Nanostructured particulate materials are expected to revolutionize diagnostics and the delivery of therapeutics for healthcare. To date, chemistry-derived solutions have been the major focus of materials design to control interactions with biological systems. Only recently has control over a new set of physical parameters, including size, shape and rigidity, been explored to optimize biological response and the in vivo performance of nanoengineered delivery vectors. This review highlights the methods used to manipulate the physical properties of particles, and the relevance of these physical properties to cellular and circulatory interactions. Finally, we discuss the importance of future work to synergistically tailor both physical and chemical properties of particulate materials, with the aim of improving control over particle interactions in the biological domain.

1. Introduction

In the past few decades a diverse spectrum of particulate delivery systems has been developed, with sizes ranging from a few nanometers to micrometers. Among these delivery systems, drug-loaded liposomes and albumin nanoparticles have reached clinical application and have shown increased efficacy and minimized toxic side effects in the treatment of cancer.\textsuperscript{[1]} These successes promise momentous advances in healthcare, as these particulate systems offer a number of advantages over traditional therapy,\textsuperscript{[2]} including: improved single or multiple
therapeutic loading while shielding potentially fragile or toxic components; tailored pharmacokinetics to evade mononuclear phagocytic system (MPS) clearance,[3,4] allowing for optimized biodistribution,[5] subcellular targeting to deliver therapeutics to the intracellular site of action; overcoming subcellular barriers, such as drug pump-related multidrug resistance (MDR);[6] and triggered therapeutic release in response to specific physiological stimuli.[7]

The preparation of particulate carriers typically involves ‘bottom-up’ approaches, due to the inherent high degree of molecular-level specificity afforded. Polymersomes and liposomes,[8-12] polymer capsules,[7,13,14] mesoporous particles,[15] along with polymer,[16-18] gold,[19,20] carbon,[21,22] and silica[21,23] nanoparticles have been fabricated using this approach. Only recently have several ‘top-down’ methods, such as PRINT (particle replication in non-wetting template) or microlithography, been explored to fabricate delivery systems for biomedical applications, largely owing to the ability to precisely control shape, size, and scalable production.[24-26] Efforts have been directed to the control of chemical properties such as surface functionality and cargo release. Surface functionalization is achieved using a variety of conjugation chemistries, including thiol/disulfide, biotin/avidin and alkyne/azide coupling, and affords increased control over particle interactions in biological systems. Hydrophilic polymer brushes, such as poly(ethylene glycol) (PEG), may be used to improve circulation times due to reduced bio-fouling and opsonization,[27,28] while surface-conjugated targeting moieties such as ligands or antibodies can selectively promote the interaction with specific tissues or cells.[29,30] Coupling chemistries have also been employed to engineer triggered release in particulate systems,[13,31-33] with reducible disulfide linkages under cellular concentrations of glutathione,[34,35] and cleavage of hydrazone-doxorubicin conjugates under acidic conditions[36] being notable examples. Using these approaches, a range of therapeutics, including small cytotoxic compounds, peptides and siRNA, have been successfully delivered \textit{in vitro} and \textit{in vivo}.[1,35,37-39]
In addition to the effect of bulk and surface chemistry, more recent studies have shown that the physical properties of particle delivery vectors also have a profound effect on the blood circulation dynamics and lifetime, biodistribution, and cellular interaction and uptake.\cite{40} To ultimately control the \textit{in vivo} performance of particle systems, both theoretical and experimental biological studies of material elasticity and deformability,\cite{41-48} and geometry\cite{40,46,48-52} have been undertaken. This review focuses exclusively on the fabrication and characterization of advanced particle delivery vectors with variable physical properties, such as size, shape and elasticity, and implications of these properties upon biological interactions and material performance.

\section*{2. Particle Geometric Ratio}

While the influence of particle size on circulation clearance, cell uptake mechanisms and flow effects has been studied in detail,\cite{7,19,53-55} only recently has it been reported that shape, roughness and the resulting surface area of particles may also have a significant impact upon biological response.\cite{40,56} Because these geometric features, including size, shape and surface area interdependently affect biodistribution and cellular uptake, an integrative concept, namely the surface area-to-volume ratio (SAV), may be used in analyzing these systems. It can be noted that the SAV increases when particle size decreases and the geometry becomes more complex, as can be seen for the systems in \textbf{Figure 1}.

A trend can be noted in the literature describing a logarithmic decrease in phagocyte uptake with increasing SAV due to flow alignment effects, whilst cellular uptake into target cells increases logarithmically due to improved surface interaction. Therefore, this chapter will first address progress in fabricating non-spherical particles, followed by an analysis of recent literature on how the geometric components of particulate systems affect blood flow dynamics, cellular uptake and intracellular dynamics.
2.1. Non-spherical Particle Fabrication

Fabrication of particles with controlled sizes can be achieved with relative ease through adjustment of discrete fabrication parameters on both micro- and nanoscales.[57-60] Although good examples exist of non-spherical particle systems (Figure 2), obtaining a high degree of control over particle shape still remains a challenge, limiting investigation into high SAV particulate drug-delivery systems. Recently, review articles have focused on the fabrication of non-spherical drug delivery carriers,[61,62] and as such, synthetic methods will not be discussed in great detail here, but will be briefly addressed to provide context for the current review.

The biological interactions of metal nanoparticles of various shapes have received much attention,[63,64] with gold nanorods being the most widely investigated due to their extensive synthesis library.[65,66] Gold nanorods have also shown potential for ablation therapy due to their strong surface plasmon resonance effects.[67] Gold nanorods are generally synthesized using a surfactant-based stabilizer,[68] and can be simply modified with polyelectrolytes[69] or PEG for low bio-fouling.[20] Similarly, chemically-stabilized silica-,[70,71] carbon-,[72] and palladium-[73] based rod structures have been fabricated with biological applications in mind.

