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Recent developments in nanoparticle-based drug delivery and targeting systems with emphasis on protein-based nanoparticles

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Background: Drug delivery systems with nm dimensions (nanoparticles [NPs]) are attracting increasing attention because they can sequester drugs in systemic circulation, prevent non-specific biodistribution, and target to specific tissues. *Objective*: We reviewed the recent literature pertinent to NP-based drug delivery, primarily emphasizing NPs fabricated from proteins. *Methods*: A summary of common NP fabrication techniques is provided along with the range of sizes and functional properties obtained. The NP properties critical for injectable drug delivery are reviewed, as well as the attempts to design 'tissue-specific' NPs. *Results/conclusions*: It has been possible to design > 100 nm NPs from different biomaterials, and further understanding of *in vivo* stability and interactions with physiologic systems will lead to improved drug delivery systems.

Keywords: drug delivery, nanoparticles, nanoparticle fabrication, particle size, phagocytosis, surface modification, tissue targeting

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1. Overview of nanoparticulate drug carriers

Injectable colloidal systems (hereon referred to as nanoparticles [NPs]) hold promise for systemic administration of different therapeutic agents, including conventional pharmacologic agents, disease-modifying bioactive factors (e.g., interleukins) and protein growth factors, as well as nucleic acid-based agents intended for gene-based therapies. Nanoparticulate drug delivery systems in the form of solid spheres, micellar emulsions and liposomes (this review does not focus on micellar and liposomal systems and the reader is referred to other reviews on these topics) have been used in the past 20 years as injectable formulations for systemic and tissue-specific delivery of drugs. Several types of biodegradable macromolecules have served as the foundation of drug carriers (**Table 1**). Conventional synthetic polyesters, whose prototypical examples include poly(lactic acid) (PLA) and poly(lactic-co-glycolic acid) (PLGA), have been extensively used: i) their long history of clinical use brings comfort to their tolerability in the body; ii) their well-understood degradation pattern allows better engineering of drug-release kinetics; and iii) established processing methodologies enable a relatively 'homogenous' product whose performance is better predicted and controlled.

Proteins, primarily members of the albumin family, have served as an alternative biomaterial for carriers, facilitated by the obvious compatibility of this material in physiologic systems. Administration of albumin in the body should not raise any adverse effects as long as the source of the protein is free of transmissible diseases.

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Table 1. A representative summary of biomaterials used for the preparation of nanoparticulate delivery systems and drugs used for delivery.

Biomaterial	Drugs	Particle size (nm)	Author [ref.]
Polyester			
Poly(lactic acid)	Protein C	200 – 250	Zambaux [75]
Poly(lactic-co-glycolic acid)	Estrogen	40 – 60	Choi [54]
Poly(ε-caprolactone)	Tamoxifen	250 – 300	Chawla [6]
Protein			
Human serum albumin	Doxorubicin	150 – 500	Dreis [29]
Bovine serum albumin	IFN-γ	300 – 340	Segura [26]
β-Lactoglobulin	–	60 – 130	Ko [21]
Protamine	Oligonucleotides	80 – 200	Lochmann [89]
Gelatin	Clodronate	300 – 500	Li [67]
Gliadin	α-Tocopherol/benzalkonium chloride/linalool and linalyl acetate	900 – 950	Duclairoir [90]
Legumin	–	250 – 300	Irache [91]
Polysaccharide			
Chitosan	Ovalbumin	350	Amidi [43]
Polycyanoacrylate			
Poly(isobutylcyanoacrylate), Poly(isohexylcyanoacrylate)	Oligonuclceotides	N/A	Fattal [92]
Poly(butylcyanoacrylate)	Doxorubicin	178	Reddy [93]
Poly(hexadecylcyanoacrylate)	–	150	Peracchia [94]
Lipids			
Trimyristin	Paclitaxel	200 – 250	Lee [95]
Glycerol monostearin	Mifepristone	27	Yuan [96]
Glyceryl behenate	Vitamin a	300 – 500	Jenning [97]
Polyelectrolyte			
Polyethylenimine	Ciclosporin	100 – 300	Cheng [98]
Poly(styrene sulfonate)/poly(allylamine.HCl)	Chymotrypsin	100 – 300	Balabushevitch [41]
Poly(L-maleic acid)/Chitosan	Insulin	100 – 250	Fan [42]

Two albumin-based particulate formulations – Albunex™ as an ultrasound contrast agent and Abraxane™ as a carrier of anticancer drug paclitaxel [1] – have been accepted for use in humans. Some plant proteins, such as legumin and gliadin, have also been attempted in the development of NP-based drug delivery systems. Due to the unique structure of proteins with multifunctional moieties and/or domains, it might be possible to tailor unique carrier-drug combinations when proteins are used as carriers. For example, albumin – with its hydrophobic core – displays a high affinity for hydrophobic drugs and such combinations may form exceptionally slow-release formulations under physiologic situations. As the primary structure of proteins contains -NH_2, -COOH, -SH and other functional groups, protein NPs can offer various possibilities for covalent as well as non-covalent (electrostatic) modification of particulate surfaces. The latter is critical for the design of tissue-specific carriers or when physiologic responses to carriers need to be controlled. This is an advantage over polymeric biomaterials, which are rather homogenous and typically provide only a single functional group for modifications. Multifunctional polymers from copolymerization strategies might need to be alternatively used to accommodate various modifications for improved drug delivery.

In addition to proteins and polyesters, other macromolecules used to formulate NP carriers include polysaccharides, synthetic degradable acrylates (e.g., polycyanoacrylate)

and natural/synthetic polyelectrolytes (e.g., chitosan and polyethylenimine [PEI]). The biologic fate of the latter macromolecules is less understood and may pose a challenge for their clinical development. Another attractive idea to achieve controlled drug delivery and release is the use of solid lipid NPs (SLNs), which are primarily derived from middle-chain triglycerides (e.g., trimyristin) or partial glycerides (e.g., Imvitor® 900 and Compritol® 888 ATO) as lipid phase, with other ingredients including emulsifier and water [2].

This review summarizes the recent developments in NP-based drug delivery systems for systemic administration of therapeutic agents, with emphasis on the NPs fabricated from PLA (or PLGA) and albumin, which are two of the most widely used families of macromolecules. The methods for formulating NP carriers, the surface modification of the NPs and the physicochemical properties in the design of drug delivery systems are evaluated; and finally the functionalization of specific tissue-targeting delivery is discussed in detail.

2. Principal preparation methods for nanoparticles

Several distinct methods developed for the preparation of NPs include emulsification solvent extraction, 'salting out' or precipitation, spray drying, solvent displacement, and coacervation and complex formation. Significant overlap exists among some of these methods and it is common to combine more than one process to obtain NPs tailored for specific applications. Each method usually needs to be optimized for entrapment of a drug of interest at high efficiency and retention of the desired pharmacologic activity (especially important for protein therapeutics). An additional requirement for process success is the control over final NP size, usually the diameter. A survey of the literature indicated that most reported NPs are between 100 and 1000 nm. **Figure 1** summarizes typical sizes reported for NPs prepared from polyesters, proteins and chitosan as representative biomaterials. Although some processes are able to yield sub-100-nm sizes, this is rare. Three of the more promising methods are emulsion/solvent evaporation, coacervation or desolvation, and polyelectrolyte complexation; **Figure 2** schematically outlines the principles underlying these methods.

2.1 Emulsion/solvent extraction process

The emulsification and solvent extraction process is a widely used method for polymeric NPs. It is possible to adopt this technique for protein NPs, although particle sizes are generally larger as compared to the coacervation process described in Section 2.2 [3]. In its simplest form, a polymer solution (in organic solvent [O]) or protein solution (in aqueous buffer [W]) is dispersed in an appropriate non-solvent to form O/W or W/O emulsions, after which the solvents/non-solvents are removed to form the NPs (**Figure 2A**). Use of certain organic solvents, such as ethylacetate and chloroform, and surface-active agents, such as poly(vinyl alcohol) (PVA) and polysorbate-80, is not desirable because they may alter the bioactivity of protein therapeutics [4,5]. They may additionally elicit undesired reactions against NPs and need to be reduced to insignificant levels. Surfactant are critical to control particle size: surfactants such as Pluronics™ stabilize the formation of smaller fluid particles in emulsions that lead to smaller solid particles [6]. Such a stabilization results from an interaction of the surfactants with the NP matrix. Surfactants are likely to influence drug–matrix interactions and possibly the release rate of drugs in the physiologic milieu [6]. It should be noted that it was possible to prepare PLGA NPs without surfactants [7]. The W/O method was recently used to prepare surface-functionalized NPs after the addition of the desired molecules into the aqueous phase [8]. The W/O/W double-emulsion method can particularly be used for the encapsulation of proteins and hydrophilic drugs [9]; the primary W/O emulsion is introduced into a second water phase to form a double emulsion by using a surfactant for emulsion stabilization. The organic solvent O is then removed and the NPs are finally retained in the aqueous medium.

For PLGA NPs, polymer concentration and solvent composition were the primary factors affecting size [10]. Encapsulation of conventional drugs (e.g., doxorubicin) could be optimized by controlling the ionization state of the drugs [11]. Proteins can be also encapsulated in PLGA NPs with high efficiency (e.g., > 50% [12] or 30 – 50% [13]). For protein NPs, protein concentration and relative volume of solvent:non-solvent during emulsification were the critical parameters [3]. Bovine serum albumin (BSA) NPs with sizes in the range of 100 – 800 nm were reported in a W/O method, dependent on BSA concentration and relative W:O volume ratio [14]. Excellent encapsulation efficiency (> 80%) could be obtained with model macromolecular compounds (e.g., Fluorescein isothiocyanide (FITC)-dextran), partly due to minimal solubility of the compound in the non-aqueous phase. Encapsulation efficiency of conventional drugs is usually lower (e.g., 10 – 60% for tamoxifen) [6]. The bone-inducing morphogen BMP-2 was formulated with PLGA/heparin NPs [15], but protein loading was performed by adsorption post-fabrication, even though prior evidence suggests compatibility of the emulsion process with growth factors such as BMPs [16] and IGFs [17].

