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Particle shape effects *in vitro* and *in vivo*

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1. ABSTRACT

Particles that have the potential to deliver imaging agents and drugs to cells and tissue now have many different shapes and sizes. This diversity in particle shape could provide new options for potential treatments of diseases because geometry affects biodistribution. However, the myriad of particle shapes now available increases the number of variables or parameters that must be taken into consideration for the drug delivery field to understand particle-cell interactions. This is especially true when the shape of a particle is a tunable parameter along with particle chemistry, charge, and hydrophobicity. Here we review the impact of shape on particle-cell interactions *in vitro* and the ramifications of different particle geometries on circulation, biodistribution, localization to tumors, and toxicology in rodents.

2. INTRODUCTION

Nano- and micron-scale particles are of emerging importance for diagnostic and therapeutic applications due to their many attributes which include the ability to carry imaging agents and drugs, stability, persistent circulation, reduced toxicity compared to naked drugs, and potential targeting functionality. The combination of these characteristics results in the possibility for increased delivery to target areas in the body and reduced damage to healthy tissues compared to current treatment options.

Original particulate carriers for drugs and imaging agents were spherical (low aspect ratio) liposomes (1,2), which have a center that can hold water-soluble agents and a membrane that can carry lipid-soluble agents. Unfortunately, the stability of liposomes in the body is

somewhat low so their contents tend to leak (3). They also attract attention from the immune response system and are cleared rapidly. Therefore a need arose for drug delivery carriers that did not release their contents prematurely and remained in circulation for long periods of time. These issues have, in part, been rectified by conjugating poly(ethylene-glycol) (PEG) to the head groups of the lipids (3,4). This technique is called "PEGylation." PEGylated liposomes that carry the anti-cancer drug doxorubicin are on the market for treating Kaposi's sarcoma, recurrent breast cancer, and ovarian cancer (5).

Around the time liposomes were being PEGylated, it was shown that nanometer sized spheres made from synthetic crosslinked polymers could persist in the circulation of rodents without causing major toxic effects (6). These original nanospheres had cores composed of biodegradable and hydrophobic poly(lactic-co-glycolic acid), poly(caprolactone), and their copolymers. PEG was covalently attached to these polymers to solubilize the nanospheres in aqueous solvents and in blood. This breakthrough showed that stable alternatives to liposomes could be constructed exclusively from polymers.

Extensions of these two technologies, liposomes and cross-linked polymeric particles, have been prevalent (7). In the case of liposomes, or more generally speaking, assemblies of amphiphiles, hydrophilic and hydrophobic blocks that comprise an amphiphile can be made through polymer synthesis techniques. Popular chemistries for the hydrophilic block include poly(ethylene-oxide)/poly(ethylene-glycol) or poly(styrene); poly(acrylic acid), poly(butadiene), poly(caprolactone), or poly(ethyl-ethylene) are typically used for the hydrophobic block (8). In many cases, the weight fractions of the blocks drive the geometry of the resulting assembly. These diblock copolymers can form polymer vesicles, cylindrical micelles, or spherical micelles in aqueous solvents when the blocks have specific weight ratios (9,10). Assemblies of all three geometries were originally formed without drug delivery applications in mind, but in initial experiments, the polymer vesicles in particular proved to be 5-50 times more elastic than liposomes (9,11). It was thought that this could possibly overcome issues encountered with the fragility of liposomes in circulation. Thus, it was natural to consider the polymer vesicles for delivery applications. Indeed, 100 nm polymer vesicles circulated for two days in rodents and their cylindrical-micelle analogs, "filomicelles," did so for at least one week (12). These circulation times surpassed those of any other synthetic particle to date (13,14).

