size is in the order of 5–10 nm). There are alternative nanoscale approaches for solubilization of water insoluble drugs too (Rabinow, 2004). One approach is to mill the substance and then stabilize smaller particles with a coating; this forms nanocrystals in size ranges suitable for oral delivery, as well as for intravenous injection (Rabinow, 2004; Liversidge & Cundy, 1995). Thus, the reduced particle size entails high surface area and hence a strategy for faster drug release. Pharmacokinetic profiles of injectable nanocrystals may vary from rapidly soluble in the blood to slowly dissolving. Second, by virtue of their small size and by functionalizing their surface with synthetic polymers and appropriate ligands, nanoparticulate carriers can be targeted to specific cells and locations within the body after intravenous and subcutaneous routes of injection (Allen & Cullis, 2004; Moghimi *et al*., 2001; Sahoo & Labhasetwar, 2003; Krämer *et al*., 2004; Raja, 2003; Fenske *et al*., 2001; Allen, 2002; Sudimack & Lee, 2000; Torchillin *et al*., 2003). Such approaches, may enhance detection sensitivity in medical imaging, improve therapeutic effectiveness, and decrease side effects. Some of the carriers can be engineered in such a way that they can be activated by changes in the environmental pH, chemical stimuli, by the application of a rapidly oscillating magnetic field, or by application of an external heat source (Moghimi *et al*., 2001; Drummond *et al*., 2000; Panyam *et al*., 2002; Clark *et al*., 1999). Such modifications offer control over particle integrity, drug delivery rates, and the location of drug release**,** for example within specific organelles. Some are being designed with the focus on multifunctionality; these carriers target cell receptors and delivers simultaneously drugs and biological sensors (Quintana *et al*., 2000). Some include the incorporation of one or more nanosystems within other carriers, as in micellar encapsulation of QDs; this delineates the inherent nonspecific adsorption and aggregation of QDs in biological environments (Dubertert *et al*., 2002). In addition to these, nanoscale-based delivery strategies are beginning to make a significant impact on global pharmaceutical planning and marketing (Allen & Cullis, 2004; Sahoo & Labhasetwar, 2003; Moghimi *et al*., 2005).

Micelles for drug delivery

Micelles are self-assemblies of amphiphiles that form supramolecular core-shell structures in the aqueous environment. Hydrophobic interactions are the predominant driving force in the assembly of the amphiphiles in the aqueous medium when their concentrations exceed the critical micelle concentration (CMC) (Tanford, 1991). In this review we cover only the micelles that fall into the nanosize range that are formed with amphiphilic polymers. Typical classical surfactant micelles are not included in this review. Most nanosized micellar delivery systems are made up of amphiphilic polymers that consist of PEG and a low-molecular-weight hydrophobic core-forming block. Usually, the molecular weight of PEG (the outer corona component) is higher than the molecular weight of the hydrophobic core-forming block (Kwon, 2003). These types of micelles are generally smaller than 100 nm (Koo *et al.*, 2005; Kwon, 2003; Torchilin 2002; Ashoke *et al.*, 2004) and have CMC in the micromolar range (Torchilin, 2002; Ashoke *et al.*, 2004). Due to low monomer concentration in equilibrium with the micelles, these micellar delivery systems have reduced toxicity and are more thermodynamically stable to dilution compared to classical micelles formed with traditional surfactants that have CMCs orders of magnitude higher. Furthermore, nanosized micelles have polarity gradients from the highly hydrated corona to the hydrophobic core (Torchilin, 2002), and are used for solubilization of hydrophobic compounds of varying polarities by physical association with different regions within the micelles without drug modification. Finally, the biodistribution and pharmacokinetics of drugs such as doxorubicin, cisplatin, and paclitaxel are altered favorably, such as increased circulation half-life and tumor accumulation, when compared to free drug (Garrec *et al.*, 2004).

Hollow nanocrystals

Hollow nanocrystals can be synthesized through a mechanism analogous to the Kirkendall Effect, in which pores form because of the difference in diffusion rates between two components in a diffusion couple. It has been shown that reaction of cobalt nanocrystals in solution with oxygen and either sulfur or selenium leads to the formation of hollow nanocrystals of the resulting oxide and chalcogenides. This process provides a general route to the synthesis of hollow nanostructures of a large number of compounds. A simple extension of the process yielded platinum–cobalt oxide yolk-shell nanostructures, which may serve as nanoscale reactors in catalytic applications (Yin *et al.*, 2004).

