Engineering Biodegradable Polyester Particles With Specific Drug Targeting and Drug Release Properties

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ABSTRACT: Poly(lactic acid) (PLA) and poly(lactic-co-glycolic acid) (PLGA) microspheres and nanoparticles remain the focus of intensive research effort directed to the controlled release and *in vivo* localization of drugs. In recent years engineering approaches have been devised to create novel micro- and nano-particles which provide greater control over the drug release profile and present opportunities for drug targeting at the tissue and cellular levels. This has been possible with better understanding and manipulation of the fabrication and degradation processes, particularly emulsion-solvent extraction, and conjugation of polyesters with ligands or other polymers before or after particle formation. As a result, particle surface and internal porosity have been designed to meet criteria-facilitating passive targeting (e.g., for pulmonary delivery), modification of the drug release profile (e.g., attenuation of the burst release) and active targeting via ligand binding to specific cell receptors. It is now possible to envisage adventurous applications for polyester microparticles beyond their inherent role as biodegradable, controlled drug delivery vehicles. These may include drug delivery vehicles for the treatment of cerebral disease and tumor targeting, and codelivery of drugs in a pulsatile and/or time-delayed fashion. © 2007 Wiley-Liss, Inc. and the American Pharmacists Association J Pharm Sci 97:71–87, 2008

Keywords: controlled delivery; targeted drug delivery; poly(lactic/glycolic) acid(PLGA or PLA); nanoparticles; microspheres; microencapsulation

INTRODUCTION AND SCOPE OF REVIEW

Advanced drug delivery vehicles based on biodegradable polyesters primarily use poly(ε-caprolactone) (PCL), poly(lactic acid) (PLA) and poly (lactic-co-glycolic acid) (PLGA) matrices.[1] The low immunogenicity and toxicity of these polyesters offers a biocompatible vehicle[2,3] whose physicochemical and mechanical properties can be tailored via selection of polymer molecular weight, copolymerization, and functionalization. The number of commercial formulations employing PLGA or PLA matrices for drug delivery is growing and is expected to continue to do so in line with the promise of further peptide, protein, and DNA-based drugs emerging from the biotechnology sector. It is clear that isolated knowledge of polymer chemistry, materials characterization, or the pathophysiology and cellular mechanism of the diseases is not sufficient to achieve novel, useful particles with therapeutic potential. Specific drug targeting and release properties have been accomplished via collaborative work bridging the materials and life sciences.

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This review discusses the current methodologies used to engineer PLGA and PLA particles with specific external and internal morphologies and derivatization of particles with other polymers or ligands. The utility of the particles is then discussed, that is, in passive or active targeting and controlled release strategies, alongside the corresponding disease target or envisaged application. This review does not focus on general fabrication techniques and novel methods such a microchannel mixing, which are reviewed in detail elsewhere.[4] Similarly, there is a large volume of data describing PLGA and PLA bioresorbable matrices for use as tissue engineering scaffolds, whose fabrication processes, material, and drug release properties inevitably overlap somewhat with those for micro- and nanoparticles.[5,6] The area of "Polymeric Prodrugs"[7] has been used to describe colloidal copolymer systems which may involve the grafting of one polymer, for example, an anhydride, onto a polyester. This area of research is very broad ranging, including nanoparticles, liposomes, micelles, and dendrimers, and discussion here will be limited to polyester copolymers yielding distinct, solid particles. The term "microsphere" as used below refers to a small, porous sphere typically from 1 to 500 μm in diameter. Alternative terms such as microcapsules and microparticles are also encountered in the literature, with nanospheres or nanoparticles referring to particles 10–1000 nm in size.

GENERAL FACTORS AFFECTING PARTICLE MORPHOLOGY

During the fabrication of polyester microspheres, slight changes in the formulation parameters can have radical effects on microsphere morphology and, in turn, drug release.[8] With respect to double emulsion-solvent extraction, the mechanisms controlling microsphere internal porosity have been established for some time.[9] Internal porosity is dependent on the stability of the primary emulsion.[10] More viscous primary emulsions due to high oil-phase volumes or polyester concentrations are less easy to break by homogenization and yield larger microspheres;[8,11] high shear rates and low molecular weight PLGA reduce microsphere size.[12] During the secondary emulsion, high concentrations of emulsifier or low volumes of continuous phase, minimize microsphere size via increased emulsion stability and reduced solvent evaporation, respectively.[13] Emulsification at low temperatures minimizes solvent evaporation and the outer shell of the embryonic microspheres pushes the inner water-droplets toward the soft core as it hardens, facilitating droplet coalescence.[14]

Less is understood regarding the mechanisms underpinning the microsphere external morphology, although the formation of the surface pores is thought to occur on water extrusion from osmotic imbalance.[8] Surface porosity appears to be influenced by the PLGA concentration, the volume of the inner water phase and substitution of nonionic surfactants for PVA.[15] The release of encapsulated drug during solvent evaporation, which is dependent upon its physico-chemical properties, was further observed to be a critical parameter of microsphere morphology.[15] A "defective skin surface" has been described as a consequence of using low oil-phase volumes,[8] and use of tricaprin oil (a medium-chain triacylglycerol) in emulsion-solvent extraction microsphere fabrication unexpectedly yielded microspheres with dimpled surfaces.[16] Substitution of nonionic surfactants for PVA in the primary emulsion has been empirically observed to change surface roughness according to the surfactant's hydrophile–lipophile balance.[17] Surface charge and hydrophobicity influence adhesion and uptake of microspheres by the intestinal M-cells and enterocytes[18] and surface engineering may yield microspheres with favorable dispersion and flow properties for pulmonary delivery, discussed below.

DRUG TARGETING

Passive Targeting

Pulmonary Delivery

An attractive utilization of PGLA microspheres is the formulation of small organic drugs or biopharmaceuticals for inhalation devices, not least because of the medical need for inhaled insulin. PLGA microspheres are suitable for engineering into aerosols so long as several criteria can be met: (i) an aerodynamic diameter (d_a) of around 3 μm with a narrow size distribution to present the particles to the lung periphery, (ii) a small surface contact area for good dispersion and flow characteristics, (iii) a minimal hygroscopic nature to reduce capillary interaction.[19] To date, this has been largely achieved by PLGA "large porous particles" using simple preparation techniques based on double (w/o/w) and single (o/w) emulsion

extraction.[20] Key to the success of the particles is their highly porous nature which reduces particle mass density (ρ) to $\sim$0.4 g/cm^3, such that, although the mean geometric diameter (d) is $>$5 μm, d_a becomes 3 μm (given that $d_a = d^*\sqrt{\rho}$).