Progress in drug delivery has also been made by using the successors to the nanospheres of Gref *et al.* (6) as carriers. Although those original objects were spherical, particles have been created recently that have asymmetries in their shape (15,16,17), the effect of which on cells is the focus of this review. These were formed by techniques such as colloid stretching and Particle Replication In Non-wetting Templates (PRINT), which allow for the production of monodisperse, shape-specific particles (18,19). We focus on the properties of particles after they are formed. Other reviews address formation techniques (20). With the above fluid assemblies, these solid particles form a vast array of options for drug delivery vehicles. But the explosion in the different shapes of these particles requires a deeper understanding of how variables such as aspect ratio and particle flexibility affect particle-cell interactions both in culture (*in vitro*) and in live animals (*in vivo*).

3. PARTICLE-CELL INTERACTIONS *IN VITRO*

Reductionist experiments that retain only certain components of a system allow for control over select variables. Since the behavior of particles in the body is so complex, experiments on cells in culture are necessary to help the field understand how particles distributed throughout the body interact with cells. Several particle parameters have been tested using such simplified conditions. In this section we review the findings from experiments where particles of different shapes and sizes were incubated with either immune response cells or target cells. Some trends are beginning to become clear from these experiments and they are matching results from *in vivo* work.

3.1. Particle engulfment by immune response cells

One of the most important advances in our understanding of immune response cell reaction to synthetic asymmetric foreign objects has been through experiments where particles of different shapes were incubated with cells and the particle engulfment and internalization capabilities of the cells were monitored. Through these experiments, it was discovered that particle shape plays an important role in the phagocytosis of particles.

In one study, the alignment of the particle at the initial contact point with the macrophage membrane seemed to dictate whether phagocytosis events would be triggered (Figure 1A) (15). Spheres, ellipsoids, and disks were incubated with macrophages and events for 1) unsuccessful internalization, 2) successful internalization, or 3) cell spreading were scored. Particles with a low volume and a low angle between the particle and the macrophage were successfully internalized. Particles with large volumes and low values for the contact angle were unsuccessfully internalized and cells simply spread over particles with high values for the contact angle independent of particle volume (15). Nanometer size particles showed similar trends: 50 nm spheres were taken up in 4-fold higher quantities than rod analogs (45 x 10 nm) by macrophages *in vitro* (21).

Follow-up studies to the above work used the same assay with particles having larger aspect ratios (16,17). Generally speaking, particles with high aspect ratios avoided being phagocytosed by macrophages *in vitro*. It is not known how the local curvature at the particle–cell contact point would affect the signaling cascade that activates cytoskeletal rearrangement leading to phagocytosis (22), nor is it clear how this theory would extend to flexible particles that have geometries similar to the above particles. Incubating high aspect ratio particles of

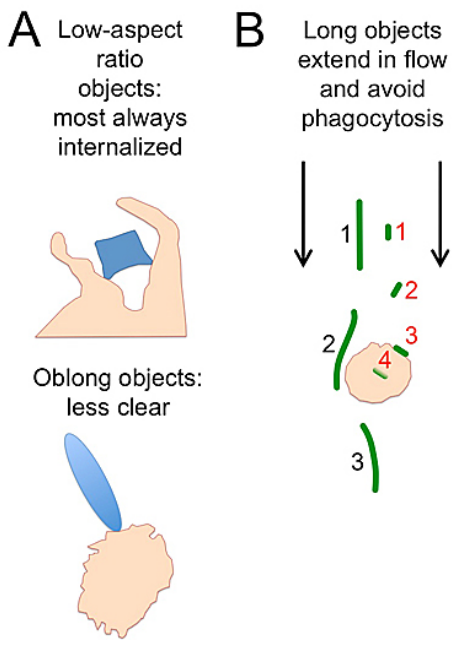

Figure 1. The internalization of particles by immune response cells is a function of particle shape. (A) Sketches of particle-macrophage experiments where internalization of particles is scored. Blue objects represent particles and tan objects represent macrophages or parts thereof. The internalization of large particles is a function of the angle of contact with the macrophage membrane. A key question is what is keeping the actin cytoskeleton and the plasma membrane from surrounding high aspect ratio particles. (B) In flow long flexible particles – called filomicelles - conform to streamlines around objects such as macrophages. Shorter particles tend to tumble and bind to the same immune response cells. This may explain why filomicelles persist in the circulation of rodents for longer periods than shorter particles. Arrows represent blood flow.

varying flexibilities and degrees of crosslinking with these cells would seem to be the next logical step for determining mechanisms of particle-macrophage interactions.

