Published in final edited form as:

Biomaterials. 2012 August ; 33(22): 5504–5513. doi:10.1016/j.biomaterials.2012.04.027.

The preferential targeting of the diseased microvasculature by disk-like particles

Giulia Adriani^{a,b}, Marco D. de Tullio^a, Mauro Ferrari^b, Fazle Hussain^b, Giuseppe Pascasio^a, Xuewu Liu^b, and Paolo Decuzzi^{b,c,*}

^aDepartment of Mechanical and Management Engineering Politecnico di Bari Bari, 70125, Italy

^bDepartments of Translational Imaging & Nanomedicine The Methodist Hospital Research Institute Houston/Texas, 77030, USA

^cDepartment of Experimental and Clinical Medicine University of Magna Graecia Catanzaro, 88100, Italy

Abstract

Different classes of nanoparticles (NPs) have been developed for controlling and improving the systemic administration of therapeutic and contrast agents. Particle shape has been shown to be crucial in the vascular transport and adhesion of NPs. Here, we use mesoporous silicon non-spherical particles, of disk and rod shapes, ranging in size from 200 nm to 1800 nm. The fabrication process of the mesoporous particles is described in detail, and their transport and adhesion properties under flow are studied using a parallel plate flow chamber. Numerical simulations predict the hydrodynamic forces on the particles and help in interpreting their distinctive behaviors. Under microvascular flow conditions, for disk-like shape, 1000×400 nm particles show maximum adhesion, whereas smaller (600×200 nm) and larger (1800×600 nm) particles adhere less by a factor of about two. Larger rods (1800×400 nm) are observed to adhere at least 3 times more than smaller ones (1500×200 nm). For particles of equal volumes, disks adhere about 2 times more than rods. Maximum adhesion for intermediate sized disks reflects the balance between adhesive interfacial interactions and hydrodynamic dislodging forces. In view of the growing evidence on vascular molecular heterogeneity, the present data suggest that thin disk-like particles could more effectively target the diseased microvasculature as compared to spheres and slender rods.

Keywords

Nanoparticle shape; mesoporous silicon; vascular adhesion; rational design; hydrodynamic forces

1. Introduction

Since the first liposomal formulations in the 70s [1,2], several different classes of nanoparticles (NPs) have been developed for controlling and improving the systemic administration of therapeutic and contrast agents [2-5]. NPs offer distinctive advantages

© 2012 Elsevier Ltd. All rights reserved.

*Corresponding author: pdecuzzi@tmhs.org.

Publisher's Disclaimer: This is a PDF file of an unedited manuscript that has been accepted for publication. As a service to our customers we are providing this early version of the manuscript. The manuscript will undergo copyediting, typesetting, and review of the resulting proof before it is published in its final citable form. Please note that during the production process errors may be discovered which could affect the content, and all legal disclaimers that apply to the journal pertain.

over systemically administered individual therapeutic and imaging agents. First, NPs can simultaneously carry multiple molecules to the diseased site in a controlled manner. Second, different agents can be simultaneously loaded into NPs and co-delivered, for multi-modal imaging and poly-pharmacy. Finally, NPs can be engineered to enhance accumulation at biological targets and change the biodistribution of the active agents [6-10].

Most NPs were developed for the treatment and imaging of tumors. In this context, the commonly used strategy to maximize accumulation at the biological target (tumor mass) relies on the enhanced permeation and retention (EPR) effect [11,12]. The tumor vasculature is discontinuous with fenestrations of a few hundreds of nanometers in size. Therefore, sufficiently small NPs could passively cross the fenestrations and eventually accumulate in the tumor interstitium. Curiously enough, this same mechanism is used by organs of the mononuclear phagocyte system (MPS), such as the liver and the spleen, to sequester circulating foreign objects, including pathogens, macromolecules and NPs. Not surprisingly, then, a large portion of systemically injected NPs end up in the MPS. Also, it should be emphasized that the EPR effect strongly depends on the tumor type, stage and location [13].

The notion that endothelial cells (ECs) are inhomogenous and may express organ-specific receptor molecules appeared in the early 90s [14]. Since then, several studies have focused on identifying vascular molecules, and the corresponding ligands, expressed specifically on the diseased vascular districts and with a low, or even null, expression on normal ECs [15]. These specific receptors can be used as vascular docking sites for systemically injected NPs, whose surface has been decorated with ligand molecules. Indeed, exploiting the biochemical affinity of the ligands for the corresponding receptors, circulating NPs would recognize and firmly adhere to the diseased ECs. Thence, NPs can preferentially release their cargo toward the extravascular matrix or enhance the local tissue contrast for imaging. Note that whilst EPR is specific to cancer, the vascular molecular heterogeneity has been documented for several diseases, including cardiovascular, neurodegenerative and hemorrhagic [16,17].

NPs targeted to the diseased vasculature can be effectively used for i) antiangiogenesis therapies, by delivering drug molecules directly to the endothelial cells; ii) inducing vascular damage and collapse through thermal ablation; and iii) deploying large number of drug molecules, contrast agents and secondary nanoparticles, unbiased by the tumor fenestration size [18-20]. However, most currently available NPs are not engineered for vascular targeting but rather for passively crossing tumor fenestrations. In the latter case, the most stringent requirement for NPs is to be sufficiently small in comparison with the fenestration size. On the other hand, vascular targeting would require NPs capable of sensing and recognizing the over expression of specific molecules, and firmly adhering to the walls of the diseased vasculature withstanding the dislodging hydrodynamic forces. The authors have previously shown, via mathematical modeling, that these objectives can be more efficiently achieved using non-spherical particles [21,22].

