



Novel approaches for drug delivery systems in nanomedicine: effects of particle design and shape

Nicole Daum,¹ Clemens Tscheka,² Andrea Neumeyer³ and Marc Schneider^{2*}

The identification of novel drug candidates for the treatment of diseases like cancer, infectious diseases, or allergies (especially asthma) assigns new tasks for pharmaceutical technology. With respect to drug delivery several problems occur such as low solubility and hence low bioavailability or restriction to inconvenient routes of administration. Nanotechnological approaches promise to circumvent some of these problems, therefore being well suited for future applications as nanomedicines. Furthermore, efficient and sufficient loading is a critical issue that is approached through mesoporous particles and/or through nonspherical particles both offering larger volumes and surfaces. Special interest is laid on the effect of shape of particulate materials on the body and related physiological mechanisms. The modified response of biological systems on different shapes opens a new dimension to adjust particle system interaction. Finally, the biological response to these systems will determine the fate with respect to their therapeutic value. Therefore, the interaction pattern between nonspherical particulate materials and biological systems as well as the production processes are highlighted. © 2011 Wiley Periodicals, Inc.

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INTRODUCTION

In recent years, much work has been done on the investigation of nanoparticles for pharmaceutical and medical applications. The potential of these nanosized particulate materials as drug delivery vehicles is considered to be enormous. A key element in drug delivery is the transport of the drug to a specific target. Drug solubilization as well as the interaction of the carrier with biological barriers, both influencing bioavailability, are in this context also of outmost importance. Especially this field is assumed to profit most from nanotechnological approaches.

Hitherto, different strategies have been used in the field of nanomedicines based on polymers, polymer-based particles, as well as solid inorganic particles highlighting the potential applications (Figure 1).

Numerous nanoparticle-based drug formulations and diagnostic agents have already been developed, e.g., for the treatment of cancer, diabetes, pain, allergy (in particular asthma), and infections.^{1–3} A predominant parameter of nanoparticulate carriers is their large surface. Therefore, relevant descriptors for drug delivery systems have always been size and surface chemistry (of the respective carrier). Having a future application in mind a pivotal role will depend on the delivered drug dose. Both the drug loading and the encapsulation efficiency will play a key role for an efficient delivery of drugs and especially the delivery of a certain drug amount (dose). Two possible ways to increase the loading capacity of nanoparticles are porous systems (mainly from silica, keeping the size) or nonspherical systems (keeping 2 dimensions

*Correspondence to: marc.schneider@mx.uni-saarland.de

¹Drug Delivery, Helmholtz Institute for Pharmaceutical Research Saarland, Helmholtz Center for Infection Research, Saarland University, Saarbrücken, Germany

²Pharmaceutical Nanotechnology, Saarland University, Saarbrücken, Germany

³Biopharmaceutics and Pharmaceutical Technology, Saarland University, Saarbrücken, Germany

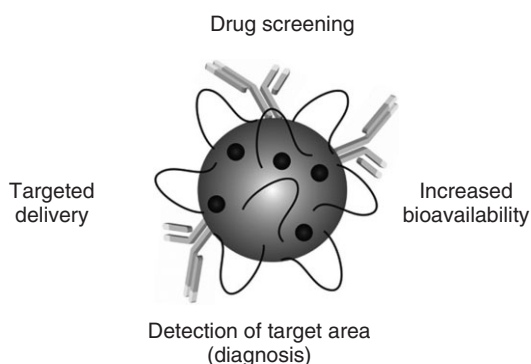


FIGURE 1 | Applications of functionalized nanoparticles and research aims in nanomedicine. The surface of nanoparticles can be modified with various molecules such as fluorescence dyes or biological ligands (e.g., antibodies). Furthermore, drug molecules can be encapsulated or adsorbed to nanoparticles. Adding parts or all of these functionalities turns the nanoparticle into a sensor, an imaging aid for diagnosis, or a directed carrier, respectively.

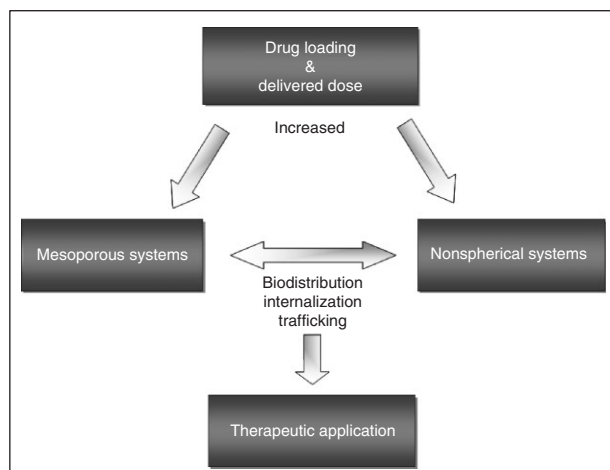


FIGURE 2 | Sketch of the possible approaches to increase the drug loading capacity of nanoparticles which is offered by porous and nonspherical particles in comparison to spherical particles. Different biological responses and subsequent application schemes are the consequence.

but increasing the third) and the combination of both (Figure 2).

Typically, the shape of nano- and microparticles has been spherical. The primary reason for the spherical shape is the optimization of the ratio between volume and surface. This property in effect minimizes surface tension and spherical shapes can form automatically, given appropriate conditions and production protocols. However, different shapes will exhibit a preferential biodistribution for the target tissue.⁴ Therefore, nonspherical shapes as found also in natural systems such as certain pathogens or erythrocytes promise to allow altering the interaction. As a consequence, the production of nonspherical

carrier systems and their impact on biological processes such as uptake, distribution and clearance are currently of specific interest for these potential drug delivery systems. The first efforts in order to elucidate the specific effects of complex morphology on biological processes have been undertaken and will be described.