Diffusion of nanoparticles in agarose gel

The blood-brain barrier (BBB) is the bottleneck in brain drug development and is the single most important factor limiting the future growth of neurotherapeutics. Histamine with a molecular mass of ~100 Da readily crosses the porous capillaries perfusing all peripheral tissues but is excluded from entry into the brain or spinal cord by the BBB (Pardridge, 2005).

Size effect on the diffusion properties of various media, more particularly, gels have extensively been studied Diffusion properties in random media such as soils (Sahimi, 1993), gels (Starchev *et al*., 1997; Pluen *et al*., 1999) bacterial cytoplasm (Berland *et al*., 1995; Schwille *et al*., 1999), membranes (Saffman & Delbrück, 1975; Peters & Cherry, 1982; Ghosh & Webb, 1988) and channels (Wei *et al*., 2000) are subject to considerable theoretical and experimental interest. Diffusion in gels is an important component in many biological (Ottenbrite & Huang, 1996), pharmaceutical (De Rossi *et al*., 1991), and environmental applications (Buffle, 1988). The hindered diffusion of solutes in gels has been modeled (Amsden, 1998) and is well-documented for agarose gels (Johnson *et al*., 1996; Pluen *et al*., 1999). Agarose gel is the preferred chromatographic medium used for separating biological molecules of molecular mass >250 kDa, for which minimal nonspecific binding and retention of the biological activity is required.

To investigate diffusion processes in agarose gel, nanoparticles with sizes in the range between 1 and 140 nm have been tested by means of fluorescence correlation spectroscopy. Understanding the diffusion properties in agarose gels is interesting, because such gels are good models for microbial biofilms and cells cytoplasm. The largest hydrodynamic radius of trapped particles that displayed local mobility was estimated to be 70 nm for a 1.5% agarose gel. The results showed that diffusion of particles in agarose gel is anomalous, with a diverging fractal dimension of diffusion when the large particles become entrapped in the pores of the gel. The latter situation occurs when the reduced size of the diffusing particle, *A*, is >0.4. Variations of the fractal exponent of diffusion with the reduced particle size were in agreement with three-dimensional Monte Carlo simulations in porous media (Fatin-Rouge *et al.*, 2004).

Large porous nanoparticles

Large porous nanoparticle (LPNP) systems can be made of diverse materials, prepared in a variety of different conditions, and designed to deliver drugs to specific sites of the body by using nanoparticles (NPs) with diameters ranging from 25 nm to several hundred nm. They appear to be robust drug delivery systems that may be useful for encapsulating drugs of varying chemistry and molecular weight into biodegradable NPs (Lamprecht *et al.,* 1999), thereby combining the persistence advantages of NPs with the delivery convenience of large porous particles (LPPs). Their ultimate practical utility for drug delivery requires incorporation of drug, exploration of the use of other biocompatible materials, and delivery to human and animals.

Nanoparticle systems having drug release and delivery potential with the ease of flow, processing, and aerosolization have been reported having potential of large porous particle (LPP) systems by spray drying solutions of polymeric and nonpolymeric NPs into extremely thin-walled macroscale structures. These hybrid LPPs exhibit much better flow and aerosolization properties than the NPs; yet, unlike the LPPs, which dissolve in physiological conditions to produce molecular constituents, the hybrid LPPs dissolve to produce NPs, with the drug release and delivery advantages associated with NP delivery systems.

LPPs are characterized by geometric sizes larger than 5 µm and mass densities around 0.1 g/cm^3 or less, have achieved popularity as carriers of drugs to the lungs for local and systemic applications (Edwards *et al.*, 1997; Edwards, 2002). A principal advantage of LPPs relative to conventional inhaled therapeutic aerosol particles is their aerosolization efficiency (French *et al.*, 1996); in addition, LPPs possess the potential for avoidance of alveolar macrophage clearance (Kawaguchi *et al.*, 1986; Krenis & Strauss, 1961; Rudt & Muller, 1992), enabling sustained drug release in the lungs (Vanbever *et al.*, 1999).

