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The margination propensity of ellipsoidal micro/nanoparticles to the endothelium in human blood flow

Alex J. Thompson, Eric M. Mastria, Omolola Eniola-Adefeso*

Department of Chemical Engineering, University of Michigan, 2300 Hayward Street, 3074 H. H. Dow Building, Ann Arbor, MI 48109, USA

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ABSTRACT

Particle shape is becoming increasingly recognized as an important parameter for the development of vascular-targeted carriers (VTCs) for disease treatment and diagnosis. However, limited research exists that investigates how particle shape coupled with hemodynamics affects VTC margination (localization and adhesion). In this study, we investigate the effects of particle shape parameters (volume, aspect ratio, axis length) on the margination efficacy of targeted spheres and prolate ellipsoids (rods) to an inflamed endothelial wall from human blood flow in an *in vitro* model of human vasculature. Overall, particles with 2 μm equivalent spherical diameters (ESD) display higher margination than particles with either 1 μm or 500 nm ESDs. Interestingly, rod-shaped microparticles (1 μm or 2 μm ESD) with high aspect ratios display significantly improved margination compared to spheres of equal volume, particularly under high shear rates and disturbed flow profiles. Nanorods (500 nm ESD), even with high aspect ratio, do not display enhanced margination compared to that of equivalent spheres, which suggests that nanorods, like nanospheres, display minimal margination due to their inability to effectively localize to the vessel wall in the presence of RBCs.

1. Introduction

Vascular-targeted carriers (VTCs) offer unique opportunities to improve disease diagnosis and treatment by providing highly localized delivery of therapeutics/imaging agents. Regardless of the carrier type (e.g. polymeric, liposomes, dendrimers, or micelles), the typical geometry for a VTC is spherical with diameters on the nanometer scale. However, recent publications have shown that nanospheres may be suboptimal for targeting diseases affecting larger vessels with bulk blood flow, such as atherosclerosis [1,2]. In bulk blood flow, red blood cells (RBC) tend to move to the center of flow, resulting in a RBC core that forces leukocytes and platelets to concentrate into the red blood "cell free layer" (CFL) adjacent to the vascular wall. This enhanced concentration at the wall, i.e. "near wall excess", then allows for these cells to optimally interact with the endothelium despite large vessel diameters. Microspheres (2–10 μm diameters) can also take advantage of the near wall excess mechanism [1–4], allowing for their efficient margination (localization and adhesion) to the endothelium from bulk blood flow. Nanospheres, however, are not similarly displaced to the CFL, and thus exhibit minimal margination from bulk blood flow [1–5]. Therefore, one major challenge in targeting atherosclerosis is in designing carriers that have the capacity to localize to and have a high affinity for binding to the vascular wall from bulk blood flow. Recent published works have suggested that deviating from the typical spherical shape of VTCs could potentially improve VTC performance. Specifically, macrophages are reported to be less efficient at engulfing elongated particles of sufficient volume than spheres, which could translate to reduced clearance and lower accumulation in non-targeted organs [6]. Indeed, differently shaped microparticles of similar volume have been shown to have different *in vivo* biodistribution profiles in tumor-bearing mice [7]. Drug loaded filamentous micelles have also been shown to persist in circulation for much longer than spherical micelles in mice [8], and ICAM-1 targeted elliptical disks were shown to have a longer circulation half-life and a better targeting specificity than their spherical counterparts [9]. Theoretical works have also predicted that ellipsoidal particles display a preferential lateral drift in shear flow near a wall (analogous to the CFL) [10,11]. Also, disk-shaped particles are predicted to better adhere to endothelial cells in the presence of flow-induced shear stress, due to both a higher contact surface with the endothelium and a streamlined shape [12]. Recent experimental works have also demonstrated that particle elongation can increase particle adhesion to protein-coated surfaces in microchannels; however these works were done with particles in low shear buffer flows and thus do not provide indication as to

* Corresponding author. Tel.: +1 734 936 0856; fax: +1 734 764 1761.
E-mail address: lolaa@umich.edu (O. Eniola-Adefeso).