2.2 Coacervation process

The coacervation or desolvation process under mild conditions is preferable for the preparation of protein-based NPs and encapsulation of protein drugs. A colloidal system is formed when the solvent used for dissolving the NP matrix is extracted into a non-solvent phase, forming a phase with a colloidal component or coacervate, and a second phase with a solvent/non-solvent mixture (**Figure 2B**). The particle formation initially proceeds with an increase in size until

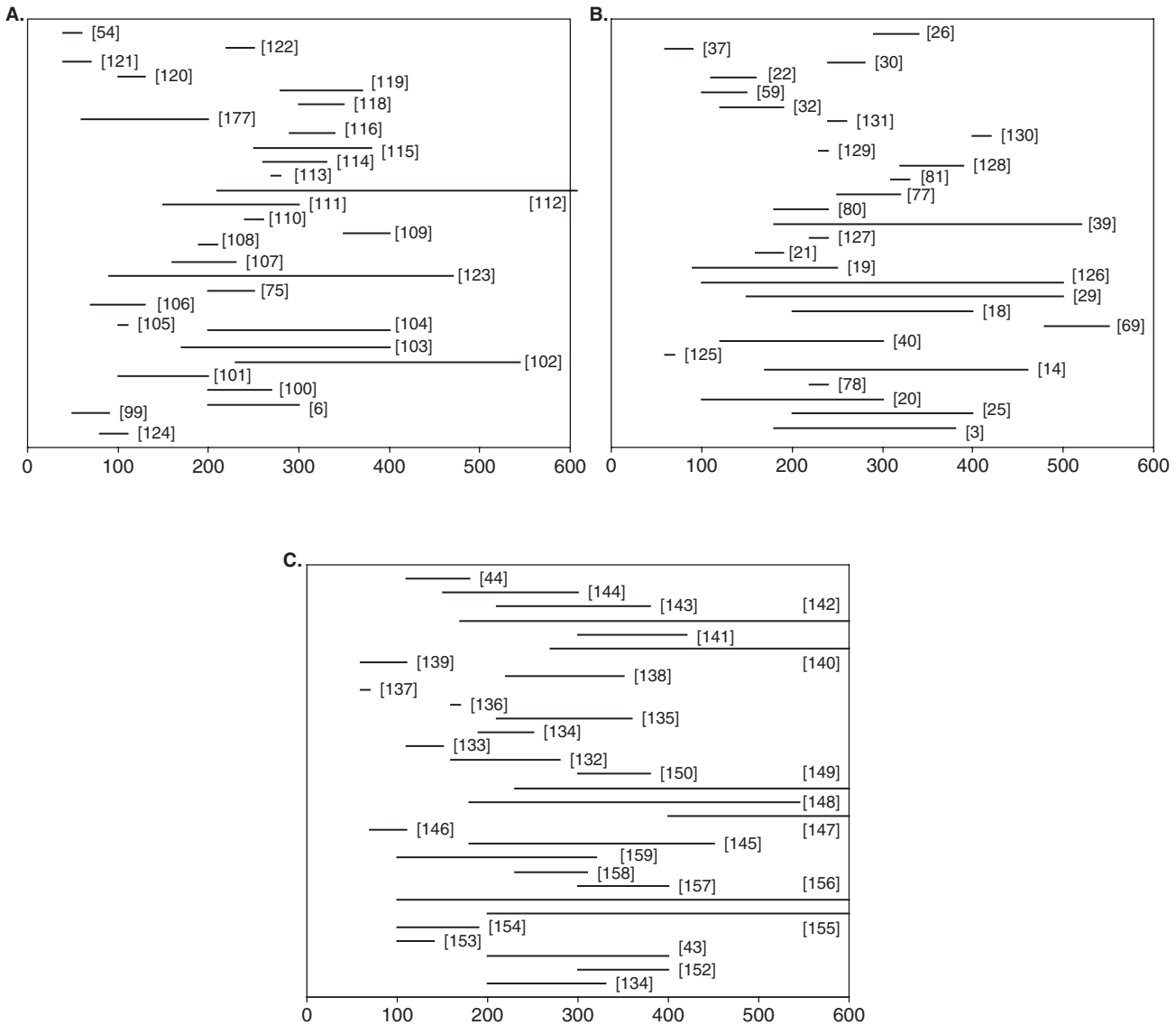

Figure 1. Typical size (nm) ranges of (A) polyester, (B) protein and (C) chitosan nanoparticles reported in the literature. Note that the chosen references were not intended to be exhaustive, but rather representative of common size ranges reported. Chitosan was chosen as a representative polyelectrolyte-based nanoparticle because it has recently received attention for a diverse range of biomedical applications.

a stable size is reached, after which the number of particles gradually increase with increasing desolvation [18]. Lin *et al.* [19] described a pH-controlled coacervation method to prepare ~ 100 nm human serum albumin (HSA) NPs. The particles were prepared by acetone addition to an aqueous HSA solution at pH 7 – 9, and followed by stabilization of the particles by glutaraldehyde (GA) cross-linking. Langer *et al.* [18,20] prepared HSA NPs via desolvation with ethanol; process parameters such as HSA concentration, rate of non-solvent (ethanol) addition, pH of coacervation phase and purification conditions were evaluated and optimized for particle sizes between 100 and 300 nm. The pH prior to desolvation procedure was identified as the main factor affecting the size, with high pH values leading to smaller particles [20]. This was independently confirmed in our laboratories using ethanol as the non-solvent and BSA as the matrix (**Figure 3**). The pH and, to a lesser degree, osmolarity were important in maintaining the stability (size) of the particles because conditions leading to net-zero surface charge were more conducive to particle aggregation [20]. Replacing BSA with the smaller β-lactoglobulin (a similar isoelectric point [pI] to BSA) led to a reduction in particle sizes (from 170 to 130 nm with acetone as the non-solvent), presumably due to lower hydrophobicity of

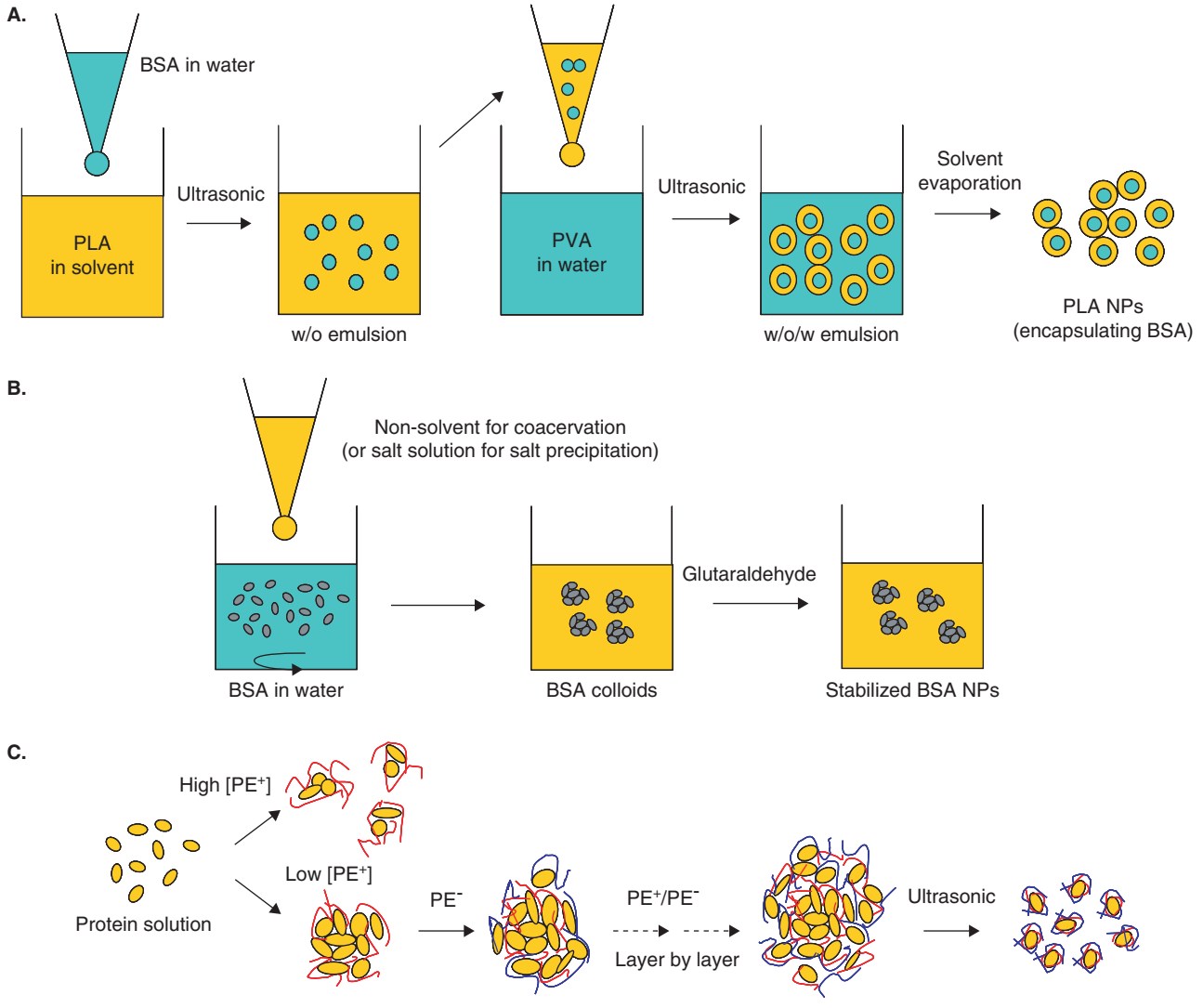

Figure 2. Commonly used methods to prepare nanoparticles: (A) double emulsion; (B) coacervation (and salt precipitation); and (C) polyelectrolyte complexation.

BSA: Bovine serum albumin; o: Oil; PE^+: Cationic polyelectrolyte; PE^-: Anionic polyelectrolyte; PLA: Poly(lactic acid); PVA: Poly(vinyl alcohol); w: Water.

the β-lactoglobulin [21]. Denaturation of β-lactoglobulin by heat treatment further reduced the NP sizes to ~ 60 nm and the authors attributed this result to reduced hydrophobic interactions among the extended proteins [21]. Chemically modified proteins may also be used as the NP matrix (e.g., polyethylene glycol-modified BSA [PEG-BSA]), but the use of modified proteins may require changes in the choice of solvents and non-solvents [22].

BSA NPs can achieve very high encapsulation efficiencies for protein drugs. Encapsulation efficiencies in excess of 95% were reported for IFN-γ [23], and Zhang *et al.* observed high entrapment efficiencies (~ 90%) for BMP-2 in BSA NPs [24]. It was interesting that high entrapment was obtained even with simple absorption of IFN-γ onto NPs, suggesting a favorable interaction between the NP matrix (BSA) and IFN-γ. This effect is expected to be drug dependent and, in the case of ganciclovir, entrapment during NP formation was more beneficial than simple adsorption [25].

Despite its known carcinogenicity and, hence, the need for its complete removal before human use, GA has been traditionally used as a cross-linker to stabilize albumin NPs after coacervation. As expected [18], Segura *et al.* [23,26] observed GA cross-linking to reduce the extent of $-NH_2$ groups at the particle surfaces, affecting biodegradability and drug release from the NPs. GA may cross-link the protein amines to BSA matrix, which was also noted for the encapsulation of recombinant IFN-γ, leading to complete abrogation of its bioactivity, while IFN-γ adsorbed on GA-cross-linked NPs retained its bioactivity [23,26]. The GA cross-linking may also be problematic for small molecular drugs, such as doxorubicin [27] and adriamycin [28].