Both sets of experiments described above were done in stagnant culture conditions. Of course, flow is an important factor in particle-cell interactions in the vasculature, especially for flexible, high-aspect ratio particles (23). Simple *in vitro* particle-cell experiments were devised that tried to emulate flow conditions in the body. In one such experiment, macrophages were placed in a capillary tube and filomicelles with lengths between 100 – 10000 nm and diameters of 10 - 20 nm were driven past the cells by flow rates comparable to those found in the body (12). Filomicelles of lengths greater than a few microns conformed to streamlines around the macrophage, but smaller filomicelles bound to the macrophage and were subsequently internalized (Figure 1B) (12). It would be very informative to observe a macrophage binding a long filomicelle. Then it would be possible to observe how a cell tries to engulf a high aspect ratio particle that is both flexible and fluid. All of the above experiments are solid starting points for achieving the long-term goal of increasing our knowledge of particle-cell interactions.

3.2. Particle binding to target cells

One of the key goals in attempting to use particles to deliver imaging agents and drugs to cells is to avoid particle engulfment by immune response cells while maximizing particle binding to target cells. The cellular mechanisms of immune response cell particle engulfment and target cell internalization are different and need to be explored separately.

Two topics must be considered when studying high aspect ratio particle interactions with target cells. The first is the binding of the particle to the cell. The shape of an object should affect its binding to a target cell (24). Particles that are the size of the targeting moiety that will bind a receptor on the cell surface will probably exhibit a one-step binding mechanism. As the size of the particle increases - with respect to its targeting moiety - the binding behavior should become more complex based on the flexibility of the particle and also the flow environment.

Indeed, the entropic energy of particles with high enough aspect ratios and flexibilities may be of the same order as the binding energy of the particle for a cell surface. If the entropic energy dominates, the particle will bind the cell surface at non-contiguous points. Conversely, if the binding energy dominates, the particle should zip up along the cell surface (Figure 2A). The interplay between these two behaviors should be a function of the distribution of the targeting moiety on the surface of long flexible particles. If the distance between targeting moieties is greater than the relevant length scale of bending of the particle, then the entropy of the particle should define much of its interaction behavior with cells. In the opposite case, the concentration of the targeting moiety should be the important variable.