Although good growth control of nanorod systems has been demonstrated, their use is restricted by rapid renal clearance, in some cases inherent toxicities, and challenges in producing monodisperse suspensions of nanoparticles with a major-axis greater than 200 nm. In contrast to inorganic systems, the volume of recent literature describing the fabrication of non-spherical polymer particles is limited. Many techniques focus on lithographic, microfluidic, and PRINT methods, often incorporating photopolymerization,[74-80] or through the self-assembly of colloids,[81,82] or polymer chains to form filomicelles.[83-87] In addition, mechanically modified polystyrene (PS) and poly(lactide-co-glycolide) (PLGA) particles with a broad range of possible geometries have been fabricated through the controlled stretching of a hydrogel polymer matrix containing a dispersion of the particles.[49,62,88-92] Although anisotropic hollow polymer capsule systems have been reported,[93,94] capsules with non-
spherical shapes have not received much attention. Studies include polyelectrolyte adsorption, and then dissolution of either red blood cell, \( ^{[95-100]} \) bacterial, \( ^{[97]} \) or large glass fiber \( ^{[101]} \) micro-templates, along with the controlled deposition of polypyrrole onto hydrogen bubbles with variable detachment geometries. \( ^{[102]} \) While on the nanoscale, groups have fabricated hollow polyelectrolyte nanotubes using pressure-filter, \( ^{[103]} \) nickel nanorod, \( ^{[104]} \) and porous templates, \( ^{[105]} \) while Muller et al. produced hollow DNA nanotubes using an electrospun fiber template soluble in tetrahydrofuran. \( ^{[106]} \)

### 2.2. Biological Effects

Particles of variable size and shape have been increasingly investigated in the literature due to their effect on biological systems both \textit{in vitro} and \textit{in vivo}. In terms of flow-dynamics, reports have shown that the SAV may affect the propagation of particles in both interstitial and intravascular compartments, margination toward capillary walls, clearance through fenestrations, and alignment and turbulence in flow fields. For cellular association, the SAV and associated curvature effects affect antibody interaction area and adhesion strength, \( ^{[107]} \) opsonization, \( ^{[108]} \) and internalization kinetics and mechanisms. \( ^{[49]} \) The SAV also affects intracellular aspects through nuclear alignment and particle degradation. This section will therefore focus on these effects within the context of recent literature.

#### 2.2.1. Flow Dynamics and Biodistribution

According to fundamental fluid dynamic science, the movement of a particle within a flow regime is governed by both shape and size. The size of particles administered in blood vessels may dictate their velocity and diffusion, \( ^{[109]} \) while particle movement in tissue is limited by size due to steric hindrance within the extracellular matrix. \( ^{[49]} \) For non-spherical particles, angular movement under flow is often described using the rotational Peclet number, \( \text{Pe} = \dot{\gamma} / D_r \), which takes into account Brownian and non-Brownian motions of particles. The rotary diffusivity, \( D_r \), is closely related to the aspect ratio of the particle, and quantifies the extent of
Brownian motion. For high $Pe$ values, anisometric particles are not prone to Brownian effects and solely ‘tumble’ end over end periodically, as described by Jeffery.\cite{110} The rate of ‘tumbling’, $\dot{\phi}$, can be quantified according to Equation 1

$$\dot{\phi} = \dot{\gamma} \frac{1}{r_e^2 + 1} \left( r_e^2 \cos^2 \varphi + \sin^2 \varphi \right)$$

(1)

where $\dot{\gamma}$ is the strain rate, $r_e$ is the effective particle aspect ratio, and $\varphi$ is the angle between the long particle axis and the plane orthogonal to the flow direction.\cite{111,112} Larson states that for a Jeffery orbit, the period of rotation increases for increasing aspect ratio. Large aspect ratio particles decelerate in rotational velocity when the long axis is nearly parallel to the flow direction, and accelerate otherwise.\cite{112} Mueller and co-workers verified that $r_e$, which is determined experimentally and related to the actual aspect ratio, impacts dramatically upon angular velocity, and in turn determines the particle position probability density.\cite{111} This is shown in Figure 3 for prolate and oblate ellipsoidal particles. It is important to note that a non-spherical particle will therefore spend a greater amount of time aligned with a flow field, reducing the available cross-sectional area for interaction with other particles or cells. This was recently observed experimentally \textit{in vitro} by Discher and colleagues for long filomicelles, which avoid interaction with phagocytic cells, using flow rates similar to those found in the spleen.\cite{48} As the filomicelle length increased from 1 to 3 $\mu$m, uptake into macrophages decreased dramatically and logarithmically due to alignment of the filomicelle within the flow field.

When investigating the effect of the SAV on shear rate and the turbulent nature of a particle suspension within a flow regime, a modified Reynolds number, $Re_p = 4Ge^2/v$, can be adopted where $G$ is the shear rate, and $v$ the kinematic viscosity.\cite{113} Qi and Luo investigated the effect of aspect ratio on $Re_p$ and particle rotational state in Couette flow, and observed that particle shape had a clear impact upon $Re_p$. The authors observed several distinct regions of $Re_p$ where independent rotational states such as ‘tumbling’, ‘wagging’, flow aligning, ‘log-
rolling’, and ‘kayaking’ occurred.[113] In terms of particle size, Decuzzi and co-workers showed that for silica microspheres initially adhered to a flow-chamber substrate, the critical shear rate increased dramatically as the particle diameter increased from 1.3 to 5µm,[25] which is consistent with the conclusions of both Goetz and Lamprecht.[54,55] This illustrates that particle geometry has a large influence on the shear experienced in both the capillary and vasculature, affecting cellular interaction and adhesion with immune-system cells under flow conditions.