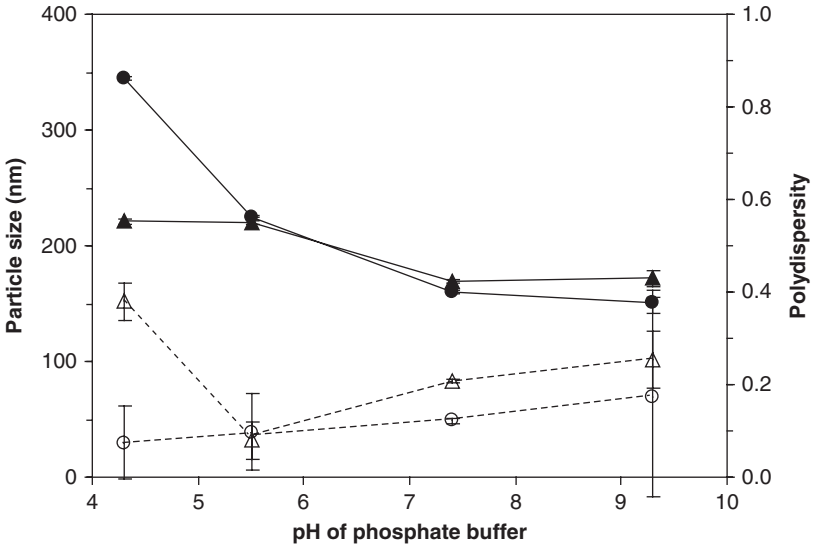

Figure 3. Influence of pH on the size (solid line) and polydispersity index (dashed line) of BSA NPs. NPs were prepared by drop-wise addition of ethanol to a mixture of equal volumes of BSA solution (circle: 10 mg/ml; triangle: 30 mg/ml) and 10 mM phosphate buffer (pH: 4.3, 5.5, 7.4 and 9.3). Size (from dynamic light scattering) and polydispersity data are expressed as mean ± SD (n = 3). Note the gradual reduction of particle size as the pH of the NP preparation solution is increased.
BSA: Bovine serum albumin; NP: Nanoparticle; SD: Standard deviation.

An independent study did not indicate any adverse effect of GA on doxorubicin-loaded HSA NPs, although drug release was not assessed in this study [29]. GA cross-linking did not also appear to affect the integrity of an antisense oligonucleotide entrapped in HSA NPs [30], suggesting that GA reaction can be controlled to minimize any adverse effects. An alternative approach is to use high temperature/low moisture cross-linking [18,31], but this may be problematic due to drug deactivation or protein denaturation at high temperature. Macromolecular cross-linkers, such as oxidized dextran, could be alternatively used to replace GA [32] because they are less likely to freely diffuse into the NP matrix and react with the functional groups on the NP surface. A different approach for NP stabilization is to use a non-covalent coating of NP surfaces; BSA NPs could be coated with cationic polymers in aqueous buffers to create a coating that not only improves particle stability, but also controls the release rate of a protein. The feasibility of this approach was demonstrated with BMP-2 entrapped in BSA NPs and coated with PEI [24].

2.3 Polyelectrolyte complexation (complex coacervation)

Macromolecular interactions under aqueous conditions due to electrostatic forces can lead to coacervation complexes (**Figure 2C**). Formation of insoluble complexes between proteins and oppositely charged polymers will strongly depend on medium pH; use of polyanions for NP formation will require a pH lower than the protein pI, and vise versa for polycations [33]. Cationic polymers, such as poly-L-lysine (PLL) and PEI, are commonly used for condensing long, string-like DNA molecules into NPs by complexation with anionic oxygen atoms in the phosphodiester backbone of DNA [34]. The driving force for this type of interaction is usually entropic due to the release of the small counterions rather than enthalpic [35]. Comb-type polyelectrolytes were proposed to be particularly suitable for the preparation of water-soluble complexes [35,36] because hydrophilic segments facilitate the aqueous solubility that is essential for systemic application [35]. Serefoglou *et al.* [37] described the preparation of soluble NPs with hydrodynamic radii of 65 – 83 nm through coulombic interaction of BSA with anionic copolymers at a pH lower than the pI of BSA (~ 4.5). These NPs contained, on average, 13 – 14 BSA molecules held together by two polymeric molecules. They were proposed to bear a hydrophobic core of BSA/anionic polymer moieties (~ 20 nm in diameter) and a polymeric corona. In addition to electrostatic interactions, H-bonding or hydrophobic interactions can also drive albumin/polymer complexation [38], the balance between the polymer–polymer and polymer–protein interactions dictating the number of proteins/polymer in a complex. The size of intermolecular complexes was dependent on the number of bound protein/polymer, but its dependence was variable for individual polymers (and possibly interaction mechanisms within the complexes). Rhaese *et al.* [39] prepared HSA-PEI-DNA NPs using complex coacervation induced by charge neutralization as a delivery system for gene therapy. These NPs were much larger in size (300 – 1000 nm) as compared to BSA particles formed at low pH.

Smaller particles with a much broader distribution (30 – 300 nm) were reported by using a combination of protamine, oligonucleotides and albumin; incorporating albumin into such particles was achieved by electrostatic assembly and seemed to significantly influence intracellular distribution of the internalized oligonucleotides [40].

Polyelectrolyte complexation can also be used for coating of protein aggregates by layer-by-layer assembly (**Figure 2C** [41,42]). This process may provide exquisite control over the structure of the NPs formed, but reported results indicated primary aggregates of 100 – 300 nm in size (similar to BSA NPs) that was further assembled to form microscopic structures as a result of coating. Prevention of larger aggregates in a reproducible way (e.g., with sonication [42]) is paramount to obtaining uniform nano-sized particles. It was possible to control the release rate of proteins from such NPs by controlling the coating layer (i.e., nature of the polymer and the order of coating), providing a convenient method for controlled release [41]. Distinguishing the underlying release mechanism (i.e., protein diffusion versus NP disintegration in a destabilizing environment) will be important for any therapeutic effect obtained.

NP formation by complexation is particularly suited for naturally occurring polymers such as alginate and chitosan. Chitosan offers a unique biologic matrix because it is mucoadhesive and acts as a good substrate for cellular binding. In addition to water-soluble deacetylated chitosan, successful NPs were prepared with *N*-trimethylated (200 – 400 nm [43]) and *N*-(2-hydroxy)-propyl-3-trimethyated chitosan (110 – 180 nm [44]) using tripolyphosphate as the counter polyion. Model proteins (albumin and ovalbumin) were encapsulated with ≤ 80 – 100% efficiency under optimized conditions. As much as 50% of NPs could be made with the protein fraction, indicating the possibility of obtaining protein NPs as a result of polyelectrolyte-mediated aggregation.

2.4 Salt precipitation

A simple approach to the preparation of protein NPs is simply 'salting out' a protein solution to form protein coacervates. The simplicity of this approach is attractive, provided that the precipitation process does not change the bioactivity or conformational structure of the protein. For example, insulin particles were prepared in this way by exposing the protein solution to high NaCl concentration (> 0.5 M) at low pH (< 2.0) [42]. The particle sizes were 100 – 1000 nm depending on the pH and appeared to display a large heterogeneity compared to other NP fabrication methods. Coating of such particles with polyelectrolytes can be easily achieved and this helps to stabilize the NPs, as well as to facilitate control under certain conditions [42]. The latter will depend on the pI of encapsulated proteins and the net charge of coating polyelelectrolyte(s).

3. Surface modification of nanoparticles with PEG

Considerable effort has been spent to obtain 'stealth' NPs with reduced reticuloendothelial system (RES) uptake and prolonged circulation time. One widely used method is surface adsorption or grafting with PEG to create a hydrophilic barrier [45-47], which can block electrostatic and hydrophobic interactions responsible for opsonization. PEG-functionalized PLGA NPs were prepared from PLA-PEG and PLGA-PEG copolymers [48-51]. By using di-block PEG-PLA, PEG-PLGA and PEG-PCL polymers, the influence of PEG molecular weight (MW) and content on the plasma protein adsorption and phagocytosis was investigated [52]. The sterically stabilized PEG surface was found to reduce protein adsorption but not eliminate it. The PEG MW critical for significant reduction of protein adsorption varied between 1.5 and 5 kDa [52,53], and it appeared that the critical MW to prevent protein adsorption might depend on specific protein; that is, a different length of PEG is needed for different proteins. Methoxy-PEG (mPEG)-PLGA block copolymers with different sizes of mPEG block (M_n 550, 750 and 2000 Da) were used for the hydrophilic layer on PLGA NPs [54]. The average particle size was slightly increased with increasing mPEG MW due to extended PEG corona extending into aqueous medium.

Surface coating by adsorption is especially attractive for NP modification because the process can be separately controlled from the fabrication process. Based on a comb polymer of PLL-g-PEG designed for the modification of metal oxide surfaces for protein resistance [55-57], PLL-g-PEG polymers were used to coat PLGA microspheres through electrostatic interactions between cationic PLL backbone and anionic PLGA surface [58]. A drastic decrease in protein adsorption (by two orders of magnitude) was the result. Similarly, protein (HSA) NPs were stabilized with an oxidized dextran-PEG conjugate, ultimately leading to surface grafting of PEG chains [32].

As an alternative to coating or grafting, it is possible to obtain surface-modified NPs by using derivatized proteins in the fabrication process. Lin *et al.* [32] were the first to report HSA NPs prepared from HSA modified with mPEG (HSA-mPEG), poly(amidoamine)-PEG (HSA-PAA-PEG) and poly(thioetheramidoacid)-PEG (HSA-PTAAC-PEG) [22,59]. The existence of a hydrated barrier surrounding the NPs was confirmed and the surface-modified particles showed a reduced plasma protein adsorption. The PEG MW was again important for the prevention of protein adsorption, where a 2-kDa (but not 5-kDa) PEG was most effective. The particles made from PEG-BSA displayed increased stability on salt/pH challenge [22]. The fact that a reduction in protein adsorption was not always proportional to PEG size or the extent of engraftment indicated the possibility of unique interactions with the matrix of the albumin NPs.

Affecting the release rate of entrapped drugs as a result of surface modification is always a concern, but it was possible to achieve NP modification with PEG without affecting the release pattern of drugs [14].