Fabrication of rifampicin-loaded PLGA microspheres for inhalation compared double emulsion-solvent extraction and spray drying techniques, taking eight variables for the emulsification in a factorial design and using a Box Behnken statistical approach to optimize against drug loading and particle size.[21] Interestingly, the surface of the spray dried microparticles appeared to have collapsed inwards (and had smaller volume median diameter ca. 2 μm), in contrast to spherical particles from the emulsion–extraction method (ca. 4 μm diameter). *In vivo* clearance of *Mycobacterium tuberculosis* for the solvent extraction formulation in a guinea pig model of infection, administered via nebulization and insufflation showed dose-dependent bacterial load, alongside fewer lung lesions.[22] Other studies aiming to target the lung using PLGA microspheres for cisplatin therapy have quoted large size ranges of 5–30 μm for smooth microspheres stable over a 3 month period. Although no corresponding particle density was measured, these spheres would appear to be too large for effective deposition to the peripheral lung and, unusually, *in vivo* distribution and pharmacokinetics were measured following intravenous injection rather than following pulmonary administration.[23]

Further engineering of the particle surface may improve dispersion and flow of PLGA aerosols by minimizing particle–particle contact area. Using atomic force microscopy (AFM), direct measurement of the interaction between aerosol particles can be made to inform a fundamental understanding of adhesion–cohesion and how this effects *in vitro* performance of carrier-based formulations from dry powder inhalers.[24] Minimization of the particle surface area is aptly demonstrated by Nature in fungal spores which have surface asperities or dimples,[25] and also by research directed to particle surface engineering. Chew et al.[26,27] produced albumin particles of d_a $\sim$2.8 μm with a corrugated surface, minimizing particle cohesive forces. Relating surface corrugation (quantified by light scattering and Rayleigh–Gans–Debye theory to give a "fractal dimension") to the fine particle fraction (the mass of particles $\leq$5 μm in the aerosol) showed that improvement in aerosol performance can be achieved for minimal surface corrugation via reduced contact areas and increased particle separation.[27]

Therefore, an alternative route to achieving PGLA aerosols would be to focus on engineering-specific surfaces. Establishing primary emulsions with poor stability using blends of poly(ethylene oxide)/poly(propylene oxide)/poly(ethylene oxide) (PEO/PPO/PEO) triblock copolymers (Pluronics®) generated PLGA microspheres with hollow interiors: "microcapsules" of low density, 0.24 g/cm^3, and 3.43 μm geometric mean weight diameter, suitable for inhalation (Fig. 1).[28] The hollow nature of the particles was due to the poor stability of the primary emulsion, leading to internal water droplet coalescence, while maintaining a stable secondary emulsion to prevent oil droplet coalescence. Triblock copolymers self-assemble into colloidal systems in aqueous media with and without additional surfactants.[29] These colloids have long been exploited in the templating of mesoporous silica microspheres, similar in morphology to polyester microspheres.[30] While the chemistry behind the formation of the particles is entirely different, it is likely that the Pluronics imprinted the embryonic PLGA microcapsules to generate the dimpled surfaces, whose depth and definition were greatest for Pluronics with high MW hydrophobe blocks.[28]

It is apparent that porous PLGA microspheres with surface asperities mimicking fungal spores are rarely reported, if at all. Alternative engineering of PLGA microspheres could address this drawback. The question of how to proceed experimentally may be found in work describing "biocolloids" which self-assemble during emulsification to yield patterned microspheres remarkably similar to spores.[31,32] In these reports, interparticulate van der Waal's attraction and electrostatic repulsion were controlled via the ionic strength and Hamaker constant, respectively, to stabilize styrene aggregates in o/w emulsions, but their application to polyester microsphere fabrication may be limited. Other considerations may be more applicable: (1) superposition of excess surfactant, forming discreet lamellae microstructures in a ternary system with an oil and water, on emulsion droplets within a certain timescale[32] (the requirement for an appropriate timescale over which colloidal particles can migrate to an interface is elegantly shown for the formation of "Trojan particles"[33]), (2) the addition of a strongly adsorbing polymer but in insufficient quantities such that bridging-flocculation occurs.

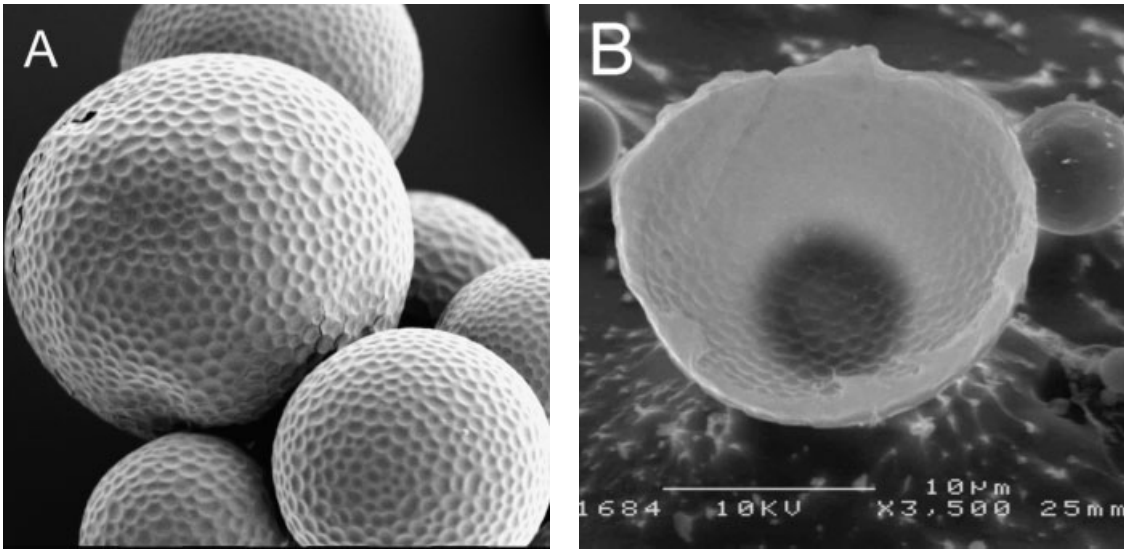

Figure 1. SEM micrographs for (A) surface morphology of the microcapsules fabricated using Pluronic L92 in the primary emulsion (magnification ×3500) and (B) the corresponding internal morphology for microcapsules prepared using freeze-fracture techniques. Adapted from Mohamed & van der Walle.[28]