Here, the transport and adhesive performance under flow of disk-like and rod-like particles are analyzed. Mesoporous silicon particles are used as a reference system in that they have been already proven as carriers of therapeutic and imaging agents and their size and shape can be accurately controlled during manufacturing [23,24]. Three different disks are considered, with diameters and heights of 600×200 nm, 1000×400 nm and 1800×600 nm; and two different rods with lengths and heights of 1500×200 nm and 1800×400 nm. In vitro transport and adhesion experiments are performed using a parallel plate flow chamber to assess the number of adhering particles under different hydrodynamic conditions. Computational modeling is used to estimate the hydrodynamic forces exerted over the particles and elucidate the mechanisms regulating their distinctive behavior.

2. Materials and methods

2.1 Fabrication and Surface Modification of Mesoporous Silicon Particles

Starting with a heavily doped P-type (100) wafer with resistivity of 0.005 ohmcm as the substrate (Silicon Quest, Inc, Santa Clara, CA), the wafer was exposed to 1:3 HF (49%): ethanol solution, and electrochemically etched with applied current density of 7 mA/cm² for 65 s and 125 s to form 200 nm and 400 nm thick porous silicon layers, respectively. The current density was then increased to 76 mA/cm², and applied for 6 s to form a second porous layer, the high porosity release layer. The double-layer films were washed and spin-dried. A 40 nm thick low-temperature oxide (LTO) was deposited on the porous film in a LPCVD furnace. The photolithography process was carried out using an i-line contact aligner (KARL SUSS MA6 mask aligner) and NR9-250P photoresist (Futurrex Franklin, NJ, USA). A dark-field photomask (Chrome on quartz) consisting of a pattern array of 1800×400 nm bars with 2500×1000 nm pitch were obtained from HTA Photomask. A thin NR9-250P photoresist layer was spun on the double-layer film at 3000 RPM and baked. The resist exposure time and development process were optimized to get clean 1800×400 nm pattern on 400nm thick film. Using the same mask, the exposure time was cut into half to obtain 1500×200 nm pattern on 200 nm thick film. The patterns were transferred into the double-layer porous films by dry etch in CF₄ plasma (Plasmatherm BatchTop, 15sccm CF₄, 100 mTorr, 200W RF). After striping the LTO in dilute HF, the as-made porous silicon particles were retained on the substrate allowing go through washing steps, and were released by ultrasound in isopropyl alcohol (IPA). The particles were oxidized in a 35% H₂O₂ solution at 100 °C for 2 h. The oxidized silicon particles are sonicated in IPA containing 2% (v/v) APTES (Sigma) for 3 h at 55 °C. The APTES modified particles are washed three times in IPA and dried in vacuum desiccator for 2.5h. Finally, the dried particles are placed 2h in the oven at 75°C. APTES modification is evaluated by measuring the zeta potential. The APTES modified particles are conjugated with the dye Alexa Fluor 488 (Fluorescence absorbance $\lambda_{\text{max}} = 495$ nm, Emission maxima = 519 nm) to obtain green-fluorescent conjugates that result brighter and more photostable than fluorescein isothiocyanate (FITC) conjugates. The geometrical properties of the considered particles are listed Table.1.

2.2 Parallel Plate Flow Chamber System

The parallel plate flow chamber by GlycoTech Corporation is the main part of the experimental system constituted by a syringe pump (Harvard Apparatus, MA), a Nikon Ti-Eclipse epi-fluorescence inverted microscope and a computer. The flow chamber is obtained by assembling the PMMA flow deck and the silicon rubber gasket using an appropriate amount of vacuum grease and then adding the collagen-coated glass cover slip. The collagen coating procedure consists of cleaning the cover slip in a Petri dish with 70% Ethanol and adding the Collagen solution Sigma C8919, Type 1 obtained from calf skin at a concentration of about 50 µg/cm². After about 5 h at room temperature the cover slip is rinsed with PBS and it is left under the bio hood to dry. Once the deck, the gasket and the cover slip are assembled, they are tightly attached, in order to avoid leakages from the chamber, by sucking the air with a syringe. The inlet and outlet bores on the flow deck are connected, through silastic tubing, to a syringe mounted on the syringe pump and to an empty tube to contain the waste solution. The inlet particle solution is obtained by suspending the mesoporous silicon particles in Millipore water and measuring the particle concentration with a Multisizer 4 Coulter Particle Counter (Beckman Coulter). Before the experiment, the particle solution is sonicated for a few minutes and mixed for some seconds in order to avoid particle aggregates. The gasket defines the dimensions of the channel, i.e. thickness $h = 0.254$ mm, length $l = 20$ mm and width $w = 10$ mm, and determines the flow characteristics of the media. In the channel the flow between the two parallel walls is

laminar with a parabolic velocity profile and the wall shear rates S studied are 10 s^{-1} , 50 s^{-1} , 100 s^{-1} and 200 s^{-1} . Knowing the equation relating the wall shear stress to the volumetric flow rate: $\tau_w = \mu S = 6\mu Q/h^2 w$, the volumetric flow rates Q , corresponding to the desired S , are obtained and set as a parameter in the syringe pump: $64.516 \mu\text{l/min}$, $322.58 \mu\text{l/min}$, $645.16 \mu\text{l/min}$ and $1290.3 \mu\text{l/min}$. The flow chamber is mounted on the stage of a Nikon Ti-Eclipse inverted microscope. The particle motion is seen using dry microscope objectives with different magnification, 10x, 20x and 40x and in this way the particles whose size ranges between 200 and 1800 nm are clearly visible with the FITC filter. The Andor's Luca^{EM} S camera utilizes a 658×496 'interline frame transfer' EMCCD sensor to acquire the region of interest (ROI) and allows the real time following of the dynamic events happening in the flow chamber. Tuning the appropriate focus, exposure time and look up tables (LUTs), an optimal visualization of the collagen layer can be reached in order to set the z position of the microscope stage for the experiment. The ROI of 658×496 px is captured at different time steps with an acquisition rate as high as possible, in relation with the exposure time necessary to see the brightness of the fluorescent particles. The resulting movie or the single captured frames are exported in the preferred format and analyzed to understand the adhesion propensity of the differently shaped and sized particles towards the collagen substrate. The number of adherent particles is counted and normalized with the number of particles injected and the considered surface in order to presents homogeneous results.