Particles with geometric diameters less than a few hundred nanometers (Oberdörster, 2001) represent an even more tenacious resident of the lungs. Once deposited, nanoparticles (NPs) or "ultrafine" particles often remain in the lung lining fluid until dissolution (assuming they are soluble), escaping both phagocytic and mucociliary clearance mechanisms (Kawaguchi *et al.*, 1986; Krenis & Strauss, 1961; Rudt & Muller, 1992; Vanbever *et al.*, 1999). Thus, deposition of drug-bearing NPs in the lungs may offer the potential for sustained drug action and release throughout the lumen of the lungs, where macrophage clearance occurs. However, the utility of NPs for drug release is severely limited because of their low inertia, which causes them to be predominantly exhaled from the lungs after inspiration (Heyder *et al.*, 1986). Moreover, their small size leads to particle–particle aggregation, making physical handling of NPs difficult in liquid and dry powder forms. This is a common practical problem that must be overcome before using NPs for oral drug delivery (Kabbaj & Phillips, 2001). As a result of these limitations, NPs are not presently being explored commercially or clinically as vehicles for drug delivery in the lungs.

Recently, a form of particle for drug delivery have been developed that combines the advantages of LPPs and NPs while avoiding their limitations. Tsapis *et al*. (2002) used spray drying to form LPPs comprised of NPs held together by physical means, such as Van der Waals forces, or within a matrix of additional ingredients such as biopolymers or phospholipids. These large porous NP (LPNP) aggregates have the same physical and delivery properties as LPPs, yet, once deposited in the lungs (or placed in a physiological or physiological-like environment), they disassociate to yield NPs, with their inherent attractive features for drug delivery.

Formation of the large porous NP (LPNP) aggregates occurs via a spray-drying process that ensures the drying time of the sprayed droplet, which is sufficiently shorter than the characteristic time for redistribution of NPs by diffusion within the drying droplet, implying a local peclet number much greater than unity. Additional control over LPNPs physical characteristics is achieved by adding other components to the spray-dried solutions, including sugars, lipids, polymers, and proteins. The ability to produce LPNPs appears to be largely independent of molecular component type as well as the size or chemical nature of the NPs.

The LPNPs tend to be spherical and hollow as indicated by the SEM pictures in figures 1 *a* and *b*, with a wall thickness of approximately 400 nm, consisting of three distinct layers of NPs. These LPNPs have several attractive features: they are comprised solely of NPs; are readily redispersed as NPs in solution (figure 1*c*), yet the LPNPs are readily dispersed as aerosols.

LPNPs can also be formed with NPs of smaller sizes. The SEM pictures (figure 2 *Upper*) show a LPNP particle structure with 25 nm NPs, similar to the one obtained with the 170-nm NPs. However, in this case, the shell thickness is approximately 200 nm and consists of eight layers of NPs.

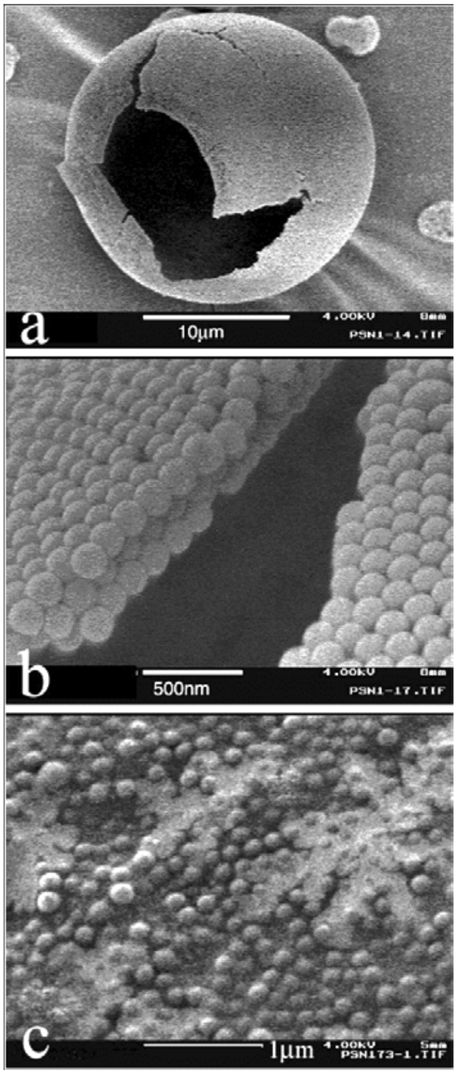

Fig. 1: SEM images of (*a*) a typical hollow sphere LPNP observed from the spray drying of a solution of PS NPs (170 nm), (*b*) a magnified view of the particle surface in *a*, and (*c*) the NPs in solution after redissolving the LPNPs in a mixture of 70:30 ethanol/water (vol/vol). LPNPs dissolve readily into the NPs once in solution.