Two further considerations may be made toward the optimization of microparticulate systems for lung targeting. The first is size uniformity for maximum lung deposition. The membrane emulsification technique may be most applicable to the fabrication of uniform particles with a d_a of 3–5 μm.[34,35] However, the procedure is not rapid since the primary emulsion must be forced under pressure through the porous glass membrane. This requires good primary emulsion stability, which may be difficult to achieve and then compromise the integrity of biopharmaceuticals. The second is mucoadhesion. PLGA and PLA are both poorly adhesive in comparison with polyanhydrides and polymers of fumaric and sebacic acid, possibly due to limited hydrogen bonding potential with mucus glycoproteins.[36] The engineering of mucoadhesive microparticulate systems has been described in detail elsewhere[37] but there are interesting applications to lung targeting. For example, PLGA nanoparticles were made mucoadhesive either by the inclusion of chitosan into the aqueous phase of the emulsion (with poly vinylalcohol, PVA), or mixing the nanoparticles with chitosan, keeping polymer concentrations $\leq$0.5% to prevent particle aggregation.[38] The mucoadhesive PLGA nanoparticles were subsequently nebulized and showed increased retention in the lung, compared to rapid mucociliary clearance of noncoated particles, and prolonged drug action.[39] A recent study by the same group using the same formulation suggested that increased drug absorption was due to chitosan-mediated transient loosening of the tight junctions between cells[40]; the mechanism of which is now partially understood to involve protein kinase Cα migration to the cell membrane without activation and, unspecified, second messenger pathways.[41]

Nanoparticles

Classical emulsion–solvent extraction protocols yield microspheres around 100 μm which are suitable for oral delivery[18] and may be further modified for mucosal adhesion, as above. For parenteral administration or for the production of particles intended for cell internalization via endocytosis, a reduction in size by around three to four orders of magnitude, respectively, is clearly required. Suitable protocols have included "Phase Inversion Nanoencapsulation", which is similar to coacervation but involves a primary dispersion of solid-in-dichloromethane poured into petroleum ether without stirring, causing phase separation of the polymer and nanoparticle formation.[1] The cytokine interleukin-12 has been encapsulated in such nanoparticles and injected intratumorally to tumor-bearing mice, resulting in tumor regression and metastatic suppression.[42] Nanoparticles are nonspecifically internalized into cells via adsorptive endocytosis, initially

observed for endothelial cells.[43] Since particles internalized into endosomes are subject to acidification of the endosome environment and enzymatic activity ultimately in the lysosome, this can be problematic for drug stability. Therefore, it was interesting that rapid escape of nanoparticles from the endosome was observed,[44,45] dependent upon particle surface charge and fusion with the endosomal membrane. The mechanism by which nanoparticles are taken up is likely to involve the clathrin- or calveolin-mediated pathway for particles <200 nm and >200 nm diameter, respectively,[46] although nonspecific adsorptive endocytosis has also been reported.[47]

Polyester nanoparticulates with and without the inclusion of polycations such as chitosan for condensation of DNA and application to gene or DNA vaccine therapies have also been tested.[48] However, it is worth noting that the use of distinct, solid, polyester nanoparticles in gene and tumor therapy is little reported compared to other polymeric aggregates.[49,50] Nevertheless, there are some pertinent examples. He et al.,[51] prepared PLGA nanoparticles of ca. 70 nm encapsulating the thymidine kinase gene, expressed as a fusion protein targeting hepatocellular carcinomas. Following intravenous administration the greatest distribution of the particles was in the liver, in this case exploiting the reticuloendothelial system, more specifically, phagocytic cells residing in the peripheral lymphoid tissue including the bronchus-associated lymphoid tissue and liver Kupffer cells. Since surface chemistry and particle size determine the rate and primary site of nanoparticulate elimination following intravenous administration, this may represent a good strategy for targeting hepatocytes; nanoparticulate targeting of related macrophages is reviewed elsewhere.[52] Another interesting application using PLA nanoparticles to exploit the reticuloendothelial system was described for the targeting of macrophages within the spleen for delivery of arjunglucoside I in hamster models of leishmaniasis.[53] Although the targeting mechanism remained undefined, since both hydrophobic PLA and hydrophilic nanoparticles were equally efficient in their clearance of spleen parasitic loads, the work is a good example of how PLA nanoparticles can be used in passive targeting to increase drug efficacy while concomitantly decreasing toxicity (in this case hepatoxicity and nephrotoxicity of arjunglucoside I).

In a further demonstration of DNA encapsulation into PLGA nanoparticles, delivery of plasmid DNA encoding the p53 tumor suppressor gene resulted in marked reduction in the proliferation of breast cancer cells.[54] The formulation was suggested to be superior to polyplex (DNA complexation with cationic polymer) possibly as a result of favorable release kinetics from the endosome following cell uptake. Interestingly, extensive particle washing following emulsion–solvent extraction should be observed since residual PVA was demonstrated, among other variables such as polymer type and DNA release, to reduce efficient cellular internalization.[55] Breast tumors have also been passively targeted *in vivo* using PCL nanoparticles coated with PEO–PPO–PEO copolymers following intravenous administration. Here, PCL was dissolved in acetone and homogenized with an aqueous solution of Pluronic, the strength of adsorption being dependent on the PEO:PPO ratio (thicker coatings were obtained with Pluronic F-108 than F-68).[56] Tail vein injection of the PEO–PCL nanoparticles and also PEO-poly(β-amino ester) nanoparticles into female mice with/without tumor load, showed both breast tumor and liver accumulation.[57] However, while the PEO increased circulation time *in vivo*, the poly(β-amino ester) microspheres localized more efficiently to the tumor and released their DNA load more rapidly, being pH sensitive polymers.