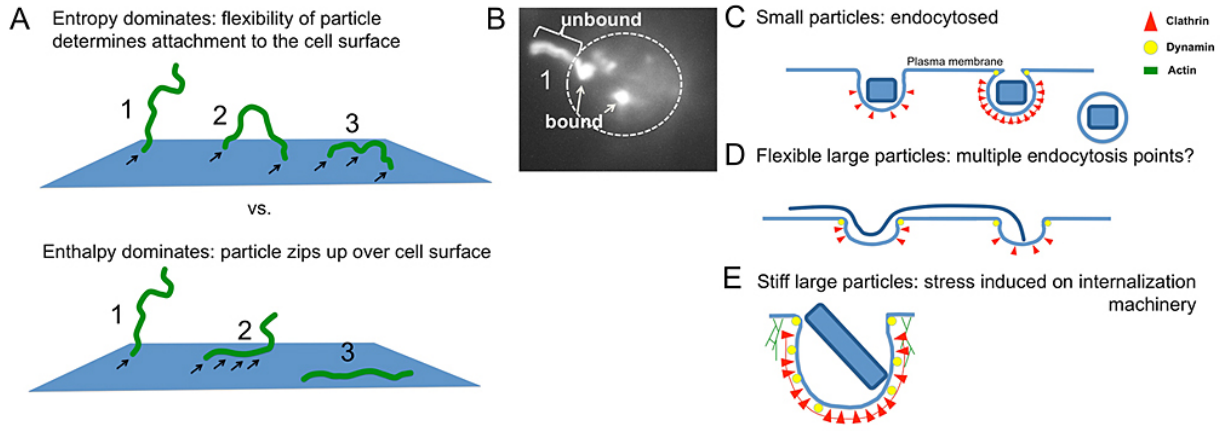

Figure 2. The binding and internalization mechanisms of individual particles to target cells should be a function of particle size, shape, and flexibility. (A) As the length of the particle increases, the binding mechanism to the surface of the cell will become more complicated. The numbers represent time points over the course of attachment. (B) Fluorescent micrograph highlighting two filomicelles loaded with imaging agent with different degrees of attachment to a malignant B-lymphocyte (traced by dotted line). The longer filomicelle (left) is bound to the cell at one end while the rest is dynamic. This filomicelle is represented by the particle at time 1 in (A). The shorter filomicelle (center) is completely bound to the cell, showing no movement. (C) Smaller particles are endocytosed more rapidly and with fewer cellular factors needed to bend the plasma membrane than larger particles. (D) Long, flexible particles will probably have more complicated entry mechanisms possibly involving multiple endocytosis points. (E) Large stiff particles of high aspect ratio present difficulties for cellular internalization mechanisms.

These topics are crucial because factors that are targeted on cells are usually present on target and healthy cells. Thus, particles whose surfaces are saturated with targeting moieties will bind and cause apoptosis in healthy cells. There should be a relationship between the number of targeting moieties on the particle, particle flexibility, and the ratio of target / healthy cells that will increase the effectiveness of delivery via particles. This has yet to be determined.

3.3. Particle internalization

The second topic that must be addressed is the internalization of a high aspect ratio particle after binding to a cell not capable of phagocytosis. Receptor mediated endocytosis via clatherin-coated pits is the most characterized internalization mechanism of foreign objects into cells. Thus it is the starting point for understanding how particles enter target cells.

Viruses have been tremendously useful model systems in the study of "particle" internalization because of their rich geometric diversity. Vesicular stomatitis virus (VSV) is bullet-shaped (dimensions: 200 x 70 nm) and proved too large to enter cells by clathrin-dependent endocytosis alone because the virus exceeds the spatial capacity of typical clathrin-coated vesicles (25). Instead, it entered cells through endocytic carriers that had a partial clathrin coat. It is hypothesized that dynamin is responsible for the additional bending of the membrane to accommodate the asymmetric virus. This internalization pathway also required local actin polymerization (25). A smaller analog to VSV (75 nm long) was internalized in complete clathrin-coated vesicles without the need for actin polymerization (26). This suggests that there is a strong correlation between endocytic pathway and particle size and shape. These observations should extend to synthetic particles (Figure 2C-E).

Indeed, the internalization rates of synthetic particles were also found to be a function of size. A series of synthetic cubic particles with diameters from 200 to 5000 nm were incubated with HeLa cells (18,19,27,28). Smaller particles were internalized faster and in higher quantities than larger particles (27). This is not surprising: smaller objects should be readily endocytosed by cells whereas larger objects present problems for cells attempting to internalize them (Figure 2C). Also, larger particles of complicated geometries (29) would especially seem to need many factors for internalization. They will probably have to be surrounded by actin filaments, combinations of membrane-curving factors such as clathrin and/or BAR (Bin/Amphiphysin/Rvs) domain proteins, and of course the membrane itself. This combined mechanism is likely to be complicated and will take careful cell biology and biochemical assays to uncover.

Along with the viruses and particles mentioned above, carbon nanotubes are an ideal system for understanding the effects of shape – in this case aspect ratio - on particle–cell interactions. Single-walled carbon nanotubes (SWCTs) have lengths of 20 – 1000 nm and diameters from 0.4 to 2.0 nm; multi-walled carbon nanotubes (MWCTs) have lengths greater than 1000 nm and diameters from 1.4 – 100 nm. This span in aspect ratio would be particularly useful to determine its role on particle-cell interactions.

