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Unique size and shape-dependent uptake behaviors of non-spherical nanoparticles by endothelial cells due to a shearing flow

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ABSTRACT

The size and shape of nanoparticle (NP) drug carriers can potentially be manipulated to increase the drug delivery efficacy because of their effects on particle margination and interactions with various cells *in vivo*. It is found in this work that the presence of a physiologically relevant shearing flow rate results in very different size and shape-dependent uptake behavior of negatively charged, non-spherical polyethylene glycol (PEG) hydrogel NPs by endothelial cells (ECs) cultured in a microchannel compared to uptake of either identical NPs in static culture or spherical particles in a shear flow. In particular, larger rod- and disk-shaped PEG NPs show more uptake than smaller ones, opposite to the size effect observed for spherical particles in a flow. Moreover, the trend observed in this dynamic uptake experiment also differs from that reported for uptake of similar PEG NPs by ECs in a static culture, where the smaller disks were found to be uptaken the most. These differences suggest that the increasing rotational and tumbling motions of larger-size non-spherical NPs in the flow play a dominant role in NP margination and cell interaction, compared to Brownian motion, gravity, and cell membrane deformation energy. These findings suggest that the coupling between NP geometry and shear flow is an important factor that needs to be accounted for in the design of the size and shape of nanocarriers.

1. Introduction

Nanoparticles (NPs) have shown promise as drug carriers to cancer cells [1–3]. Targeted delivery of nanocarriers specifically to diseased cells can help to shield collateral cells from harmful cytotoxic drugs as well as reduce the many harmful side effects associated with traditional chemotherapy. The size, shape, and surface chemistry of NPs can all influence their delivery efficacy to cancerous tissues. NPs, because of their size, cannot enter cells by diffusion alone and rely on active uptake by cells, providing more control over cellular uptake and undesired sequestration by the body [4]. Spherical particles smaller than approximately 10 nm are rapidly cleared by the kidney glomerulus [5]. In addition, the liver effectively removes particles with a characteristic dimension larger than 500 nm and the spleen filters particles larger than 200 nm [6,7]. NPs with a characteristic dimension <500 nm can be used to exploit the fenestrated endothelium associated with tumor angiogenesis. This phenomenon along with the reduced lymphatic drainage in tumors is known as the enhanced permeation and retention (EPR) effect and has been investigated extensively in order to increase NP delivery efficacy [8]. In addition, endothelial cells (ECs) offer a logical delivery target for many NP-based therapeutics. Spherical NP uptake by ECs has been shown to increase with decreasing size down to approximately 100 nm for most studies in a static culture, [9,10] and down to 200 nm under shear stress [11,12].

In addition to size, the evolutionarily conserved shapes of viruses and bacteria motivate exploring NP shape as a potentially key factor in enhancing the delivery efficacy [13–17]. Several studies have reported that rod-like particles are more uptaken than their lower aspect ratio (length divided by width), disk-like, counterparts and that both tend to be uptaken more and more quickly than volumetrically equivalent spherical particles [18–21]. However some conflicting trends have been reported for gold NPs as well as long worm-like polymeric particles of ultra-high aspect ratio where increasing the aspect ratio of the particles decreases uptake efficiency [22–27]. In a recent study of the uptake of negatively charged, hydrophilic, polyethylene glycol (PEG) NPs of varying aspect ratios, it was reported that mammalian epithelial and immune cells preferentially internalized disk-shaped PEG NPs compared to rod-like and smaller disk-like PEG NPs [28]. In the same study, ECs internalized intermediate-sized disks followed by higher aspect ratio rods, then low aspect ratio rods and larger to smaller disks. ECs also showed significantly higher overall uptake efficiency compared to epithelial cells [28]. These complex and cell line-specific shape/size dependencies were attributed to a competition between three factors: particle-cell membrane adhesion, energy of membrane deformation,

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and local particle concentration. However, another important factor in NP uptake is the shear stress experienced by ECs, which has been neglected in prior studies of shape-dependent uptake of polymeric rod and disk-like NPs.

Here, we report the shape-dependent uptake of negatively charged, hydrophilic, PEG NPs of varying aspect ratios under physiological shear. Uptake in 100 μm high channels was found to differ qualitatively from uptake of identical particles by ECs in static conditions, and be influenced by gravity for the two relatively large-volume disks and rods and the relatively small disks but not the rods of a similarly small volume. Larger NPs and NPs with higher aspect ratios show more interaction with, and uptake by, cells because of NP rotation and tumbling in the flow. This factor dominates the other factors that were found to be important in static culture.

2. Material and methods

2.1. Particle synthesis and properties

The four particle shapes used in this study are illustrated in Fig. 1. The two largest particles: 800 × 100 × 100 nm^3 rods and 325 nm diameter × 100 nm high disks, have volumes within 4% of each other. The relatively small 400 × 100 × 100 nm^3 rods and 220 nm diameter × 100 nm high disks have volumes within 5% of one another. All particles were made up of poly(ethylene glycol) diacrylate (PEGDA) with Fluorescein isothiocyanate (FITC) encapsulated in the polymer matrix and manufactured using Jet and Flash Imprint Lithography (J-FIL™) as described previously [28–30]. The zeta potentials of all four particle shapes were measured and found to be approximately −57 mV in PBS [28].