APPROACHES FOR NONSPHERICAL PARTICLE PREPARATION

The fabrication of nanosized objects in various shapes has already been mastered for a long time. At the beginning of the last decade, a multitude of methods were available, ranging from lithographic to chemical synthesis approaches.^{5,6} The efforts encompass many fundamentally different approaches, each being suitable for certain materials and sizes. The formation of particles critically depends on the material influencing particle sizes and their applicability. Looking at the literature, inorganic and elemental carbon-based particles were found to be generated in smaller dimensions than tailored, complex polymeric objects. However, the latter exhibits a higher freedom of design with respect to adapting the form. As a consequence, the potential utility for drug delivery and hence medical application is increased.

As the fabrication of nonspherical particles is a crucial aspect for application, relevant procedures will be shortly summarized below. For more detailed descriptions regarding production other review articles are recommended for further reading.^{5–8} Thereby, a focus is laid on several approaches that might be suited for drug delivery, but there are more methods available which are not discussed herein.

FORMATION OF NONSPHERICAL PARTICLES BY CHEMICAL SYNTHESIS

A straightforward and classical bottom-up approach is the synthesis of particles. Gold nanoparticles (AuNP) have attracted considerable attention and are suitable for drug delivery.^{9,10} They can also be prepared in nonspherical form by selection of appropriate reaction conditions. Rods with variable length-to-width ratios (aspect ratio) below 10 can be created smaller than 100 nm,^{11,12} even complex forms like 'urchins' are possible.^{13,14} Drug loading of these systems can be performed, e.g., by the layer-by-layer technique incorporating the active agent in the coating. Advantages of AuNP encompass ease of synthesis of monodisperse nanoparticles with different sizes (1–100 nm), surface functionalization, and detection based on their optical

properties.⁹ Nevertheless, these biocompatible particles cannot be degraded in the body and the long-term toxicity is still widely unknown.

Silica nanoparticles can be prepared by two general synthetic routes: the Stöber¹⁵ and the microemulsion process.¹⁶ These methods allow a controlled generation of spherical silica particles with uniform sizes ranging from 50 to 2000 nm in diameter and also to produce dye-doped particles for detection. Mesoporous silica particles (MSN), previously developed as molecular sieves, are hollow nanocapsules with well-defined and controllable morphology and porosity. They can be synthesized as described by Di Renzo et al.¹⁷ A previously reported modified synthesis allows the preparation of differently shaped MSN (e.g., nanoworms, nanocylinders) by altering the molar ratios of different chemicals during synthesis.^{18,19}

NONSPHERICAL PARTICLES FORMED BY SELF-ASSEMBLY METHODS

Classical self-assembly of surfactants allows the adjustment of the form (lamellae, cylinders, or vesicles) of the assembled objects limited within these thermodynamically stable mesophases. The chemical composition as well as the ratio and dimension of the polar and the apolar segments result in different macrostructures.^{20,21} Moreover, the technique is not limited to amphiphilic fatty acid derivatives but also allows the use of amphiphilic polymers. Block copolymers can be utilized and fundamental parameters such as stability, release properties, and size can be tailored.²² These filamentous micelles made from block copolymers can be produced with diameters below 25 nm and a length of more than 10 μm (Figure 3(c) and (d))⁴; even janus cylinder architectures are feasible.²³ However, the mechanical stiffness of noncrosslinked objects is low and the structure of the noncovalent associates depends on external factors (surrounding media, temperature, etc.) in addition to the structural parameters of the monomers,²⁰ restricting a straightforward design of the desired geometry.²¹ In this context, amphiphilic DNA block copolymers, single-stranded-oligonucleotides covalently bound to a hydrophobic core segment of biocompatible polymer was found to form rod-like micelles through hybridization with longer DNA sequences. The rods consist of two parallel aligned double helices and have the dimension $3\text{--}4 \times 30\text{ nm}$.²⁴

Other anisometric nanoobjects consisting of lipids include solid lipid nanoplatelets²⁶ in a size below 200 nm, ellipsoidal micelles,²⁷ and rod- or thread-like micelles,²⁸ among others.

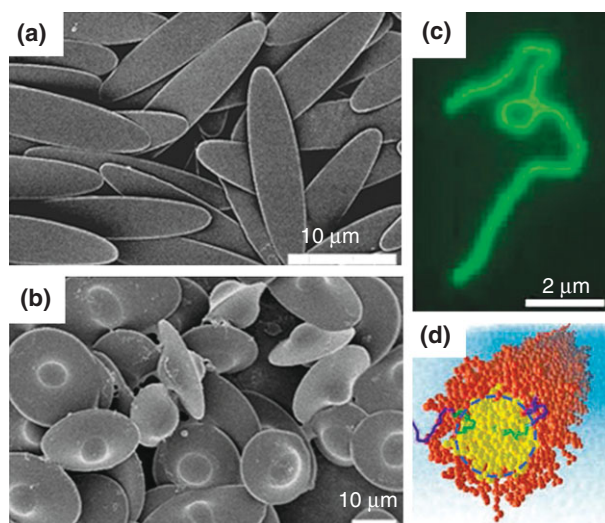


FIGURE 3 | Selection of nonspherical particles fabricated using different strategies: SEM images of nonspherical polystyrene (PS) particles created by a stretching technique in the form of (a) elliptical disks and (b) UFOs. (Reprinted with permission from Ref 25. Copyright 2006 NCBI) (c) Fluorescence microscopy shows an isolated filamentous block copolymer associate (filomicelle). (Reprinted with permission from Ref 4. Copyright 2007 Nature Publishing Group) (d) Schematic of the filomicelle structure: yellow/green indicates hydrophobic polymer, orange/blue is hydrophilic. (Reprinted with permission from Ref 4. Copyright 2007 Nature Publishing Group)

Micellar systems have already been loaded with active substances and can serve as carriers for hydrophilic and hydrophobic molecules because of their amphiphilic nature.