2.3 Numerical Simulations

A computational model is considered in order to predict forces and torques acting on wall attached particles under a laminar shear flow at low Reynolds number (Fig. 1). The numerical approach considers the three-dimensional unsteady incompressible continuity and Navier-Stokes equations in their dimensionless form:

$$\nabla \cdot \mathbf{u} = 0 \quad (1)$$

$$\frac{D\mathbf{u}}{Dt} = -\nabla p + \frac{1}{\text{Re}} \nabla^2 \mathbf{u} + \mathbf{f} \quad (2)$$

where D/Dt is the material derivative, $\mathbf{u}$ is fluid velocity, p is pressure, $\mathbf{f}$ is mass force field

and possible local forces and $\text{Re} = \frac{\rho_{\text{ref}} u_{\text{ref}} d_{\text{ref}}}{\mu}$ is the Reynolds number, with ρ_{ref} as the reference density, u_{ref} as the reference velocity and d_{ref} as the reference length. All quantities are expressed in their dimensionless form.

An immersed boundary method (IBM) [25] is introduced to take in account the particle presence in the fluid domain. In particular, an underlying Eulerian grid is laid on regardless of the shape of the particle immersed into the computational domain which is discretized by a Lagrangian triangulated grid (Standard Triangulation Language format). (Fig. 1B)

In the STL format, for each triangle, there are three spatial coordinates of any vertex (here used to evaluate the three centroid spatial coordinates) and three director cosines of the normal to the surface so defined.

The Navier-Stokes equations governing the fluid flow are, in this way, solved on a fixed Cartesian grid using a second-order-accurate finite difference discretization coupled with a fractional-step integration consisting of an explicit Adam-Bashforth scheme to discretize the nonlinear terms and an implicit Crank-Nicolson scheme to discretize the linear terms and the terms related to the external body forces [26,27]. The resulting equation is:

$$\frac{\widehat{\mathbf{u}} - \mathbf{u}^n}{\Delta t} = -\alpha_n \nabla p^n + [\gamma_n H^n + \rho_n H^{n-1}] + \frac{\alpha_n}{2\text{Re}} \nabla^2 (\widehat{\mathbf{u}} + \mathbf{u}^n) + \mathbf{f}^{n+1/2} \quad (3)$$

where $\mathbf{u}^n$ denotes the velocity at the time n , Δt is the time step, H contains the nonlinear terms, α_n , γ_n and ρ_n are the constants of the hybrid third-order Adam-Bashforth/Crank Nicolson scheme and $\mathbf{f}$ is the forcing term.

Equation (3) can be written in terms of the solution increment and solved by a factorization technique, giving an intermediate non-solenoidal velocity field $\widehat{\mathbf{u}}$. A second step is necessary to get a solenoidal velocity field. For this purpose, a scalar quantity ϕ can evaluate the velocity at the new time step $n+1$ by:

$$\mathbf{u}^{n+1} = \widehat{\mathbf{u}} + \alpha_b \Delta t \nabla \phi \quad (4)$$

If the discrete divergence operator is applied to Equation (4) and $\Delta \mathbf{u}^{n+1} = 0$ is imposed, an elliptic Poisson equation is obtained:

$$\nabla^2 \phi = -\frac{\nabla \cdot \widehat{\mathbf{u}}}{\alpha_n \Delta t} \quad (5)$$

The scalar quantity ϕ is calculated with the Fast Fourier Transform (FFT) using the package FISHPACK [28].

The pressure field at the new time $n+1$ is finally defined as:

$$p^{n+1} = p^n + \phi - \frac{\alpha_n \Delta t}{2\text{Re}} \nabla^2 \phi \quad (6)$$

All variables are located on a staggered grid: at each face centre one defines the velocity component perpendicular to the face, whereas the pressure is defined in the cell centre.

Concerning the IBM, in this work a direct forcing approach [25,29] is used to impose the boundary condition at the fluid/body interface, by means of a forcing term $\mathbf{f}$ evaluated using a versatile moving-least-squares (MLS) approximation [30] to build the transfer functions between the Eulerian and Lagrangian grids. When solving the Navier-Stokes equation and continuity equation, the following boundary conditions are applied: at inlet points, a linear velocity profile is imposed for the streamwise component of the velocity vector whereas the wall-normal and spanwise components are set to zero; at outlet points, a standard convective condition is employed [31]; in the spanwise direction, periodicity is imposed for the three velocity components; at the upper-boundary points the corresponding streamwise velocity component is imposed while the wall normal and spanwise velocity components are set to zero. Finally, at the bottom boundary the no-slip boundary condition is prescribed.

Once the flow equation has been solved, the total force $\mathbf{F}_{tot}$ and the total torque $\mathbf{T}_{tot}$ acting on the body can be evaluated as:

$$\mathbf{F}_{tot} = \mathbf{F}_p + \mathbf{F}_v + \mathbf{F}_g \quad (7)$$

$$\mathbf{T}_{tot} = \mathbf{T}_p + \mathbf{T}_v \quad (8)$$

where F_p and T_p are the pressure force and torque, F_v and T_v are the viscous force and torque and F_g is the gravity force.