It is clear that to dissect the underlying mechanisms involved in *in vivo* localization of particles, an understanding of the particle-tissue macroscale interface is necessary, which can be acquired through detailed imaging studies. To this end, one report has described the synthesis of PLGA-fluorescein and PLGA-biotin for nanoparticle preparation using carboxyl-terminal PLGA and activation with carbodiimide and N-hydroxysuccinimide.[58] As proof of concept, following femoral and brain perfusion of a suspension of these nanoparticles, their distribution could directly be determined by fluorescence microscopy or light microscopy with streptavidin-peroxidase staining. These polymers could therefore be employed in conjunction with surface engineering approaches to more readily visualize particle–tissue interactions. A further application has been the recent report describing the inclusion of magnetite powder (Fe_3O_4) into nanoparticles (100–200 nm) of poly ethyleneglycol (PEG)–PLA block copolymers of varying mass ratios (PLA:-PEG 2 to 12).[59] The magnetic nanoparticles were proposed to provide controlled release reservoirs with drug targeting via magnetic localization but

may also be useful for imaging through electron microscopy. The use of PEG to provide a highly hydrated surface and thereby minimize nonspecific adsorption of the coated particle with serum proteins and surfaces is well established. In addition to the ring-opening synthesis of PEG–PLA copolymer described by Ren et al.,[59] simple electrostatic coating of spray-dried PLGA particles with poly-L-lysine (PLL)-*g*-PEG in low ionic strength buffers has been used to generate protein repellent particles.[60] Characterization of the PLL-*g*-PEG-coated particles showed that they retained <1% of the protein adherent properties of the noncoated particles and may therefore be of use for increasing circulation times *in vivo*.

Active Targeting

Functional Surfaces as Platforms for Ligand Derivatization

As described above, the terminal carboxyl group of PLGA or PLA (assuming they are not end-capped with an alkyl ester) can be activated using carbodiimide for ligand derivatization. However, this route is inherently inefficient because the mole fraction of free acid to monomer is low. To overcome this obstacle, one strategy has been to coat polyester microparticles with polymers harboring high densities of functional groups (e.g., polyamines), either via covalent conjugation with the polyester before particle fabrication or nonspecific or ionic binding with the fabricated particles.[60] Recently, Keegan et al.[61] exploited the observation that a residual layer of PVA emulsifier remains coated to washed particles produced by emulsion–solvent extraction. By substituting poly(ethylene-alt-maleic acid) for PVA, it was possible to present a high density of carboxylic groups on the particle surface. The poly(ethylene-alt-maleic acid) layer was stable enough to facilitate carbodiimide coupling of proteins to the surface, while retaining the basic properties of PLGA particle biodegradation, drug encapsulation, and controlled release.[61] The partial negative charge of the polyester monomers can also be exploited for microsphere surface derivatization with cationic polymers. Chitosan-polyester blends have proven popular, not only to alter the surface charge of the particle[48] but also to incorporate functional amine groups for further ligand conjugation.[62] In the latter, PLGA (oil phase) and chitosan (aqueous phase) were homogenized using a micromixer technique, with zeta potential measurements confirming a polycationic layer; proof of concept for derivatization of the chitosan layer was obtained by the formation of a N-hydroxysuccinimide–PEG–biotin adduct.

Work from different groups has also demonstrated that PLGA/PLA particles can be coated with avidin to yield a "bridge" by which the particle surface may bind biotinylated ligands. Although noncovalent protein–ligand interactions may be considered unsuitable for cell targeting, the avidin–biotin bond is almost irreversible (K_d 10^{-15} M) and its strength has been directly measured as 3–170 pN.[63] One route to surface modification with avidin involved addition of fatty acid–avidin conjugates into the emulsion–solvent extraction protocol, wherein the hydrophobic properties of the fatty acid chain caused partitioning of the conjugate to within oil phase and thereby the relatively hydrophobic PLGA matrix interior (Fig. 2).[64] In doing so, it was shown that avidin remained displayed by the microsphere during hydrolysis. The mechanism of microsphere erosion and degradation therefore becomes important, microspheres generally undergoing bulk erosion on account of their size (discussed below). The functional activity of the avidin was preserved during microsphere fabrication, although more detailed structural analysis was not undertaken to determine the protein fraction denatured, given that the o/w interface is highly destabilizing to proteins.[65,66] An alternative route to particle coating was with avidin-employed thiolation of free carboxyl groups on the surface of PLA particles using water-soluble carbodiimide and cystamine dihydrochloride, followed by reduction of the disulphide (Fig. 3).[67] The reactive cross-linking agent, *m*-maleimidobenzoyl-*N*-hydroxysulfosuccinimide ester, was then used to covalently link avidin via its amine side chains to the thiol groups at the particle surface. (Alternatively,

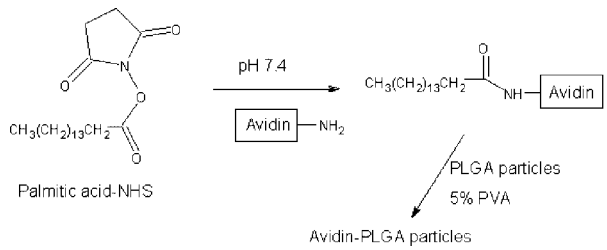

Figure 2. Conjugation of palmitic acid to avidin and incorporation of avidin–palmitate on the surface of PLGA particles using the double emulsion–solvent extraction protocol. NHS, N-hydroxysuccinimide. Adapted from Fahmy et al.[64]

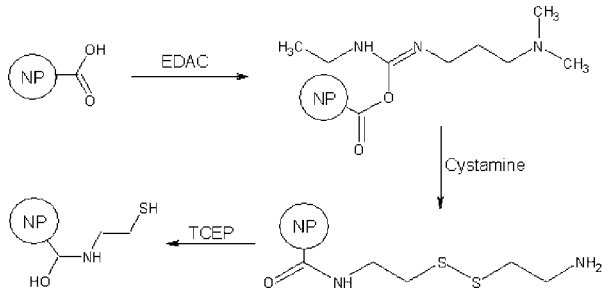

Figure 3. Schematic presentation of the coupling of thiol groups on a nanoparticle (NP) using cystamine as substrate. EDAC, 1-ethyl-3-(3-dimetylaminopropyl) carbodiimide; TCEP, Tris(2-carboxyethyl)phosphine hydrochloride. Adapted from Nobs et al.[67]

protein coupling could proceed via disulphide formation). Although the density of thiol groups, and more so avidin, at the particle surface was low, the protocol was considered sufficiently robust to be of use in microsphere-targeting strategies.