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whether these particles can better localize to the CFL from the RBC core than spheres [13,14]. Thus, it largely remains unclear how particle shape affects margination in human blood flow, and in conditions relevant to targeting the endothelium in blood vessels with bulk blood flows. This study seeks to evaluate the efficiency of rod-shaped, relative to spherical, model VTCs in marginating to the vascular wall from high shear human blood flows via parallel plate flow chamber (PPFC) adhesion assays with the central goal of identifying particle shape parameters (volume, aspect ratio, major/minor axis length), if any, that are paramount in determining the capacity for VTCs to readily marginate in physiological human blood flow conditions. Pulsatile and recirculation blood flow patterns were used in adhesion assays for relevance in large arteries and areas of sudden expansion within these arteries, respectively, that are typically afflicted with atherosclerosis. Steady laminar blood flow was explored for their relevance in small arteries (i.e. arterioles) that are important in cancer and some cardiovascular diseases, including arteriolosclerosis and specific types of vasculitis.

2. Materials and methods

2.1. Rod shaped particle fabrication and characterization

Polystyrene (PS) rods of various aspect ratio and volume were fabricated using a previously developed polymer film stretching method [15,16]. Briefly, Fluoresbrite® YG Carboxylate Microspheres (Polysciences, Inc) were suspended in a solution of 10% w/v aqueous polyvinyl alcohol (PVA) (Sigma Aldrich) and cast into a film. The films were dried overnight and placed in a 1-D stretching device composed of a pair of parallel clamps, one movable and the other stationary. The films were heated in an oven and stretched uniaxially to produce rods. The stretched films were dissolved in 30% v/v isopropanol (Sigma Aldrich) overnight and subsequently washed/centrifuged multiple times to ensure minimal residual PVA. Samples of recovered particles were dried and imaged using a Philips XL30 FEG SEM (courtesy of University of Michigan EMAL, Fig. 1). Particle dimensions were obtained from SEM images via Metamorph® Software (Molecular Devices, LLC). Prolate rods having equivalent spherical diameters (ESD) of 2 μm, 1 μm, and 500 nm were used with aspect ratios ranging from 2 to 11. The dimensions of the particles used in this study are given in Table 1, where rods are denoted by their aspect ratios (AR) – the ratio of the major axis length to the minor axis length. Commercially obtained spherical particles having the same ESD as rods of different volume served as control for all assays.

2.2. Conjugation with targeting ligands

Polystyrene rods and spheres were conjugated with Neutravidin® Biotin-Binding Protein (Thermo Scientific) via carbodiimide coupling chemistry and stored at 4 °C in phosphate buffer [1]. Avidin-coated particles were conjugated with biotinylated Sialyl-Lewis A (Glycotech; Gaithersburg, MD) for 20 min and stored in phosphate buffer with 1% bovine serum albumin until ready to use. Unless otherwise stated, a fixed sLe^a site density of 970 ± 40 (SE) sites μm^{-2} was used for rods and spheres as determined via fluorescent staining and analysis using a BD Biosciences FACSCalibur flow cytometer (courtesy of the University of Michigan BRCF).

2.3. HUVEC isolation

Human umbilical vein endothelial cells (HUVEC) were obtained from human umbilical cords (University of Michigan C.S. Mott Children's Hospital) according to a previously described protocol [17]. Isolated cells were pooled from multiple donors and were grown in culture flasks until confluent. Subsequent passages of HUVEC were grown on gelatin-coated coverslips at confluent density for use in parallel plate flow chamber (PPFC) assays.

Table 1
Dimensions for spherical and rod-shaped particles used in adhesion experiments. (ESD = equivalent spherical diameter).