Table 1 shows the hydrodynamic diameter, volume fraction, and sedimentation velocity of each nanoparticle size and shape. The

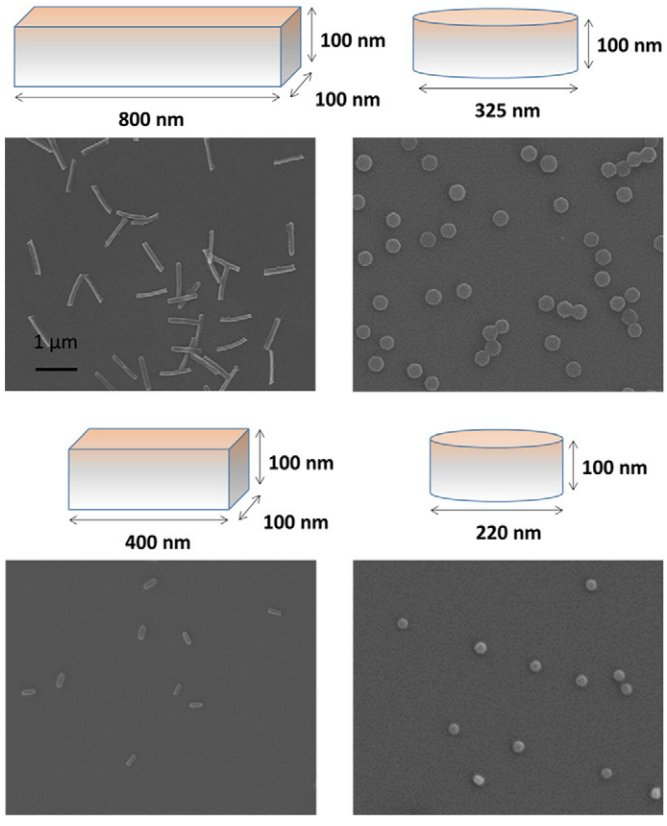

Fig. 1. SEMs and geometries of the four particle shapes manufactured using the J-FIL process.

sedimentation velocity of the particles in a quiescent fluid was calculated from the following equation

$$V_s = \frac{2g\left(\rho_p - \rho_m\right){d_h}^2}{2\mu} \quad (1)$$

where g is the acceleration due to gravity, ρ_p is the apparent density of the PEG nanoparticles, ρ_m is the density of the culture medium, d_h is the hydrodynamic diameter of the nanoparticle, and μ is the viscosity of the culture medium. For the calculation, ρ_p was found according to the procedure in a prior report [31]. Briefly, the apparent particle density must include both the volume of the solid PEG as well as the volume containing serum proteins adsorbed onto the surface of the particle. In order to calculate this volume fraction (V_f) the experimentally determined d_h (Malvern Inc.) was used to calculate an apparent volume and compared to the known PEG volume obtained from SEM images of the nanoparticles. Using the known density of PEG (ρ_{PEG}) and assuming the density of all organic layers and solution occupying the hydrated nanoparticles is 1000 kg/m^3, the apparent nanoparticle density was calculated from the following relation

$$\rho_p = (1 - V_f) + \rho_{PEG} * V_f \quad (2)$$

2.2. Cell culture

HUVEC (ATCC) cells were cultured in MCDB 131 medium (Life Technologies) supplemented with 10% fetal bovine serum (Characterized FBS, Hyclone) and 1% antibiotics (penicillin and streptomycin, Invitrogen) and further supplemented with EGM2 Supplement and growth factor kit (Lonza Inc.). All experiments were performed with cells between passages 4–8. Once confluence was reached in a cell culture flask, cells were removed using 0.25% trypsin-EDTA (Life Technologies), pelleted and seeded into each of six rectangular cross-section microchannels (μ-slide VI$^{0.1}$, ibidi, LLC) at a density of 5 × 10^5 cells/ml. The seeded microchannels were then placed in an incubator at 37 °C and 5% CO_2 for 1 h to allow the cells to adhere to the bottom channel wall. Sixty milliliter of cell media was then placed in each channel reservoir and replenished at least every 24 h until cells reached confluence. Once confluence was observed, the channels were connected to a multichannel peristaltic pump with a pulse dampening system [32] as shown in Fig. 2. The entire system was then placed in an incubator at 37 °C and 5% CO_2 and flow of cell media was applied for 24 h to allow for conformational changes in the cells.

In order to deliver NPs to an inverted culture, ECs were first cultured to confluence identically to the procedure described above. However when connecting the peristaltic pump and delivery system, the entire chip containing the six microchannels was inverted 180 degrees and secured such that the ECs were above the flow of media and NPs relative to the direction of the gravitational force.

2.3. Flow system

A chip containing six microchannels with a height ($2h$) of 100 μm and width ($2w$) of 1 mm each was connected to a multichannel

Table 1
Hydrodynamic properties and sedimentation velocities of the nanoparticle sizes and shapes.

	d_h (nm)	V_f	v_s (nm/s)
800 × 100 × 100 nm^3 Rods	652	0.055	2.94
325 nm diameter × 100 nm high Disks	710	0.044	2.79
400 × 100 × 100 nm^3 Rods	385	0.134	2.49
220 nm diameter × 100 nm high Disks	418	0.100	2.18