These observations can be put further into a biological context by examining literature describing the margination properties of particles. Margination is defined as the movement and interaction of particles toward the endothelial wall in blood capillary channels. This is a critical aspect of therapeutic delivery to endothelial cells and also in being able to exploit the enhanced retention and permeability (EPR) effect for passive delivery to tumor sites, where EPR is a size dependent process with a maximum limit of approximately 500 nm.[114] Decuzzi and co-workers discussed the margination propensity of particles with different geometries, where due to the core movement of red blood cells in capillaries, there exists a cell-free layer within close proximity of the endothelial wall in which particles with little propensity for longitudinal and lateral drift would remain.[115] This result was also found by Goldman et al. for spherical particles, which were found to reside within this laminar phase layer unless an external lateral force was applied.[116] Non-spherical particles, however, exhibit an intrinsic hydrodynamic lateral force and torque, meaning that these particles marginate highly, interacting with the endothelial wall to a greater extent than spherical particles. This has been validated by experiments performed by Gentile and co-workers using a parallel plate flow chamber to measure the margination propensity of silica particles with different sizes, densities, and shapes.[117] It was reported that while higher mass particles were overcome by gravity and moved towards the walls as expected, as the aspect ratio and SAV increased, margination increased and a greater number of particles adhered to the chamber substrate.
Similarly, this is found in a biological setting for the margnination of leukocytes due to platelet aggregation altering their co-geometry,[118] as well as interactions between the endothelial wall and irregularly shaped platelets as shown in Figure 3.[119]

The action of natural fenestrations during circulation is inherently connected to particle geometry. Small particles with a hydrodynamic radius of 5.5 nm[120] and free molecules with a size limit of 5 kDa[121] are often removed in the kidney through glomerular filtration, while particles with a diameter less than 100 nm pass through fenestrations in the endothelial lining,[3] and rigid particles larger than 200 nm may be filtered in the spleen.[5] Larger microparticles are often cleared through phagocytic interactions with Kupffer cells in the liver or trapped in capillary beds; however, smaller particles can also be removed via endocytosis by phagocytic or non-phagocytic cells.[3] Kostarelos and co-workers reported that carbon nanotube bundles with a diameter of 10 to 40 nm and length of 0.3 to 1 µm showed rapid clearance, predominantly due to renal excretion via active glomerular filtration.[72] However, it was shown by Bhatia and co-workers that renal excretion of gold nanorods with a similar minor axis length could be comprehensively reduced by attaching a 5 kDa PEG outer layer using thiol chemistry, thereby increasing the hydrodynamic radius.[20] In addition to particle clearance through fenestrations, an increased SAV can help exploit passive tumor targeting via the EPR effect. Bhatia and co-workers also showed that passive tumor accumulation was high for the gold nanorods due to size effects and reduced renal clearance,[20] while Decuzzi et al. reasoned that particle shape impacts heavily upon the rational design of particulate systems in the exploitation of the EPR effect.[115]

All of these geometry-dependant flow properties culminate in affecting biodistribution outcomes in vivo. Discher and co-workers observed that the alignment of long filomicelles in flow fields affecting macrophage interaction in vitro paralleled the in vivo behavior of long filomicelles.[48] Increased length resulted in higher blood circulation half-lives and passive accumulation in the lung. When compared to spherical PEGylated stealth vehicles, which are
generally cleared within a day, long filomicelles exhibited circulation lifetimes of up to five days. Muro showed that elliptical disks were able to better target the lung using anti-ICAM targeting moieties compared to a range of sphere sizes, evidenced through a high immunospecificity index (ISI).\textsuperscript{[122]} Interestingly for the anti-ICAM functionalized PS spheres, the smaller the diameter the better the ISI and consequently the percentage of particles in the blood was also greater, which also correlates well with the impact of SAV on critical shear and flow-alignment, as previously mentioned. Devarajan \textit{et al.} found that irregular shaped glycerylmonostearate polymer lipid nanostructures (LIPOMERs) avoided macrophages in circulation, achieving high concentrations in the spleen when compared to spherical LIPOMERs, which accumulated in the liver.\textsuperscript{[51]} This could have important consequences for splenotropic drug delivery, due to avoiding Kupffer cell clearance while still demonstrating high spleen retention. Decuzzi \textit{et al.} also recently showed that discoidal particles actively avoided Kupffer cells and consequently liver accumulation, boosting accumulation in non-MPS organs.\textsuperscript{[25]}

2.2.2. Cellular Interaction and Uptake

It is generally suggested that phagocytosis of particles is predominantly mediated by molecular recognition between cells and particles. One important example is the presence of integrin-associated protein CD47 on red blood cells, preventing phagocytosis. Lindberg and co-workers showed that CD47 interacts with a corresponding protein on macrophages, halting internalization.\textsuperscript{[123]} Besides molecular recognition, the SAV of particles also plays an important role in both phagocytosis and target cell interaction. Long filomicelles align in flow fields reducing phagocytic association,\textsuperscript{[48]} while Decuzzi and colleagues observed that firm adhesion between particles and cells is obtained when hydrodynamic and dislodging forces are balanced by ligand-receptor interactions combined with other adhesion forces.\textsuperscript{[115]} These forces have been shown to depend on shape,\textsuperscript{[107,115]} and have also been extensively proven to depend on size.\textsuperscript{[107,124]} Decuzzi \textit{et al.} and Gao \textit{et al.} both note that geometry and ligand density,
in a similar vein to membrane adhesion, will determine the rate of receptor-mediated endocytosis of particles into a cell,[107,124] while Muro and co-workers showed that high SAV elliptical disks achieve high targeting specificity for endothelial cells due to improved interaction when compared to spheres.[122] Cellular uptake of particles involves endocytosis, a process of internalizing materials by engulfing them with the cell membrane. Several mechanisms, including phagocytosis, macropinocytosis, clathrin-mediated endocytosis, and caveolae-mediated endocytosis can be used for particle internalization depending on the physicochemical properties of the particles and the cell physiology. Generally these endocytic pathways differ with regard to the size of the particles. Particles larger than 0.5 µm are more likely internalized by macropinocytosis or phagocytosis, as the inherent portal size for clathrin-mediated endocytosis is about 120 nm, and approximately 90 nm for caveolae-mediated endocytosis. Foged et al. investigated the uptake of PS spheres with diameters ranging between 0.04 and 15 µm, some with modified surfaces, into dendritic cells.[53] It was found that as the diameter of the PS spheres decreased the cellular uptake rate increased, similar to the results of Muro et al. who reported more rapid uptake, and lysosomal trafficking, of 0.1 µm diameter PS spheres when compared to 1 µm particles.[122] Muro et al. postulated that this was due to increased small particle surface interaction witnessed through double-positive flow cytometry experiments, combined with a macropinocytosis uptake mechanism compared to a phagocytic mechanism for the larger spheres. This size effect was also observed by Rejman et al. in a study of the compartmentalization of both 200 and 500 nm PS beads, where only the 200 nm particles were able to accumulate in late endosomal and lysosomal compartments.[125] Although to a large extent particle size determines the internalization mechanism from the viewpoint of the space limits in each endocytic pathway, varied findings have suggested the interplay between size, shape and chemical properties of particles on observed internalization mechanisms. Hoekstra and colleagues found that clathrin-mediated endocytosis dominated for PS-based
microspheres with a diameter less than 200 nm incubated with eukaryotic cells, with a shift
towards caveolae-mediated internalization as the microsphere size increased.[125]