In the numerical simulations for a sphere with $d = 0.48$, a rod with $d = 0.2$ and $h = 1.5$ and a disk with $d = 0.6$ and $h = 0.2$, a linear shear flow is imposed in the fluid domain with a wall shear rate $S = 5$; the Reynolds number, Re , based on the diameter of the volume-equivalent sphere and on the undisturbed fluid velocity at the center of the volume-equivalent sphere, is equal to 5.76. In the numerical simulations for a sphere with $d = 0.84$, a rod with $d = 0.4$ and $h = 1.8$ and a disk with $d = 1$ and $h = 0.4$, a linear shear flow is imposed in the fluid domain with a wall shear rate $S = 5$; in this case, Re is 17.64. The geometrical parameters for the particles and the dimensionless numerical results are summarized in Table 2.

2.4 The Statistical Analysis

The F-test is used for the equality of variances followed by the two-sample t-test for the equality of means. The preliminary F-test indicates if the variances of the two compared groups are significantly different. Generally, if the one-tail p-value associated with the F-test is less than 0.05 the variances are assumed to be different and a t-test that does not assume equal variances is performed. On the contrary, if the one-tail p-value associated with the F-test is greater than 0.05 the variances are assumed to be equal and, therefore, a t-test that assumes equal variances is performed.

3. Results

3.1 Mesoporous Silicon Particles Fabrication and Characterization

Mesoporous silicon particles were fabricated using a process consisting of three major steps: porosification of a silicon film by electrochemical etching; particle patterning on the porous film by photolithography; and reactive ion etching (RIE) [23]. Photolithography allowed us to precisely tailor the size and shape of the particles independently of their porosity, which is instead controlled by the specific properties of the silicon film (doping) and by the porosification parameters (etching current, etching solution and temperature). This is an extremely flexible strategy supporting the fabrication of particles with a characteristic size > 200 nm, a disk-like and rod-like shape in a variety of aspect ratios, and a porosity ranging from 30 to 70%. In this study, three disks were fabricated, namely 600×200 nm, 1000×400 nm and 1800×600 nm; and two different rods, namely 1500×200 nm, 1800×400 nm. Note that with these characteristic sizes the porous silicon particles can safely navigate the vascular system while exposing a sufficiently large area for vascular adhesion. The two smallest disks and the rods have a similar volume (Table 1).

A schematic of the fabrication process is presented in Fig. 2, for the case of rod-like nanoparticles. From a blank silicon wafer, a porous silicon film, consisting of a device layer and a release layer (light blue and cyan in Fig. 2A, respectively), was formed using step-current electrochemical etching in HF/ethanol solution. The particles are generated from the device layer. The release layer has a larger porosity as compared to the device layer which is sufficient to firmly link the particles to the wafer during handling and stripping, but would collapse rapidly upon sonication for the final separation step. This bi-layer film (device and release layers) is capped with 40 nm low temperature oxide layer (LTO, dark blue in Fig. 2B), deposited by Low Pressure Chemical Vapor Deposition (LPCVD). This capping technique is fundamental to the direct photolithographic patterning of particle arrays on the porous film (green in Fig. 2C), in that it reduces the local variations in optical reflectance and excludes the photoresist from permeating the pores. The particle patterns are thereby transferred into bi-layer porous film by one-step RIE in CF_4 plasma (Fig. 2D). After cleaning the residual photoresist and removing the LTO, the particles are retained on the

wafer as an array, allowing for further surface treatments (Fig. 2E,H where a SEM image shows 1800×400 nm particles still linked to the wafer through the release layer).

All this was possible because of a proper mask design, selection of a thin negative resist and optimization of the photolithographic process. In particular, for the 1800×400 nm rods, a 5 inch dark-field photomask (for a 4 inch wafer) was designed consisting of high-density arrays of 1800×400 nm corner-rounded strips at a 2500×1000 nm period, but with a small offset from line to line to eliminate possible grating effects. Using a 250 nm thick negative-tone photoresist and vacuum contact mode, the photolithographic processing parameters were optimized to obtain clean particle patterns (Fig. 2F). Then, the optimized exposure time was cut in half while keeping other parameters unchanged, and 1500×200 nm particles were obtained using the same photomask (Fig. 2G). The rods are eventually separated by the original wafer by ultrasonic treatment.

Representative scanning electron micrographs of the rod-like and disk-like nanoparticles are shown in Fig. 2I and Fig. 2J, respectively. The particle porosity ($\sim 65\%$, with ~ 30 nm pore size) can be appreciated from the top view, whereas particle thickness and aspect ratio are clear in the lateral view.

3.2 Particle Margination and Adhesion under Flow

The dynamics of the mesoporous silicon particles under microvascular flow conditions is studied using a parallel plate flow apparatus, traditionally used for the analysis of leukocyte adhesion and rolling [32]. A schematic representation of the in vitro apparatus (parallel plate flow chamber) is shown in Fig. 3A. It comprises a parallel plate chamber, an inverted fluorescence microscope, a tubing system for connecting the chamber to an external syringe pump and the waste. The main constituents of the parallel plate flow chamber are the PMMA flow deck (top), the gasket (middle) and the microscope cover slip (bottom), where a layer of collagen is distributed. These constituents are held together by circumferential low pressure, generated through an additional external vacuum syringe, and a layer of vacuum grease, interposed between the gasket and the deck to improve sealing. The hydrodynamic conditions in the chamber (wall shear rate S) are carefully controlled by changing the flow rate of the syringe pump and the geometry of the gasket.

Fluorescently labeled, untargeted mesoporous silicon particles are injected in the flow chamber and imaged continuously using a microscope set-up (Fig. 3B). Via off-line image analysis, the number of particles adhering on the substrate is quantified and from there two main parameters can be extracted: the total number of adhering particles at the end of the experiment (n_{adh}) and the rate of adhesion over time. The typical outcome of the parallel plate flow experiment is presented in Fig. 3C: the number of adhering particles grows steadily with time at the beginning and then it plateaus until the end of the experiment.

