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Shaping the Future of Nanomedicine: Anisotropy in Polymeric Nanoparticle Design

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Abstract

Nanofabrication and biomedical applications of polymeric nanoparticles have become important areas of research. Biocompatible polymeric nanoparticles have been investigated for their use as delivery vehicles for therapeutic and diagnostic agents. Although polymeric nanoconstructs have traditionally been fabricated as isotropic spheres, anisotropic, non-spherical nanoparticles have gained interest in the biomaterials community due to their unique interactions with biological systems. Polymeric nanoparticles with different forms of anisotropy have been manufactured utilizing a variety of novel methods in recent years. In addition, they have enhanced physical, chemical, and biological properties compared to spherical nanoparticles, including increased targeting avidity and decreased non-specific *in vivo* clearance. With these desirable properties, anisotropic nanoparticles have been successfully utilized in many biomedical settings and have performed superiorly to analogous spherical nanoparticles. We summarize the current state-of-the-art fabrication methods for anisotropic polymeric nanoparticles including top-down, bottom-up, and microfluidic design approaches. We also summarize the current and potential future applications of these nanoparticles, including drug delivery, biological targeting, immunoengineering, and tissue engineering. Ongoing research into the properties and utility of anisotropic polymeric nanoparticles will prove critical to realizing their potential in nanomedicine.

Introduction

Polymeric nanoparticles are finding increasing success in nanomedicine applications as both therapeutics and diagnostics. Due to their biocompatibility, their capability to circumvent normal biological barriers to small molecules, and their targeting abilities, polymeric nanoparticles have been shown effective in numerous functions. These applications include most prominently drug delivery vehicles for various types of chemical and biological therapeutics[1–4] and contrast agents for diagnostic and imaging purposes.[5–7] A wide range of fabrication methods exist for polymeric nanoparticles including bulk emulsions,[8] microfluidics,[9] and self-assembly.[10] These methods have been successful at synthesizing

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spherical nanoparticles with advantageous properties including biodegradability, drug release, biological targeting, and evasion of *in vivo* elimination.

One property of nanoparticles that has been investigated extensively in the literature for controlling biological interactions is particle size.[11–13] Nanodimensional polymeric constructs have been synthesized across the full range of 1–1000 nm depending on their desired application. Although particle size has been shown to have a significant effect on properties such as biodistribution and cellular uptake of polymeric nanomedicines, it is not the only parameter that should be considered in the design of a nanotherapetic or nanodiagnostics. Interest in nanoparticle shape has emerged in the past several years as a novel strategy to control the interface between particles and biological systems and to enhance efficacy of polymeric nanomedicines.[14] Anisotropy and shape specificity in biological interactions have been shown to be a critical parameters at the molecular,[15] cellular,[16] and tissue levels.[17] As such, to enable superior biological interaction between a nanoparticle and its target, the shape of the nanoparticle should be rationally engineered for its biological function. In this review, shape has been considered in a variety of settings including nanoparticle drug delivery, targeting, cellular uptake, biodistribution, immunoengineering, and tissue engineering.

This review describes the state-of-the-art of polymeric anisotropic nanoparticles and summarizes the main fabrication methods and applications of anisotropic nanoparticles in the literature over the past decade. The primary focus of this review is on polymeric nanoparticles and on engineered nanoscale features. While larger, micron scale polymeric particles and inorganic nanoparticles are also of interest, they are beyond the scope of this review and a reader is referred to other manuscripts[18,19] that discuss these circumstances. Future research into the fabrication and application of anisotropic polymeric nanoparticles will provide insight into the benefits of their utilization and optimize their use in nanomedicine.

Fabrication of Anisotropic Nanoparticles and Nanofeatures

Top-Down Methods

Top-down assembly methods are widely applicable and controllable for the fabrication of nanostructures including nonspherical anisotropic particles. Through macroscopic manipulation to environments containing preformed nanoscale objects, a wide variety of particle shapes and morphologies can be produced. The main top-down fabrication methods utilized for the fabrication of anisotropic nanoparticles and creation of nanofeatures include mechanical deformation by thin film stretching, particle replication in non-wetting templates, and micro/nanoscale lithography.

A widely applied method to generate particles of non-spherical shape is the thin film stretching method pioneered by Ho et. al.[20] The method consists of synthesizing spherical polymeric nanoparticles and casting them into a thin film of polyvinyl alcohol. The film is then heated above the glass transition temperature of the polymer so that the particles can be easily deformed and the thin film is stretched utilizing a single dimensional mechanical tension application device. Upon cooling, the resulting particles are ellipsoidal in shape and

have been demonstrated to have an aspect ratio of 2–5.[20] Alternatively, rather than increasing temperature, a solvent can also be used to enable particle deformation within a film. In recent years, this method has been adapted and further developed to produce polymeric micro and nanoparticles consisting of a wide repertoire of shapes. By translating the method to two dimensions and modifying particle deformation procedures, Champion et. al. demonstrated the capability to generate many different shapes including rods, discs, worms, bullets, barrels, as well as porous morphological variants of these shapes (Figure 1).[21]

Particles fabricated by thin film stretching method have also been of recent interest for their shape memory properties. Yoo et. al. published a study investigating the effect of various environmental stimuli on the shape retraction of ellipsoidal PLGA particles,[22] rendered anisotropic by the thin film stretching method. The authors demonstrated complete reversion to a spherical form in the presence of liquefying factors including increased temperature, decreased pH, and chemical treatment. In addition, upon incubating shape-switching opsonized microparticles with macrophages, the authors demonstrated that the particle resisted uptake until it assumed a spherical form.[22] Wischke et. al. examined the capability of copolymers composed of polycaprolactone (PCL) and polypentadecalactone (PPDL) to undergo shape programming and reversion.[23] Utilizing the stretching method and taking advantage of the fact that the polymers possessed a “permanent reprogramming” melting temperature and a “temporary reprogramming” melting temperature, the authors were able to induce shape change from oblate ellipsoid to prolate ellipsoid as well as reversion of the ellipsoids to the spherical form (Figure 2).[23]

Another method utilized for the production of nonspherical nanoparticles is the particle replication in non-wetting templates (PRINT) technique. Pioneered by Rolland et. al. this method allows for excellent top-down control over particle morphology.[24] The method consists of first synthesizing a silicon mold with nanoscale features by e-beam lithography. From this fabricated template, a photocurable non wetting polymer perfluoropolyether (PFPE) is deposited and solidified to form the mold. The non-wetting nature of the polymer allows for individual, discrete particles to be molded as opposed to a film smear. The authors demonstrated wide versatility of this method through the synthesis of poly (lactic acid), poly(pyrole), and poly(ethylene glycol) (PEG) particles of various shapes including cones, rods, and arrows.[24] The method was combined with mechanical elongation of the PFPE mold to produce rods with a higher aspect ratio as well as disc shaped particles.[25] In addition, this procedure has been recently combined with layer by layer spray-on technology to generate biologically active nanoparticles.[26]