Direct Conjugation to Specific Ligands

There are several reviews describing the selection and use of appropriate ligands for active targeting purposes, including intracellular targeting, for drug delivery.[68,69] Generic routes by which such ligands may be conjugated to functionalized polyester microspheres are discussed above. Direct conjugation of ligands to PLGA microparticles has also been demonstrated to be feasible and successful in particle-targeting strategies. A pulmonary epithelial lectin-binding protein, wheat germ agglutinin, was conjugated to PLGA nanoparticles fabricated via an emulsion–extraction protocol wherein the drug, paclitaxel, was added to the oil phase of the emulsion with isopropyl myristate to increase drug loading.[70] Wheat germ agglutinin was covalently bound to free surface carboxyl groups using carbodiimide/N-hydroxysuccinimide, although the addition of the long-chained fatty acid decreased coupling efficiency. An improved cytotoxic activity was then demonstrated to be due to receptor-mediated endocytosis upon lectin binding and internalization into the lung epithelial A549 cells.

The tripeptide arginine–glycine–aspartic acid (RGD) has gained widespread employment for cell adhesive surfaces and as a targeting ligand to the integrin receptors on mammalian cell surfaces. RGD peptides have been conjugated to PLA microcapsules (in this case purposefully engineered to be hollow, i.e., encapsulated microbubbles, to form an effective ultrasound contrast media) which were then able to bind breast cancer cells in culture.[71] However, *in vivo* studies employing RGD-targeted particles may be problematic since integrins are ubiquitous, particularly at the basolateral surface of epithelium and apical surface of endothelium, and RGD cannot be used to distinguish between different integrin subtypes, which is acknowledged by the authors. RGD binding to cells expressing both αvβ3 and α5β1 integrin, favors αvβ3 binding over α5β1 on account of peptide conformational restriction.[72] Near-maximal α5β1 integrin-binding activity requires the "synergy site" (PHSRN) on the 9th type III fibronectin domain (^{9}FNIII) in addition to RGD on ^{10}FNIII, whereas maximal αvβ3-binding activity is seen with RGD alone.[73] The spatial relationship and conformation of the $^{9–10}$FNIII domain pair is also critical to α5β1 integrin-binding affinity,[74,75] therefore, it is not possible to simply incorporate a PHSRN peptide in addition to RGD, as a minimalist system. Although $^{9–10}$FNIII has not been conjugated to microspheres as a targeting ligand, only having been encapsulated,[17] a potential application would be targeting of tumor blood vessels which overexpress α5β1 integrin.[76]

An alternative to integrin targeting of the endothelium has been the design of a two-ligand system directing microspheres to E-selectins. Of particular interest was that derivatizing the surface of microspheres with E-selectin ligand (sialyl Lewis(x)) in combination with antibodies against intercellular cell adhesion molecule-1, enabled the microspheres to mimic the *in vivo* leukocyte adhesion response to inflamed vasculature, delivering encapsulated drug to locally inflamed tissue, as during disease.[77] Recently, PLGA nanoparticles have been targeted to the brain via synthetic opioid peptide analogs coupled to the nanoparticles using carbodiimide/N-hydroxysuccinimide.[78] Importantly, the peptides were exposed outward from the particle surface, rather than tightly adsorbed, and using *in vivo* brain perfusion models and fluorescent microscopy, the peptide-derived nanoparticles were shown to be able to cross the blood–brain barrier. However, as for RGD and avidin–sulphydryl coupling, above, the issue of ligand density remains problematic. Dendrimeric polyamines, often used in gene delivery studies,[79] may overcome this obstacle since their conjugation to the end-carboxyl of

PLGA greatly increases the number of functional groups for ligand coupling. This strategy was used by the same group to improve surface derivatization of PLGA nanoparticles for targeting nanoparticles across the blood–brain barrier.[80] The dendrimers could also be employed to render polyvalent ligand displays on the particle surface which would be particularly useful for integrin-targeting systems.[81] Other strategies for the targeting of polyester nanoparticles across the blood–brain barrier may be applied from work with poly(butyl cyanoacrylate) nanoparticles. Here, nanoparticles were coated with polysorbate 80 and the mechanism for uptake across the blood–brain barrier was determined to be mediated by apolipoproteins B and E, possibly mimicking receptor-mediated endocytosis of lipoproteins.[82]

DRUG RELEASE

Microsphere Surface Porosity and Drug Burst Release

Although polyester microspheres are purported to facilitate a steady, prolonged release of encapsulated drug, this is generally not the case. The biggest problem in this respect is the so-called "burst release" wherein >60% of the drug load is released within the first 24 h, though this may depend on whether w/o/w or s/o/w emulsions are chosen.[83] Surface porosity has naturally been suspected of directly determining the drug release profile and some studies have shown this experimentally and by computer modeling.[8,84] An interesting observation of the surface morphology for microspheres immersed in aqueous buffer was reported wherein a "skin" enveloped the outer surface of the microspheres within 24 h.[85] The concomitant loss of external pores was suggested to result in the subsequent termination of the burst release period. The mechanism can be attributed to the plasticizing effect of water (or moisture) on amorphous polyesters such as PLGA and PLA, seen as a concomitant fall in the glass transition temperature (T_g) of the polymer by differential scanning calorimetry (DSC).[86] By understanding moisture effects on the T_g of the matrix, chain mobility and surface structure, it may be possible to modulate the surface properties of PLGA microspheres for controlled release. Recently, PLGA plasticization was achieved through temporary storage at high humidity environments to bring about collapse of the pore walls in order to "seal" the microsphere surface and so attenuate the burst release of encapsulated protein (Fig. 4).[87] A first-order release profile was not observed, rather, a second burst release phase was recorded after 8 weeks, equivalent in terms of protein release to the primary burst release phase. Although the secondary release phase at around 8 weeks is generally accounted to erosion of the PLGA matrix, the unusually rapid nature of this release phase probably points to remodeling of the internal pores.