The introduction of a second nonvolatile species, such as lactose a common material in spray-dried particles, gives an insight into the formation mechanism of the LPNPs (Hennigs *et al*, 2001). Addition of 70% (wt/wt) polystyrene NPs (170 nm) to the lactose in solution produces high-quality LPNPs, with aerodynamic diameter 4 µm ± 2 µm and geometric diameter $d = 8 \pm 3$ µm, as illustrated in figure 2 *Lower*. LPNPs can also be formed with other molecular species as well. In addition to lactose, LPNPs have been described by using hydroxypropylcellulose, BSA, and lipids (figures 3 and 4). The chemical nature of the NPs also seems to be of little importance, since Tsapis *et al.* (2002) produced LPNPs by replacing polystyrene NPs with colloidal silica NPs, as shown in figure 3 *Bottom Right*. In all cases, the LPNPs had a solid deformable shell, consisting of several layers of NPs, and had a wrinkled structure indicative of a low relative density, making their aerodynamic properties highly favorable.

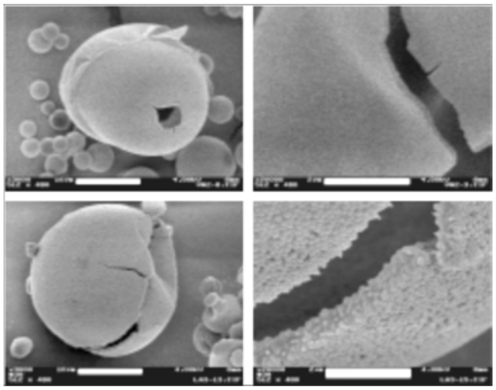

Fig. 2: SEM images of typical hollow spheres observed from the spray drying of a solution of PS NPs (25 nm, *Upper*) and a solution of lactose and PS NPs (170 nm, 70% of total solid contents in weight, *Lower*) [Scale bars: 10 µm (*Left*) and 2 µm (*Right*)].

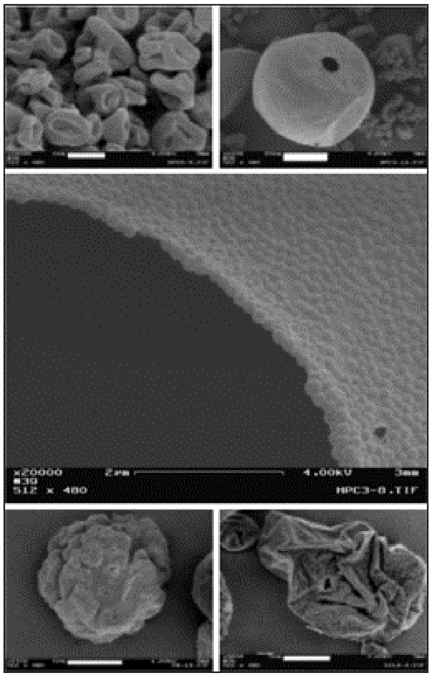

Fig. 3: SEM images of a typical hydroxypropylcellulose spray-dried particle without (*Top Left*) and with (*Top Right*) NPs. (*Middle*) A magnification of the particle surface (*Top Right*). (*Bottom*) A typical particle observed from the spray drying of a solution of BSA and PS NPs (170 nm, 80% of total solid contents in weight, *Bottom Left*), and a typical particle observed from the spray drying of a solution of lipids/lactose and colloidal silica (□100 nm, 88% of total solid contents in weight, *Bottom Right*). [Scale bars: 2 µm (*Top Left* and *Middle*), 20 µm (*Top Right*), and 5 µm (*Bottom*).]

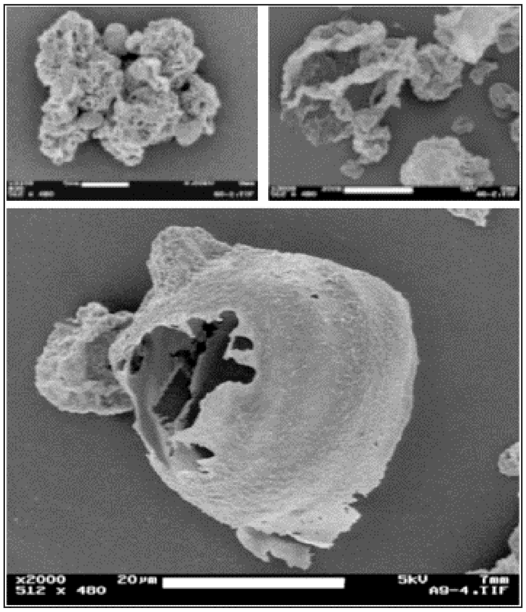

Fig. 4: SEM pictures of typical particles from the spray drying of a solution of lipids and lactose with increasing concentration of PS NPs (170 nm): 0% (*Upper Left*), 35% (*Upper Right*), and 82% of total solid content (*Lower*). [Scale bars: 5 µm (*Upper Left*), 10 µm (*Upper Right*), and 20 µm (*Lower*)].