The ratio P between the number of particles adhering at the end of the experiment (n_{adh}) and the total number of injected particles n_i , further normalized by the area A of the region of interest, is given in the bar charts of Fig. 4, for the 5 different used particles. The quantity P ($= n_{adh}/(n_i A)$) can be interpreted as the particle adhesion efficiency: a larger P implies a larger proportion of injected particles firmly adhering at the chamber substrate. Fig. 4A provides P for the three disks as a function of the wall shear rate S ranging from 10 to 200 s^{-1} . The larger shear rates ($> 100 \text{ s}^{-1}$) are typically found in the microcirculation of a healthy tissue, whereas the smaller S are relevant to the tumor microcirculation ($< 100 \text{ s}^{-1}$) [13]. Fig. 4B presents similar data for the two analyzed rods; and Fig. 4C directly compares disks and rods of equivalent volume. As expected, the number of adhering particles decreases steadily as the wall shear rate increases, regardless of the particle size and shape (Fig. 4A,B). Indeed, the hydrodynamic forces exerted over the particle grow linearly with

the wall shear rate S , thus reducing the likelihood of particle deposition and adhesion. More interestingly, for the disk-like shape a neat maximum in adhesion occurs for the 1000×400 nm particles, for all four tested wall shear rates (Fig. 4A). The difference with the 600×200 and 1800×600 nm particles is statistically significant up to $S < 100 \text{ s}^{-1}$ ($p < 0.05$). For larger S , the number of adhering particles is generally small ($< 2 \text{ \#/m}^2$). Even for the rod-like shape, the data in Fig. 4B document the larger particles (1800×400 nm) adhering more than the smaller ones (1500×200 nm). The difference in P between the two rods is statistically significant for $S < 200 \text{ s}^{-1}$ ($p < 0.05$). The occurrence of a maximum in the adhesion curve was already predicted by a mathematical model developed by the authors in the more general case of oblate spheroidal particles [22]. Finally, Fig. 4C directly compares the two differently shaped particles, of equivalent volume. The disks adhere more than the rods with a ratio $P_{\text{disk}}/P_{\text{rod}}$ which is ~ 2 and the difference is significant up to about $S = 100 \text{ s}^{-1}$. For larger wall shear rates, the number of adhering particles is nearly zero regardless of their geometry ($< 2 \text{ \#/m}^2$). The specific values of n_{adh} are listed in the Supplementary Table 1.

3.3 Analysis of the Hydrodynamic Forces on the particles

A computational model was developed to estimate the hydrodynamic forces exerted on firmly adhering particles. Note that the adhesive interactions arising at the particle/substrate interface are non-specific, resembling those that untargeted, systemically administered particles would experience in vivo. Fig. 1A-C shows a schematic for the problem at hand and provides details on the computational grid in a small neighborhood of the particle. The fluid domain was discretized using a regular Cartesian grid, whereas a triangulated grid (STL format) was used for the particle. Fig. 1D shows representative velocity streamlines and pressure contours for the 1000×400 nm disks. Similar contours are presented in the Supplementary Fig. 1 also for rods, differently oriented with respect to the flow field.

The normalized drag force D and torque T exerted on the disks (600×200, 1000×400 nm) and rods (1500×200, 1800×400 nm) are presented in Fig. 5. The convergence analysis for the numerical calculations is presented in Supplementary Fig. 2. The normalization was performed with respect to the forces exerted over a spherical particle sitting on the substrate, exposed to the same flow conditions and exhibiting the same volume. Note that the comparison between particles with different shapes is always performed for a fixed volume, hence fixed payload carried by the particle. For the rods, three different orientations with respect to the flow direction were considered, namely 0° , 45° and 90° . It immediately appears that the normalized values for D and T are smaller than unity for all sizes, shapes and orientations. This implies that, for a fixed volume, the hydrodynamic drag and torque exerted over disks and rods are smaller than for spherical particles. Also, spherical particles offer a much smaller surface of adhesion as compared with rods and disks. These two conditions confirm that non-spherical particles are more appropriate than spherical beads for recognizing and firmly adhering to the vascular walls under flow. The drag force exerted over a rod is orientation dependent, and it reaches a maximum for a rod laying with its major axis orthogonal to the flow ($\theta = 90^\circ$). Disks and rods, aligned with the flow ($\theta = 0^\circ$), experience the lowest drag force D . For the hydrodynamic torque T , small variations were observed among the different particles. As expected, the trends in T and D are very similar for both sizes (Fig. 5A,B).

4. Discussions

Particle firm adhesion under flow results from two sequential events: i) approaching the surface from the bulk flow and ii) establishing sufficiently strong adhesive interactions. For both events, the authors have previously shown that particle geometry plays a crucial role [21,22]. Non-spherical particles have been shown to drift laterally (margination) within the flow, rather than following the streamlines as a spherical particle would do. This has been

predicted for ellipsoidal, disk-like and hemispherical particles, and it has been demonstrated that particles with lower aspect ratios tend to drift with larger lateral velocities [21]. Mathematical models have also predicted that oblate spheroidal particles would more avidly adhere to a substrate, resisting the dislodging hydrodynamic forces, as compared to spherical particles of equivalent volume. This mainly happens because of the larger surface of adhesion exposed by the particle to the substrate [22].