It has also been reported that internal and external porosity vary according to drug loading, with high drug loading increasing pore tortuosity and decreasing pore size to bring about a reduction in the burst release.[88] However, this observation may have been peculiar to the drug encapsulated (propafenone) and the unusual addition of base (triethylamine) to the emulsion–solvent extraction protocol. Nevertheless, the principle of altering the drug diffusion coefficient by modulating pore tortuosity and pore size distribution is consistent with the above studies. Burst release of encapsulated drug from PLGA or PLA particles has also been attenuated by combining nanoprecipitation or emulsion–solvent extraction protocols with a novel gel encapsulation technique.[89] The method exploits the thermo-reversibility of triblock copolymers, and is unusual in that drug and Pluronic F127 are allowed to gel (at 37°C) and then homogenized with polyester dissolved in acetone, after which the mixture is cooled such that Pluronic F127 undergoes gel-sol transition to a liquid, extracting the acetone and forming the particles; the reduced burst release being attributed to the localization of the drug to the particle core.[90]

A similar route to the formation of particles from gel networks is described for the production of composites of PLGA and mesoporous silicas.[91] Mesoporous silicas, such as SBA-15 are well established in the catalysis and petrochemical industries[92] and recently have been applied to drug delivery, particularly for gene therapy.[93] Their homogenous porous morphologies are particularly attractive in that drugs may be entrapped according to molecular weight and charge. Xue and Shi,[91] prepared mesoporous silica particles using a classic acid-catalyzed hydrolysis polymerization of an alkylsilane precursor (tetraethoxysilane). The templating surfactant, Pluronic P123, was then removed by calcination and gentamycin loaded into mesoporous silica particles; these silica particles were then dispersed in

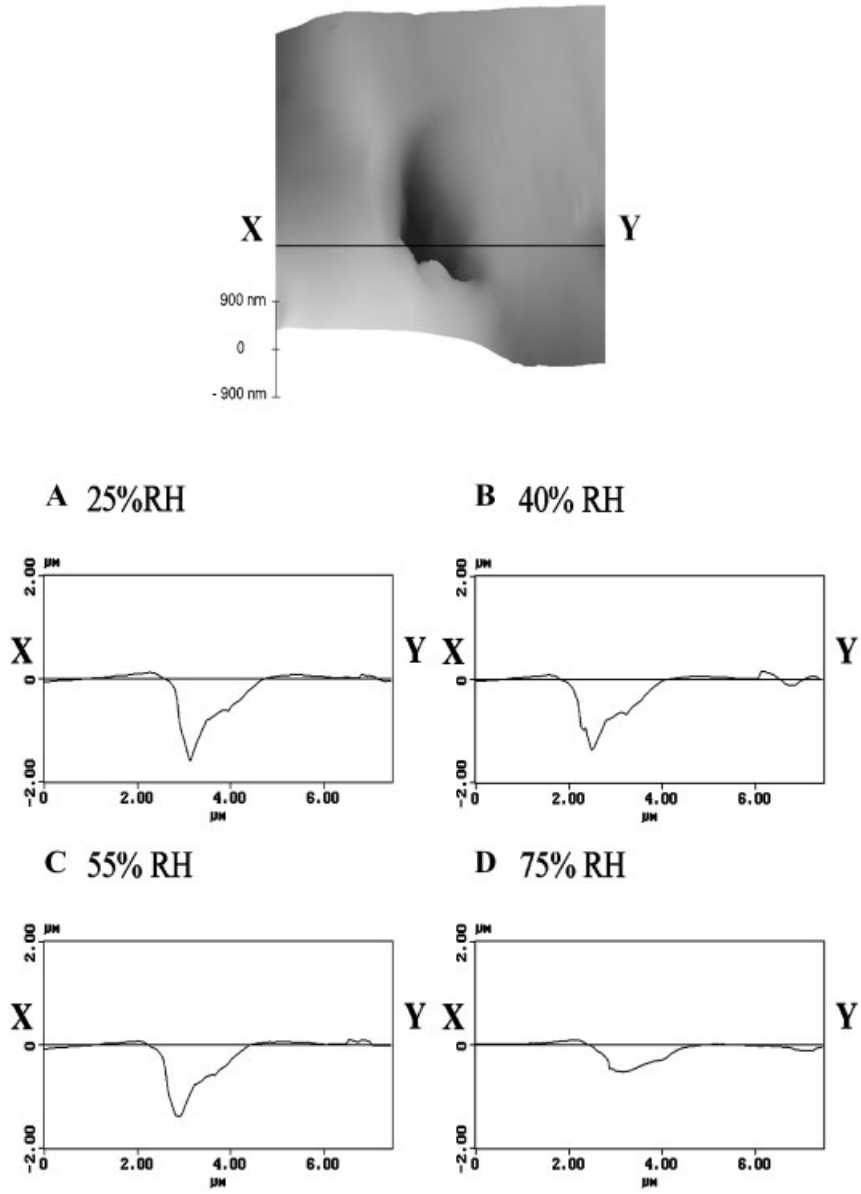

Figure 4. AFM topography of a surface pore for a microsphere containing FIII9'-10 and Triton X-100 under ambient humidity at 20°C. A cross-section along the line marked X-Y revealed the modification of the pore's profile under relative humidities of 25% (A), 40% (B), 55% (C), and 75% (D). Adpated from Bouissou et al.[87]

the oil phase of a typical emulsion–solvent extraction protocol for PLGA microsphere production (Fig. 5).[91] The silica-PLGA hybrids showed decreased burst release, presumably because gentamycin release from the silica particle required diffusion through the enveloping PLGA matrix.

A practical example wherein surface remodeling can be used to attenuate the burst release and so prevent release of otherwise toxic doses is the encapsulation of dexamethasone in PLGA microparticles. Here the PLGA particle surface was cross-linked by UV-induced polymerization of a tri(ethylene glycol) dimethacrylate monomer,[94] although toxic metabolites and drug stability to UV exposure may be a concern to widespread application. As for closure of surface pores, cross-linking of the particle surface with second polymer formed a diffusional barrier to drug release. Attenuation of the burst release was observed