However, carbon nanotubes are highly hydrophobic necessitating the addition of amphiphiles or water-soluble materials to their exteriors to make them

usable in aqueous environments. Carbon nanotubes seem to show two modes of entry into cells based on the functionalization of their exteriors. It has been proposed that carbon nanotubes covered with proteins or genes can be internalized into cells via endocytosis (30), whereas ammonium- and acetamido-functionalized carbon nanotubes tend to behave as nanoneedles that can pierce through cell membranes, thereby allowing for entry into cells (31). These entry pathways should be a function of the length of the carbon nanotubes, but to our knowledge, this has not been shown. At this point, carbon nanotubes have not been fully harnessed as a model system for shape effects on particle-cell interactions.

3.4. Particle distribution in cells after internalization

The final destination of a particle in a target cell is most likely a function of its internalization mechanism. This is why particle escape from endosomes is such an important topic since many particles are hypothesized to be endocytosed. There is little evidence showing that particle shape plays a strong role in cellular distribution. However, uncovering any such relationships would be useful since localization of particles to targets in cells may increase the effectiveness of delivery. It is worth noting that initial studies in the area of particle distribution in cells (32) were somewhat tenuous and spurred debate (33).

Like the endocytosis studies that utilized viruses, insights into the distribution and trafficking of particles come mostly from naturally occurring invaders. A well-studied model system is the gram-positive bacterium *Lysteria monocytogenes* (34). These bacteria actively move in the cytoplasms of mammalian cells, with actin polymerization providing the driving force (34). The size of L. monocytogenes is similar to many synthetic particles, thus its movement in cells is applicable to the drug delivery field. L. monocytogenes utilize a factor, ActA, to activate Arp2/3 complex, which is an actin branch nucleator (35). ActA is a surface protein with a transmembrane motif (36), which can reproduce the intra-cellular movement of the bacteria when coated on polystyrene beads (37). Interestingly, smaller beads (< 0.5 microns) initiate movement readily; larger beads (up to 2 microns) are mobile only if they are coated with ActA on one hemisphere. Such asymmetries may have to be kept in mind with particles that are used to target areas of the cell.

Time-lapse microscopy with dual labeling schemes will be paramount for determining particle distribution in cells. But the distance between a particle and multiple organelles can be less than the wavelength of light. Thus, care should be taken when stating that a particle localizes to a particular organelle since it may be equidistant from several. High-resolution microscopy may be necessary for quantifying particle localization (38).

4. THE BEHAVIOR OF PARTICLES IN RODENTS

In vitro assays are useful for determining particle binding and internalization on the single cell level, but particles need to be administered to live animals to determine circulation times, biodistribution, localization to target areas, and toxicology. These assays shed light on how a particle behaves in the body, but also how the body responds to the particle. Below we focus on the effect of particle shape on the above four metrics. Ideally, particles would avoid healthy and immune response cells but still be able to localize to target cells. Shape has been shown to be a key factor in achieving this goal.

4.1.Circulation

Circulation time is a function of particle shape, fluidity, and flexibility. Particles with aspect ratios less than ~100 are cleared from the circulation of rodents within two days of injection (12,15). However, recent experiments have shown that even within this two day time window, short particles of aspect ratio ~5 remain in circulation longer than spheres (21). Particles with aspect ratios greater than ~100, such as filomicelles, can remain in the circulation of rodents for up to one-week after injection (Figure 3A) (12). It is not known if the aspect ratio or the micron lengths of the filomicelles are responsible for the increase in circulation time.

The fluidity of high aspect ratio particles seems critical for their extended circulation times (Figure 3B,C). Filomicelles were cleared at the same rate as spheres when their cores are cross-linked to form a solid (12). The cross-linking was performed so that the flexibility of the filomicelles did not change (12,23,39). A cross-linked filomicelle can be thought of as a soluble carbon nanotube. This may help explain why carbon nanotubes are cleared from circulation on the same time scales as spheres even though they have aspect ratios similar to filomicelles (31,40).