In Fig. 4A, the adhesion efficiency of the three disks is presented. A maximum occurs for the 1000×400 nm particles, whereas smaller and larger particles consistently adhere less. A similar biphasic behavior was predicted [22] and is in agreement with the current literature on particles adhesion under flow. In particular, the existence of an optimal size for maximizing vascular adhesion has been elegantly demonstrated *in vitro* by the group of Eniola-Adefeso [33], documenting on the adhesion dynamics of sLe^a-targeted spherical beads over a layer of endothelial cells. Their experiments have shown that an increase in the surface density of ligands and blood hematocrit (volume concentration of RBCs in the flow) would induce an increase in the number of adhering particles and a shift of the optimal size towards larger diameters, with no change in the overall biphasic behavior. These have also been shown by the authors using untargeted spherical beads nonspecifically interacting with a collagen layer, demonstrating again the shear dependence of the optimal particle size [34]. Finally, the same disk-like particles presented here were recently shown to target the tumor microvasculature in melanoma-bearing mice at unprecedented levels, exhibiting exactly the same biphasic behavior for both untargeted and RGD-4C targeted particles [35]. This can be explained with the fine balance between adhesive and hydrodynamic forces. Both these two forces grow with the particle size, as documented in Table 2. Small particles (600×200 nm) expose an insufficiently large surface area to the substrate; whereas large particles (1800×600 nm) experience excessively high dislodging forces. Consequently they both have a smaller probability of adhesion as compared with the 1000×400 nm [22]. It should be also noted that although the particles have different masses because of their different sizes, the gravitational force is 4 orders of magnitude smaller than the hydrodynamic forces (Table 2). Similar reasoning can be used to interpret the size-dependent adhesion efficiency of the two rods, as presented in Fig. 4B.

The direct comparison between disks and rods is given in Fig. 4C. Here, the ratio between the number of adhering particles is ~ 2 for the larger particles (1000×400 nm disk over 1800×400 nm rod) and ~ 2.3 for the smaller particles (600×400 nm disk over 1500×200 nm rod). Looking at the diagrams in Fig. 5, it appears that the hydrodynamics forces exerted over the rods are slightly larger than for the disks. Indeed, D and T depend on the orientation of the rods, for which no preferential direction was observed in the flow chamber experiments. Also, the base areas of disks and rods differ of less than 15% (Table.1).

These slight differences in hydrodynamic forces and size of the adhesion area would only partially explain the larger adhesive efficiency observed for the disks. Indeed, the near wall dynamics of the rods and disks is expected to be different [21]. In particular, the rotational inertia and the instantaneous area exposed to the substrate depend strongly on the particle shape. For rods, the rotational inertia is equal to $1/12mL^2$, with m the mass and L the length; whereas for a disk the same quantity takes the form $1/16mD^2$, with D the diameter. The ratios between the two rotational inertia is $4/3(L/D)^2$ which for the specific sizes considered here would be equal to ~ 4 and ~ 8 for the larger and smaller particles, respectively. Also, the instantaneous surface of adhesion exposed by the disks is expected to be larger than that provided by the long rods. This would imply that rods are more difficult to decelerate from the bulk flow velocity and eventually arrest because of the higher rotational inertia and smaller adhesion interactions that can instantaneously be developed during the near wall particle dynamics, just preceding firm adhesion.

It is important to emphasize here that the range of wall shear rates considered in the present analysis ($10 - 200 \text{ s}^{-1}$) are typical for the microcirculation and the subphysiological flow conditions met in the tumor vasculature [13], where untargeted particles can efficiently lodge. For higher shear rates or turbulent flow, additional analysis should be considered which are beyond the scope of the present work. Finally, the rod-like particles considered here are very slender and have a large aspect ratio (> 4.5) in order to provide a loading volume comparable with the disk-like particles. Indeed, shorter rods could behave differently and possibly limit the difference with the disk-like particles.

5. Conclusions

The behavior of mesoporous silicon particles, with disk-like and rod-like shapes and with diameters ranging from 200 to 1800 nm, was analyzed in a parallel flow chamber system under different hydrodynamic conditions. As the wall shear rate S increased, the number of particles adhering firmly to the substrate decreased steadily, being smaller than 0.5 particles per m^2 at physiologically relevant wall shear rate S ($S > 100 \text{ s}^{-1}$). For disks, maximum adhesion is observed for the intermediate sized particles ($1000 \times 400 \text{ nm}$), in agreement with early theoretical predictions. Similarly, for the two rod-like particles, the largest rods adhere at least 5 times more than the smaller particles. Using computational modeling, it was shown that spherical particles experience the largest dislodging forces and exhibit the smallest area of adhesion, followed by rods and then disks.

In the design and development of NPs intended to preferentially target the diseased microvasculature, the present data suggest that thin disk-like particles would be more effective than slender rods and spheres in the systemic delivery of relevant doses of therapeutic and contrast agents.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

This work was supported by the Cancer Prevention Research Institute of Texas through the grant CPRIT RP110262. This work was also partially supported through grants from the National Institutes of Health (USA) (NIH) U54CA143837 and U54CA151668. GA acknowledges the Interpolytechnic Doctoral School of Turin (Italy) for travel support. MF acknowledges the additional supports from DoD/BCRP (W81XWH-09-1-0212) and the Ernest Cockrell Jr. Distinguished Endowed Chair. PD acknowledges also the partial support of the European Science Foundation EUROCORES Programme FANAS, through funds by the Consiglio Nazionale delle Ricerche and the EC Sixth Framework Programme under contract N. ERAS-CT-2003-980409FANAS. The photolithography work was performed in Microelectronic Research Center at University of Texas at Austin.