An additional technique that has been investigated extensively in the literature for the fabrication of anisotropic nanoparticles and nanofeatures is particle lithography. Through the use of particle-surface interactions, various methods have been developed to add nanoscale features anisotropically onto micron scale particles. Contact printing has been utilized to induce Janus-like “two faced” anisotropy in micron sized latex particles.[27] Micron scale particle lithography has been utilized to add nanoscale anisotropic features to chemically modified polystyrene particles. By immobilizing the particles on a charged glass surface, the authors were able to block the functionalization of a nanoscale region on the surface of the

particle. Upon release from the surface, the previously blocked nanoscale region could be further modified.[28] Nanoscale patches of gold have also been deposited on tightly packed lattices of microparticles.[29] The morphology of these particles is controlled through the crystal structure of the multilayer particle lattices.

In addition to particle lithography, nanoimprint lithography has recently been appropriated for the fabrication of anisotropic polymeric nanoparticles. Direct fabrication of nanorods has also be reported through a procedure in which a nanoetched silicon is utilized as a template to mold a photoresist in order to produce rod shaped particles less than a micron in size.[31] This procedure could theoretically be translated to any photocurable polymer and aspect ratio can be regulated by the depth of silicon etching. Nanoimprint lithography has also been applied to synthesize anisotropic particles made of a crosslinked peptide that can be utilized to encapsulate antibodies and nucleic acids.[32] The authors demonstrated that these particles could be degraded and release their cargo through the addition of a protease. Thus, there are multiple approaches to successful top-down fabrication of nanoparticles designed to have various anisotropic shapes.

Bottom-Up Methods

Nonspherical nanoparticles have also been synthesized from a variety of directed self-assembly methods. Generally these procedures are more experimental in nature and have not found widespread application due to the difficulty of controlling self-assembly. However, the simplicity of these bottom-up approaches makes them attractive for the synthesis of anisotropic nanoparticles. These methods include block co-polymer aggregation, phase separation by polymerization, and particle core destabilization.

Block copolymers offer the capability to design particle shape at the molecular level. By varying the length and composition of the individual blocks in a block copolymer, anisotropic nonspherical particles can be synthesized by self-assembly. One example of the use of block copolymers in non-spherical particle synthesis was a study published by Jiang et. al. which demonstrated the self-assembly of PEG and polyphosphoaramidate (PPA) block copolymers into long string like micelles in the presence of DNA plasmids.[33] The resultant nanoparticles demonstrated an enhanced stability in aqueous media as well as reduced toxicity for *in vivo* applications. Other approaches have yielded better control over the rod-like shape of the particles. Petzetakis et. al. demonstrated the use of enantiomerically pure poly lactide (as opposed to the mixture of D and L enantiomers which is commonly used) in a block copolymer with polyacrylic acid resulted in the formation of self-assembled cylindrical micelles.[34] The aspect ratio was shown to be a function of the time of self-assembly. Stripped non-spherical particles have been synthesized by block copolymers of polystyrene and poly(2-vinylpyridine) (P2VP) along with a surfactant gold nanoparticle.[35] The block copolymer forms an alternating layer structure and this was exploited to produce ellipsoidal particles with stripes by utilizing gold nanoparticles that neutralized the preferential interaction of the polystyrene with the emulsion interface (Figure 3).

Phase separation emulsions offer the potential to synthesize Janus particles as well as non-spherical anisotropic polymeric particles. Kaewsaneha et. al. demonstrated the production of polymeric nanoparticles with a single magnetic face.[36] Starting with an emulsion of styrene,

acrylic acid, and oleic acid coated magnetic nanoparticles, the polymerization of the two organics resulted in a polymer matrix that excluded the magnetic nanoparticles, resulting in the uneven distribution on the surface. Emulsions of liquid crystal materials with a polymerizable monomer have enabled the synthesis of non-spherical microparticles with nanocolloids at their poles.[37] By taking advantage of the positional preference for surface defects in the liquid crystal, the authors demonstrated a polar arrangement of polystyrene nanoparticles on the surface of the microparticle. In addition, upon removal of the liquid crystal, the polymerized material assumed a non-spherical shape due to the deswelling of the polymer matrix.[37] Anisotropic bulging nanoparticles have been reported utilizing the polymerizable nature of surface styrene monomers. Park et. al. demonstrated the formation of dumbbell shaped particles through the initiation of styrene surface polymerization with a core-shell polystyrene/poly(styrene-co-trimethoxysilylpropylacrylate) particle.[38] Multibulge anisotropic particles have also been synthesized with seed particles of poly(vinyl chloride-co-acetoacetoxyethyl methacrylate).[39] Induced polymerization of surface adsorbed polystyrene resulted in a bulging morphology of these particles that could be controlled by increasing the concentration of the acetoacetocyetyhl monomer in the seed particle.

Block copolymer nanoparticles have also been investigated for a shape memory effect. Yang et. al. studied the capability of the copolymer poly(9,9dioctylfluorene-co-benzothiadiazole) to achieve a native ellipsoidal shape after bulk synthesis by emulsion.[40] Upon heating this liquid crystalline polymer above its nematic transition temperature, it attained a temporary spherical shape. Reversion to ellipsoidal shape was evident upon cooling as well. The stripped ellipsoidal nanoparticles described above[35] have also been utilized for a shape memory application. By crosslinking the P2VP layer the authors demonstrated a pH dependent, reversible swelling and deswelling property of the polymer matrix to produce ellipsoidal shapes with different aspect ratios.[41]

Particle core destabilization also offers the bottom-up capability to produce anisotropically shaped particles. By either starting with a hollow core template, or chemically destabilizing the core of a core shell particle, various shapes have been produced. In the case of a hollow particle precursor, liquefaction alone has also been shown to produce particles of red blood cell (RBC) shape. Doshi et. al. demonstrated how hollow polystyrene particles could be heated, thereby inducing a collapse of the particle into an RBC shape.[42] In the same study, RBC shaped particles were shown to be produced from poly (lactic-co- glycolic) acid through solvent based liquefaction. Chemical destabilization of spherical core polystyrene particles has also been shown to produce rod shaped nanoparticles of bovine serum albumin (BSA) and poly L lysine (PLL).[43] Zhou et. al. demonstrated that coating a spherical polystyrene template with BSA and PLL followed by solvent based extraction of the polystyrene core resulted in the fracturing of the surface layer and the production rod shaped nanoparticles.[43]

Microfluidic Methods

Microfluidics technology has revolutionized many fields of research including particle synthesis. Although the majority of microfluidic particle synthesis has been completed on the micron scale (to which the reader is referred to a more comprehensive review[44–46]),

there has been some research on the synthesis of nanoparticles and nanoscale features utilizing microfluidics technology. The predominant methods that have emerged for the synthesis of nanoscale particles on a microfluidic chip include electrojetting and nanoprecipitation.