It is not known how the fluidity of a high aspect ratio particle extends its circulation time. One possibility is that immune response cells bind only part of the particle while the rest extends in blood flow. As phagocytosis is initiated, the fluid components of the particle are able to rearrange and detach from the cell. It is also possible that the shear of blood flow is breaking up the fluid particles. However, neither theory has been proven true.

In addition to the need for fluidity, it stands to reason that particle flexibility should be no greater than particle length since highly flexible cylinders will coil into spheres, and would seem to be cleared on the same time scales. In the case of flexible circulating particles with high aspect ratios, it is instructive to consider the Weissenberg number, *Wi*, which describes the extension of a polymer in flow. $Wi = v^*t / d$, where v denotes the velocity of flow, t, the relaxation time of the polymer, and d, the diameter of a (spherical) cell past which the polymer is flowing. When $Wi > 1$, the polymer is elongated in flow; when $Wi < 1$, the polymer has the shape of a random coil and is able to "relax" in the flow. The particle should tumble like a sphere in the latter situation. But particles with relaxation times on the order of one second such as filomicelles should be extended around immune response cells in flows of $v > 5$ microns / sec. The flow in most blood vessels and the spleen is greater than this value (41). Thus, the Weissenberg number may be an important indicator of

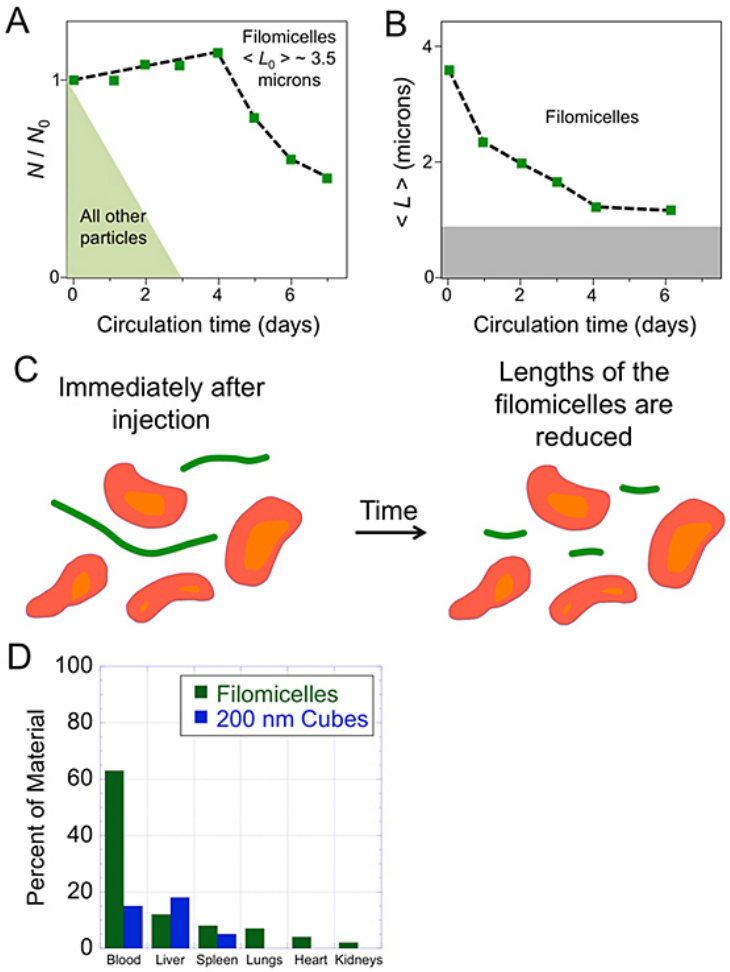

Figure 3. Circulation time of particles in rodents is a function of their shape and in some cases fluidity. (A) Plot of the number of particles in the blood of rats versus time. The average initial length of the filomicelles is about 3.5 microns as indicated. (B) The length of the filomicelles is reduced over time in circulation. Thus, fluidity is key for the extended circulation times as solid filomicelles are cleared within two days of injection. (C) Cartoons of what long, flexible particles may look like in blood flow. Particle lengths of filomicelles have been observed to shrink over time. This is indicated in the cartoon on the right. (D) Comparison of the biodistribution of filomicelles versus 200 nm cubic particles after 24 hours in rodents. The plots in (A) and (B) are traced from Geng *et al.* (12).

extended circulation time for flexible particles with high aspect ratios.

4.2. Biodistribution of particles in healthy rodents

Biodistribution is a function of particle shape. Filomicelles with lengths up to 10 microns were distributed as follows 24 hours after injection in rodents: 63% blood, 12% liver, 8% spleen, 7% lungs, 4% heart, 2% kidney (42). By comparison, 200 nm cubic particles were distributed as follows in rodents: 15% serum (blood), 18% liver, 5% spleen, and trace amounts in other organs one hour after injection (29) (Figure 3D). Thus, elongation of one of the dimensions of a particle seems to extend its presence in the blood as discussed above. Unfortunately, the particle samples in Christian *et al.* (42) have a significant percentage of filomicelles with lengths smaller than those reported. Thus, it is impossible to precisely say how the biodistribution was affected by the aspect ratios of the filomicelles.