References

- [1]. Drummond DC, Meyer O, Hong K, Kirpotin DB, Papahadjopoulos D. Optimizing liposomes for delivery of chemotherapeutic agents to solid tumors. *Pharmacol Rev.* 1999; 51(4):691–743. [PubMed: 10581328]
- [2]. Torchilin VP. Recent advances with liposomes as pharmaceutical carriers. *Nat Rev Drug Discov.* 2005; 4(2):145–60. [PubMed: 15688077]
- [3]. Allen TM, Cullis PR. Drug delivery systems: entering the mainstream. *Science.* 2004; 303(5665):1818–22. [PubMed: 15031496]
- [4]. Ferrari M. Cancer nanotechnology: opportunities and challenges. *Nat Rev Cancer.* 2005; 5(3):161–71. [PubMed: 15738981]
- [5]. Peer D, Karp JM, Hong S, Farokhzad OC, Margalit R, Langer R. Nanocarriers as an emerging platform for cancer therapy. *Nat Nanotechnol.* 2007; 2:751–60. [PubMed: 18654426]

- [6]. Elbayoumi TA, Torchilin VP. Enhanced accumulation of long-circulating liposomes modified with the nucleosome-specific monoclonal antibody 2C5 in various tumours in mice: gamma-imaging studies. *Eur J Nucl Med Mol Imaging*. 2006; 33:1196–205. [PubMed: 16763815]
- [7]. Huang X, Peng X, Wang Y, Wang Y, Shin DM, El-Sayed MA, et al. A reexamination of active and passive tumor targeting by using rod-shaped gold nanocrystals and covalently conjugated peptide ligands. *ACS Nano*. 2010; 4(10):5887–96. [PubMed: 20863096]
- [8]. Decuzzi P, Pasqualini R, Arap W, Ferrari M. Intravascular delivery of particulate systems: does geometry really matter? *Pharm Res*. 2009; 26(1):235–43. [PubMed: 18712584]
- [9]. Wang J, Byrne JD, Napier ME, DeSimone JM. More effective nanomedicines through particle design. *Small*. 2011; 7(14):1919–31. [PubMed: 21695781]
- [10]. Mitragotri S, Lahann J. Physical approaches to biomaterial design. *Nat Mater*. 2009; 8(1):15–23. [PubMed: 19096389]
- [11]. Matsumura Y, Maeda H. A new concept for macromolecular therapeutics in cancer chemotherapy: mechanism of tumorotropic accumulation of proteins and the antitumor agent smancs. *Cancer Res*. 1986; 46(12 Pt 1):6387–92. [PubMed: 2946403]
- [12]. Maeda H, Wu J, Sawa T, Matsumura Y, Hori K. Tumor vascular permeability and the EPR effect in macromolecular therapeutics: a review. *J Control Release*. 2000; 65(1-2):271–84. [PubMed: 10699287]
- [13]. Jain RK, Stylianopoulos T. Delivering nanomedicine to solid tumors. *Nat Rev Clin Oncol*. 2010; 7(11):653–64. [PubMed: 20838415]
- [14]. Auerbach R. Vascular endothelial cell differentiation: organ-specificity and selective affinities as the basis for developing anti-cancer strategies. *Int J Radiat Biol*. 1991; 60(1-2):1–10. [PubMed: 1677953]
- [15]. Sergeeva A, Kolonin MG, Molldrem JJ, Pasqualini R, Arap W. Display technologies: application for the discovery of drug and gene delivery agents. *Adv Drug Deliv Rev*. 2006; 58(15):1622–54. [PubMed: 17123658]
- [16]. Folkman J. Angiogenesis in cancer, vascular, rheumatoid and other disease. *Nat Med*. 1995; 1(1):27–31. [PubMed: 7584949]
- [17]. Hajitou A, Pasqualini R, Arap W. Vascular targeting: recent advances and therapeutic perspectives. *Trends Cardiovasc Med*. 2006; 16(3):80–8. [PubMed: 16546688]
- [18]. Sengupta S, Eavarone D, Capila I, Zhao GL, Watson N, Kiziltepe T, et al. Temporal targeting of tumour cells and neovasculature with a nanoscale delivery system. *Nature*. 2005; 436(7050):568–72. [PubMed: 16049491]
- [19]. West JL, Halas NJ. Engineered nanomaterials for biophotonics applications: improving sensing, imaging, and therapeutics. *Annu Rev Biomed Eng*. 2003; 5:285–92. [PubMed: 14527314]
- [20]. Pasqualini R, Arap W, McDonald DM. Probing the structural and molecular diversity of tumor vasculature. *Trends Mol Med*. 2002; 8(12):563–71. [PubMed: 12470989]
- [21]. Lee SY, Ferrari M, Decuzzi P. Shaping nano-/micro-particles for enhanced vascular interaction in laminar flows. *Nanotechnology*. 2009; 20(49):495101. [PubMed: 19904027]
- [22]. Decuzzi P, Ferrari M. The adhesive strength of non-spherical particles mediated by specific interactions. *Biomaterials*. 2006; 27(30):5307–14. [PubMed: 16797691]
- [23]. Ananta JS, Godin B, Sethi R, Moriggi L, Liu X, Serda RE, et al. Geometrical confinement of gadolinium-based contrast agents in nanoporous particles enhances T1 contrast. *Nat Nanotechnol*. 2010; 5(11):815–21. [PubMed: 20972435]
- [24]. Tanaka T, Mangala LS, Vivas-Mejia PE, Nieves-Alicea R, Mann AP, Mora E, et al. Sustained small interfering RNA delivery by mesoporous silicon particles. *Cancer Res*. 2010; 70(9):3687–96. [PubMed: 20430760]
- [25]. Mittal R, Iaccarino G. Immersed boundary methods. *Annu Rev Fluid Mech*. 2005; 37:239–61.
- [26]. Verzicco R, Orlandi P. A finite-difference scheme for three-dimensional incompressible flows in cylindrical coordinates. *J Comput Phys*. 1996; 123(2):402–14.
- [27]. Orlandi, P. *Fluid Flow Phenomena: A numerical toolkit*. Kluwer Academic Publishers; 1999.
- [28]. Swartzrauber PN. A direct method for the discrete solution of separable elliptic equations. *SIAM J Numer Anal*. 1974; 11:1136–50.