4. Nanoparticle properties critical for drug delivery

Delivery of pharmacologic agents formulated in NPs can be broadly classified into two categories (i.e., those intended for local versus systemic administration). In the case of local administration, the NP formulation is expected to act as a 'depot', where a local concentration of the drug is maintained while preserving the agent in active form, usually in a hostile environment. The NP may also act as a controlled-release formulation, where NP properties precisely control the drug release rate. In the case of systemic administration, the NP formulation is expected to circumvent the initial clearance mechanisms in circulation and preferably localize at the site of action of the drug. Once successfully localized to a desired site, the NP formulation becomes a local depot formulation. Understanding relationships among the physicochemical properties of NPs and their physiologic performance is paramount for successful delivery. Towards this end, a summary of the pertinent literature follows, specifically focusing on NP properties critical for systemic and local delivery.

4.1 Particle size and circulation time

Long-circulating carriers have a better chance of reaching their target and leading to a therapeutic benefit. Larger NPs are more likely to sediment in circulation or filtered in capillary beds and it is generally accepted that the NPs should not exceed 200 nm for this reason [60]. Despite several reports with exquisite control over NP size, the effect of size on circulation time is understudied. Using relatively small BSA NPs (110 – 125 nm), PEG functionalization of NPs was shown to improve the circulation time (systemic mean retention time increased by ~ 1.5-fold) as well as localization in some tissues, in particular the brain [14]. Harashima *et al.* [61] reported the size dependence of opsonization (see Section 4.2 for more details on opsonization process) for liposomes, suggested that smaller carriers led to a reduced adsorption of proteins and opsonins, and accordingly reduced uptake by phagocytic cells. The extent of opsonization decreased with a reduction in size from 800 to 200 nm, and no enhancement of phagocytic uptake was recorded at < 200 nm. Besides systemic clearance, biodistribution of the NPs is also affected by their size. Using PEG-decorated NPs in rabbits, Porter *et al.* [62] showed that particles < 150 nm exhibited an increased localization in bone marrow, while particles ~ 250 nm were mostly sequestered in the spleen and liver, presumably by selective filtration in these organs [60]. In the bone marrow, a membrane of lining cells functions as a marrow-blood barrier and not all NP localized to bone are actually accessible to bone-resident cells [63]. It is suggested that the vasculature in bone has pores of 80 – 100 nm [54,64] and NPs need to have a hydrodynamic size of < 80 nm to extravasate into bone tissue. However, particles < 100 nm may also pass through fenestrated hepatic sinusoidal endothelium [65] and may get entrapped in the liver, ultimately reducing their availability to the bone-resident cells.

For some applications, it is desirable to engineer NPs that can be taken up by the phagocytic cells. Immunostimulation of macrophages against microbial infections or immunosuppression of macrophages in auto-inflammatory diseases may be achieved with large NPs of 200 – 300 nm [23]. Gelatin NPs containing the cytotoxic drug clodronate, a bisphosphonate drug usually formulated with liposomes [66], were successfully used for the elimination of macrophages [67].

4.2 Nanoparticle opsonization and phagocytosis

A major obstacle for particulate drug carriers is the opsonization and clearance by the RES. The opsonization process is the adsorption and/or activation of proteins capable of interacting with specific receptors on monocytes and various subsets of tissue macrophages, thus promoting particle recognition and uptake [65]. As much as 95% of foreign particles may undergo opsonization and clearance by the RES [45]. Opsonization typically takes place in the blood circulation and can take from a few seconds to many days to complete [68]. The exact mechanism through which this process is activated is not yet fully understood. The small size of the NPs is no impediment for cellular uptake, and sizes and surface characteristics have been repeatedly emphasized as important factors affecting clearance behavior and tissue distribution of particulate carriers [65]. Neutral and hydrophilic particles have a much lower opsonization rate than the charged and hydrophobic particles [69]. The core properties of the NPs, although generally considered shielded from the physiologic contact, may also affect the extent and nature of protein adsorption [52]. PEGylation is the standard approach to reduce protein adsorption to NPs (see below), but some degree of protein adsorption might be beneficial to prevent NP aggregation if the surface properties alone are not sufficient (e.g., low PEG density) to prevent aggregation [32]. Recent studies with poly(isobutylcyanoacrylate) NPs indicated that the particle size (100 – 300 nm) do not influence the extent of complement activation [70]. However, surface chemistry, specifically the choice of polysaccharide coating, was important with chitosan coating giving relatively low complement activation that could be reduced to background levels with high-MW chitosans. Whereas the size of naked poly(cyanoacrylate-co-n-hexadecyl cyanoacrylate) NPs did not influence protein adsorption, PEGylation of such NPs resulted in lower protein adsorption and phagocytic uptake *in vitro* with decreasing particle size [71]. However, the ideal

PEG MW for effective reduction of opsonization was variable in different NP preparations and this was likely to reflect the exact configuration of the PEG on the NP surfaces [72].

With BSA NPs, the negative ζ-potential (-15 to -30 mV) was considered a significant advantage because it can reduce non-specific interaction with cellular surfaces. However, despite an anionic surface, ~ 200 nm HSA NPs were readily internalized by primary human macrophages [20]. Cationic gelatin NPs (300 – 500 nm) were also internalized by the rat macrophage-like cells [67]. The effect of particle size and internalization was not systematically investigated for protein NPs, but having highly anionic and cationic surfaces was equally detrimental for uptake in macrophage-like U937 cells and mouse macrophages [69]. Internalization of cationic NPs was not surprising, but anionic NPs were presumably opsonized for such a stimulated uptake [69]. Unlike *in vitro* results, *in vivo* uptake into the major clearance organ (liver) was not affected by the ζ-potential of these particular NPs (size ~ 500 nm) [69].

4.3 Factors controlling drug release

Mechanism(s) controlling drug release from NPs may include: i) dissolution of drugs from a solid state; ii) displacement of adsorbed drugs from NP matrix; iii) diffusion through NP matrix or surface coating (if any); and iv) degradation of NP matrix or surface coating [46]. For drugs strongly interacting with a NP matrix [6], displacement with buffer components (*in vitro*) or endogenous solutes (*in vivo*) may be a critical factor. With conventional drugs of high diffusivity, such as ganciclovir [25] and clodronate [67], release patterns indicated two types of drug fractions: a weakly and a strongly bound fraction. The former fraction is released following changes in medium composition, where the drug solubility is enhanced or displacing moieties appear in the new medium. A rapid release could be observed without a distinctive 'burst' versus 'sustained-release' pattern [14] and the extent of the initial release is likely to depend on the loading method [25]. NPs prepared from synthetic polyesters, as compared to proteins, might be better engineered for a smaller burst release [73,74], and longer duration of release [12,75,76] by controlling the MW and/or composition of polymers used for NP fabrication [74,75]. Although sustained release is usually desired, a 'burst' release might be sufficient for some indications; IFN-γ released within a 24-h period was sufficient for lasting immunostimulatory activity [23].

Enzymatic degradation is an important mechanism for protein and polyester NPs. It is usually surface restricted [6] and may cause accelerated NP degradation due to a large surface area:volume ratio. Little work has been performed in assessing the degradation of NPs under physiologic conditions. Wartlick *et al.* [77] showed a correlation between the extent of GA cross-linking and rate of enzymatic degradation of HSA NPs *in vitro*. This was also the case after NP internalization, where HSA particles remained intact when NPs were excessively cross-linked, but the NP cargo was dispersed in cytoplasm for undercrosslinked particles [77]. Because these studies relied on fluorescently labeled molecules and the integrity of the label was not explored, these results need to be considered with caution. BSA and β-lactoglobulin particles also displayed a relatively rapid (half life [$t_{1/2}$] ~ 8 – 15 h) enzymatic degradation *in vitro* [21]. The effect of degradation on pharmacologic activity needs to be probed for individual indications, as well as the correlation between the drug release rate and enzymatic degradation.

5. Designing nanoparticles to target specific tissues

An exciting possibility for NPs could be realized if they can be made 'tissue seeking'. Although particle size and surface characteristics may alter biodistribution *in vivo*, tissue specificity can only arise if NPs display a preferential affinity to a target tissue. To accomplish this goal, NPs are functionalized with tissue-specific molecular ligands. These ligands usually display an affinity to tissue-specific cell surface molecules and they improve the retention of NPs due to enhanced local affinity and slowing of NP loss from the tissues. Alternatively, substrates for tissue-specific transporters have been used for NPs to improve cellular uptake (i.e., cells responsible for barrier functions, such as the brain–blood-barrier [BBB]) into the desired tissues. While natural ligands have been preferred, synthetic ligands with no biologic function have also been used for this purpose. The impetus for the latter ligands is the superior affinity of the ligands to target tissues (e.g., bisphosphonates for bone), or an ability to tailor target affinity and specificity via chemical engineering (e.g., aptamers). A summary of current efforts for tissue-targeting NPs is provided in **Table 2** and a select set of applications is discussed below.

5.1 Brain targeting

Targeting NPs to the brain has relied on ligands that display facilitated transport at the BBB. Transferrin is one such ligand and Mishra *et al.* [14] described modification of BSA NPs with maleimide-PEG-*N*-hydroxysuccinimide (MAL-PEG-NHS) so as to functionalize the NP surfaces with transferrin. Only a small fraction of surface PEG groups were coupled to transferrin (3 – 4%) so that an effective PEG coating for improved biodistribution could be achieved by this method. Delivering azidothymidine (AZT) to the brain in transferrin-PEG-HSA NPs was two- to threefold more effective than the PEG-HSA NPs, which was more effective as compared to free AZT or AZT entrapped in unmodified NPs [14]. Liver uptake was also enhanced for transferrin-functionalized NP and these two organs – at times – accounted for ~ 50% of the administered dose [14]. NPs functionalized with apolipoprotein E were also prepared

Table 2. A summary of ligands used for NP derivatization for targeting NPs to specific tissues.

Ligand	NP derivatized	Target cells/tissue	Evaluation	Ref.
Folate	BSA	Cancerous cells	*In vitro*	[125]
Folate	PLGA	Cancerous cells	*In vitro*	[160]
Aptamer against PSMA	PLGA	Tumor	*In vivo*	[83,84]
Wheat germ agglutinin	PLGA	Tumor	*In vivo*	[161]
HER2	HSA	Cancerous cells	*In vitro*	[80,81]
HER2	PLA or PLGA	Cancerous cells	*In vitro*	[76,82]
GFdTGFLS-glucose	PLGA	Brain	*In vivo*	[162]
Apolipoprotein E	HSA	Brain	*In vivo*	[78,79]
Synthetic opioid peptides	PLGA	Brain	*In vivo*	[163]
Transferrin	HSA	Brain	*In vivo*	[14]
Bisphosphonate	Liposome	Bone	*In vitro*	[87]
Bisphosphonate	PLGA	Bone	*In vitro*	[54]
Cyclic RGD	PLGA	Endothelial cells	*In vitro*	[164]
Linear GRGDS	PLGA/PLGA-PEG/PCL-PEG	M cells	*In vitro*	[13]
Tetanus toxin C	PLGA-PEG	Neuronal cells	*In vitro*	[50]

GRGDS: ; HSA: Human serum albumin; NP: Nanoparticle; PCL: ; PEG: Polyethylene glycol; PLA: Poly(lactic acid); PLGA: Poly(lactic-co-glycolic acid); PSMA: Prostate specific membrane antigen.

for brain targeting by direct coupling with the MAL-PEG-NHS linker [78,79] or indirect coupling via an avidin/biotin bridge [79]. A desired pharmacologic effect was observed only when the drug of interest, loperamide, which is impermeable to the BBB, was formulated in apolipoprotein E-decorated NPs [78,79]. The extent of NP targeting as a result of apolipoprotein E coupling remains to be quantified. By using a variety of isoforms, the mechanism of NP transport appeared to be specific for apolipoprotein E and it relied on the transcytosis pathway undertaken by lipoprotein particles.