Electrojetting has been utilized in a variety of applications and has been used with microfluidics technology to produce Janus particles. Roh et. al. demonstrated the synthesis of biphasic particles that have Janus surface characteristics.[47] By electrojetting two different solutions adjacent to each other, nanoparticles and microparticles made of dextran were formed with nanoscale features. In addition, this method was utilized with poly acrylic acid to generate nonspherical Janus nanoparticles.[47] This method was extended to produce micron scale Janus particles encapsulating nanoscale superparamagnetic particles and titanium dioxide particles for imaging.[48, 49] The authors demonstrate localized distribution of these nanoparticles within the larger nano and micron scale structures.

Nanoprecipitation in a microfluidic device has been recently investigated for its capability of fabrication of nonspherical nanoparticles as well as anisotropic micron sized structures with nanoparticulate features. Hasani Sadrabadi et. al. demonstrated direct fabrication of anisotropic polybenzimidazole (PBI) nanoparticles by focused hydrodynamic flow of a solution containing the polymer.[50] As the solvent exchange took place at the flow interface, nanoparticles precipitated out of the focused flow (Figure 4). The anisotropy of the particles was controlled by changing the ratio of the inlet focusing flow.[50] Lan et. al. utilized a similar hydrodynamic focusing scheme to synthesize microparticles with an anisotropic coating of nanoparticles by the use of coinjection of a photocurable phase and a nonphotocurable phase.[49] By dispersing nanoparticles in the nonphotocurable phase a single face of spherical nanoparticles was formed on the surface.[49] Another method developed by Suh et. al. demonstrated the capability to induce growth of magnetic nanoparticles on the surface of anisotropically fabricated microparticles synthesized by a stop flow photolithography process.[51] The shape of the particles could be directed by a photomask and the Janus nature was achieved by a side by side laminar of two polymer solutions. Polyethylene glycol (PEG) and poly(acrylic acid) (PAA) were utilized for these studies and the authors demonstrated subsequent growth of magnetic nanoparticles directed by the anionic nature of the PAA.[51] Nanoprecipitation of particles into micro scale molds has also been utilized for production of anisotropic microstructures. Angly et. al. demonstrated the capability to form densely packed nonspherical arrays of nanoparticles through a selective permeable microfluidic chamber.[52] Water droplets containing PEG and silicon dioxide coated gold nanoparticles were assembled into lithographically specified superstructures by evaporation of the water phase through convection of dry gas.

Characterization of Anisotropic Nanoparticles

Nonspherical anisotropic microparticles and nanoparticles are routinely characterized by microscopy methods including scanning electron microscopy (SEM), transmission electron microscopy (TEM), atomic force microscopy (AFM), and optical microscopy. Although these procedures work suitably for a thorough characterization of anisotropic nature of these constructs, they typically require substantial preparation and expensive microscopes. As a

result there is active research in developing alternative methods of characterizing non-spherical nanoparticles.

Mathaes et. al. made a comparison of different standard methods utilized in particle and cell characterization to detect differences between spherical and non-spherical microparticles and nanoparticles.[53] These protocols included flow cytometry/coulter counter for microparticles and asymmetrical flow field flow fraction for nanoparticles. The authors were able to detect differences between the spherical and nonspherical of aspect ratio 3–5 microparticles and aspect ratio 4 nanoparticles that were 40 nm in size in each of the assays. In addition, they were able to record characteristic data that could be used to predict the shape of an unknown sample of particles.[53]

Innovative light scattering methods have also been developed for the characterization of non-spherical nano and microparticles. Wang et. al. utilized predictive dipole modeling of holograms projected by particles scattering a laser beam.[54] The resulting approximation method was able to characterize 3D diffusion and rotation of non-spherical microparticles of aspect ratio 2 and size 2 microns. In addition, this method was utilized to characterize the content and anisotropy of spherical Janus nanoparticles of size 900 nm.[54] Methods based on light scattering of gold nanorods at different wavelengths of light[55] and light scattering detecting differences of electrophoretic mobility of nanorods vs nanospheres[56] have also been reported.

Biomedical Applications of Anisotropic Nanoparticles

Shape Specific Targeting

Among the most useful properties that have been demonstrated for nonspherical nanoparticles are the inhibition of non-specific cellular uptake leading to enhanced *in vivo* biodistribution[57] and the increased targeting capabilities due to the higher radius of curvature.[14] These two properties have been investigated extensively in recent years and have been characterized in a wide variety of systems. These characteristics of anisotropic nanoparticles make the technology an attractive platform for biomedical applications (see Table 1).

Inhibition of non-specific cellular uptake is an important attribute for *in vivo* therapeutics as the reticuloendothelial (RES) system's clearance of nanoparticles prevent the majority of the administered dose from reaching its target. Sharma et. al. investigated the capability of non-spherical micro and nanoparticles to resist cellular uptake by macrophages, the primary cells responsible for RES clearance.[58] By utilizing confocal microscopy image analysis, their results demonstrated that prolate ellipsoids (AR 2) attached to the cells more efficiently than oblate ellipsoids (AR 2) which in turn attached more efficiently than spheres. However, uptake of prolate ellipsoids was inhibited 50% compared to spheres whereas uptake of oblate ellipsoids was enhanced nearly 300% compared to spheres.[58] Similar trends were demonstrated with mesenchymal stem cells (MSCs) and HeLa cells.[59] It was shown that not only particle shape, but also the aspect ratio of the ellipsoidal particle had an impact on cellular uptake as a particle with an aspect ratio of 4 was internalized at a rate nearly 3-fold higher than an ellipsoid with an aspect ratio of 2.[59] Orientation of the particle once it is