While the cellular uptake of particles is influenced by the size, more recent studies have also
demonstrated the significant impact of the shape on cell dynamics. DeSimone and co-workers
investigated cationic PRINT particles with various sizes and shapes, examining both uptake
kinetics and mechanisms.[126] Cylinders (150 x 450 nm) were internalized quicker and to a
greater extent than 200 nm diameter spherical particles in HeLa cells, even though the internal
volume of the cylinders was greater. By using endocytic inhibitors, it was found that the
cylinders utilized multiple mechanisms to a greater extent than the spheres to enter the cells,
correlating with other extensive studies by DeSimone and co-workers for 1 µm cylindrical
PRINT particles with both positive and negative charges, which showed both dual clathrin-
mediated endocytosis and macropinocytosis uptake routes were predominantly utilized in a
range of cell lines.[24] This is consistent with the observation that the increased SAV of
cylinders would further promote interaction between the cationic surface and the cell
membrane proteins. Mitragotri and co-workers compared the cellular internalization of 1 µm
PS spherical and elliptical particles with the same internal volume in endothelial cells.[52] It
was found that the spheres internalized much more rapidly, however this difference was seen
to decrease over time. This work was extended by examining the effect of the alignment of
the particle major axis to the cellular membrane, quantified by the initial contact angle (Ω), on
cell membrane penetration velocity and internalization using rat alveolar macrophages.[127]

Particles with their major axis oriented tangentially to the cell membrane, that is as Ω
approaches 90°, exhibited dramatically reduced internalization. This was mainly attributed to
the necessary expansion required to form an actin cup for phagocytosis, while similar
internalization trends were seen for the static incubation of long worm-like polymer particles
with phagocytes, as seen in Figure 4.[15,48] Similar time dependant high aspect ratio particle
alignment was also seen by Mitragotri and co-workers for elliptical disk shaped PLGA
particles tangentially aligning in the cytoplasm with the nucleus of pooled human umbilical vein endothelial cells.\cite{52} Over long time scales spherical particles were found to have a shorter average distance to the cell nucleus, having important implications for the delivery of therapeutics that have limited diffusion coefficients in the cell cytoplasm.

Yang and Ma also simulated the effect of $\Omega$ for different SAV nanoparticles on the penetration of a model lipid bilayer.\cite{128} They showed that the minimum driving force of the ellipsoidal particle for breaching the lipid bilayer was dependent upon internal volume, aspect ratio, and approach angle. It was also shown that the process was time dependent; with time, the ellipsoid aligned itself tangentially with the bilayer, altering the angle $\Omega$. Placing this in context with experimental gold nanoparticle interactions with cell-lines, Chan and co-workers showed that 14 x 40 nm and 14 x 74 nm gold nanorods internalized into HeLa cells at a much slower rate than spherical nanoparticles with a diameter of 74 nm,\cite{19} correlating well with the modeling of Yang\cite{128} and experimental work on surfactant-stabilized gold nanorod internalization into human breast adenocarcinoma cells, where increasing the aspect ratio slowed uptake.\cite{129} Chan and co-workers also demonstrated that gold nanoparticles with a diameter of 50 nm were optimal for HeLa internalization, an effect most likely due to harnessing multi-mechanism internalization, and differences in adsorption of serum proteins due to curvature effects.\cite{19} In addition to the work on gold nanorods, several groups have also studied DNA or protein delivery using carbon nanorods, and have found them to deliver effectively into the cellular cytoplasm via an endocytotic mechanism.\cite{130,131}

In therapeutic delivery applications, cargo must be often released in a controlled manner, and delivered into the cytoplasm in order to interact with the cell nucleus. In some cases, degradation and cargo release may depend upon particle SAV. Dunne and co-workers investigated PLGA microsphere mass loss due to hydrolysis, and found that larger spheres degraded at almost twice the rate of smaller ones, primarily due to longer diffusion path lengths allowing autocatalytic degradation.\cite{132} However for a similar system, Labhasetwar
and co-workers found that a 100-fold decrease in particle diameter had limited effect on bovine serum albumin release.[133]

3. Stiffness and Deformability

The assembly or arrangement of a material within a structure is of design importance. The Young’s Modulus ($E_Y$) can be used to characterize the intrinsic mechanical properties of the constituent materials. The stiffness or rigidity of particle systems can be affected by material variations, such as the porosity of solid particles, as well as the diameter and shell thickness of hollow systems. There are a number of approaches to tune the mechanical properties: for instance, co-assemblies affect the mechanics of colloidal structures; cholesterol incorporating liposomes[51] and polymer systems doped with metal nanoparticles[134,135] exhibit increased rigidity. More frequently, the extent of chemical bonding or crosslinking in polymer systems has been used to control rigidity and $E_Y$. In separate work, the Giaison and DeSimone groups showed for hydrogel nanoparticles and microparticles, respectively, that through adjustment of the crosslinker concentration, control over $E_Y$ and system stiffness could be afforded.[41,46] Fery and co-workers also demonstrated that by controlling hydrogen bonding interactions in microcapsules, that the stiffness could be controlled using pH, where the stiffness was seen to reproducibly increase and decrease by two orders of magnitude by changing the pH from 2 to 6.[136] This precise control over the mechanical properties of particulate systems has lead to an improvement in the characterization methods available, along with an early understanding as to how particle stiffness and deformability affect cellular interaction. These will be discussed further in Sections 3.1 and 3.3.