5.2 Tumor targeting

Surface modification of BSA [80,81], gelatin [81], PLA [82] and PLGA NPs [76] with anti-human epidermal growth factor receptor 2 (anti-HER2) antibodies was attempted to target NPs to breast cancers. The uptake of antibody-coupled NPs was facilitated by HER2-expressing tumor cell lines *in vitro*. However, on prolonged incubation, even non-specific NPs (e.g., PEG-HSA) were internalized [80]. This was unexpected because: i) the NPs displayed a ζ-potential of ~ -40 mV (repulsive to anionic cell surfaces); and ii) PEG molecules should have reduced non-specific cell surface interactions. Sedimentation might be one mechanism for depositing NPs on the cell surface, but how this translates to the *in vivo* situation remains to be seen. PLA NPs functionalized with a control (non-internalizing) antibody gave little uptake as compared to HER2-mediated uptake [82], suggesting that the internalization was specific and not due to chemical derivatization on the surface. *In vivo* studies on these NPs remain to be reported. On the other hand, PLGA NPs functionalized with aptamers against prostate-specific membrane antigen yielded approximately fourfold improved targeting of the carriers in a xenograft prostate graft model [83] and significantly improved the survival in a xenograft model as compared to non-targeted chemotherapeutic delivery [84].

5.3 Bone targeting

The structural qualities of bone – especially the presence of hydroxyapatite (HA) – and specific affinity of certain molecules to this mineral provide a unique opportunity to target drugs to bone tissue. Molecules with diverse structural features were reported with bone affinity [85,86]. Unlike natural molecules used for targeting to other tissues, bone-targeting ligands are generally synthetic entities, among which bisphosphonates (BPs) play a prominent role. The BPs are a class of synthetic compounds structurally related to pyrophosphates, but they feature a geminal diphosphonate (P-C-P) instead of the hydrolyzable P-O-P bond of pyrophosphate. BPs are highly water-soluble, acidic compounds at physiologic pH, and remain bound to bone mineral after systemic administration. BPs have been traditionally conjugated to therapeutic molecules for bone targeting, but using NPs functionalized with BPs might be a superior alternative; bone targeting can be achieved without modifying the pharmacologic agent *per se*, an important consideration particularly for bioactive protein therapeutics.

The design of bone-seeking PLGA NPs (micelles) was reported by using a PLGA polymer grafted with alendronate [54]. The NPs were 40 – 60 nm in size (a suitable size for vascular penetration at bones) and were able to entrap and release a hydrophobic drug (estrogen) from its PLGA core. Although the alendronate-incorporated NPs displayed hydroxyapatite affinity, it was not clear whether the binding was due to alendronate *per se*, because binding of unfunctionalized NPs were not reported. BPs were also incorporated into liposomes by using a lipophilic BP derivative, cholesteryl-trisoxyethylene-bisphosphonic acid (CHOL-TOE-BP) [87]. Such liposomes were 100 – 135 nm in size, and displayed an *in vitro* hydroxyapatite affinity that was dependent on the extent of BP incorporated in liposomes. The pharmacokinetics and tissue distribution of the BP-conjugated NPs and liposomes remain to be probed to explore their full potential to act as bone-seeking NPs.

Future studies in bone targeting are likely to be accelerated because the basic foundation of this approach is being steadily established in independent laboratories. Synthetic polymers with BP moieties have been synthesized and their superior bone affinity demonstrated [64]. Such polymers can be easily fabricated into bone-seeking NP formulations. Our extensive experience on protein derivatization with BPs could yield bone-seeking protein NPs if the derivatized proteins can be fabricated into NPs. Cationic polymers, such as PLL and PEI, were recently reported to display a hydroxyapatite affinity that was equivalent to BP-mediated affinity [88]. Such polymers can be used in coating NPs for bone targeting.

6. Conclusions

Pharmacologic agents entrapped in NPs are attracting increasing attention to overcome delivery challenges posed *in vivo*. Well-established approaches to fabricate NPs are continually optimized to improve the encapsulation efficiency while preserving the bioactivity of therapeutic agents. New methods to formulate NPs are being proposed; molecular assembly along with the precise control of biomaterial structures are leading the way in this effort. While the size of NPs from several fabrication processes is now routinely controlled, understanding the impact of size on various facets of drug delivery – such as phagocytosis, circulation time and drug release kinetics – remains understudied. Several avenues have been taken to functionalize the surface of NPs with desired ligands. These include NP fabrication from modified biomaterials, coating NPs with designed polymers, surface-grafting post-fabrication and using non-covalent 'affinity' bridges to place ligands on NP surfaces. These efforts are establishing the foundation of tissue-specific drug delivery by NPs. Therapeutic applications of tissue targeting are expected to broaden and more efforts will be needed to better understand the *in situ* fate of NPs, as well as the relationship among NP degradation, drug release and observed therapeutic effects.

7. Expert opinion

NPs fabricated from native proteins are attractive due to the existence of natural mechanisms for metabolizing such particles. Once internalized, enzymatic degradation of such particles is expected to be rapid. *In vitro* studies indicated rapid particle degradation (within 24 h), but it is not possible to readily extrapolate these results to the *in vivo* situation mainly due to an arbitrary choice of the components of the *in vitro* system (i.e., choice of enzymes and concentrations, biologic milieu and so on). Rate of *in vivo* degradation of NPs remains to be fully explored and this will shed light on drug pharmacokinetics delivered with NPs [74]. One important aspect related to protein NPs is the possibility of inducing an autoimmune response, whereby inflammatory or immune response against synthetically produced NPs may turn against the host. This is especially important for application where NPs need to be administered repeatedly. *In vivo* residence time of the NPs is expected to be one important determinant of such a reaction, because rapidly metabolizing systems are less likely to elicit such a response. Clinical use of recombinant proteins had raised the same issue initially, but long history with such proteins has clarified the acceptable benefit–risk relationships for such drugs. However, two additional concerns in the case of NPs are: i) solidified/bulk nature of administered proteins, unlike recombinant proteins that are administered as soluble proteins; and ii) possibility of significant denaturation during NP fabrication. It is difficult to predict the chances of NPs raising an autoimmune reaction based on animal studies, but should it be a limiting concern, synthetic polymers are expected to provide an alternative in this regard. Our survey of the literature indicates that particles with desired sizes could be made from synthetic polymers, proteins and natural polymers (**Figure 1**), so that a wide variety of biomaterials is available for a given size of NP preparation.

From a technologic perspective, a better understanding of the factors controlling release rate as well as the ways to prolong the release will be greatly beneficial. It is recognized that 'burst' release could be sufficient for some applications in preclinical models; however, even for these applications, a sustained release will translate into clinical benefit if the administration frequency could be reduced. Based on published reports from independent laboratories, NPs from synthetic biomaterials appear to provide a longer-release duration, whereas NPs prepared from protein and natural polyelectrolytes provide a large burst-release and shorter sustained-release period. The sustained release, which is usually quantified under *in vitro* conditions, cannot be translated to the *in vivo* situation due to a lack of realistic degradative mechanisms

in vitro. As highlighted earlier (see Section 4.3), determining the physiologic half-life of NPs in various tissues will provide a good measure of the maximal sustained release that can be achieved. Understanding the factors that determine NP stability in various organs is likely to lead to engineering NPs with tailored degradation rates and, hence, sustained release. Very little, if any, work has been performed that probes the internal structure of the NPs. It is likely that significant heterogeneity exists in NPs and that release rates are closely linked to the internal structure. This is particularly relevant to tissue-targeting efforts; *in situ* residence of a NP coupled with practical release rates/duration will dictate the success of the tissue-targeting efforts.

Declaration of interest

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Bibliography

Papers of special note have been highlighted as either of interest (•) or of considerable interest (••) to readers.