attached to the cell membrane appears to play a role in phagocytosis. Champion et. al. demonstrated that prolate and oblate microellipsoids that attached to cells on their long axis were not phagocytosed as readily as ellipsoidal particles attached on their short axis.[60] The orientation was shown to be important for "UFO" shaped particles as particles approaching at a 45° angle were not internalized at all compared to particles approaching at 0° and 90° angles.[60] The work was translated to a theoretical model of shape dependent uptake presented by Dasgupta et. al.[61] Computation results based on the minimization of free energy of binding and membrane deformation indicated that nanoellipsoids attach on their long axis. The particles must then undergo a transition to attachment on the short axis in order to be internalized completely by a cell.[61] Particle internalization pathways have also been investigated for anisotropic particles. It has been shown that smaller (150–200 nm) anisotropic cylindrical nanoparticles are taken up by clatharin-mediated endocytosis and caveolae-mediated endocytosis.[62] The importance of which uptake pathway nanoparticles take has been investigated[63] and can directly impact the efficacy of intracellular therapeutics. Surface density of 5 kDa PEG (PEG_{5K}) has also been shown to be an important factor in the macrophage uptake and cellular biodistribution of anisotropic 320 nm nanoparticle therapeutics.[64] Reduced PEGylation surface density (0.028 PEG_{5K}/nm^2) resulted in higher macrophage uptake, lower *in vivo* circulation time, and higher accumulation in the liver compared to higher surface density of PEG_{5K} (0.083 PEG_{5K}/nm^2).[64]

In addition to the altered cellular uptake patterns exhibited by anisotropic particles, shape appears to confer increased specific uptake mediated by stronger avidity of surface bound targeting ligands. Antibody targeting efficacy and specificity was directly demonstrated *in vitro* by Barua et. al.[65] The authors utilized Trastuzumab (an antibody specific for the human epidermal growth factor receptor HER2) and cell lines that were HER2 + and HER2−. Cell uptake was shown to be increased 1.5–3 fold for nanodimensional prolate and 1.5–2.5 fold for oblate ellipsoids compared to spheres for only the HER2+ cell lines.[65] Circular disk particles have also been shown to have greater targeted adhesion efficiency than rod-like disk particles under various flow shear rates.[66] Substantial investigation has also been conducted into the role of size and shape for *in vivo* targeting as well. Muro et. al. demonstrated anti ICAM-1 surface bound discs had 30 fold higher targeting specificity for endothelial cells and longer circulation time than spheres.[67] However, the spheres were taken up by targeted cells more readily than the discs. Prolate ellipsoids have also been shown to have targeting enhancement over spherical particles. Kolhar et. al. investigated the *in vitro* and *in vivo* accumulation of targeted rods vs. spheres.[68] The authors found that under flow *in vitro* there was about a 2-fold increase in specific adhesion of rods compared to spheres under shear rates ranging from 15 s^{-1} to 250 s^{-1}. Similarly there was close to a 2 fold decrease non-specific adhesion of rods compared to spheres at lower shear rates (15 s^{-1}). *In vivo* experiments demonstrated that rods had greater accumulation in the organs they were targeted to compared to spheres. For lung targeted rods, there was a 2 fold increase in accumulation of rods vs. spheres. For brain targeted rods there was a 7.5 fold increase in the accumulation of rods compared to spheres (Figure 5).[68]

Drug Delivery

With the added benefits of reduced non-specific cell uptake, longer circulation time *in vivo* and higher specific targeting, anisotropic non-spherical particles have been utilized for a wide variety of applications. One of the most prominent uses for non-spherical nanoparticles in recent years has been for delivery of small molecule drugs such as chemotherapeutics and genetic material such as siRNA. Many of the fabrication methods presented in this review have been extended to produce non-spherical nanoparticles with unique properties that are tailored for specific drug delivery applications.

The PRINT technology has been utilized in multiple applications for non-spherical particle drug delivery. Hasan et. al. demonstrated a novel approach to the delivery of short interfering RNA (siRNA) for gene knockdown.[69] The authors utilized a modified emulsion technique to encapsulate siRNA in PLGA and then induced biomimicry in their nanoparticle production through the addition of a cationic lipid coat. Although there was no comparison to a spherical particle, the authors demonstrated comparable efficacy to Lipofectamine 2000 for gene delivery to a variety of cell lines. In addition, there was a 60–80% knockdown of KIF11 in prostate cancer cells by the cylindrical particles.[69] A similar study was published by Xu et. al. utilizing lipid coated PRINT BSA particles with a bioreducible cross-linker for RNA replicon delivery for vaccination purposes.[70] There was a 2 fold increase in transfection efficacy observed by the formulated particles compared to the commercially available TransIT reagent. The authors were also able to demonstrate the utility of this platform as a genetic vaccine through the enhanced delivery of RNA encoding the influenza hemaglutinin gene.[70]

PRINT based PLGA particles have also been utilized in chemotherapeutic applications as well. Chu et. al. demonstrated the favorable pharmacokinetics of a non-spherical PLGA particle loaded with Docetaxel.[71] There was greater tumor accumulation over the initial time points and lower clearance by spleen and liver of the non-spherical particles compared to the spherical particles. The same group also synthesized PRINT PLGA non-spherical particles with acid sensitive prodrug of Docetaxel to enable higher dosing and antitumor therapeutic effect.[72] The prodrug encapsulated in the particle was shown to be able to be delivered at higher effective doses in the particle to mediate significant antitumor effects in a subcutaneous cancer model, without excess toxicity. The enhanced pharmacokinetic profiles of the non-spherical particle enabled them to remain just as effective as the free chemotherapeutic drug even though they had reduced toxicity.[72]

Another class of PRINT based particles utilized in drug delivery carriers have been in RBC mimicking particles for oxygen transport.[73, 74] Although these particles are micron in size (due to the desire to achieve complete biomimicry of the RBC), the studies resulted in important implications for the design of nanoparticle therapeutics to achieve the same goal of oxygen transport. RBC mimicking hydrogels were utilized to conjugate hemoglobin internally without the loss of protein activity.[73] Also, due to a low elastic modulus, the particles could be sheared at physiologically relevant rates without loss of structures. In addition to the proof of principle, these hydrogel microparticles were utilized to investigate the role of particle modulus in the administration and clearance of therapeutics.[74] By

controlling the modulus of the hydrogel microparticles, the authors demonstrated that these therapeutics could avoid entrapment in the lung and elimination in the spleen and liver. Decreasing the modulus by 8 fold also led to a 30 fold increase in circulation time of the particles. Further investigations into how the role of modulus plays into the biodistribution of analogous nanoparticles would be of great interest.