Eventually, the fabrication strategy for iron oxide nanoworms below 100 nm in length²⁹ is based on the linear assembly of magnetic nanoparticles, including iron oxide, along strands of high molecular weight dextrans.³⁰ These segmented nanopearl chains can also be formed from different biotemplates,³¹ however, they cannot be fabricated in perfect accuracy in terms of homogeneity with this method.

TEMPLATE-ASSISTED APPROACHES

The most prominent approach resulting in the largest variety of particles, including fiber formation, is the template-assisted method. Concerning the template method two general approaches are used: the direct template method based on the coating of a core material³² and the inverted template method based on the coating of inner walls. Recently, biocompatible particles mimicking key aspects of red blood cells, including the shape, could be fabricated with a layer-by-layer (LbL) approach (Figure 4(g)).³³ Also in this case active substances can be incorporated in the layers or used as layer material itself.

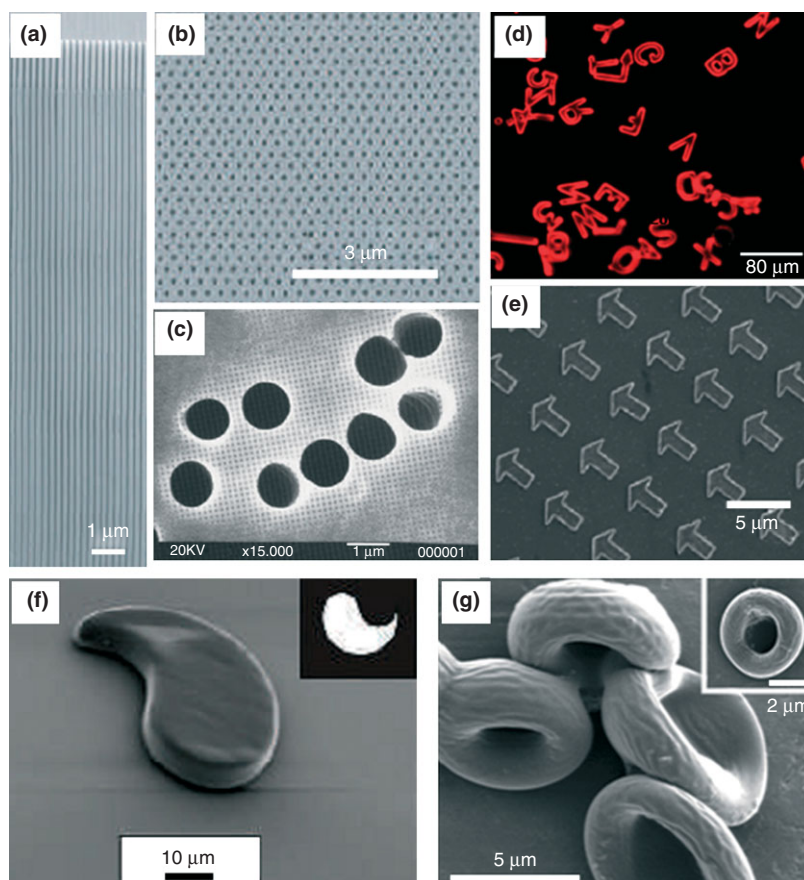


FIGURE 4 | Membranes used for the formation of cylindrical and nonspherical particles formed via molds, or template-assisted techniques. (a) SEM image of a cross section of an alumina membrane (internal diameter = 210 nm) with an extremely high aspect ratio (>1000) of uniform nanopores. (Reprinted with permission from Ref 38. Copyright 2006 ACS Publications) (b) SEM image showing the (top) surface of the alumina membrane with a perfect hexagonal arrangement of the nanopores. (Reprinted with permission from Ref 38. Copyright 2006 ACS Publications) (c) SEM image of a track-etched polycarbonate membrane (PC) with a pore diameter of 1 μm . (Reprinted with permission from Ref 36. Copyright 1997 RSC Publishing) (d) Fluorescence micrograph of poly(lactic-co-glycolic acid) (PLGA) particles created through the hydrogel template approach representing some symbols of a computer keyboard. (Reprinted with permission from Ref 44. Copyright 2010 Springer) (e) SEM images of 3 μm arrow-shaped poly(ethylene glycol) (PEG) particles fabricated by the particles replication in nonwetting templates (PRINT) technique. (Reprinted with permission from Ref 45. Copyright 2005 ACS Publications) (f) SEM image of a polymeric particle that was made in a microfluidic device polymerized by light. The inset shows the transparency mask feature that was used to make the corresponding particle. (Reprinted with permission from Ref 46. Copyright 2006 Nature Publishing Group) (g) SEM image of artificial red blood cells which were formed using the layer-by-layer (LbL) technique. Inset shows close up image. (Reprinted with permission from Ref 33. Copyright 2009 NCBI)

The well-established indirect template-assisted method was pioneered by Martin³⁴ based on a membrane with uniform pores enabling the formation of cylindrical objects within the confined space. Porous alumina membranes (Figure 4(a) and (b)) and track-etched membranes (Figure 4(c)) are the two most common templates for this approach.³⁵ The internal geometry and structure of the membrane template dictates the resulting geometry (track-etched membranes $\geq \sim 10$ nm and alumina membranes $\geq \sim 5$ nm³⁶), while both can be produced in a thickness of a few microns. Both types have parallel, straight, uniform pores³⁷ allowing the fabrication of fibers with very high aspect ratios of high

precision and abundance.³⁸ The variety of feasible materials for this approach is enormous, ranging from inorganic nanostructures such as gold nanorods³⁹ or nanotubes,⁴⁰ silica tubes,^{41,42} and objects made from semiconductors^{34,35} to organic compounds, including hydrophobic, hydrophilic, biocompatible, and biodegradable polymers.^{35,43} Regarding the drug, it can either be loaded in the material forming the cylinder or bound to the surface. The large surface implies a higher loading capacity in comparison to spheres that can successively be modified, allowing the introduction of additional functionalities.