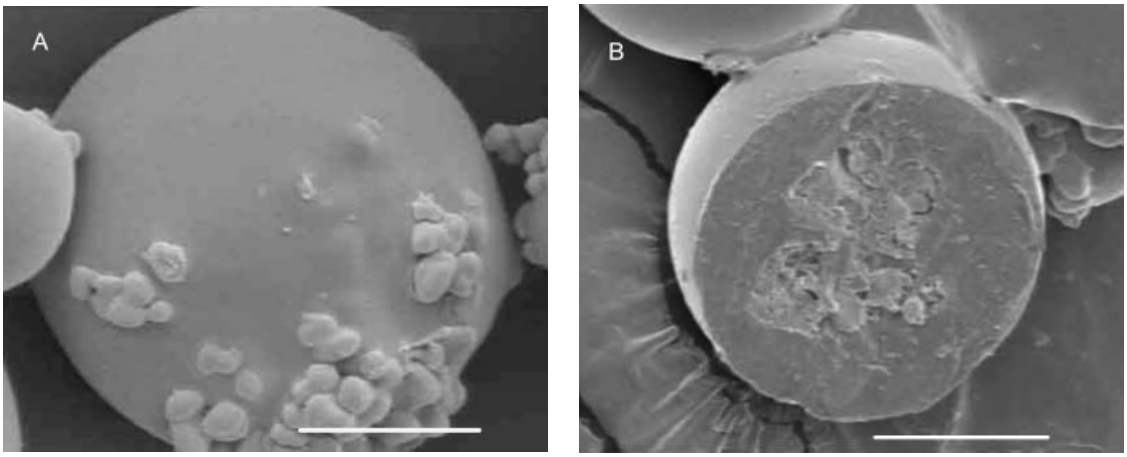

Figure 5. SEM micrographs showing the external (A) and internal (B) microstructures of PLGA-silica hybrid microspheres fabricated at a mesoporous silica/polymer weight ratio of 0.133. Adapted from Xue and Shi.[91]

for both dexamethasone and its salt form, the latter using phase separation/coacervation for drug encapsulation, rather than simple o/w emulsion. Since optimizing formulations can be complex given the number of variables to consider, it is perhaps not surprising that recent work has employed a statistical approach using Box Behnken design, to achieve controlled drug release, underpinned by data relating to each of the parameters chosen.[95] Another simple protocol, addressing the requirement for an homogenous dispersion of drug throughout the particle matrix rather than secondary diffusional barriers, involved spontaneous emulsification and coprecipitation of protein and PLGA.[96] Here, the charge of the surfactant was paired against that of the encapsulated protein dissolved with PLGA in the oil phase, the reported decrease in the protein burst release was demonstrated *in vitro* and following *in vivo* administration. However, it is worth noting that release profiles may be complicated by adsorption of drug to the particle surfaces[97] and the aqueous buffers typically used for determination of the drug release profile may not relate to the final formulation in which the polyester particles are included.[98]

Core-Shell Microspheres

Instead of remodeling the microsphere surface, the burst release may be attenuated by coating a (core) polyester microsphere with another polymer. The fabrication of double-walled microspheres for this purpose is well established[99] and, moreover, by careful selection of PLGA and PLA molecular weights (e.g., ~50 kDa and 20 kDa, respectively) and concentration in the oil phase (~20% w/v), their formation can be directed in a one-pot protocol via phase separation of the two polymers.[100] Varying the ratio of PLGA:PLA facilitates control over the encapsulation of the protein to the inner core or the outer shell. Recently, the phase separation method for the preparation of double-walled PLA/PGLA microspheres has been applied to the localization of cisplatin to an inner PLGA core surrounded by a PLA layer.[101] The release of cisplatin was dependent on the initial drug distribution, with near zero-order release from the PLGA core governed chiefly by erosion of the outer PLA layer but also by microsphere swelling during water ingress and integrity of the outer layer.[102]

More recent advances in coating technologies include layer-by-layer deposition of polymers to generate microcapsules with controlled wall thickness and chemical nature to give specific mechanical and permeability properties.[103] While layer-by-layer assembly relating directly to polyester microspheres is scarce, there are some good examples how this technology could be applied. A layer-by-layer self-assembly protocol, using ionic interaction between the two polymers, was used to build up a shell of alternate multiple polymeric layers surrounding core porous calcium carbonate ($CaCO_3$) microparticles to encapsulate drug and slow the rate of release.[104] The same group have produced novel capsules using the layer-by-layer process for chitosan and sodium alginate polymers assembled on melamine formaldehyde micropar-

ticle "templates", the latter dissolved in mild acid to leave a microcapsule.[105] However, drug loading relied on ionic interaction, in this case, positively charged drugs adsorbing to the anionic polymeric capsule. Although the technique needs to become more routinely applied to polyester microparticles, the concept is promising.

Other groups have designed alternative methodologies for the production of core-shell particles. Various ratios of PCL–PEG:poly(isobutylcyanoacrylate) (PIBCA) were coprecipitated and imaging of the nanoparticles showed distinct distributions of the two polymers (Fig. 6).[106] The PIBCA appeared to localize to the particle interior (the core) with a surface layer of PCL–PEG (the shell), the latter potentially increasing blood circulation time. The utility of these particles was demonstrated for controlled release of busulfan, potentially overcoming toxic side effects of current intravenous therapies. An adaptation of the PLGA/PLA phase separation techniques, above, but for synthesized poly(orthoester) (POE, Mw ~24 kDa) and PLGA (~50 kDa) generated a PEO core with porous PLGA outer layer (the shell) using a double emulsion–solvent extraction protocol.[107] Interestingly, the POE core eroded faster than the PLGA layer on account of the acidic PLGA breakdown products, leaving a hollow PLGA microcapsule (Fig. 7).[108] Simultaneous encapsulation of a hydrophilic protein (albumin) and a hydrophobic peptide (cyclosporin A) during the same fabrication protocol resulted in their distribution to the shell and core, respectively, presumably on account of their solubility and polymer phase separation.[108] However, surface porosity was dependent on drug loading which in turn governed the release profile of cyclosporin A, which was biphasic and more prolonged than the relatively rapid and complete release of albumin.

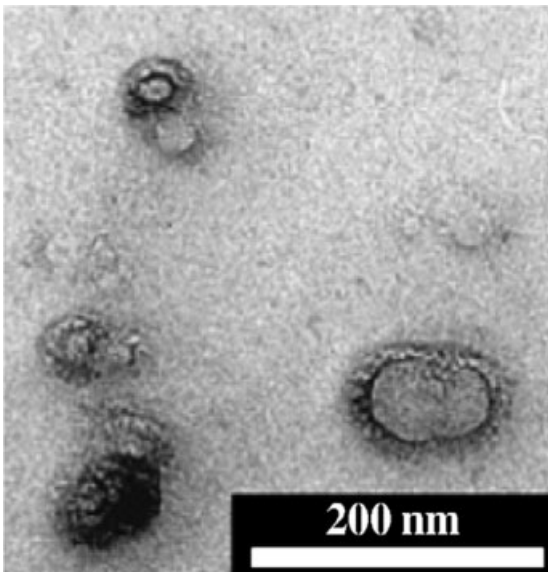

Figure 6. Transmission electronic microscopy after freeze-fracture images of PCL–PEG:PIBCA (50:50) nanoparticles. Adapted from Layre et al.[106]