Self-assembled nonspherical particles by block copolymer micelle aggregation have also been utilized for drug delivery applications. As described above, Jiang et. al. pioneered a method to form condensed plasmid-PEG-PPA micelles that demonstrated an enhanced stability for *in vivo* applications.[33] A follow up study illustrated that the condensation shape could be controlled by the polarity of the solvent with increasing hydrophobicity corresponding to increased sphericity of the particles.[75] This study was particularly interesting because the authors were able to achieve enhanced *in vivo* luciferase transfection of hepatic cells by intrabiliary administration of the worm-like nanoparticles compared to the spherical nanoparticles. The worm-like particle was immensely superior to the spherical particle, mediating a 10000 fold increase in luciferase expression of hepatic cells.[75] Block copolymer nanoparticles of various shapes and sizes have also been successfully applied to drug delivery of chemotherpeutics. Karagoz et. al. demonstrated the capability of rod-like and worm-like micelles of copolymers containing styrene, vinyl benzaldehyde, and oligo (ethylene glycol) methacrylate to encapsulate doxorubicin via conjugation to aldehyde groups in the polymer.[76] The resulting worm-like and rod-like micelles exhibited greater capability to be taken up by target cells and thus an enhanced ability to deliver the payload of doxorubicin to mediate cellular toxicity. Geng et. al. also published promising results in the synthesis of filamentous micelles consisting of block copolymers containing PEG-polyethylethylene and PEG-polycaprolactone.[77] Particles bearing shapes with 4 fold higher length were shown to circulate *in vivo* for 2–3 days longer than micelles with a shorter length and also mediated higher tumor apoptosis than their shorter counterparts.[77]

Immunoengineering

One exciting application for anisotropic particles that has just recently been described is in the area of immunoengineering.[78, 79] Although some work has been completed on the study of immune responses to anisotropic nanoparticles,[70, 80] there has been little application of these polymeric particles toward the modulation of the immune system for a therapeutic benefit or evaluation of the role of particle shape on immunostimulation. Given the long circulation time, resistance to cellular uptake, and high specific targeting, these non-spherical nanoparticles could easily be applied to DNA or protein based vaccine delivery platforms. Almost all of the developed polymeric particle vaccines to date have been developed with spherical micro and nanoparticles,[79, 81, 82] and this is an area where an anisotropic nanoparticle strategy could make a significant difference in terms of efficacy.

Another potential application of nonspherical nanoparticles to immunoengineering is in the development of artificial antigen presenting cells (aAPCs). aAPCs attempt to recapitulate the normal APC/T-Cells interaction through a reductionist surface presentation of a “Signal 1” protein (MHC dimer, anti CD-3, etc.) that serves as an antigen target and a “Signal 2” protein (anti CD28, anti CD 40, etc.) that serves as a danger signal. It has been shown that

spherical micro aAPCs can mediate antigen specific T-Cell activation[83] or antigen specific T-Cell killing.[84] Despite the breadth of literature on the development of micro scale aAPCs,[85] these are not the ideal candidate for an *in vivo* therapeutic due to their demonstrated poor draining to the lymphatics upon subcutaneous administration.[86] Although a nano aAPC could rectify this poor pharmacokinetic profile, it has been shown that nano aAPCs are less effective at stimulating an immune response due to poor mimicry of the normal aAPC/T-Cell interaction and minimal surface contact with the lymphocyte.[87] In addition, non-spherical ellipsoidal micro aAPCs have been previously shown to mediate an antigen specific T-Cell activation more effectively than equivalent spherical micro aAPCs, including leading to improved efficacy in a melanoma mouse model.[88] Some studies have also been conducted on the utility of single walled carbon nanotubes as a nano aAPC for immune system activation, although this has been primarily utilized for *in vitro* immune cell stimulation.[89–91] A non-spherical polymeric nano aAPC is intriguing as it could potentially remedy the short comings of a spherical nano aAPC as well as retain the favorable *in vivo* pharmacokinetic and toxicity properties of anisotropic non-spherical nanoparticle systems.

Conclusion

Non-spherical polymeric nanoparticles hold promise for various biomedical applications. Although shape has been traditionally neglected with respect to polymeric nanoparticle design, in recent years it has come to light as an important parameter. With the advent of many new fabrication methods based on top-down, bottom-up, and microfluidic technologies, our understanding of how to control the shape and anisotropy of polymeric nanoparticles is continuing to expand.

Top-down technologies allow readily translatable methods for applications in the biomedical sciences due to their reliable production of uniformly anisotropic nanoparticles. These methods include thin film stretching of spherical particles, PRINT based lithography, particle lithography, and nanoimprint lithography. Among these methods, the thin film stretching protocol is the easiest and most approachable protocol to produce particles bearing anisotropic shape. Many of the current biomedical applications of anisotropic particles utilize fabrication methods based on the thin film stretching method. However, particles produced by the thin film stretching protocol are limited to prolate/oblate ellipsoidal shape and derivatives thereof. Lithography based techniques such as PRINT and nanoimprint lithography can circumvent this shape limitation, however they are more difficult to implement in practice. As efforts progress to identify the optimal shaped particle for each biomedical scenario, each of these methods can be utilized for the translatable production of anisotropic particles.

Bottom-up and microfluidic technologies offer the promise of simple platforms to rapidly synthesize large batches of anisotropic nanoparticles. Key methods in this category of fabrication include phase separation emulsion, block copolymer micelle formation, and microfluidic nanoprecipitation. Phase separation emulsion is an approachable method to generate anisotropy on spherical polymeric nanoparticles, however this technology has not been well established for biomedical applications. Block copolymer micelle formation has been utilized to produce particles of different shapes encapsulating various therapeutics and

has already shown promise for translation. However, it can be difficult to control the micelle formation as evidenced by the limited repertoire of shapes that can be produced with this method. Microfluidic nanoprecipitation shares similar advantages and disadvantages as block copolymer micelle formation, except translation of anisotropic polymeric nanoparticle produced on microfluidic device to biomedical applications is more limited. Further experimentation into the control of anisotropy in particles fabricated by the bottom up method will be of great interest in the coming years.

Given the existence of these well-established methods for synthesizing non-spherical, anisotropic nanoparticles, there are many applications in fields such as drug delivery, immunoengineering, and tissue engineering, which can benefit immensely from consideration of shape in the design of nanotherapeutics. Due to the increased *in vivo* circulation time and targeted avidity/cell uptake, non-spherical nanoparticles are a versatile, robust platform for drug delivery such as intracellular delivery of genetic therapeutics and chemotherapeutic drugs. In addition, the increased avidity for ligand targeted nanoparticles make them the ideal candidate for the application of nanomedicine to immunoengineering.