1. Socinski M. Update on nanoparticle albumin-bound paclitaxel. Clin Adv Hematol Oncol 2006;4:745-6
2. Mehnert W, Mader K. Solid lipid nanoparticles Production, characterization and applications. Adv Drug Deliv Rev 2001;47:165-96
3. Muller BG, Leuenberger H, Kissel T. Albumin nanospheres as carriers for passive drug targeting: an optimized manufacturing technique. Pharm Res 1996;13:32-7
4. Raghuvanshi RS, Goyal S, Singh O, Panda AK. Stabilization of dichloromethane-induced protein denaturation during microencapsulation. Pharm Dev Technol 1998;3:269-76
5. Sturesson C, Carlfors J. Incorporation of protein in PLG-microspheres with retention of bioactivity. J Control Rel 2000;67:171-8
6. Chawla JS, Amiji MM. Biodegradable poly(ε-caprolactone) nanoparticles for tumor-targeted delivery of tamoxifen. Int J Pharm 2002;249:127-38
7. Nehilla BJ, Bergkvist M, Popat KC, Desai TA. Purified and surfactant-free coenzyme Q10-loaded biodegradable nanoparticles. Int J Pharm 2007;348:107-14
8. Sussman EM, Clarke MB Jr, Shastri VP. Single-step process to produce surface-functionalized polymeric nanoparticles. Langmuir 2007;23:12275-9
9. Zambaux MF, Bonneaux F, Gref R, et al. Influence of experimental parameters on the characteristics of poly(lactic acid) nanoparticles prepared by a double emulsion method. J Control Rel 1998;50:31-40
10. Song X, Zhao Y, Wu W, et al. PLGA nanoparticles simultaneously loaded with vincristine sulfate and verapamil hydrochloride: systematic study of particle size and drug entrapment efficiency. Int J Pharm 2008;350:320-9
11. Tewes F, Munnier E, Antoon B, et al. Comparative study of doxorubicin-loaded poly(lactide-co-glycolide) nanoparticles prepared by single and double emulsion methods. Eur J Pharm Biopharm 2007;66:488-92
12. Li YP, Pei YY, Zhang XY, et al. PEGylated PLGA nanoparticles as protein carriers: synthesis, preparation and biodistribution in rats. J Control Rel 2001;71:203-11
13. Garinot M, Fiévez V, Pourcelle V, et al. PEGylated PLGA-based nanoparticles targeting M cells for oral vaccination. J Control Rel 2007;120:195-204
14. Mishra V, Mahor S, Rawat A, et al. Targeted brain delivery of AZT via transferrin anchored pegylated albumin nanoparticles. J Drug Target 2006;14:45-53
• **This is an adaptation of a well-established brain delivery approach (i.e., transferrin-mediated passage of the BBB) for delivery of protein NPs to brain.**
15. Chung YI, Ahn KM, Jeon SH, et al. Enhanced bone regeneration with BMP-2 loaded functional nanoparticle-hydrogel complex. J Control Rel 2007;121:91-9
16. Isobe M, Yamazaki Y, Mori M, et al. The role of recombinant human bone morphogenetic protein-2 in PLGA capsules at an extraskeletal site of the rat. J Biomed Mater Res 1999;45:36-41
17. Meinel L, Zoidis E, Zapf J, et al. Localized insulin-like growth factor I delivery to enhance new bone formation. Bone 2003;33:660-72
18. Weber C, Coester C, Kreuter J, Langer K. Desolvation process and surface characterisation of protein nanoparticles. Int J Pharm 2000;194:91-102
19. Lin W, Coombes AGA, Davies MC, et al. Preparation of sub-100 nm human serum albumin nanoparticles using a pH-coacervation method. J Drug Target 1993;1:237-43
•• **This is one of the earliest reports that used a simple approach to prepare sub-100-nm particles by simply using acetone as the non-solvent. Preparing NPs in this size range and exploring their use is relatively less studied.**
20. Langer K, Balthasar S, Vogel V, et al. Optimization of the preparation process for human serum albumin (HSA) nanoparticles. Int J Pharm 2003;257:169-80
21. Ko S, Gunasekaran S. Preparation of sub-100-nm β-lactoglobulin (BLG) nanoparticles. J Microencapsul 2006;23:887-98.:
22. Lin W, Garnett MC, Schacht E, et al. Preparation and in vitro characterization of HSA-mPEG nanoparticles. Int J Pharm 1999;189:161-70
23. Segura S, Gamazo C, Irache JM, Espuelas S. Gamma interferon loaded onto albumin nanoparticles: in vitro and in vivo activities against Brucella abortus. Antimicrob Agents Chemother 2007;51:1310-4
24. Zhang S, Wang G, Lin X, et al. Polyethylenimine-coated albumin nanopartices for BMP-2 delivery. Biotechnol Prog 2007; accepted
25. Merodio M, Arnedo A, Renedo MJ, Irache JM. Ganciclovir-loaded albumin nanoparticles: characterization and in vitro release properties. Eur J Pharm Sci 2001;12:251-9
26. Segura S, Espuelas S, Renedo MaJs, Irache JM. Potential of albumin nanoparticles as carriers for

interferon-gamma. Drug Dev Ind Pharm 2005;31:271-80

27. Leo E, Vandelli MA, Cameroni R, Forni F. Doxorubicin-loaded gelatin nanoparticles stabilized by glutaraldehyde: involvement of the drug in the cross-linking process. Int J Pharm 1997;155:75-82
28. Willmott N, Cummings J, Florence AT. In vitro release of adriamycin from drug-loaded albumin and haemoglobin microspheres. J Microencapsul 1985;2:293-304
29. Dreis S, Rothweiler F, Michaelis M, et al. Preparation, characterisation and maintenance of drug efficacy of doxorubicin-loaded human serum albumin (HSA) nanoparticles. Int J Pharm 2007;341:207-14
30. Arnedo A, Irache JM, Merodio M, Espuelas Millan MS. Albumin nanoparticles improved the stability, nuclear accumulation and anticytomegaloviral activity of a phosphodiester oligonucleotide. J Control Rel 2004;94:217-27
31. Chen CQ, Lin W, Coombes AGA, et al. Preparation of human serum albumin microspheres by a novel acetone-heat denaturation method. J Microencapsul 1994;11:395-407
32. Lin W, Coombes AGA, Garnett MC, et al. Preparation of sterically stabilized human serum albumin nanospheres using a novel dextranox-MPEG crosslinking agent. Pharm Res 1994;11:1588-92
33. Wang Y, Gao JY, Dubin PL. Protein separation via polyelectrolyte coacervation: selectivity and efficiency. Biotechnol Prog 1996;12:356-62
34. Farrell LL, Pepin J, Kucharski C, et al. A comparison of the effectiveness of cationic polymers poly-l-lysine (PLL) and polyethylenimine (PEI) for non-viral delivery of plasmid DNA to bone marrow stromal cells (BMSC). Eur J Pharm Biopharm 2007;65:388-97
35. Nisha CK, Manorama SV, Ganguli M, et al. Complexes of poly(ethylene glycol)-based cationic random copolymer and calf thymus DNA: a complete biophysical characterization. Langmuir 2004;20:2386-96
36. Dautzenberg H, Zintchenko A, Konak C, et al. Polycationic graft copolymers as carriers for oligonucleotide delivery: complexes of oligonucleotides with polycationic graft copolymers. Langmuir 2001;17:3096-102
37. Serefoglou E, Oberdisse J, Staikos G. Characterization of the soluble nanoparticles formed through coulombic interaction of bovine serum albumin with anionic graft copolymers at low pH. Biomacromolecules 2007;8:1195-9
38. Matsudo T, Ogawa K, Kokufuta E. Complex formation of protein with different water-soluble synthetic polymers. Biomacromolecules 2003;4:1794-9
39. Rhaese S, von Briesen H, Rubsamen-Waigmann H, et al. Human serum albumin- polyethylenimine nanoparticles for gene delivery. J Control Rel 2003;92:199-208
40. Vogel V, Lochmann D, Weyermann J, et al. Oligonucleotide-protamine-albumin nanoparticles: preparation, physical properties, and intracellular distribution. J Control Release 2005;103:99-111
41. Balabushevitch NG, Sukhorukov GB, Moroz NA, et al. Encapsulation of proteins by layer-by-layer adsorption of polyelectrolytes onto protein aggregates: factors regulating the protein release. Biotechnol Bioeng 2001;76:207-13
42. Fan YF, Wang YN, Fan YG, Ma JB. Preparation of insulin nanoparticles and their encapsulation with biodegradable polyelectrolytes via the layer-by-layer adsorption. Int J Pharm 2006;324:158-67
43. Amidi M, Romeijn SG, Borchard G, et al. Preparation and characterization of protein-loaded N-trimethyl chitosan nanoparticles as nasal delivery system. J Control Release 2006;111:107-16
44. Xu Y, Du Y, Huang R, Gao L. Preparation and modification of N-(2-hydroxyl) propyl-3-trimethyl ammonium chitosan chloride nanoparticle as a protein carrier. Biomaterials 2003;24:5015-22
45. Malafaya PB, Silva GA, Baran ET, Reis RL. Drug delivery therapies I: general trends and its importance on bone tissue engineering applications. Curr Opin Solid State Mater Sci 2002;6:283-95
46. Soppimath KS, Aminabhavi TM, Kulkarni AR, Rudzinski WE. Biodegradable polymeric nanoparticles as drug delivery devices. J Control Release 2001;70:1-20
47. Lee KY, Yuk SH. Polymeric protein delivery systems. Prog Polym Sci 2007;32:669-97
48. Stolnik S, Dunn SE, Garnett MC, et al. Surface modification of poly(lactide-co-glycolide) nanospheres by biodegradable poly(lactide)-poly (ethylene glycol) copolymers. Pharm Res 1994;11:1800-8
49. Gref R, Minamitake Y, Peracchia MT, et al. Biodegradable long-circulating polymeric nanospheres. Science 1994;263:1600-3
50. Townsend SA, Evrony GD, Gu FX, et al. Tetanus toxin C fragment-conjugated nanoparticles for targeted drug delivery to neurons. Biomaterials 2007;28:5176-84
51. Gryparis EC, Mattheolabakis G, Bikiaris D, Avgoustakis K. Effect of conditions of preparation on the size and encapsulation properties of PLGA-mPEG nanoparticles of cisplatin. Drug Deliv 2007;14:371-80
    - **This is an examplatory study for optimizing a NP fabrication process conditions in order to suit the needs of a particular drug.**
52. Gref R, Luck M, Quellec P, et al. 'Stealth' corona-core nanoparticles surface modified by polyethylene glycol (PEG): influences of the corona (PEG chain length and surface density) and of the core composition on phagocytic uptake and plasma protein adsorption. Colloids Surf B Biointerfaces 2000;18:301-13
53. Bergstrom K, Osterberg E, Holmberg K, et al. Effects of branching and molecular weight of surface-bound poly(ethylene oxide) on protein rejection. J Biomater Sci Polym Ed 1994;6:123-32
54. Choi SW, Kim JH. Design of surface-modified poly(D,L-lactide-co-glycolide) nanoparticles for targeted drug delivery to bone. J Control Release 2007;122:24-30
55. Kenausis GL, Voros J, Elbert DL, et al. Poly(L-lysine)-g-poly(ethylene glycol) layers on metal oxide surfaces: attachment mechanism and effects of polymer architecture on resistance to protein adsorption. J Phys Chem B 2000;104:3298-309
56. Huang NP, Michel R, Voros J, et al. Poly(L-lysine)-g-poly(ethylene glycol) layers on metal oxide surfaces: surface-analytical characterization and resistance to serum and fibrinogen adsorption. Langmuir 2001;17:489-98

57. VandeVondele S, Voros J, Hubbell JA. RGD-grafted poly-l-lysine-graft-(polyethylene glycol) copolymers block non-specific protein adsorption while promoting cell adhesion. Biotechnol Bioeng 2003;82:784-90

58. Faraasen S, Voros J, Csucs G, et al. Ligand-specific targeting of microspheres to phagocytes by surface modification with poly(L-lysine)-grafted poly(ethylene glycol) conjugate. Pharm Res 2003;20:237-46

59. Lin W, Garnett MC, Davies MC, et al. Preparation of surface-modified albumin nanospheres. Biomaterials 1997;18:559-65

60. Moghimi SM, Hedeman H, Muir IS, et al. An investigation of the filtration capacity and the fate of large filtered sterically-stabilized microspheres in rat spleen. Biochim Biophys Acta 1993;1157:233-40

61. Harashima H, Sakata K, Funato K, Kiwada H. Enhanced hepatic uptake of liposomes through complement activation depending on the size of liposomes. Pharm Res 1994;11:402-6

•• **An early study to probe the relationship between the tissue response and the particle size. Although the results of this study are regularly extrapolated, whether the observations are applicable to different NP systems remains to be better characterized.**

62. Porter CJH, Moghimi SM, Illum L, Davis SS. The polyoxyethylene/polyoxypropylene block co-polymer Poloxamer-407 selectively redirects intravenously injected microspheres to sinusoidal endothelial cells of rabbit bone marrow. FEBS Lett 1992;305:62-6