Controlling Drug Diffusion via Erosion–Degradation Mechanisms

Surface erosion of microspheres is generally considered unlikely on the basis of their size and the ingress of water into the porous matrix being more rapid than the degradation process causing bulk erosion.[109] In this scenario, the polyester particle tends to maintain its original size while eroding from within; the erosion kinetics being biphasic.[110,111] Upon immersion in aqueous media, hydrolysis of the polyester causes a decrease in the polymer molecular weight (degradation) but, initially, there is no loss in the mass (erosion) of the microsphere. Modeling drug release as a function of microsphere erosion and drug diffusion shows the interdependency of the two mechanisms in governing drug release.[112] Mechanisms underlying the biphasic release of drug from PLGA microspheres (burst release followed by zero-order release) were determined by experiment and successfully approximated to Fick's second law of diffusion, the kinetics of degradation being too slow to account for the burst release.[113]

More sophisticated modeling for 5-fluorouracil-loaded PLGA microspheres took into account pore evolution and consequent changes in drug diffusivity. This enabled prediction of drug release over the entire period encompassing the burst, zero order, and secondary burst releases phases observed.[114] A recent model used time-resolved Pulsed-Field Gradient NMR and confocal microscopy data to directly observe the free diffusion of water (relating to the internal pore structure) and drug release following immersion, respectively.[115] Pore cavities grew within 24–48 h, the rate and extent being a function of synthesis conditions, with the drug release process shown to be heterogeneous. Modeling these data to theories for molecular transport through porous matrices[92,116] defined the rate-limiting step to be the polymer swelling and/or the drug diffusion within the

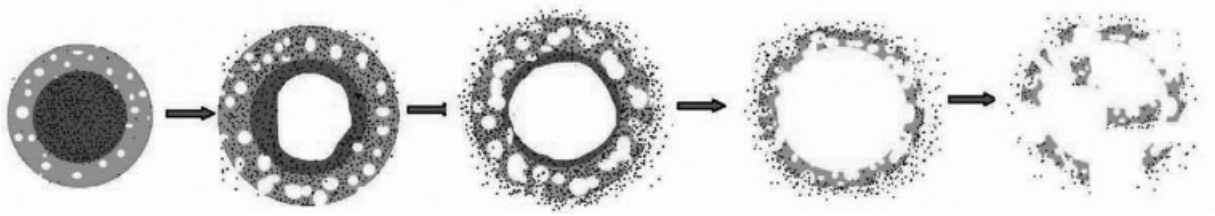

Figure 7. Schematic representation of the proposed release mechanism for the double-walled POE/PLGA microspheres; cyclosporin A (black dots) is shown encapsulated within the POE core (dark grey) and released through the PLGA shell (light grey). Adapted from Shi et al.[108]

polymer matrix, with a percolation-type mechanism suggested to control drug release. It is interesting that the field of mesoporous silica materials is somewhat more advanced than the field of polyester drug delivery matrices, in that tightly defined pore interconnectivities and pore geometries can be templated for silica using various polymers and surfactants to define surface area, diffusion, and transport.[117]

Thus, if we are to be able to control drug release, for example, to obtain zero-order release rather than a burst release, then prediction of the degradation–erosion mechanism for a certain microsphere size, porosity,[118] polymer type, and molecular weight becomes important. This may be achieved through engineering of the polymer itself (consideration of the stability of the ester bond, hydrophobicity, steric effects, autocatalysis, and T_g) to achieve a certain hydrolytic reactivity. This would therefore require selection of PLA:PGA molecular ratio and sequential arrangement, chain length, capping of the terminal carboxyl groups, or polyester conjugates. An excellent example of what can be achieved was described by Wang et al.,[119] who synthesized various poly(ortho esters) (POEs) with the intention to control polymer hydrolysis, with respect to hydrolytic rate and toxicity/acidity of degradation products, for use a DNA vaccine delivery vehicles. Interestingly, the chemical nature of the one particular POE was such that following DNA burst release at pH 7, uptake into the phagosome and fall in pH to ~5 caused its protonation and condensation of DNA, delaying further release; the biphasic release profile matching, and thereby stimulating the primary and secondary humoral and cellular immune responses *in vivo*. Work toward pH responsive drug release matrices has also investigated the homogenous blending of PLGA with polyethyleneimine (PEI) to produce microspheres, able to control drug release via polymer swelling and protonation of the PLGA/PEI matrix.[120]

FUTURE PERSPECTIVES

The last few years has seen widespread utilization of polyester particles for treatment of specific diseases including immune modulation and tumor regression. Many of the active drugs developed to treat these diseases will be biopharmaceuticals, given the continuing trends in the biotechnology sector. The formulation of peptide/protein and DNA/RNA-based molecules is notoriously problematic and it is likely that vehicles combining controlled release with passive and/or active targeting will be a necessary requirement. Engineering approaches are now emerging to meet these challenges and blurring the boundary between the (passive) delivery vehicle and active compound in therapeutic regimes. For example, clinical advantages in coencapsulating therapeutic antigens into PLGA microspheres have been attributed[121] not only to mutual stabilization of the proteins but also to the role of the microspheres as vaccine adjuvants.[122] Microspheres therefore also hold promise for the delivery of DNA-vaccines, with passive targeting to the bronchus-associated lymphoid tissue to provide a needleless system able to test a range of DNA-vaccine concepts,[123] such as in the treatment of malaria.[124] There are high hopes that polyester particles carrying therapeutic compounds can be effectively inhaled and commercially viable. However, it appears that any medical potential must be tempered against recent concerns in clinical trials and current products over local inflammation. The problem of biomedical polyester-induced inflammation is little reported in the literature; one study of PLGA implants in rodents reported mild local inflammation at the

implantation site which disappeared after implant degradation.[125] Codelivery of drugs, both small organic and biopharmaceutical drugs, would seem ideally suited to core-shell particles, and a recent, seminal example of this approach has been applied to gene delivery using drug/gene core-shell nanoparticles.[126] Investigating routes to the engineering of the particle surface to increase the *in vivo* half-life and/or specifically target cell surface and intracellular receptors is well underway. Future work addressing the molecular-particle interface, for example, understanding how polyvalent presentation of ligands at the particle surface affects receptor-binding affinity and downstream cell signaling, and the mesoscale cell-particle interface will be key future challenges.

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