The distinct topological features of anisotropic polymeric nanoparticles can also be utilized in the synthesis of novel tissue engineering scaffolds to better mimic the ECM. Among the numerous applications of nanoparticles in medicine, tissue engineering has become a prominent venue for the utilization of nanofabricated materials.[92] Although the application of nanoparticles in tissue engineering and regenerative medicine has been numerous,[93] the impact of anisotropic nanostructures is just coming to be understood for the *ex vivo* induction of various tissues. Non-spherical nanostructures can be key to the development of tissues as nanotopography has been proven to be important for the accurate delivery of ECM cues to cells in the development of tissues.[94, 95] Non-polymeric, non-particulate anisotropic nanostructures such as carbon nanotubes,[96] electrospun fibers,[97] and hydoxyapaite nanoparticles[98] have demonstrated the importance of anisotropic nanotopographical features in tissue scaffold engineering and also highlight the potential impact polymeric particles can have in this discipline.

Although the breadth of applications is vast, we have only begun to understand the benefits that non-spherical and anisotropic nanoparticles can confer compared to traditional spherical particle. Continued investigation into the properties, fabrication methods, and interactions with biological systems will elucidate the true potential of the anisotropic polymeric nanoparticle and make an immense impact in nanomedicine research.

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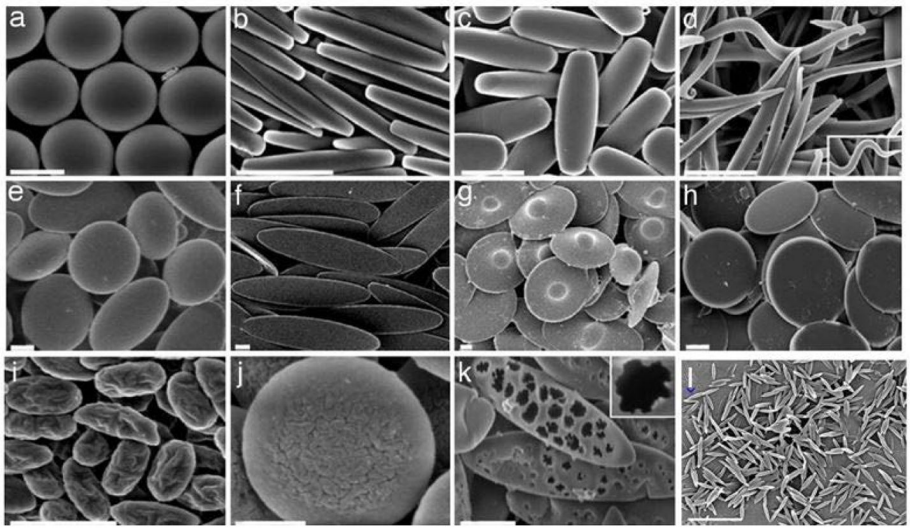

Figure 1.

A wide repertoire of particle shapes can be produced with the thin film stretching method. (a) Spherical, (b) rectangluar disk, (c) prolate ellipsoidal, (d) worm-like, (e) oblate ellipsoidal, (f) prolate ellipsoidal disk, (g) UFO-like, (h) flattened circular disk, (i) wrinkled prolate ellipsoidal, (j) wrinkled oblate ellipsoidal, and (k) porous prolate ellipsoidal particles can all be synthesized by liquefaction in a thin film and mechanical stretching. The technology is also translatable to the (l) nanoscale as the size of the particle is determined by bulk spherical particle synthesis. Scale bars are 2 μm. Adapted with permission from [21], Copyright 2007 by the National Academy of Sciences of the USA.

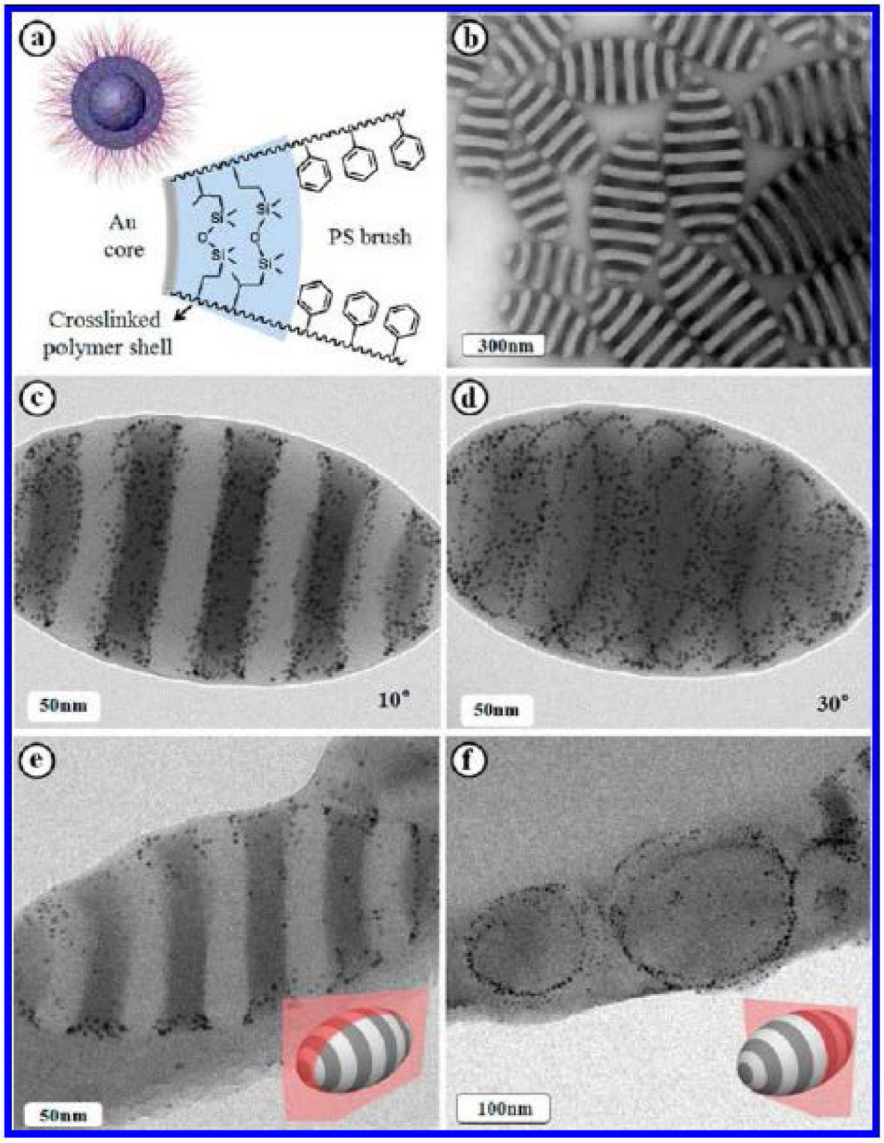

Figure 2.