63. Hirabayashi H, Fujisaki J. Bone-specific drug delivery systems: approaches via chemical modification of bone-seeking agents. Clin Pharmacokinet 2003;42:1319-30

64. Wang D, Miller S, Sima M, et al. Synthesis and evaluation of water-soluble polymeric bone-targeted drug delivery systems. Bioconjugate Chem 2003;14:853-9

65. Moghimi SM, Hunter AC, Murray JC. Long-circulating and target-specific nanoparticles: theory to practice. Pharmacol Rev 2001;53:283-318

66. van Rooijen N, van Kesteren-Hendrikx E. Clodronate liposomes: perspectives in research and therapeutics. J Liposome Res 2002;12:81-94

67. Li P, Tan Z, Zhu Y, et al. Targeting study of gelatin adsorbed clodronate in reticuloendothelial system and its potential application in immune thrombocytopenic purpura of rat model. J Control Release 2006;114:202-8

68. Owens DE III, Peppas NA. Opsonization, biodistribution, and pharmacokinetics of polymeric nanoparticles. Int J Pharm 2006;307:93-102

69. Roser M, Fischer D, Kissel T. Surface-modified biodegradable albumin nano- and microspheres. II: effect of surface charges on in vitro phagocytosis and biodistribution in rats. Eur J Pharm Biopharm 1998;46:255-63

• **This study noted a lack of correlation between the surface characteristics, specifically charge, and *in vivo* pharmacokinetic features of the albumin NPs. It was clear that *in vitro* systems did not necessarily predict the *in vivo* fate of the particles.**

70. Bertholon I, Vauthier C, Labarre D. Complement activation by core-shell poly(isobutylcyanoacrylate)-polysaccharide nanoparticles: influences of surface morphology, length, and type of polysaccharide. Pharm Res 2006;23:1313-23

71. Fang C, Shi B, Pei Y-Y, et al. In vivo tumor targeting of tumor necrosis factor-α-loaded stealth nanoparticles: effect of MePEG molecular weight and particle size. Eur J Pharm Sci 2006;27:27-36

72. Zahr AS, Davis CA, Pishko MV. Macrophage uptake of core-shell nanoparticles surface modified with poly(ethylene glycol). Langmuir 2006;22:8178-85

73. Zambaux MF, Bonneaux F, Gref R, et al. Protein C-loaded monomethoxypoly (ethylene oxide)-poly(lactic acid) nanoparticles. Int J Pharm 2001;212:1-9

74. Mittal G, Sahana DK, Bhardwaj V, Ravi Kumar MN. Estradiol loaded PLGA nanoparticles for oral administration: effect of polymer molecular weight and copolymer composition on release behavior in vitro and in vivo. J Control Rel 2007;119:77-85

75. Zambaux MF, Bonneaux F, Gref R, et al. Preparation and characterization of protein C-loaded PLA nanoparticles. J Control Rel 1999;60:179-88

76. Sun B, Ranganathan B, Feng SS. Multifunctional poly(D,L-lactide-co-glycolide)/montmorillonite (PLGA/MMT) nanoparticles decorated by trastuzumab for targeted chemotherapy of breast cancer. Biomaterials 2008;29:475-86

77. Wartlick H, Spankuch-Schmitt B, Strebhardt K, et al. Tumour cell delivery of antisense oligonuclceotides by human serum albumin nanoparticles. J Control Rel 2004;96:483-95

78. Kreuter J, Hekmatara T, Dreis S, et al. Covalent attachment of apolipoprotein A-I and apolipoprotein B-100 to albumin nanoparticles enables drug transport into the brain. J Control Release 2007;118:54-8

79. Michaelis K, Hoffmann MM, Dreis S, et al. Covalent linkage of apolipoprotein E to albumin nanoparticles strongly enhances drug transport into the brain. J Pharmacol Exp Ther 2006;317:1246-53

80. Steinhauser I, Spankuch B, Strebhardt K, Langer K. Trastuzumab-modified nanoparticles: optimisation of preparation and uptake in cancer cells. Biomaterials 2006;27:4975-83

81. Wartlick H, Michaelis K, Balthasar S, et al. Highly specific HER2-mediated cellular uptake of antibody-modified nanoparticles in tumour cells. J Drug Target 2004;12:461-71

82. Nobs L, Buchegger F, Gurny R, Allemann E. Biodegradable nanoparticles for direct or two-step tumor immunotargeting. Bioconjugate Chem 2006;17:139-45

83. Cheng J, Teply BA, Sherifi I, et al. Formulation of functionalized PLGA-PEG nanoparticles for in vivo targeted drug delivery. Biomaterials 2007;28:869-76

84. Farokhzad OC, Cheng J, Teply BA, et al. Targeted nanoparticle-aptamer bioconjugates for cancer chemotherapy in vivo. Proc Natl Acad Sci USA 2006;103:6315-20

•• **This is a study that eused a new type of targeting ligand, an aptamer, for tissue (tumor) targeting. Due to flexibility in designing aptamers for specific ligands, this approach could serve as a platform technology for tumor-specific delivery.**

85. Zhang S, Gangal G. 'Magic bullets' for bone diseases: progress in rational design of bone-seeking medicinal agents. Chem Soc Rev 2007;36:507-31

86. Gittens SA, Bansal G, Zernicke RF, Uludag H. Designing proteins for bone targeting. Adv Drug Deliv Rev 2005;57:1011-36

87. Hengst V, Oussoren C, Kissel T, Storm G. Bone targeting potential of bisphosphonate-targeted liposomes: preparation, characterization and hydroxyapatite binding in vitro. Int J Pharm 2007;331:224-7

88. Zhang S, Wright JEI, Uludag H, Ozber N. The interaction of cationic polymers and their bisphosphonate derivatives with hydroxyapatite. Macromol Biosci 2007;7:656-70

89. Lochmann D, Vogel V, Weyermann J, et al. Physicochemical characterization of protamine-phosphorothioate nanoparticles. J Microencapsul 2004;21:625-41

90. Duclairoir C, Orecchioni A-M, Depraetere P, et al. Evaluation of gliadins nanoparticles as drug delivery systems: a study of three different drugs. Int J Pharm 2003;253:133-44

91. Irache JM, Bergougnoux L, Ezpeleta I, et al. Optimization and in vitro stability of legumin nanoparticles obtained by a coacervation method. Int J Pharm 1995;126:103-9

92. Fattal E, Vauthier C, Aynie I, et al. Biodegradable polyalkylcyanoacrylate nanoparticles for the delivery of oligonucleotides. J Control Release 1998;53:137-43

93. Reddy LH, Sharma RK, Murthy RSR. Enhanced tumour uptake of doxorubicin loaded poly(butyl cyanoacrylate) nanoparticles in mice bearing Dalton's lymphoma tumour. J Drug Target 2004;12:443-51

94. Peracchia MT, Fattal E, Desmaele D, et al. Stealth® PEGylated polycyanoacrylate nanoparticles for intravenous administration and splenic targeting. J Control Rel 1999;60:121-8

95. Lee MK, Lim SJ, Kim CK. Preparation, characterization and in vitro cytotoxicity of paclitaxel-loaded sterically stabilized solid lipid nanoparticles. Biomaterials 2007;28:2137-46

96. Yuan H, Huang LF, Du YZ, et al. Solid lipid nanoparticles prepared by solvent diffusion method in a nanoreactor system. Colloids Surf B Biointerfaces 2008;61:132-7

97. Jenning V, Schafer-Korting M, Gohla S. Vitamin A-loaded solid lipid nanoparticles for topical use: drug release properties. J Control Rel 2000;66:115-26

98. Cheng WP, Gray AI, Tetley L, et al. Polyelectrolyte nanoparticles with high drug loading enhance the oral uptake of hydrophobic compounds. Biomacromolecules 2006;7:1509-20

99. Chen J, Tian B, Yin X, et al. Preparation, characterization and transfection efficiency of cationic PEGylated PLA nanoparticles as gene delivery systems. J Biotechnol 2007;130:107-13

100. Lemarchand C, Gref R, Lesieur S, et al. Physico-chemical characterization of polysaccharide-coated nanoparticles. J Control Rel 2005;108:97-111

101. Rodrigues JS, Santos-Magalhaes NS, Coelho LCBB, et al. Novel core(polyester)-shell(polysaccharide) nanoparticles: protein loading and surface modification with lectins. J Control Rel 2003;92:103-12

102. Trimaille T, Pichot C, Elaissari A, et al. Poly(D,L-lactic acid) nanoparticle preparation and colloidal characterization. Colloid Polym Sci 2003;281:1184-90

103. Gao H, Yang YW, Fan YG, Ma JB. Conjugates of poly(D,L-lactic acid) with ethylenediamino or diethylenetriamino bridged bis(β-cyclodextrin)s and their nanoparticles as protein delivery systems. J Control Rel 2006;112:301-11

104. Gao H, Wang YN, Fan YG, Ma JB. Conjugates of poly(D,L-lactide-co-glycolide) on amino cyclodextrins and their nanoparticles as protein delivery system. J Biomed Mater Res Part A 2007;80A:111-22

105. Lu W, Wan J, She Z, Jiang X. Brain delivery property and accelerated blood clearance of cationic albumin conjugated pegylated nanoparticle. J Control Rel 2007;118:38-53

106. Lu W, Zhang Y, Tan YZ, et al. Cationic albumin-conjugated pegylated nanoparticles as novel drug carrier for brain delivery. J Control Release 2005;107:428-48

107. Shenoy DB, Amiji MM. Poly(ethylene oxide)-modified poly(e-caprolactone) nanoparticles for targeted delivery of tamoxifen in breast cancer. Int J Pharm 2005;293:261-70

108. Akagi T, Kaneko T, Kida T, Akashi M. Multifunctional conjugation of proteins on/into bio-nanoparticles prepared by amphiphilic poly(γ-glutamic acid). J Biomater Sci Polym Ed 2006;17:875-92

109. Hirsjarvi S, Peltonen L, Hirvonen J. Layer-by-layer polyelectrolyte coating of low molecular weight poly(lactic acid) nanoparticles. Colloids Surf B Biointerfaces 2006;49:93-9

110. Farokhzad OC, Jon S, Khademhosseini A, et al. Nanoparticle-aptamer bioconjugates: a new approach for targeting prostate cancer cells. Cancer Res 2004;64:7668-72

111. Perez C, Sanchez A, Putnam D, et al. Poly(lactic acid)-poly(ethylene glycol) nanoparticles as new carriers for the delivery of plasmid DNA. J Control Rel 2001;75:211-24

112. Kim IS, Lee SK, Park YM, et al. Physicochemical characterization of poly(L-lactic acid) and poly(D,L-lactide-co-glycolide) nanoparticles with polyethylenimine as gene delivery carrier. Int J Pharm 2005;298:255-62