Inverted template-assisted methods are mainly based on polymeric materials. Filling a membrane

serving as a mold also allows the fabrication of cylindrical objects such as rods and fibers with high fidelity. Generally, size is accompanied by a more elaborate shape and therefore ranges primarily in micrometer size. The morphology is highly tailorable except for the open side, not facing the mold. In general, one should be aware that the presence of polymerization initiators and limitations in the scaling up may restrict the applicability of template-assisted approaches in the biomedical field.

PRINT

Among template techniques a prominent and promising approach is the PRINT technique (Particles Replication In Nonwetting Templates) that uses a PFPE (perfluoropolyether)-mold which is nonwetting to both organic and inorganic compounds.^{6,45} The liquid precursors in each cavity are less prone to form any connecting layer to their neighbors over the surface of the mold⁴⁵ which is a widespread problem. The PRINT technique is applicable for several materials, including proteins (albumin⁴⁷) and biocompatible and biodegradable polymers (e.g., poly(ethylene glycol) (PEG)^{45,48}; poly(D-lactic acid) (PLA)⁴⁵), that can be solidified by polymerization (Figure 4(e)). With this approach a resolution as low as 20 nm can be achieved with a composite mold.⁴⁹ Lately, the PRINT technique has been extended and refined for the formation of high aspect ratio objects ($AR > 50$) because of a stretching step implemented during sequential production of the flexible soft lithographic mold.⁵⁰

Step and Flash Imprint Lithography

Continuing with lithographic techniques, Glangchai et al.⁵¹ demonstrated the potential of step and flash imprint lithography (S-FIL). This nanomolding process is applicable to biocompatible polymers (e.g., PEG-derivatives of different molecular weight) and can form complex shapes in sub-100 nm dimension. As a proof of concept the potential for drug delivery of susceptible payloads was assessed by loading the particles with proteins (streptavidin⁵¹ and avidin⁴⁵). For both molecules the complex with biotin could be formed and was interpreted as an indicator for a gentle loading process maintaining the biological functionality of the proteins.

Hydrogel Template Method

In this context, the use of other mold materials has also been described in literature. Acharya et al.⁴⁴ implemented a hydrogel (gelatin) as template for the fabrication of separate structures down to 200 nm. An aqueous solution of gelatine is poured on the

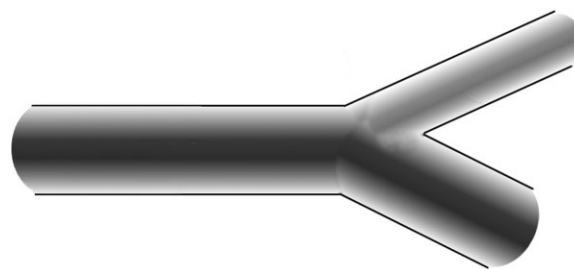


FIGURE 5 | Illustration of vessel with bifurcation. Certain nonspherical particles are reported to accumulate in close proximity to the walls and therefore can exit main vasculature more easily. This is depicted through the gradual transition of the lightness from the center towards the lateral regions. Lighter regions contain more nonspherical objects. The smaller vessel is supplied from the lateral region of the main vessel and consequently lighter.

master which has protruding geometries. After the solidification and removal the gelatine mold can be filled with a hydrophobic polymer, e.g., poly(lactic-co-glycolic acid) (PLGA) (Figure 4(d)).

Continuous Flow Lithography

Dendukuri et al. described a photolithography-based microfluidic technique allowing the production of complex shapes (Figure 4(f)).⁴⁶ An oligomer stream (PEG-diacrylate) containing a photosensitive initiator flows through a microfluidic device and is cured by pulsatile light irradiation. Hereby, particle geometry is controlled by the mask and the cross section of the channel in the microfluidic device. This technique allows for the fabrication of objects $\geq 1 \mu\text{m}$ (Figure 4(f)). Because this is a continuous technique with low polymerization times of below 0.1 second, the yield is high and should therefore be well suited for large scale production.

Overall, the indirect template-assisted techniques allow producing a wide variety of particles.

OTHER PROMISING APPROACHES TO FORM NONSPHERICAL PARTICLES

Stretching of Spheres

Mitragotri and coworkers utilized a mechanical stretching method based on the work of Ho et al.⁵² to fabricate nonspherical polymer particles consisting of polystyrene (PS)^{25,53} or PLGA (Figure 3(a) and (b)).⁵⁴ The spherical objects used as precursors are immobilized in a polymeric film and liquefied. Hereafter, stretching of the polymer film resulted in nonspherical particles of shapes in great variety⁵³ whereas size depends on the primary particles. Furthermore the particles can be loaded with active components.

Carbon Nanotubes

Carbon nanotubes (CNTs)⁵⁵ are considered as promising materials even for biomedical applications.^{56,57} They can exhibit diameters as low as a few nanometers and a length of up to a few centimeters.⁵⁸ In general, these particles are hydrophobic but can be rendered water soluble by surface modifications allowing drug loading⁵⁹ and reducing the pronounced tendency to agglomerate. Even though being a promising approach, safety issues still need to be investigated thoroughly. This is of special interest regarding the structural analogy to asbestos in particular for longer tubes.⁶⁰

Silicon Derivatives

Silicon derivatives can be fabricated in various shapes using different techniques. For cylindrical particles one fabrication method utilizes membranes. Monodisperse objects with potentially very high aspect ratios and a diameter smaller than 50 nm can be generated.^{41,42} Another technique yielding anisometric silicon-based objects is based on the conversion of spherical beads into oblate ellipsoids by means of ion beam irradiation. This technique has been successfully applied on spheres with a diameter between 125 nm and 1 μm .⁶¹ Nonspherical microparticles with a size below 10 μm can be generated as described by Decuzzi et al.⁶² The nonspherical shapes include mesoporous quasi-hemispherical silicon particles and nonporous discoidal or cylindrical silicon objects, whereas the latter are made from polycrystalline silicon (PolySi).