Particle shape	ESD [μm]	Aspect ratio	Major axis [μm]
Sphere	2.07	1.00	2.07
AR-2		2.11	3.39
AR-4		4.03	5.21
AR-9		9.09	8.97
Sphere	1.01	1.00	1.01
AR-2		1.98	1.61
AR-4		4.17	2.76
AR-11		11.23	5.90
Sphere	0.52	1.00	0.52
AR-2		2.14	0.85
AR-4		4.50	1.40
AR-10		10.15	2.41

2.4. Blood collection

Blood from human donors was drawn into syringes containing the anticoagulant acetate citrate dextrose according to IRB approved protocols and in line with the WMA Declaration of Helsinki. Red blood cells (RBC) were separated by dextran sedimentation for 2 h and were washed once with phosphate buffer before storing at 37 °C until use. All flow assays utilized reconstituted blood (i.e. RBCs reconstituted in PBS+ buffer with 1% BSA) at either 30% or 40% hematocrit.

2.5. Parallel plate flow chamber

A PPFC (Glycotech) with a straight channel having a height of 254 μm and a width of 1 cm was used for laminar and pulsatile blood flow adhesion assays. The bottom of the flow chamber is formed by a glass coverslip covered with a confluent monolayer of HUVECs. Steady blood flow was investigated at a range of physiological shear rates (200–1000 s^{-1}). Laminar flow through the chamber is generated by a syringe pump downstream of the channel, and the shear rate at the channel wall is determined using Equation (1), where Q is the volumetric flow rate, h is channel height, and w is the channel width.

$$\gamma_w = \frac{6Q}{h^2 w} \tag{1}$$

Two physiologically relevant pulsatile flow profiles were also investigated via a programmable syringe pump; forward flow at alternating low and high shear rates (120 s^{-1}–1200 s^{-1}), and alternating forward/reverse flow at high shear (1000 s^{-1}) with a net forward flow as previously described [1,2]. Also, a step channel was used to simulate regions of recirculation flow, typical of regions where atherosclerotic plaques are known to accumulate [2]. The step channel has a pre-step channel height of 125 μm and a post-step channel height of 500 μm. A region of recirculating flow is created when laminar flow through the channel passes over the step. The recirculating flow region extends to a stagnation, or reattachment, point where there is no fluid velocity in the direction parallel to the bottom of the chamber. Downstream of the reattachment point, flow develops into a 1-dimensional flow profile with fluid velocity in the direction parallel to the bottom of the chamber.

2.6. Adhesion assays

HUVECs on coverslips were activated (inflamed) to upregulate E-selectin via addition of fresh culture media containing 1 ng/mL of IL-1β (Fitzgerald; Acton, MA) to cells in static for 4 h before attaching the coverslip to the bottom of the flow deck. Targeted particles in reconstituted blood at a fixed concentration of 5 × 10^5 particles/mL were then perfused through the flow channel at the specified flow conditions for a fixed period of time. The chamber was flushed with buffer (same flow condition as blood flow) at the end of experimental time and the HUVEC monolayer was

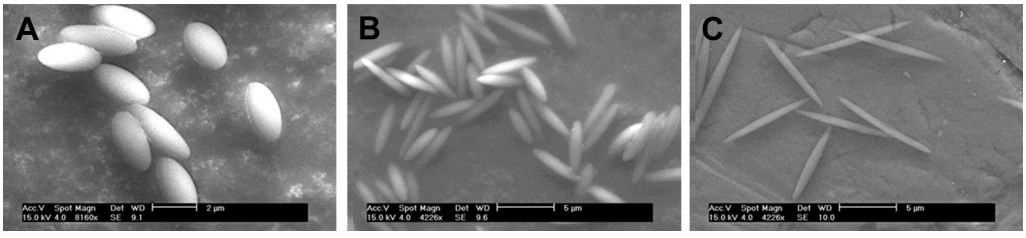

Fig. 1. SEM images of 2 μm ESD rods with AR-2 (A), AR-4 (B), and AR-9 (C). Scale bars = 2 μm for A and 5 μm for B and C.