Acta Biomaterialia 21 (2015) 99–108

Contents lists available at ScienceDirect

Acta Biomaterialia

journal homepage: www.elsevier.com/locate/actabiomat

Dense nanoparticles exhibit enhanced vascular wall targeting over neutrally buoyant nanoparticles in human blood flow

Alex J. Thompson [a], Omolola Eniola-Adefeso [a,b,*]

[a] Department of Chemical Engineering, University of Michigan, Ann Arbor, MI 48109, United States
[b] Department of Biomedical Engineering, University of Michigan, Ann Arbor, MI 48109, United States

ARTICLE INFO

Article history:
Received 28 November 2014
Received in revised form 9 March 2015
Accepted 6 April 2015
Available online 11 April 2015

Keywords:
Drug delivery
Hemodynamics
Inflammation
Nanoparticles
Vascular targeting

ABSTRACT

For vascular-targeting carrier (VTC) systems to be effective, carriers must be able to localize and adhere to the vascular wall at the target site. Research suggests that neutrally buoyant nanoparticles are limited by their inability to localize to the endothelium, making them sub-optimal as carriers. This study examines whether particle density can be exploited to improve the targeting (localization and adhesion) efficiency of nanospheres to the vasculature. Silica spheres with 500 nm diameter, which have a density roughly twice that of blood, exhibit improved adhesion to inflamed endothelium in an *in vitro* model of human vasculature compared to neutrally buoyant polystyrene spheres of the same size. Silica spheres also display better near-wall localization in the presence of red blood cells than they do in pure buffer, likely resulting in the observed improvement in adhesion. Titania spheres (4 times more dense than blood) adhere at levels higher than polystyrene, but only in conditions when gravity or centrifugal force acts in the direction of adhesion. In light of the wide array of materials proposed for use as carrier systems for drug delivery and diagnostics, particle density may be a useful tool for improving the targeting of diseased tissues.

1. Introduction

Improvements in the design of injectable carrier systems that actively target diseased tissues via the endothelium could potentially improve the diagnosis and treatment of a wide variety of human diseases, including cancer and atherosclerosis. The ability of the carrier to localize (or marginate) to the blood vessel wall is an important requisite for successful targeting to the endothelium. It has been established that particle size and geometry significantly affects the ability of a particle to marginate and adhere to the vascular wall in human blood flow [1–7]. However, it is quite possible that other particle characteristics, in addition to size, may affect the margination propensity of a carrier system. One such parameter is particle density, which can differ depending on the material makeup of the carrier system.

To date, materials with a range of densities have been proposed for use in drug delivery or diagnostics, including microbubbles [8,9], polymers [10–13], liposomes [14,15], inorganic particles (titania, gold, iron oxide) [16–18], blood cells [19,20], or combinations of multiple material types [16,21–29]. For instance, gas-filled microbubbles can have densities roughly 1% that of blood [30]. Liposomes are often neutrally buoyant, but can be fabricated with varied densities depending on the density of the medium encapsulated within the liposome [31,32]. Some common polymer-based particles which are biocompatible and FDA approved include poly(lactic acid), poly(glycolic acid), poly(methyl methacrylate), polycaprolactone, and PLGA. These polymeric particles tend to have densities either neutrally buoyant or slightly higher than blood [33]. Metal oxides, including silica and titania particles (2.0 and 3.9 g mL^{-1}, respectively, in this study) also have been proposed as potential carriers [33–35]. Particle types with even higher densities have been proposed for use as carriers as well, including iron oxide (~5 g mL^{-1}) and gold (~19 g mL^{-1}) nanoparticles. Also, combinations of different materials have been investigated which may have densities that fall between the densities of the individual materials. Such systems include liposomes or solid particles loaded with heavier materials such as gadolinium, magnetite, or gold; which provides benefits for imaging or hyperthermia therapy, for instance [16,24–29]. Despite the wide range of the VTC densities available, there is limited understanding of the role that density plays in prescribing carrier performance.