IMPLICATIONS OF PARTICLE SHAPE AND DESIGN ON BIOLOGICAL PROCESSES

Particle shape and design have been found to be crucial on various fundamental biological and biophysical processes and therefore might play an important role for future applications of particles in drug delivery. Recent publications report the influence of particle shape and design on the interaction with the target tissue by altering biodistribution, cellular internalization, and trafficking amongst others.

Circulation and Biodistribution

Principally, extended circulation in the blood stream is a major criterion for modern drug delivery systems. In particular for cytostatic therapy, permitting controlled distribution and homing into the cancer tissue can be regarded as a key parameter for the specific targeting *in vivo*. Therefore, several further studies

were conducted to elucidate the effects of particle shape and design with respect to circulation time.

Experimentally, it has been observed that elongated objects seem to affect the retention time after extravasation. Dextran-coated nanochains (length ~ 50 nm) as well as spheres were able to extravasate into tumor tissue and merely the spheres returned into circulation to a high extend because they were not physically trapped.²⁹

Nonspherical particles, such as discoidal objects or prolate spheroidal objects, show a deviating hydrodynamic behavior in comparison to spheres.³³ This influences circulation, transport in the blood flow, and finally biodistribution. Cellular components of the blood tend to accumulate in the center of the blood flow. In contrast, nonspherical objects, such as platelets, remain predominately in the proximity of the endothelium and can consequently exit the main vasculature faster toward the capillaries (Figure 5).⁷ Moreover, the unique hydrodynamic properties of filamentous, flexible micelles allow them to align with the blood flow, resulting in a substantial extension of the circulation time.⁴

Nonspherical shape of particles could be shown to enhance circulation time and influence the accumulation. The circulation time of liposomes, in the size range of 100–150 nm, could be enhanced by the transformation of the spherical vesicles into a disc-like vesicle. Polymerization of monomeric actin into a filamentous state was utilized for this morphological transition, and rats were used as a model system.⁶³ In addition, polymeric elliptical discs ($0.1 \times 1 \times 3 \mu\text{m}$) made from polystyrene⁵³ decorated with ICAM ligands to selectively target endothelium were found to remain longer in circulation in mice. They showed lower uptake by the liver and a specific targeting of lung tissue in comparison to their spherical (0.1, 1, 5, 10 μm) counterparts.⁶⁴ Other studies confirmed the results that oblate objects are taken up to a lower extent into the liver. As this organ is part of the reticuloendothelial system (RES), the lower accumulation reflects the tendency to evade phagocytosis.⁶² Additionally, filamentous micelles have been observed to exhibit a certain preferential distribution for lung tissue after intravenous administration.⁴

Furthermore, the effect of geometry on the biodistribution after systemical administration into tumor-bearing nude mice has been studied with various uncoated silica spheres (diameters ranging from 700 nm to 3.0 μm) and nonspherical objects with quasi-hemispherical, cylindrical, and discoidal shapes. A reduction in spherical particle accumulation with increasing size was evident for non-RES organs. The authors hypothesized that this was caused mainly

by adhesion to the vessel walls, than by uptake into the cells. Nonetheless, the highest accumulation for the spheres was found for the smallest diameter in the liver. Regarding the nonspherical particles, the most pronounced variation from the spherical biodistribution was observed for the disc-shaped objects, being least related to a sphere amongst the studied shapes. The discs were able to circumvent sequestration by phagocytic cells in the liver and therefore showed a lower accumulation compared to the cylindrical particles.⁶²

Internalization and Trafficking

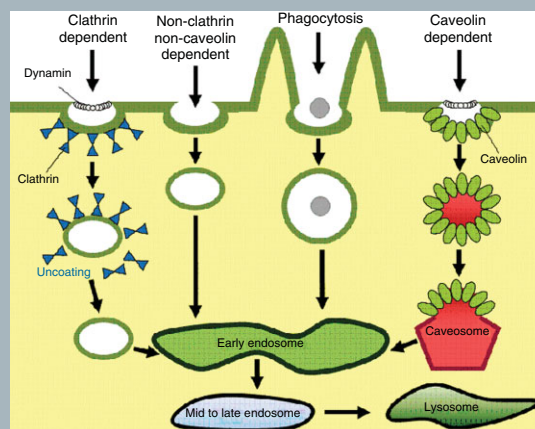
Besides the influence of the shape on the biodistribution, in general, nonspherical geometry was found to modulate internalization processes and mechanisms.^{7,25,48,54} In this context, the attachment to the cell surface is the first step of the sequential internalization processes. Adhesion can be enhanced because of a larger contact area of elongated objects with the cell membrane and polyvalent bindings can be generated.^{7,29,57} Fundamentally, this was investigated either by *in silico* models⁷ or by means of a synthetic microvascular model.³³ Observing the internalization of attached particles an oblate ellipsoid, the mild variation from spherical form, showed the highest uptake. Combining both steps, phagocytosis observed to be highest for oblate ellipsoids, even more than for spheres and lowest for the elongated shape, the prolate ellipsoid.⁶⁵ Hence, prolate ellipsoids exhibited the highest and spheres the lowest attachment.