To investigate the effect of the quantity of NPs on the final LPNP structure, Tsapis *et al* (Tsapis *et al* 2002) produced LPNPs by using a combination of three molecular constituents DPPC (1,2-dipalmitoyl-sn-glycero-3-phosphocholine), DMPE (1,2-dimyristoyl-sn-glycero-3-phosphoethanolamine) and lactose whose colloidal properties lead to LPP formation. Without NPs, the spray-dried particles show an excellent LPP structure, reminiscent of crumpled paper, as shown in figure 4. They have excellent aerodynamic properties, with $d = 8$ µm and $d_{aer} = 3$ µm. The formation mechanism of LPPs from a lipid solution is slightly different from with NPs, with the lipids spontaneously forming colloidal aggregates during the drying process. These aggregates play the role of the NPs in the shell formation process. Adding PS NPs (170 nm) to the lipid solution produces a spray-dried particle structure that is even more crumpled. At very high NP concentrations, hollow shells are formed, as shown in figure 4.

Biomineralization

Biomineralization, the production of inorganic phases (oxides, sulfides, silica, carbonates, and phosphates) by living organisms, produces metabolic energy and/or mechanical support for a variety of organisms from unicellular to mammalian. Although the starting point for the concentration and transformation of components in an aqueous medium to form crystals is generally assumed to be an aqueous solution containing dissolved ions, there is increasing evidence that clusters, nanoscale amorphous precipitates, and other more complex precursors in the aqueous phase may play an important role in crystallization (Banfield *et al.*, 2000; Furrer *et al.*, 2002; Yang & Navrotsky, 2002).

For biominerals there are several noticeable gaps in surface-energy data. The surface energies of calcite, aragonite, and vaterite are not known from direct experimental measurement. There seems to be little data for the surface energy of any of the varieties of hydroxyapatite and related phosphates, although Suzuki *et al.* (Suzuki *et al.*, 2004) recently determined the surface tension of chlorapatite. There is still some uncertainty about the surface energy of silica (Moloy *et al.*, 2002). A current working hypothesis, supported by available data, is that silica glass, amorphous silica prepared near room temperature, and zeolitic and mesoporous silicas all have a similar and rather low surface energy of $\approx$0.1 J/m^2 (Moloy *et al.*, 2002), and this value applies to both external surfaces and those on the inside of pores and channels (Moloy *et al.*, 2002; Li *et al.*, 2003). A corollary is that the energy of a zeolite changes very little after diminution of particle size, because the internal surface area dominates the external, even for particles as small as 30 nm (Li *et al.*, 2003). Similar trends may apply to porous amorphous silica materials in which neither the pores nor the frameworks are periodic.

Mesoporous silica materials, with amorphous silica frameworks but a periodic arrangement of pores in the 1- to 10-nm size range, have been synthesized by coassembly with surfactants (Kresge *et al.*, 1992). These materials are energetically similar to the larger-pore zeolite structures. Because the mesoporous materials are built by using larger molecules than for zeolite synthesis, one might ask whether natural lipids, sugars, and small proteins can also self-assemble with silica. The sizes of the pore and the silica wall are determined by the character of the surfactant micelle. Probably many failed syntheses have contained comparable porosity on the nanoscale but lacking periodicity. Do organisms self-assemble analogous mesoporous periodic, semiperiodic, or aperiodic structures? The synthesis of amorphous silica spicules in marine sponges, mediated *in vivo*, and also in laboratory studies, by protein filaments (Freer *et al.*, 2005), may be an example of such assembly.

Kinetically, the porous and typically hydrated nanophase precursor provides the right level of reactivity. It is neither too reactive, like ions in solution, nor too inert, like the final mineral (Alexandra, 2004).