113. Trimaille T, Pichot C, Delair T. Surface functionalization of poly(D,L-lactic acid) nanoparticles with poly(ethylenimine) and plasmid DNA by the layer-by-layer approach. Colloids Surf A Physicochemical Engin Aspects 2003;221:39-48

114. Messai I, Munier S, Taman-Onal Y, et al. Elaboration of poly(ethyleneimine) coated poly(D,L,-lactic acid) particles. Effect of ionic strength on the surface properties and DNA binding capabilities. Colloids Surf B Biointerfaces 2003;32:293-305

115. Lee SH, Zhang Z, Feng SS. Nanoparticles of poly(lactide) – tocopheryl polyethylene glycol succinate (PLA-TPGS) copolymers for protein drug delivery. Biomaterials 2007;28:2041-50

116. Zhang Z, Feng SS. Nanoparticles of poly(lactide)/vitamin E TPGS copolymer for cancer chemotherapy: synthesis, formulation, characterization and in vitro drug release. Biomaterials 2006;27:262-70

117. Song CX, Labhasetwar V, Murphy H, et al. Formulation and characterization of biodegradable nanoparticles for intravascular local drug delivery. J Control Rel 1997;43:197-212

118. Cegnar M, Kos J, Kristl J. Cystatin incorporated in poly(lactide-co-glycolide) nanoparticles: development and fundamental studies on preservation of its activity. Eur J Pharm Sci 2004;22:357-64

119. Davda J, Labhasetwar V. Characterization of nanoparticle uptake by endothelial cells. Int J Pharm 2002;233:51-9

120. Desai MP, Labhasetwar V, Amidon GL, Levy RJ. Gastrointestinal uptake of biodegradable microparticles: effect of particle size. Pharm Res 1996;13:1838-45

121. Astete CE, Kumar CS, Sabliov CM. Size control of poly(D,L-lactide-co-glycolide) and poly(D,L-lactide-co-glycolide)-magnetite nanoparticles synthesized by emulsion evaporation technique. Colloids Surf A Physicochemical Engin Aspects 2007;299:209-16

122. Lboutounne H, Guillaume YC, Michel L, et al. Study and development of encapsulated forms of 4, 5, 8-trimethylpsoralen for topical drug delivery. Drug Dev Res 2004;61:86-94

123. Sholes PD, Coombes AGA, Illum L, et al. The preparation of sub-200 nm poly(lactide-co-glycolide) microspheres for site-specific drug delivery. J Control Rel 1993;25:145-53

124. Dong Y, Feng SS. Methoxy poly(ethylene glycol)-poly(lactide) (MPEG-PLA) nanoparticles for controlled delivery of anticancer drugs. Biomaterials 2004;25:2843-9

125. Zhang L, Hou S, Mao S, et al. Uptake of folate-conjugated albumin nanoparticles to the SKOV3 cells. Int J Pharm 2004;287:155-62

126. Rahimnejad M, Jahanshahi M, Najafpour GD. Production of biological nanoparticles from bovine serum albumin for drug delivery. African J Biotechnol 2006;5:1918-23

127. Arbos P, Campanero MA, Arangoa MA, Irache JM. Nanoparticles with specific bioadhesive properties to circumvent the pre-systemic degradation of fluorinated pyrimidines. J Control Release 2004;96:55-65

128. Michaelis M, Langer K, Arnold S, et al. Pharmacological activity of DTPA linked to protein-based drug carrier systems. Biochem Biophys Res Commun 2004;323:1236-40

129. Brzoska M, Langer K, Coester C, et al. Incorporation of biodegradable nanoparticles into human airway epithelium cells – in vitro study of the suitability as a vehicle for drug or gene delivery in pulmonary diseases. Biochem Biophys Res Commun 2004;318:562-70

130. Langer K, Coester C, Weber C, et al. Preparation of avidin-labeled protein nanoparticles as carriers for biotinylated peptide nucleic acid. Eur J Pharm Biopharm 2000;49:303-7

• **A study describing the biotin-avidin system to immobilize ligands on NP surfaces. The versatalite nature of this system allows it to be adopted for NP engineering for different ligands and tissues.**

131. Arnedo A, Espuelas S, Irache JM. Albumin nanoparticles as carriers for a phosphodiester oligonucleotide. Int J Pharm 2002;244:59-72

132. Huang M, Vitharana SN, Peek LJ, et al. Polyelectrolyte complexes stabilize and controllably release vascular endothelial growth factor. Biomacromolecules 2007;8:1607-14

133. Lin YH, Mi FL, Chen CT, et al. Preparation and characterization of nanoparticles shelled with chitosan for oral insulin delivery. Biomacromolecules 2007;8:146-52

134. Prego C, Torres D, Fernandez-Megia E, et al. Chitosan-PEG nanocapsules as new carriers for oral peptide delivery: effect of chitosan pegylation degree. J Control Rel 2006;111:299-308

135. Bravo-Osuna I, Millotti G, Vauthier C, Ponchel G. In vitro evaluation of calcium binding capacity of chitosan and thiolated chitosan poly(isobutyl cyanoacrylate) core-shell nanoparticles. Int J Pharm 2007;338:284-90

136. Mi FL, Wu YY, Chiu YL, et al. Synthesis of a novel glycoconjugated chitosan and preparation of its derived nanoparticles for targeting HepG2 Cells. Biomacromolecules 2007;8:892-8

137. Tan WB, Huang N, Zhang Y. Ultrafine biocompatible chitosan nanoparticles encapsulating multi-coloured quantum dots for bioapplications. J Colloid Interface Sci 2007;310:464-70

138. Chen F, Zhang ZR, Huang Y. Evaluation and modification of N-trimethyl chitosan chloride nanoparticles as protein carriers. Int J Pharm 2007;336:166-73

139. Bodnar M, Hartmann JF, Borbely J. Synthesis and study of cross-linked chitosan-N-poly(ethylene glycol) nanoparticles. Biomacromolecules 2006;7:3030-6

140. Katas H, Alpar HO. Development and characterisation of chitosan nanoparticles for siRNA delivery. J Control Rel 2006;115:216-25

141. Cetin M, Aktas Y, Vural I, et al. Preparation and in vitro evaluation of bFGF-loaded chitosan nanoparticles. Drug Deliv 2007;14:525-9

142. Gan Q, Wang T. Chitosan nanoparticle as protein delivery carrier-systematic examination of fabrication conditions for efficient loading and release. Colloids Surf B Biointerfaces 2007;59:24-34

143. Zhu S, Qian F, Zhang Y, et al. Synthesis and characterization of PEG modified N-trimethylaminoethylmethacrylate chitosan nanoparticles. Eur Polym J 2007;43:2244-53

144. Zhao X, Yu SB, Wu FL, et al. Transfection of primary chondrocytes using chitosan-pEGFP nanoparticles. J Control Rel 2006;112:223-8

145. Cafaggi S, Russo E, Stefani R, et al. Preparation and evaluation of nanoparticles made of chitosan or N-trimethyl chitosan and a cisplatin-alginate complex. J Control Rel 2007;121:110-23

146. Loretz B, Thaler M, Bernkop-Schnurch A. Role of sulfhydryl groups in transfection? A case study with chitosan-NAC nanoparticles. Bioconjugate Chem 2007;18:1028-35

147. Diebold Y, Jarrin M, Saez V, et al. Ocular drug delivery by liposome-chitosan nanoparticle complexes (LCS-NP). Biomaterials 2007;28:1553-64

148. Bravo-Osuna I, Teutonico D, Arpicco S, et al. Characterization of chitosan thiolation and application to thiol quantification onto nanoparticle surface. Int J Pharm 2007;340:173-81

149. Krauland AH, Alonso MJ. Chitosan/cyclodextrin nanoparticles as macromolecular drug delivery system. Int J Pharm 2007;340:134-42

150. Grenha A, Grainger CI, Dailey LA, et al. Chitosan nanoparticles are compatible with respiratory epithelial cells in vitro. Eur J Pharm Sci 2007;31:73-84

151. Prego C, Fabre M, Torres D, Alonso M. Efficacy and mechanism of action of chitosan nanocapsules for oral peptide delivery. Pharm Res 2006;23:549-56

152. Zheng Y, Wu Y, Yang W, et al. Preparation, characterization, and drug release in vitro of chitosan-glycyrrhetic acid nanoparticles. J Pharm Sci 2007;95:181-91

153. Zhang H, Oh M, Allen C, Kumacheva E. Monodisperse chitosan nanoparticles for

mucosal drug delivery. Biomacromolecules 2004;5:2461-8

154. Chen L, Subirade M. Chitosan/β-lactoglobulin core-shell nanoparticles as nutraceutical carriers. Biomaterials 2005;26:6041-53

155. 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;14:1431-6

156. Gan Q, Wang T, Cochrane C, McCarron P. Modulation of surface charge, particle size and morphological properties of chitosan-TPP nanoparticles intended for gene delivery. Colloids Surf B Biointerfaces 2005;44:65-73

157. Sajeesh S, Sharma CP. Novel pH responsive polymethacrylic acid-chitosan-polyethylene glycol nanoparticles for oral peptide delivery. J Biomed Mater Res Part B Appl Biomater 2006;76:298-305

158. Park K, Kim JH, Nam YS, et al. Effect of polymer molecular weight on the tumor targeting characteristics of self-assembled glycol chitosan nanoparticles. J Control Rel 2007;122:305-14

159. Jintapattanakit A, Junyaprasert VB, Mao S, et al. Peroral delivery of insulin using chitosan derivatives: a comparative study of polyelectrolyte nanocomplexes and nanoparticles. Int J Pharm 2007;342:240-9

160. Kim SH, Jeong JH, Chun KW, Park TG. Target-specific cellular uptake of PLGA nanoparticles coated with poly(L-lysine)-poly(ethylene glycol)-folate conjugate. Langmuir 2005;21:8852-7

161. Mo Y, Lim LY. Paclitaxel-loaded PLGA nanoparticles: potentiation of anticancer activity by surface conjugation with wheat germ agglutinin. J Control Rel 2005;108:244-62

162. Tosi G, Costantino L, Rivasi F, et al. Targeting the central nervous system: in vivo experiments with peptide-derivatized nanoparticles loaded with loperamide and rhodamine-123. J Control Rel 2007;122:1-9

163. Costantino L, Gandolfi F, Tosi G, et al. Peptide-derivatized biodegradable nanoparticles able to cross the blood-brain barrier. J Control Rel 2005;108:84-96

164. Zhang N, Chittasupho C, Duangrat C, et al. PLGA Nanoparticle-peptide conjugate effectively targets intercellular cell-adhesion molecule-1. Bioconjug Chem 2008;19:145-52

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