Thereby, endocytosis comprises different pathways by which cells import and export selected extracellular molecules, viruses, microorganisms, and nanosized particles. The internalization pathway chosen depends on the size and nature of the extracellular cargo (Box 1). Therefore, the geometry of a given particle strongly influences its cellular internalization. Hitherto, the studies focusing on the influence of shape on cellular uptake are pointing toward different possible effects and mechanisms, as some demonstrate a reduced cellular uptake of nonspherical particles,^{11,54} while others report an increased cellular internalization^{48,66,67} or even no difference in uptake.⁶⁸ This is due to the fact, that the uptake is not only dependent on the particle geometry but also on other physicochemical properties. In some studies the particle surface of spherical and nonspherical particles is not kept constant, thereby producing a combined effect of geometry and surface chemistry or an effect which is only dependent on the surface alone. Last but not least the cell type addressed is of importance. Thereby, it is not surprising that a professional phagocytic cell

BOX 1

ENDOCYTOSIS

Various uptake mechanisms have been described as potential cell capture pathways for nanoparticles. Endocytotic mechanisms may take place in all types of cells and vary accordingly. Moreover, several endocytotic mechanisms often occur simultaneously. All these mechanisms are associated with several specific organelles involved in the sequential cellular passage of particles. In general, nanoparticles must cross one or more biological membranes before diffusing across the plasma membrane to finally enter the target organelle. Larger particles can be taken up into the cell by phagocytosis, which is a process dependent on actin-mediated remodeling of the cell membrane. Phagocytosis is a process occurring only in highly specialized cells, e.g., macrophages. However, also for other cell types a phagocytosis-like mechanism could be demonstrated. Internalized particles can be delivered to the early endosome via clathrin- or caveolin-coated vesicles that are derived from the cell membrane (clathrin- or caveolin-dependent endocytosis). Nevertheless, particles can also be endocytosed by different mechanisms that are independent of the coating proteins clathrin or dynamin (nonclathrin-/noncaveolin-dependent endocytosis). Progress in understanding the nanoparticle internalization has already allowed the tailoring of nanomedicines. Thereby, nanoparticles' physicochemical properties dramatically influence the various mechanisms of cellular internalization. To optimize the intracellular delivery of small molecules as well as of macromolecules (nucleic acids, peptides, etc.), it is indispensable to further elucidate these mechanisms and their interaction with particulate systems to design more efficient drug delivery strategies or adapt already available approaches.



(e.g., a macrophage, whose primary function is the removal of foreign material) shows other uptake patterns than nonphagocytes (e.g., epithelial cells). This may be reflected in the internalization speed as well as in the uptake rates. Thus, kinetics including the selection of reasonable endpoints should be thoroughly studied and reassessed in the future. Furthermore, the adsorption of proteins may substantially differ not only for different surface properties but also for different particle shapes, resulting in different types of orientation of adsorbed opsonins.⁶⁹

Gratton et al. studied the internalization of monodisperse hydrogel particles with well-defined geometries fabricated by PRINT technology into HeLa cells.⁴⁸ Influence of size, shape, and surface charge were evaluated. Particles with higher aspect ratio ($d = 150$ nm and $h = 450$ nm) were internalized by HeLa cells four times faster than the more compact lower aspect ratio particles ($d = 200$ nm, $h = 200$ nm). Cylindrical particles ($d = 100$ nm) were internalized to a lesser extent than the larger cylindrical particles ($d = 150$ nm) with the same aspect ratio. Similar results were obtained in a study with three different shaped MSN of similar particle diameter, chemical composition, and surface charge but with different aspect ratios.⁶⁷ Particles with larger aspect ratios were taken up into the human melanoma cell line A375 to a higher extent and had faster internalization rates. Likewise, it was also found that particles with larger aspect ratios altered cellular function including cell proliferation, apoptosis, cytoskeleton formation, adhesion, and migration.

In the seminal paper of Mitragotri and co-workers,²⁵ it was stated that the point of first contact between the phagocyte and the particle mainly governs the initiation of phagocytosis and therefore

the success of phagocytosis (Figure 6). Size, having been regarded as the crucial factor, merely directs the completion of ingestion by the overall volume ($V_{\text{particle}} < V_{\text{macrophage}}$). Roughly, the threshold for internalization at this local point is the geometrical shape of a sphere. Being more oblate, the immune cell will only spread on the object, whereas a locally more conical morphology with higher curvature will lead to ingestion. Consequently, nonspherical objects are able to control and evade clearance mechanisms. For asbestos, the consequence of frustrated clearance in combination with biopersistence is well known.

The uptake behavior of different sized and shaped colloidal AuNP was investigated in several studies. It was shown that kinetics and saturation concentrations were highly dependent not only on the physical dimensions of the nanoparticles¹¹ but also on their surface modification.⁷⁰ In summary, uptake of rod-shaped AuNP was lower than their spherical counterpart.

Further differences have been described for the kinetics of intracellular trafficking. The velocity of the approach toward the nucleus for spheres was observed to be significantly faster than for elliptical discs.⁵⁴

Besides physical implications such as adhesion and subsequent processes, Hutter et al.¹⁴ reported about a varying immune response for differently shaped particles. Shape substantially influenced the expression of toll-like receptors (TLR) and the inflammatory cytokine expression pattern, implying an altered immune response for nonspherical particles.

Taken together these studies point to the emerging influence of nanoconstruct geometry on biological processes. The understanding of the underlying mechanisms will pave the way for the tailoring of nanoparticles for drug delivery applications. Therefore, the findings from such studies will have implications on the design of nanostructures for biomedical applications, e.g., by tuning biodistribution or intracellular delivery rates and amounts using particle size and shape as parameters.

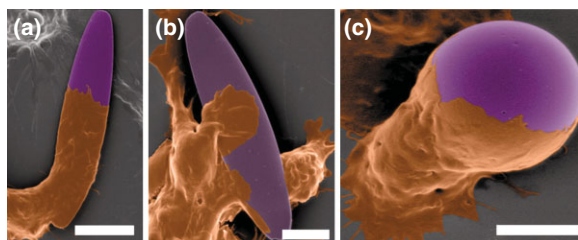


FIGURE 6 | Colored SEM images of alveolar macrophages (brown) interacting with polystyrene (PS) particles (purple). (a) The cell body (brown) can be seen at the end of an elliptical disk and the membrane has progressed down the length of the particle. (b) A cell has attached to the flat side of an elliptical disk and has spread on the particle. (c) A spherical particle has attached to the top of a cell and the membrane has progressed over approximately half the particle. Scale bar: (a) 10 μm , (b) and (c) 5 μm . (Reprinted with permission from Ref 25. Copyright 2007 NCBI)

THERAPEUTIC APPLICATIONS OF NOVEL CARRIER SYSTEMS *IN VITRO* AND *IN VIVO*

Several applications of novel carrier systems have recently been reported in the literature. However, focus is thereby laid on mesoporous silica due to the easily controllable and well-established silane chemistry. A further advantage of this system is the high loading capacity compared to other delivery systems.