3.1. Mechanical Characterization of Particulate Materials

Characterization of elastic properties can be achieved using two different approaches, either through bulk analysis of particulate suspensions, or through single particle investigation. For
single particle techniques, while detailed information can be obtained for individual system components, collecting enough data for a meaningful statistical analysis renders it a time consuming process. However, bulk analysis data can be difficult to effectively process into information on singular elastic components due to intrinsic system effects.

3.1.1. Bulk Techniques

The use of rheological methods and apparatus for measuring the bulk properties of polymeric materials is quite common for macroscopic hydrogel materials,[46] however, several groups have also shown that elastic information can be obtained for polymer nanoparticle systems.[137] Mäder and co-workers demonstrated for lipid based colloidal drug carriers that the elastic storage and loss modulus, along with the complex viscosity of the suspension, could be elucidated using both continuous shear rheometry and oscillatory testing.[138] For hollow polymer capsules, bulk elastic measurements are generally performed using either an osmotic swelling or buckling approach (Figure 5).[42,44] In this method, the osmotic pressure is controlled through the addition of polyelectrolyte either inside or outside the capsule volume, forming a polyelectrolyte-associated counterion cloud.[139] This pressure exerts a force, with direction and magnitude dependent on the concentration gradient. Using microscopy techniques and noting the critical osmotic pressure at which a capsule collapses for a given shell thickness and radius, or alternatively the degree of radial swelling, both the elastic and Young’s moduli can be evaluated. Möhwald and co-workers observed that this technique is limited in the accessible force range obtained, and that in situ force changes are not possible,[139] while Vinogradova noted that for osmotic deformation equations to apply, the capsules must not stretch, be infinitely permeable to water, and be impermeable to small ions, further limiting the technique.[44] Due to these restrictions and oversimplifications, single particle measurements for hollow polymer systems are often utilized.

Due to their small size and high rigidity, metal nanoparticles are inherently difficult to analyze using single particle techniques. Although metal nanorods and wires can be elastically...
evaluated using nanobeam mechanics,\cite{140} recent research has suggested that using a time-
resolved spectroscopic method may allow for measurement of the mechanical properties for a
wide range of metal nanoparticle suspensions.\cite{141} Using this method, the $E_Y$ and linear elastic
response can be determined through sample irradiation with femtosecond laser pulses and
consequent measurement of the acoustic response (Figure 5).\cite{141,142} While differences are
sometimes found to exist between measured nanoparticle and the bulk elastic properties due
to size and defects in the crystal lattice, it is generally found that the elastic properties
measured are similar to bulk metal properties.\cite{141}

3.1.2. Single Particle Techniques

In contrast to bulk measurements, single particle measurements allow for a high level of
control over the application of forces to each system component. One such way of achieving
this is through the use of an atomic force microscope (AFM) on a single, substrate
immobilized particle. Several groups have thoroughly investigated the mechanical properties
of films using AFM,\cite{143-145} and recently advances in optics and force control systems have
allowed for single analysis of particles. In a similar fashion to parallel-plate compression
experiments, a spherical probe with a high radius of curvature is attached to the end of an
AFM cantilever to effectively apply a controlled compression to a micro- or
nanoparticle.\cite{146,147} This technique has been applied to both hollow capsules\cite{43} and
polyelectrolyte microtubes,\cite{101} where it has been proven that system stiffness and the $E_Y$ can
be derived, along with important information on buckling forces, characterized using a
combination of AFM and reflection interference contrast microscopy (RICM) (Figure 5).\cite{148}
Polymer particles and pressurized microgel template capsules have also been investigated
using this technique,\cite{149,150} while Erath et al. were able to measure the mechanical properties
of polydimethylsiloxane microspheres adhered to a tipless cantilever.\cite{45} Similar to cell-
poking experiments using a stylus,\cite{151} it has been demonstrated that the $E_Y$ for hydrogel
nanoparticles can be obtained using AFM with a sharp, non-colloidal, probe.\cite{41} However for
a small probe, the geometry is often difficult to accurately quantify, and it may also impart an excessive axial strain leading to measurement errors in $E_Y$.

In regards to single particle investigation using a flow-field, Doyle and co-workers observed the deformability of lithographically fabricated PEG hydrogel particles with similar sizes to red blood cells, using a 4 µm microfluidic channel. Similarly, the Mitragotri and DeSimone groups independently performed capillary flow experiments to investigate the deformation extent of red blood cell (RBC) shaped particles of variable $E_Y$ within a flow regime (Figure 6). While Doyle and colleagues measured the pressure differential for particles of different shapes passing through the capillary, Mitragotri and co-workers observed the stretching of individual protein shells due to flow field effects using an optical method. Suction pressure due to micropipette aspiration was used by Hochmuth to measure the elastic properties of cells, while Barthès-Biesel and co-workers were recently able to quantify the elastic properties of large cross-linked ovalbumin microcapsules as a function of pH, using a cylindrical microchannel coupled with a high-speed optical set-up to model the deformation.

3.2. Comparison to Biological Systems

Recent approaches for the design of particulate materials for healthcare applications involve tuning the elastic properties to match that of biological particles, such as RBCs. RBCs have a circulatory lifetime of up to 120 days, are able to evade phagocytosis, and demonstrate high elastic deformation in order to pass through splenic fenestrations. However, old RBCs are removed from circulation in the spleen when the mechanical properties of the cells change, becoming more rigid. This has inspired work aimed at designing novel particulate systems to mimic healthy RBCs, in order to increase the circulation lifetime in the body. The measured elastic modulus of RBCs has been found to be consistently measured between various groups, with Lekka and co-workers, and Mitragotri and colleagues obtaining values of
26 ± 7 kPa and 15.2 ± 3.5 kPa, respectively, using AFM techniques.\textsuperscript{153,158} The elastic shear modulus for the RBC membrane has been determined to be approximately 10 μN m\textsuperscript{-1}, determined experimentally using both shear flow\textsuperscript{159} and micropipette aspiration\textsuperscript{160} techniques. The shear modulus, important to the nature of RBCs, is quite low in comparison to most healthy particle types, as they need to be able to squeeze through thin fenestrations, and demonstrate high reversible deformation under flow. For other cell types, cancer cell lines have been found to be elastically softer than healthy cells based upon scanning force microscopy measurements by Lekka \textit{et al.}\textsuperscript{161} and later through experiments by Gimzewski and co-workers for metastatic cancer cells from lung, chest, and abdominal cavities, compared to the benign cells which usually line these cavities.\textsuperscript{162} Lekka \textit{et al.} found that the $E_Y$ for normal Hu609 ureter cells was 12.9 ± 4. kPa compared to 1.0 ± 0.5 kPa for T24 bladder carcinoma cells, while it was later postulated that this decrease in elasticity allows cancer cells to metastasize or spread.\textsuperscript{163} Mooney and colleagues noted that more elastic cells were able to take up polyplexes to a greater extent, also enhancing cell proliferation and survival.\textsuperscript{164} Levental \textit{et al.} also presented a summary on the elastic moduli of different biological tissues, and the testing method used.\textsuperscript{165}