Shape memory and reprogramming applications are one application of nanoparticles produced from block copolymers. (a) Non spherical and spherical microparticles utilized as an example to explain the procedure of shape reprogramming. (b) Schematic of temporary shape reprogramming process utilized. Particles are stretched to a non-spherical shape at the “temporary reprograming” temperature and upon heating the particles reassume their spherical shape. (c) Schematic of permanent reprogramming and shape memory reversion to ellipsoidal particles. Spherical particles are first stretched to prolate ellipsoids at the “permanent reprogramming” temperature, followed by stretching to oblate ellipsoids at the “temporary reprogramming” temperature. Upon heating, the oblate ellipsoid assumes the permanently programmed prolate ellipsoid shape. Similar trends were seen with nanoparticles in the study. Adapted with permission from ref [91], Copyright 2013 by John Wiley and Sons.

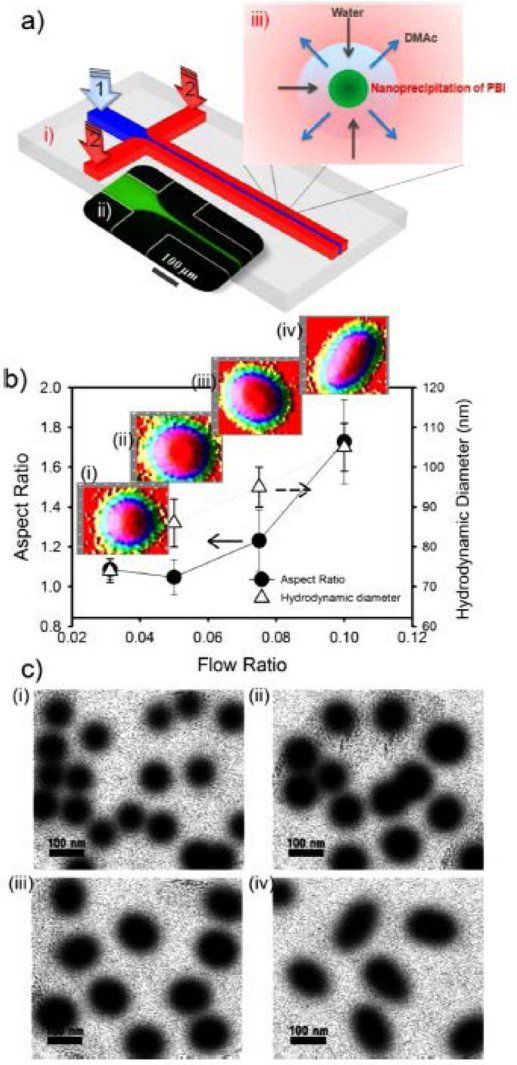

Figure 3.

Nonspherical stripped nanoparticles can be synthesized from the gold nanoparticle based surfactant dissolution of a layer block copolymer. (a) The main driving force behind the formation of this particle from a layered spherical particle is the administration of a smaller gold nanoparticle with a crosslinked polymer shell and polystyrene on the surface. (b) Stripped ellipsoidal nanoparticles can be formed through this emulsion based bottom up process. (c) and (d) Zoomed in and rotated TEM micrographs of the particle demonstrate how the gold acts as a surfactant for only one of the two polymer layers. (e) and (f) Cross-sections of the particle at different orientations illustrate the localization of the gold nanoparticle surfactant to the outside of the stripped ellipsoidal nanoparticle. Adapted with permission from [33]. Copyright 2013 American Chemical Society.

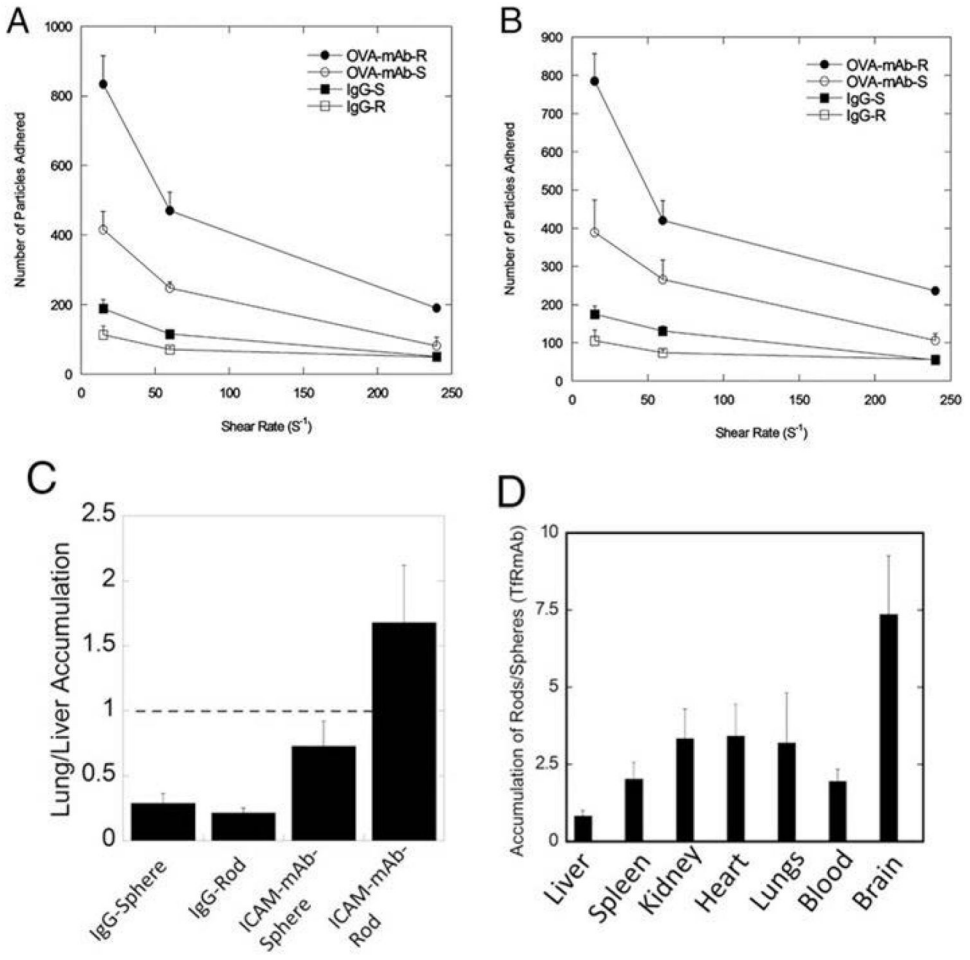

Figure 4.