Thereby, MSN showed their potential as carriers for drugs of low bioavailability, such as many anti-cancer drugs. This low bioavailability is based on the low solubility in aqueous solutions, as well as the first-pass elimination within the body. The ordered pore network of MSN with adjustable diameter allows a fine control of drug loading and release kinetics in combination with a large surface for drug adsorption and a thereby increased loading capacity.^{71,72} These characteristics make MSN a promising candidate for controlled drug delivery systems.⁷³ In this context, MSN released doxorubicin over a period of 20 days at a constant rate.⁷⁴ Furthermore, MSN were able to enhance the delivery of the hydrophobic anti-cancer drug paclitaxel to pancreatic cancer cells⁷² and allowed a targeted delivery of the chemotherapeutic agent methotrexate. Free methotrexate caused apoptosis in cancer cells as well as in healthy cells. In contrast, methotrexate bound to nanoparticles induced cell death only in cancer cells but not in normal tissue.⁷¹ In addition, a high loading of about 60% in weight for telmisartan, an angiotensin II receptor antagonist was realized. Furthermore, the release rate of telmisartan from MSNs could be effectively controlled.⁷⁵

In addition, MSN could be used as efficient and safe carrier for nucleic acids. Simple electrostatic interactions based on adsorbed cationic polymers (e.g., polyethyleneimine) allowed binding of DNA and siRNA constructs. This particle system was able to deliver siRNA to a murine hepatoma cell line (HEPA-1) which resulted in a knockdown of the green fluorescent protein (GFP) expression.⁷²

Besides the advantages of pure silica nanoparticles, the properties of the carrier might be further improved utilizing hybrid systems. The oral absorption of poorly water-soluble drugs can be enhanced via the coadministration of lipids. Thereby, lipid-based drug formulations are ranging from simple lipid solutions of drugs to more complex delivery systems such as microemulsions, solid lipid nanoparticles, and lipid-based liquid crystalline systems.⁷⁶ The combination of lipids and silica-based carriers might profit from advantages of both systems. For example, the delivery of the poorly water-soluble drug celecoxib, a selective cyclooxygenase-2 inhibitor which is classified as a biopharmaceutics classification system (BCS) class II drug (high permeability, low solubility) and which is practically insoluble at the pH of the gastrointestinal tract,⁷⁷ could be improved via the development of silica-lipid hybrid (SLH) microcapsules (Figure 7). This novel drug carrier was able to increase the oral availability of celecoxib from 62 to 93–100% when bound to SLH microcapsules.⁷⁸

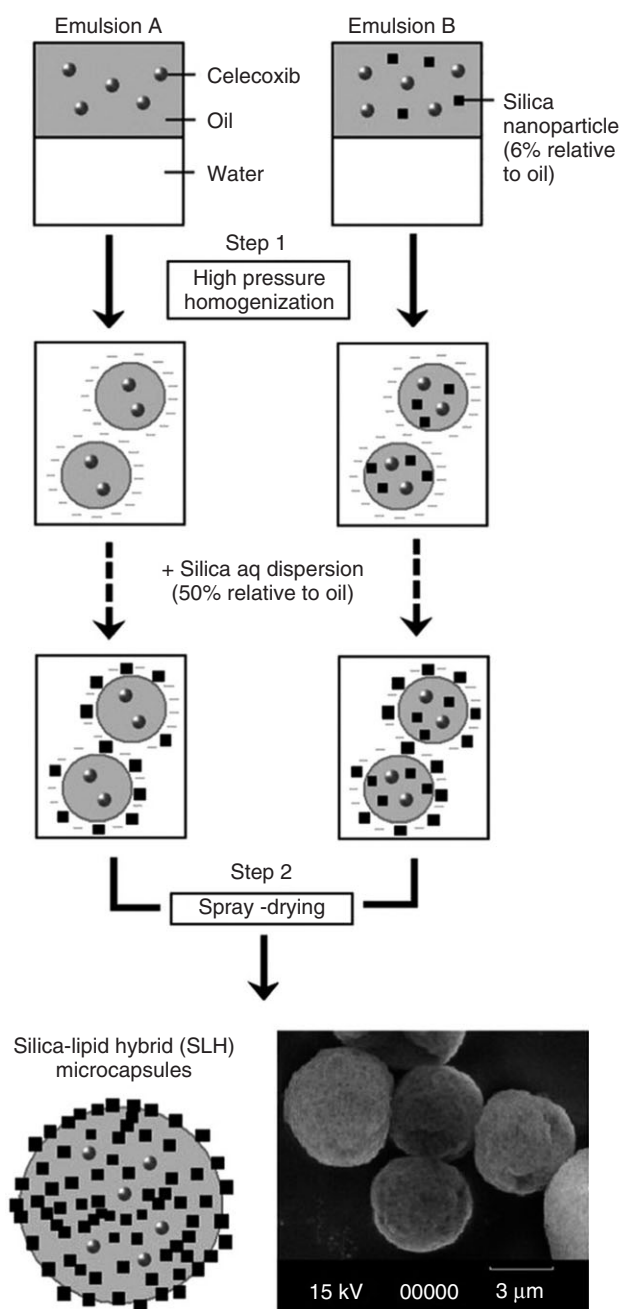


FIGURE 7 | Schematic representation of silica-lipid hybrid (SLH) microcapsule formation and scanning electron micrograph of the microcapsules. Celecoxib-containing SLH microcapsules were prepared via a two-step process including the homogenization and the following spray-drying of the silica-stabilized emulsions. (Reprinted with permission from Ref 78. Copyright 2009)

These hybrid systems offer more properties and will attract increasing attention in future (nano)medicines.