In comparison, synthetic systems demonstrate a wide variation in elastic modulus based upon AFM force spectroscopy characterization methods. Soft hydrogel particles vary between roughly 1 kPa and 1 MPa depending on the polymer type and extent of cross-linking within the system,\textsuperscript{41,46} solid polymer particles such as PLGA generally have an $E_Y$ in excess of 1 GPa,\textsuperscript{153} while synthetic surface-bound liposomes are much softer with an $E_Y$ of approximately 3 kPa.\textsuperscript{150} It is important to note that it has already been experimentally demonstrated that the $E_Y$ controls the rigidity and deformability of solid particles.\textsuperscript{46} Metal nanoparticles examined using time-resolved vibrational spectroscopy generally have an $E_Y$ in the range of 10 GPa to 1 TPa, where a value of 64 ± 8 GPa has been measured for gold nanorods. This value can be compared to the bulk gold value of 78 GPa.\textsuperscript{141} For
polyelectrolyte capsules, single particle AFM experiments reveal that the $E_Y$ generally falls within the range of 10 – 1000 MPa, which represents a transition between rigid cross-linked rubber and soft glass.[44]

3.3. Biological Effects

Similar to the effects of the SAV on biological activity both in vitro and in vivo, particle rigidity and deformability have also been found to be important factors. Control over flow dynamics, biodistribution, and cellular interactions are also dependent upon the elasticity of particle systems, although the volume of literature is more limited than in the SAV case. However, results indicate that softer particles in general will circulate longer in vivo, and will be internalized into cells at a reduced kinetic rate, or for very soft particles even completely evade phagocytosis.[166]

3.3.1. Flow Dynamics and Biodistribution

Rigidity has been found to affect clearance from the bloodstream, most notably through splenic clearance. It has been postulated that the increased splenic clearance of cholesterol-modified liposomes is due to increased stiffness compared to unmodified liposomes,[51] while nanoparticles not functionalized with a polymer brush layer are generally cleared very quickly via renal excretion.[20,72] Discher and co-workers found that long filomicelles display extended circulation lifetimes in vivo.[48] To test the effect of filomicelle rigidity on the lifetime, the micelles were crosslinked to form solid cylinders, which were found to clear within hours compared to days for flexible systems. This led to the conclusion that the circulation time is dependent on the ability of a particle to relax and fragment in a flow stream, also affecting flow-alignment and system extension within phagocyte streamlines (Figure 7).[48] Also in regards to flexibility and deformability, in order to pass through spleen fenestrations of the order of 200-500 nm wide,[108] DeSimone and Petros observed that particles must either be smaller than approximately 200 nm, or be sufficiently elastically deformable to pass through
and remain in circulation. In other work, it was shown that tuning the deformability via variation in $E_Y$ allowed for large particles to pass through narrow microchannels. Mitragotri and co-workers observed optically the deformability of particles with a similar $E_Y$ and geometry to RBCs, and found that the particles were stretched by approximately 70%, and retained their discoidal shape upon exiting the microchannel, demonstrating elastic deformation.

The only comparable study at this time, to our knowledge, which involves investigating the effect of elastic modulus on both circulation and biodistribution was performed by DeSimone and co-workers. The $E_Y$ was tuned using variable crosslinking concentrations for 6 µm diameter discoidal hydrogel particles. Similar to the results reported above, as the $E_Y$ increased, the circulation lifetime drastically decreased, correlating well with the theory that stiffer particles are cleared from circulation more rapidly. Interestingly, in regards to a 2 h biodistribution study in mice, particles with a modulus of 39.6 ± 10.4 and 63.9 ± 15.7 kPa were predominately found in capillary beds in the lungs, while particles with a lower modulus of 7.8 ± 1.0 and 16.9 ± 1.7 kPa were able to avoid clearance in the lung and were found to accumulate in the spleen. This work highlights the importance of particle rigidity in maximizing circulatory lifetimes, and also in biodistribution control.