The biodistribution of smaller particles has also shown to be a function of shape. 50 nm nanospheres accumulated in 4-fold higher amounts in the liver than nanorods of dimensions (45 x 10 nm). Both particles were made from the same materials (21). This study was particularly useful because of the consistency of the chemistries of the particles. Much work remains to be done in this area.

4.3. Localization of particles to tumors in rodents

It is hypothesized that particles localize to the leaky vasculature of tumors via the enhanced permeability and retention (EPR) effect (43). It is not clear what role shape or targeting moiety on the surface of the particle play

in tumor localization (44). The vast majority of particles still accumulate in the healthy organs of animals with tumors. For example, only 3% of the filomicelles localized to A549 tumors in mice, the rest of the material was distributed as shown in Figure 3D (42).

Also relevant here are recent studies that have focused on strategies for localizing carbon nanotubes to tumors in mice (45). SWCTs were solubilized with poly(ethylene-glycol) and functionalized with the alphavbeta3-integrin-binding RGD peptide (46). Mice bearing subcutaneous integrin alphavbeta3-positive U87MG tumors were intravenously injected with these SWCTs. The SWCTs localized to the tumor in high numbers. In fact, only the liver had more SWCTs after 24 hours than the tumor. But the size of the poly(ethylene-glycol) on the surface of the nanotube – and not the aspect ratio of the SWCT or the RGD peptide - was the main determinant of the effectiveness of localization to the tumor. However, no measure of the subsequent shrinkage of the tumor was reported. In any case, this is an interesting result and coincides with recent doubts about whether targeting moieties are necessary for localization to target areas (44).

Additional evidence showed that drugs carried by filomicelles localize to tumors without the need for targeting moieties (12,47). In this case, length of the particle and amount of drug loaded into the particle were the key parameters. Filomicelles with maximum lengths of ~8 microns loaded with (8 mg paclitaxel) / (kg mouse) shrank the size of tumor xenografts on nude mice over filomicelles with maximum lengths of ~8 microns loaded with (1 mg paclitaxel) / (kg mouse) and filomicelles with maximum lengths of ~1 microns loaded with (1 mg paclitaxel) / (kg mouse) (12). The extended circulation times of long filomicelles may allow for more chances for localization to the tumor over shorter filomicelles that are mostly cleared. To test this, samples of filomicelles with distinct lengths that are carrying paclitaxel would have to be formed and injected into separate mice. The sizes of the tumors could then be compared as a function of filomicelle size. It is also not known if the paclitaxel is leaking out of the filomicelles and subsequently localizing to the actively proliferating tumor cells independent of the localization of the filomicelles. Paclitaxel would have to be covalently attached to the filomicelles to determine whether this phenomenon is occurring.