However, a pivotal question for future silica applications is still, where those nanoparticles remain after their administration. Hitherto, there is a lack of

publications dealing with the fate of silica nanoparticles in the body. There are some studies regarding the dissolution behavior of such particles^{79,80} but this needs to be investigated in more detail. Findings about fate and accumulation of those carriers and their corresponding toxicity are essential and require intensive research for a thorough risk evaluation in the future.

Only few studies have been published dealing with therapeutic applications of novel systems other than silica. Discher and co-workers report about filamentous micelles that are formed from biocompatible and degradable block copolymers loaded with paclitaxel to the hydrophobic core.⁴ In rodents, a circulation of up to 1 week, which is more than ten times longer than the compared spherical liposomes and twice the time examined for PEGylated stealth liposomes, was observed. This remarkable property of the so-called filomicelles (Figure 3(c) and (d)) was attributed to the geometry (the diameter of ~20–60 nm allows unhindered passage of the spleen) in combination with the inherent flexibility. The carrier shows a favorable hydrodynamic behavior in the blood circulation. The structure allows the filomicelles to evade phagocytosis of the mononuclear phagocyte system. Captured

micelles are pulled away from the grip of the immune cells by the shear forces exerted by the flowing blood. These structural advantages of the carrier gave rise to a significant reduction of tumor in a xenografted model in comparison to free paclitaxel. In addition, a moderately higher accumulation in the lung leads to the speculation about implications for the localization of viral infections of the lung with respect to (nonspherical and) filamentous virions, namely the Ebola and the influenza virus H5N1.

Utilizing the pronounced adhesion of nonspherical particles to the target region for diagnostic applications, the targeting efficiency of so-called magnetic iron oxide nanoworms was assessed. Iron oxide nanoparticles (~5 nm in diameter) were interconnected with high molecular weight dextran in order to form chain-like structures with a length of 50–80 nm and studied in mice and in the MDA-MB-435 tumor cell line. The particles were functionalized with the internalizing peptide F3 providing the possibility to create multivalent specific binding. It was observed that the receptor-mediated uptake of rods was increased compared to spheres composed of 1–2 iron oxide cores with dextran coating (~25–35 nm diameter) and a constant ratio of ligands per iron atom (because of their larger surface area and multiple

TABLE 1 | Short Overview on the Different Materials and Fabrication Methods Including the Morphology and the Size Range Covered

Fabrication Strategy	Drug Delivery System	Dimension	Morphology	Shape Adjustment
Synthesis	Gold nanoparticles	5–100 nm	Rods (aspect ratio < 10) triangles, 'urchins'	+
	Spherical and nonspherical silica-NP	5 nm–μm range (spherical) ~200 nm Ø to ~1.5 μm	Spheres, rods, curved cylinders (mesoporous)	++
Self-assembly	Filomicelles	20–60 nm Ø × 5–50 μm length	Linear, filamentous	++
	Nonspherical liposomes and lipid particles	~100 nm–2 μm Ø × <100 μm length	Cylinders, tubes, ribbons, spirals	++
	Nanoworms (NW) with anorganic core	~5 nm Ø × 100 nm length	Linear, chain-like	+
Template-assisted	Particles formed within membranes	5 nm Ø × <100 μm length	Tubes, rods	+
	Micro- and nanofabrication using molds (e.g., PRINT)	≥20 nm	Complex shapes	++++
	Polymeric particles in microfluidic channels	≥5 μm	Complex shapes	+++
Various approaches	Silicon-based particles	≥300 nm	Complex shapes	+++
	Carbon nanotube	≤5 nm Ø × ≤5 cm length	Cylinders, tubes	+
	Stretched polymer particles	~500 nm Ø × 50 μm length	Variable, incl. cylinders, discs, related symmetrical shapes	+++

attachment points). These attributes have given rise to a higher targeting efficiency of dextrane coated iron oxide nanoworms in comparison to their spherical counterparts.²⁹

These structural advantages allow for a better 'sensing' of the target surface and can be used for the design of progressive multifunctional carrier systems, combining imaging and therapeutic functionalities.

CONCLUSION

Shape of nano- and microparticles (Table 1) essentially influences interactions with biological systems and the subsequent biological processing relevant for modern drug delivery systems.

Certain nonspherical shapes, such as elongated rods and discs, clearly outperform their spherical counterparts in terms of escape from phagocytosis and firm binding to the target tissue. Furthermore, alterations in biodistribution, cellular trafficking, immune response, and longevity of circulation have been reported.

However, these findings must be seen in context with other parameters. The implications of shape are highly multifactorial and parameters such as size, texture, surface chemistry, and mechanical properties need to be considered in parallel. Shape adds yet another dimension for the design of progressive drug delivery systems broadening the possibilities compared to spherical systems. This potential has been exploited and more and more methods for the fabrication of nonspherical objects are currently under

development. As a matter of fact, a more complex shape goes along with a larger dimension of the carrier compared to the spherical counterparts. In addition, not every material can be utilized over the entire size spectrum. Depending on the approach various challenges still need to be overcome, some of which include the scaling up, homogeneity of the particles, reproducibility, presence of initiators, separation of particles, amongst others. Nevertheless, significant progress has been made so far during the development of nonspherical drug delivery systems.

Besides understanding and utilizing the modulation of biological responses by varying particulate shape, the increase of bioavailability of novel drugs is a main, challenging problem in modern drug delivery. A solution can be given by the use of nanoparticulate materials. While the potential of silica particles, e.g., as gene carriers, has been shown extensively, the recently developed mesoporous silica nanoparticles allow the delivery of drug compounds with poor solubility or the controlled release of a drug over a longer time period. Also with regard to the use of more effective and convenient routes of administration these developments will contribute to a more efficient therapy in the future. This includes attempts to minimize therapeutic toxicity accompanied by a higher patient compliance and reduced health care costs. Therefore, nanomedicine based on novel approaches for drug delivery systems is expected to become a well-established term in modern medicine.

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