3.3.2. Cellular Interaction and Uptake

Compared to literature on the effect of geometry and size on cellular interaction, studies on the effect of elasticity and rigidity on this area are rather limited. While not a cell study, Fery and colleagues investigated the adhesion of hollow polyelectrolyte shells onto both flat and polyelectrolyte-covered glass substrates. It was found that as the shell thickness decreased, the radius adhesion area increased dramatically due to the shell becoming more flexible and deformable. This has broader implications in regards to the ligand/receptor-mediated adhesion interactions between particles and cells, where an increased adhesion area correlates toward increased particle/cell interaction. Based on recent fundamental studies, Gao et al. found that
the elastic energy changes in a cell membrane mean that stiffer particles would be internalized favorably via cell wrapping when compared to softer particles, which tend to spread along the membrane.\cite{168} This factor probably influenced results observed by Wang and Beningo for interactions between opsonized microparticles with variable $E_Y$, and mouse bone-marrow macrophages.\cite{169} The polyacrylamide particles were 1 to 6 µm in diameter and coated with bovine serum albumin, and it was found that as the bis-acrylamide crosslinker concentration, and hence $E_Y$, increased, so did the preference for phagocytosis (Figure 8). As both surface chemical and geometric properties were kept constant between the two systems, the effect was solely ascribed to phagocytosis being a mechanosensitive process. Similarly for 150 nm hydrogel particles, Giasson and co-workers investigated the effect of rigidity on macrophage internalization rates and mechanisms.\cite{41} Stiffer nanoparticles were seen to be taken up faster than softer particles, and by different mechanisms; particles with an $E_Y$ of 18.0 ± 5.0 kPa were exclusively taken up by the RAW 264.7 macrophage cells using macropinocytosis, while particles with an $E_Y$ of 211.4 ± 43.3 kPa were exclusively taken up via a clathrin-mediated endocytosis route. Interestingly, hydrogel nanoparticles with a modulus between these two values were able to be taken up via dual effective pathways, and thus internalized at a quicker rate. In terms of intracellular trafficking, Giasson and co-workers also found that accumulation into lysosomal compartments is dependent on the nanoparticle elastic modulus.\cite{41} An increasing elastic modulus generally led to increasing colocalization with FITC labeled dextran, a common lysosomal marker, and nanoparticles were found not to be released into the cytosol. It was also found that the kinetics of early endosomal entry was elasticity dependent, where only the stiffest nanoparticle was found to be colocalized to the early endosome marker after 15 minutes.

It can also be noted that a macropinocytosis pathway for the internalization of polyelectrolyte capsules has been observed.\cite{35,170,171} This is an interesting finding, as polyelectrolyte capsules generally have a material $E_Y$ at least an order of magnitude greater than the hydrogel
nanoparticles studied by Giasson and colleagues, indicating that the particle structure and size will strongly mediate cellular uptake.

Regarding intracellular therapeutic release, Fernandes and co-workers investigated the forces and deformations required for polyelectrolyte capsules to release their cargo,\textsuperscript{[172]} whilst Delcea \textit{et al.} extended the work to examine polyelectrolyte capsules with different shell thicknesses, and consequently, rigidity.\textsuperscript{[173]} It was found that the capsule stiffness had a pronounced effect upon the applied force at which the cargo was released, and that the forces applied (0-2 µN) were within literature estimates for an applied intracellular force range. This was coupled with \textit{ex situ} electroporation experiments where it was estimated that the African green monkey kidney cells used exert at least 0.2 µN upon intracellular incorporation of the capsules,\textsuperscript{[173]} confirming that particles will be affected by mechanical intracellular action to different extents depending on their resistance to deformation.

4. Perspectives

Recent developments in both ‘top down’ and ‘bottom up’ fabrication strategies for advanced particulate systems allow for improved control over several particle physical properties, such as size, shape, and rigidity. Increasingly, evidence has shown that the geometric and physical characteristics of particles have a pronounced effect on biological interactions, where increasing either the deformability or SAV allows particles to: align in flow fields more effectively and reducing interaction with macrophages; improve cellular surface interaction under low shear conditions; and generally reducing the rate of cell uptake. Although these studies have opened new ideas and routes to control biological responses, comprehensive knowledge gaps remain. In many instances, a precise knowledge of how particle chemistry affects these physical properties remains deficient, so that tuning of particle shape and mechanics remains difficult. Further exploration of particle biomechanics will require the development of robust synthetic methodologies to allow the tuning of physical properties in a
wide variety of particle delivery systems. Moreover, a detailed understanding of the complex mechanisms governing interactions between particles and biological systems, such as internalization and sub-cellular trafficking, still remain unclear.

While this review focuses solely upon the affect of physical parameters on biological interactions, it is clear that approaches based upon controlling both physical and chemical properties are required, extending work by Gratton et al. on cylindrical polymeric particles with variable surface charge.\cite{Gratton2009} While physical properties strongly influence circulatory and cellular interactions, equally important to the success of particulate healthcare materials are chemical-based properties, such as stealth, targeting, and triggered degradation.\cite{Stellacci2013} This is highlighted by work on the surface patterning of colloidal materials, allowing for dual chemical and active surface area modification.\cite{Stellacci2013b} Of particular relevance is the work of Stellacci, Irvine and co-workers, which demonstrated that the patterning of alternating subnanometer striations of anionic and hydrophobic groups on gold nanoparticles, as seen in Figure 9, modulated the intracellular fate. Patterned particles accumulated in the cell cytosol as opposed to being trapped in an endosome, likely due to the patterning and related surface roughness providing a resistance to non-specific protein adsorption.\cite{Stellacci2014} Although significant challenges to the field remain, we expect that future multidisciplinary work will afford an improved understanding of the mechanisms and parameters governing the interactions between biological systems and particle delivery vehicles at the circulatory, cellular and sub-cellular level. This work will provide the foundations toward the rational design of advanced particle therapeutics with specific and optimized \emph{in vivo} behavior, resulting in the more effective treatment of diseases and enhanced patient outcomes.

\textbf{Acknowledgements}
This work was supported by the Australian Research Council under the Federation Fellowship scheme (FF0776078) (F.C.) and by the National Health and Medical Research Council (NHMRC) Program Grant 487922 (F.C.).

Received: (will be filled in by the editorial staff)
Revised: (will be filled in by the editorial staff)
Published online: (will be filled in by the editorial staff)