Nanoparticles in therapy of restenosis

Restenosis is a serious complication of coronary angioplasty that involves the proliferation and migration of vascular smooth muscle cells (VSMC) from the media to the intima, synthesis of extracellular matrix, and remodeling (Reidy, 1985; Clowes *et al.*, 1989). Lanza *et al.* (2002) studied the concept of VSMC-targeted nanoparticles as a drug-delivery platform for the prevention of restenosis after angioplasty is studied. Early local administration of numerous therapeutic agents into injured vessel walls poorly inhibited smooth muscle cell proliferation (Lincoff *et al.*, 1997; Strecker *et al.*, 1998; Liu *et al.*, 1997; Axel *et al.*, 1997; Kalinowski *et al.*, 1999; Nozawa *et al.*, 1999; Oberhoff *et al.*, 1997; Song *et al.*, 1998; Suh *et al.*, 1998; Valero *et al.*, 1998). The newer stent-based drug-delivery systems, particularly devices incorporating hydrophobic antiproliferative agents, have been successful in the clinic (Sousa *et al*, 2001; Sousa *et al.*, 2001a; Rensing *et al.*, 2001). These promising results indicate that local deposition and prolonged release of appropriate antiproliferative agents can effectively ameliorate restenosis. These results highlight an opportunity for drug-delivery systems that achieve similar prolonged release of appropriate therapeutics directly within the tunica media to provide effective antirestenotic treatment, particularly in vessels less amenable to stent therapy.

Ligand-directed nanoparticles are a novel, site-specific agent that can be administered locally and can penetrate into the tunica media via microfractures created by balloon overstretch injury. Lanza *et al* (Lanza *et al.,* 2002) demonstrated that tissue factor (TF)–targeted nanoparticles specifically bind with high avidity to smooth muscle cell membranes *in vivo* (Lanza *et al*., 2000; Lanza *et al*., 2000a) and may be readily detected with intravascular ultrasound. Targeted nanoparticles bound to medial smooth muscle cells could also provide a unique vehicle to deliver antiproliferative chemotherapeutic agents, like those eluting off stents in the lumen, directly within the balloon-injured vascular wall.

The potential of site-directed nanoparticles to incorporate and deliver potent lipophilic antiproliferative agents with different water solubilities, i.e., doxorubicin (highly water soluble) and paclitaxel (poorly water soluble) have been studied. Moreover, the unique potential to utilize T_1-weighted MRI to visualize nanoparticle delivery and the opportunity to quantify local drug dosimetry with ^{19}fluorine spectroscopy are demonstrated. Collectively, these experiments illustrate that targeted therapeutic nanoparticles could provide visualizable and quantifiable therapy to prevent restenosis after percutaneous revascularization.

Mesoporous nanoparticles in stem cell tracking

Tracking the distribution of stem cells is crucial to their therapeutic use. A cellular labeling approach with a novel vector composed of mesoporous silica nanoparticles (MSNs) conjugated with fluorescein isothiocyanate in human bone marrow mesenchymal stem cells and 3T3-L1 cells has been reported. The mechanism about fluorescein isothiocyanate-conjugated MSNs (FITC-MSNs) internalization was also studied. FITC-MSNs were efficiently internalized into mesenchymal stem cells and 3T3-L1 cells even in short-term incubation. The process displayed a time- and concentration-dependent manner and was dependent on clathrin-mediated endocytosis. In addition, clathrin-dependent endocytosis seemed to play a decisive role on more internalization and longer stay of FITC-MSNs in mesenchymal stem cells than in 3T3-L1 cells. The internalization of FITC-MSNs did not affect the cell viability, proliferation, immunophenotype, and differentiation potential of mesenchymal stem cells, and 3T3-L1 cells. Finally, FITC-MSNs could escape from endolysosomal vesicles and retained the architectonic integrity after internalization. In conclusion, the advantages of biocompatibility, durability, and higher efficiency in internalization suit MSNs to be a better vector for stem cell tracking than others currently used (Hauang *et al.*, 2005).

Nanoparticle drug delivery systems in chemotherapy of tuberculosis

Nanoparticle-based drug delivery systems have considerable potential for treatment of tuberculosis (TB). The important technological advantages of nanoparticles used as drug carriers are high stability, high carrier capacity, feasibility of incorporation of both hydrophilic and hydrophobic substances, and feasibility of variable routes of administration, including oral application and inhalation. Nanoparticles can also be designed to allow controlled (sustained) drug release from the matrix. These properties of nanoparticles enable improvement of drug bioavailability and reduction of the dosing frequency, and may resolve the problem of nonadherence to prescribed therapy, which is one of the major obstacles in the control of TB epidemics.

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Received: 15-04-2006 – Accepted: 15-05-2006