The most useful attribute of high aspect ratio particles may be their ability to reach areas of the body that particles with larger girth cannot access (48). This is particularly important for penetrating epithelium and for possibly reaching target sites in the brain. High aspect ratio particles can also carry more material than low aspect ratio particles when their diameters are comparable (12). It would be beneficial to observe the penetration depth of particles in various tissues in animals based on particle shape.

4.4.Toxicology

The Food and Drug Administration has approved many of the materials that particles are made of for use in the body. Poly(ethylene-glycol) is the most common example. The question becomes: how does the high concentration of such materials in a particle augment toxic effects on the body? Since many particles are highly cross-linked (15,16,17,27) - most famously MWCTs (49) – concerns have been raised over their stress on organs that have factors that break such bonds (7). Thus, the chemical composition and the large surface area of particles are the two main concerns about potential harmful effects circulating particles could have on the body (50). What role does particle shape play in the health of the animals to which they were administered?

Naturally, there has been much focus on the toxicology of carbon nanotubes because of their widespread study, but also because of their unique high aspect ratios. The possibility that carbon nanotubes will be produced in large quantities in manufacturing facilities has demanded that their effects on the respiratory system be studied. Carbon nanotubes appear to be highly toxic to the lungs. They cause epithelioid granulomas and in some cases, interstitial inflammation at ~ (25 mg carbon nanotubes) / (kg mouse). They are more toxic than quartz, which is considered a serious occupational health hazard in chronic inhalation exposures. Nanotubes resemble asbestos since they both have high aspect ratios (51,52). Most cells that internalize carbon nanotubes are not viable after short time periods. Also, PEGylated sphere-shaped gold nanoparticles displayed improved biocompatibility over cube-shaped and rod-shaped particles (53). In sum, these results suggest that solid high aspect ratio particles are more toxic than solid low aspect ratio particles. It is noteworthy that fluid filomicelles have lower toxicities than carbon nanotubes: mice showed no ill effects after injection with (300 mg filomicelles) / (kg mouse) (12). Determination of toxicology as a function of high aspect ratio particle crosslinking would be highly useful.

5. CONCLUSIONS AND PERSPECTIVES

The formation of nanometer- to micron-size particles of many different shapes has mainly been possible by increasingly sophisticated fabrication techniques. Since these techniques are relatively new, our understanding of how each shape affects particle-cell interactions is limited, but growing. What is clear is that high aspect ratio particles tend to avoid phagocytosis *in vitro* and clearance *in vivo*. They are also able to shrink the sizes of tumors *in vivo* although the mechanism is not clear and may be no different from the successes observed for low aspect ratio particles. Conversely, toxicity of solid particles seems positively correlated with aspect ratio.

For the field to progress there is tremendous need for continuity among the chemistries and shapes of the particles. This will allow testable hypotheses to be formed on particle-cell interactions. Also, cell lines and animal models should be selected for experiments that will maximize the impact of the results. For example, cells that express fluorescent proteins conjugated to proteins involved in the binding of particles to cells, the internalization of particles, or the distribution of particles throughout cells would allow for much needed co-

localization studies. Equally useful would be the use of cell lines that have genes knocked out or knocked down that have implications for particle binding, internalization, and distribution. One could then design experiments where cellular factors and particle properties are under strict control.

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Abbreviations: Arp2/3: Actin-related protein 2-3, EPR: enhanced permeability and retention, MWCT: multi walled carbon nanotube, PEG, poly(ethylene-glycol), PRINT: Particle Replication In Non-wetting Templates, RGD: arginine-glycine-aspartate sequence, SWCT: single walled carbon nanotube, VSV: vesicular stomatitis virus, Wi: Weissenberg number

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