Figure 1. Impact of particle geometry upon the surface area to volume ratio (SAV). A 5.5 μm diameter sphere (top left, SAV → 1) displays a reduced SAV when compared to biconcave RBCs (top right), while the internal volume (V₁) is held constant. Additionally, as particle size decreases toward a 350 nm diameter nanosphere (bottom left), the SAV increases dramatically, and even more so for a long filomicelle (bottom right) with an equal internal volume to the nanosphere (V₂).
Figure 2. Fabrication of non-spherical particles. a) TEM image of cylindrical 1 µm diameter polymer particles in HeLa cells fabricated using PRINT [24]. b) SEM images of RBC shaped PLGA particles generated using electrohydrodynamic jetting [153]. c) high aspect ratio triangular prisms fabricated using flow lithography [26]. d) long PLGA particles fabricated using mechanical stretching [52]. e) discoidal particles fabricated using a combination of microlithography and reactive ion etching [25]. f) PS vase fabricated using direct replica transcription from silica [177]. g-j) TEM images of surfactant-stabilized gold nanorods with increasing aspect ratio [129]. Scale bars are 100 nm (g, h, i, j), 1 µm (f), 5 µm (b, d), 6 µm (e), 10 µm (c). Images are reproduced with permission. Copyright 2008 Springer (a), 2009 National Academy of Sciences (b), 2006 Nature (c, f), 2010 Elsevier (e, g, h, i, j).
Figure 3. The effect of particle geometry on capillary flow behavior. a) The aspect ratio ($r_e$) governs the magnitude of angular velocity under simple shearing flow [111]. b) Platelets interact heavily with the endothelial wall due to their irregular shape. Tethering 1, rolling angular velocity and activation 2, and finally firm adhesion with the wall 3, are all affected by the platelet geometry. Image is reproduced with permission. Copyright 2010 Royal Society Publications (a).
Figure 4. Phagocytosis of non-spherical particles. a) SEM images of PS disks orientated end-on [127], b) disks side-on [127], c) spheres [127], d) and IgG adsorbed worms [15] fabricated via mechanical stretching of spherical beads. Scale bars 10 µm (a), 5 µm (a, b, c). Images are reproduced with permission. Copyright 2006 National Academy of Sciences (a, b, c), 2009 Springer (d).
Figure 5. Mechanical characterization methods for nanoparticles and hollow capsules. a) Schematic diagram of a nanoparticle acoustic response to energy absorption, and associated vibrational spectra [176]. From thermal equilibrium 1, thermalization occurs upon excitation with an ultrafast electron 2, giving off acoustic vibration 3 and subsequent cooling 4. b) Confocal fluorescence images of osmotically buckled (left, scale 10 µm) and ruptured (right, scale 5 µm) polyelectrolyte capsules [42], and c) using RICM in tandem with a colloidal-probe AFM set-up to characterize buckling events for polyelectrolyte microcapsules [42]. Where region A relates to a small deformation regime, B relates to an adhesion area increase, and C is capsule buckling. Images are reproduced with permission. Copyright 2010 American Chemical Society (a), and 2004 IOP Publishing Ltd and Deutsche Physikalische Gesellschaft (b, c).
Figure 6. Studying particle deformation using microfluidics. a) A syringe pump drives the flow through the device. b) Particles with low $E_Y$ are highly deformed as they pass through the fenestration, c) while particles with a high $E_Y$ are not able to deform and therefore cannot pass through (scale bar 30 µm). Reproduced with permission from [46]. Copyright 2011 National Academy of Sciences.
Figure 7. Flow and deformation of filomicelles \textit{in vivo} and \textit{in vitro}. a) \textit{In vivo} filomicelle circulation time increases with particle length, b) while long filomicelles have been shown to deform and align in flow fields in order to avoid phagocytic interaction. Scale bar 5 µm. Reproduced with permission from [48]. Copyright 2007 Nature.
Figure 8. Phagocytic internalization of both antibody (Ab) functionalized stiff and soft polyacrylamide beads. a) Stiff Ab functionalized beads (left two bars) were six times more likely to be internalized than soft beads (right two bars). This was confirmed optically for both stiff b) and soft beads c) where stiff beads were more readily internalized. Reproduced with permission from [169]. Copyright 2002 The Company of Biologists Ltd.
Figure 9. Surface-patterning on gold nanoparticles affects cell internalization and intracellular fate. Schematic diagrams and STM images of both disordered a) and ordered b) patterning of hydrophilic and hydrophobic regions on gold nanoparticles. Dendritic cells show much lower levels of cytosolic accumulation for disordered c) than ordered d) particles incubated at 4 °C. Scale bar 4 µm for (a, b). Reproduced with permission from [176]. Copyright 2008 Nature.
James Best is a Ph.D. student in the Department of Chemical and Biomolecular Engineering at The University of Melbourne, and works under the supervision of Prof. Frank Caruso. He received his Bachelor's degree in Chemical Engineering (Hons.) and Science (Chemistry) also from The University of Melbourne in 2008. His current research interests include the mechanical analysis of nanoparticles for biomedical applications using atomic force microscopy.

Yan Yan received her Ph.D. in Biochemistry and Molecular Biology from Peking University (China) in 2008. Currently, she is a post-doctoral research fellow in the Nanostructured Interfaces & Materials group headed by Prof. Frank Caruso at The University of Melbourne. Her research focuses on the interface between materials science and biology.

Frank Caruso received his Ph.D. degree in 1994 from The University of Melbourne, and then moved to the CSIRO Division of Chemicals and Polymers in Melbourne to study the interfacial alignment of receptor molecules for biosensor applications. He was an Alexander von Humboldt Research Fellow and then group leader at the Max Planck Institute of Colloids and Interfaces (Berlin, Germany) from 1997 to 2002. Since 2003 he has been a professor, an Australian Research Council Federation Fellow in the Department of Chemical and Biomolecular Engineering at The University of Melbourne. His research focuses on polymers at interfaces, nanostructured colloidal systems, nanocomposite thin films, and biomaterials.
The effect of particulate physical properties on biological processes is a burgeoning research field. In this review, the influence of geometry and mechanics on movement under flow conditions, biocirculation, and cellular adhesion and uptake are discussed. An emphasis is placed on the ultimate use of particles in biomedical applications.

Keywords: drug delivery, particle geometry, particle elasticity, nanotechnology

J. P. Best, Y. Yan, F. Caruso*

The Role of Particle Geometry and Mechanics in the Biological Domain
Author/s:
Best, JP; Yan, Y; Caruso, F

Title:
The Role of Particle Geometry and Mechanics in the Biological Domain

Date:
2012-01-11

Citation:
Best, JP; Yan, Y; Caruso, F, The Role of Particle Geometry and Mechanics in the Biological Domain, ADVANCED HEALTHCARE MATERIALS, 2012, 1 (1), pp. 35 - 47

Persistent Link:
http://hdl.handle.net/11343/123315

File Description:
Accepted version