Nonspherical polymeric nanoparticles can be synthesized by nanoprecipitation of polymer in a focus flow microfluidic device. (a) A solution of polymer is injected into the inlet along with two other flanking streams to focus polymer solution. Subsequent solvent exchange results in the nanoprecipitation of particles. (b) By controlling the ratio of focus solution flow and polymer solution flow, the aspect ratio and size of the nanoparticles can be tuned as desired. (c) TEM images of particles produced with increasing flow ratios of the two inlet solutions. Non-spherical particles of nanoscale size are successfully produced by this method. Adapted with permission from [46]. Copyright 2012 American Chemical Society.

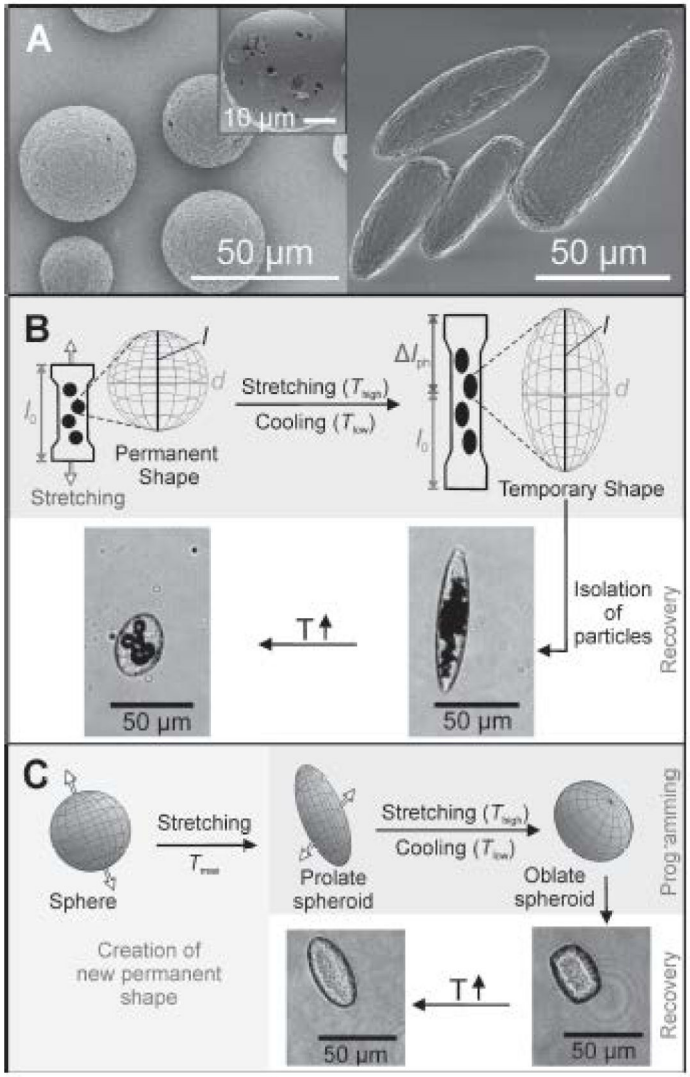

Figure 5.

Non-spherical particles mediate better specific adhesion under flow and enable enhanced *in vivo* targeting. (a) Number of particles adhered at the inlet of a microfluidic device of targeted (OVA-mAb) and nontargeted (IgG) rods (R) and spheres (S) under different shear rates. (b) Number of particles attached at the bifurcation of the device to simulate the bifurcation of a blood vessel. Increased specific adhesion and decreased non-specific adhesion are evident for rods vs. spheres. (c) Increased accumulation of ICAM targeted rods in the liver compared to spheres measured by lung to liver accumulation ratio. (d) Increased ratio of rod shaped transferrin receptor targeted particle to equivalent spherical particle accumulation in the brain indicates enhanced *in vivo* targeting capabilities of rods compared to spheres. Adapted with permission from [64], Copyright 2013 by the National Academy of Sciences of the USA.

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Table 1

Summary of applications of various anisotropic polymeric nanoparticles

Fabrication Method	Application	Material*	Size	Shape**	Result	Ref Number
Thin Film Stretching	Inhibition of macrophage uptake	PS	500 nm – 4 μm	Prolate ellipsoid (AR 2), Oblate ellipsoid (AR 2)	Prolate ellipsoids attached 4 times more, oblate ellipsoids phagocytosed 3 fold more than spheres.	54
	Inhibition of MSC/HeLa uptake	PS	100 nm	Prolate ellipsoid (AR 2–4)	AR 2 particles taken up 2–5 times more than AR 4 particles	55
	Enhanced antibody specificity	PS	200 nm	Prolate/oblate ellipsoid (AR 3),	Targeted prolate ellipsoid particles taken up 3 times more than spheres	61
	Endothelial targeting	PS	100 nm – 10 μm	Oblate ellipsoid (AR 3)	30 fold organ specificity of targeted ellipsoids vs. spheres	63
	In vivo brain and lung targeting	PS	200 nm	Prolate ellipsoid (AR 3)	Targeted ellipsoids accumulated 2 fold more in lung and 7.5 fold more in brain compared to sphere	64
	Artificial antigen presenting cells	PLGA	4.5 μm	Prolate ellipsoid (AR 2–7)	20 fold increase in antigen specific proliferation by ellipsoid aAPC compared to spherical.	84
Particle replication in non-wetting templates (PRINT)	siRNA delivery	PLGA	320 nm	Rod shaped (AR 4)	60–80% knockdown of KIF11 in cancer cells over 72 hrs.	65
	RNA replicon delivery	BSA	1 μm	Cylindrical	2 fold increase in target protein expression over Trans IT	66
	Docetaxel delivery	PLGA	320 nm	Cylindrical (AR 4)	Reduced tumor size and toxicity vs free drug	68
Self-Assembly	Plasmid delivery	PEG-PPA	40–70 nm	Rod like (AR 3)	10000 fold increase of *in vivo* expression of target gene by rods compared to spheres	71
	Doxirubicin delivery	POEGMA- P(ST-co- VBA)	20–200 nm	Rod and worm shaped	7 fold increase of cancer cell killing of worms over spheres	72
	Enhanced biodistribution	PEG- PEE/PCL	1–8 μm	Worm shaped	2 fold increase in circulation time for 4 fold increase in micelle length	73

* PS = polystyrene, PLGA = poly(lactic-co-glycolic acid), BSA = bovine serum albumin, PPA = polyphosphoramidate. POEGMA-P(ST-co-VBA) = poly[oligo(ethyleneglycol) methacrylate]-block-[poly(styrene)-co-poly(vinyl benzaldehyde)]. PEE = polyethylethylene. PCL = polycaprolactone.

** AR = aspect ratio