- [29]. Fadlun EA, Verzicco R, Orlandi P, Mohd-Yusof J. Combined immersed boundary finite-difference methods for three-dimensional complex flow simulations. *J Comput Phys*. 2000; 161:35–60.
- [30]. Vanella M, Balaras E. A moving-least-squares reconstruction for embedded boundary formulations. *J Comput Phys*. 2009; 228(18):6617–28.
- [31]. Bottaro A. Note on open boundary conditions for elliptic flows. *Num Heat Transfer, Part B*. 1990; 18:243–56.
- [32]. Lawrence MB, McIntire LV, Eskin SG. Effect of flow on polymorphonuclear leukocyte/endothelial cell adhesion. *Blood*. 1987; 70(5):1284–90. [PubMed: 3663936]
- [33]. Charoenphol P, Huang RB, Eniola-Adefeso O. Potential role of size and hemodynamics in the efficacy of vascular-targeted spherical drug carriers. *Biomaterials*. 2010; 31(6):1392–402. [PubMed: 19954839]
- [34]. Boso DP, Lee SY, Ferrari M, Schrefler BA, Decuzzi P. Optimizing particle size for targeting diseased microvasculature: from experiments to artificial neural networks. *Int J Nanomedicine*. 2011; 6:1517–26. [PubMed: 21845041]
- [35]. van de Ven AL, Kim P, Haley O, Fakhoury JR, Adriani G, Schmulen J, et al. Rapid tumorotropic accumulation of systemically injected plateloid particles and their biodistribution. *J Control Release*. 2012; 158(1):148–55. [PubMed: 22062689]

Fig. 1. Numerical simulations

A) A firmly adhering disk-like particle immersed in a linear shear flow. B) Computational grid used for the discretization of the flow field (regular Cartesian grid) and particle (triangulated grid – STL format). C) Drag force D and the torque T exerted over the adhering particle. D) Velocity streamlines (left) and pressure contours (right) on a 1000×400 nm disk.

Fig. 2. Mesoporous silicon particles

Description of the fabrication process: A) the bi-layer porous silicon film with the device layer (light blue) and the release layer (cyan). B) Capping of the porous film with a LTO (low temperature oxide) layer (dark blue). C) Photolithographic patterning of the particle array. D) Reactive ion etching of the particles in the porous film. E) Stripping of the photoresist and LTO layer to yield arrays of rod-like particles. F) SEM top view image of 1800×400 nm rods. G) SEM 45° view of 1500×200 nm rod-like particle array, corresponding to Fig. 2E. H) SEM close-up view of 1800×400nm rods, showing particles still linked to the wafer substrate through the hyperporous release layer (blue arrow). Scale bars 1000 nm. I) SEM images of 1800×400 nm rods. Scale bars 200 nm. J) SEM images of 1000×400 nm disks. Scale bars 200 nm.

Fig. 3. Adhesion experiments in a parallel plate flow chamber

A) Schematic representation of the experimental system. B) Images of fluorescently-labeled $1000 \times 400 \text{ nm}$ disks firmly adhering to the chamber substrate, taken at three different time points post infusion. C) Typical outcome of a flow chamber experiment showing the variation over time of the number of adhering particles.

Fig. 4. Adhesion efficiency of the mesoporous silicon particles under flow

A) The adhesion efficiency P for the three disk-like particles measured at four different shear rates. B) The adhesion efficiency P for the two rod-like particles measured at four different shear rates. C) The comparison in adhesion efficiency P for disks and rods with equivalent volumes at four different shear rates. The adhesion efficiency P is the ratio between the total number of adhering particles n_{adh} and the number of injected particles n_i times the area A of the region of interest ($P = n_{adh} / (n_i A)$). Data are plotted as mean $\pm$ SD. n 3. The star symbol * means not significant difference ($p > 0.05$). In all other cases the difference is statistically significant ($p \leq 0.05$).

Fig. 5. Calculated hydrodynamic forces

A) The drag force exerted over disk-like and rod-like particles divided by the drag force experienced by a spherical particle of equivalent volume. B) The torque exerted over disk-like and rod-like particles divided by the torque experienced by a spherical particle of equivalent volume. The rod-like particles have three different orientations with respect to the flow: 0° (major axis parallel to the flow), 45° and 90° (major axis orthogonal to the flow).

Table 1
The geometrical properties of the mesoporous silicon particles

A) Disk-like particles. B) Rod-like particles.

A			
Shape	Disk	Disk	Disk
Diameter [nm]	600	1000	1800
Height [nm]	200	400	600
Volume [μm^3]	0.057	0.314	1.526
Lateral surface [μm^2]	0.377	1.257	3.393
Base surface [μm^2]	0.283	0.785	2.545

B		
Shape	Red	Red
Length [nm]	1500	1800
Height [nm]	200	400
Volume [μm^3]	0.058	0.274
Lateral surface [μm^2]	0.646	1.623
Base surface [μm^2]	0.291	0.686

The geometrical properties and characteristic forces for the simulated particles
A) Comparison among the smaller spherical, rod-like and disk-like particles. B) Comparison among the larger spherical, rod-like and disk-like particles.

A
	Sphere	Cylinder 0°	Disk
	Size $d \times h$	0.200×1.500	0.200×1.500
	Volume	0.047	0.057
	Frontal area	0.031	0.120
	Drag Force	0.566	0.643
	Torque	0.034	0.040
Gravity Force		0.853	

B				
Shape	Sphere	Cylinder 0°	Cylinder 45°	Cylinder 90°
Size $d \times h$	0.840×1.800	0.400×1.800	0.400×1.800	1.000×0.400
Volume	0.310	0.226	0.226	0.314
Frontal area	0.554	0.126	0.598	0.400
Drag Force	5.213	1.973	2.911	2.734
Torque	0.487	0.213	0.217	0.274
Gravity Force		3.326		
		4.621		