In order for a targeted delivery system to be effective, it should be able to marginate to the vascular wall effectively in blood flow. It is widely known that red blood cells (RBC) play a crucial role in

* Corresponding author at: Department of Chemical Engineering, University of Michigan, 2800 Plymouth Road, NCRC B28-G046E, Ann Arbor, MI 48109, United States.
E-mail address: lolaa@umich.edu (O. Eniola-Adefeso).

http://dx.doi.org/10.1016/j.actbio.2015.04.005
1742-7061/

the margination of leukocytes and platelets. By congregating in the center of flow, RBCs force white cells and platelets to concentrate in the "cell-free layer" (CFL) adjacent to the endothelium [36]. This effect aids in the margination and interaction of white cells and platelets with the vascular wall, and is in part due to the size of platelets (≈2–3 μm diameter) and white cells (8–12 μm diameter) [37]. Studies utilizing *in vitro* models of human vasculature have shown that neutrally-buoyant microspheres of sufficient size (⩾2 μm diameter) also can take advantage of this phenomenon [1–5,38,39]. However, previous experimental and computational works have reported that neutrally-buoyant particles with diameters in the 100–500 nm size range are not preferentially distributed to the vessel wall via this RBC effect. This inefficient margination results in sub-optimal targeting to endothelium in the presence of physiological levels of RBCs [3,5,38,39]. Still, particles in this size range are attractive as VTCs in that they are able to carry a larger payload than ultra-small nanoparticles while still being able to safely navigate microvasculature [40–47]. To date, it is unclear whether particle density affects the margination of nanoparticles or sub-micron sized particles to the CFL via interactions with RBCs in blood flow. While a recent study shows that increasing particle density negatively affects nanoparticle margination in a microchannel, this work was done in the absence of blood components and as such did not account for the critical step of particle margination to the CFL [31].

Particle density forces seemingly could be regarded as negligible in describing particle motion relevant to physiological blood flow, since the hydrodynamic forces due to flow (for microparticles) and Brownian forces (for nanoparticles) are often orders of magnitude higher than density-dependent body forces such as gravitational and centrifugal forces [48,49]. However, it is possible that particle density plays a role particle margination in the presence of RBCs, which will drastically affect targeting affinity from blood flow. Then, particles that have localized to the CFL will only need to travel laterally a short distance to initiate contact with the endothelium, since the CFL has a height on the order of a few micrometers [36]. Thus perhaps over the short distance of CFL thickness, density-dependent forces may be non-negligible.

Evidence exists in the literature that density may have a role on how efficiently platelets can interact with the endothelium in blood flow. Even in the small density range reported for sub-populations of platelets (1.040–1.080 g mL^{-1}), it was found that the most dense platelets exhibited a roughly 4 times higher initial adhesion than the least dense platelets [50]. Materials which are proposed for diagnostics and therapeutic delivery have a much wider range of particle densities, including bubbles with densities less than blood, neutrally and non-neutrally buoyant polymers or lipid-based particles, and more dense metal-based particles [51]. Therefore, it is of interest to investigate how the interaction between nanoparticles with red blood cells (RBC) affects margination when particle density is considered.

In this study, we investigate how the density-dependent forces (gravitational, centrifugal), density-independent forces (hydrodynamic, Brownian), momentum, and adhesion dynamics combined with the presence of blood components affect the targeting of 500 nm diameter nanospheres to inflamed endothelium from human blood flow in a parallel plate flow chamber (PPFC). This *in vitro* model of blood flow in a vessel is a useful tool to predict the *in vivo* targeting ability of a carrier system to a multitude of diseased states. In this work, we test the targeting ability of polystyrene, silica, and titania particles, which have densities of 1.05, 2.0, and 3.9 g mL^{-1}, respectively. Polystyrene is used as a model drug carrier with a density comparable to typically proposed polymeric systems (such as polycaprolactone or PMMA) or neutrally buoyant liposomes. The more dense silica and titania have been proposed themselves as potential delivery vehicles or diagnostics, but can possibly also be applied to loaded liposomes or polymers which are of the same density as pure silica or titania [31,33–35]. Particles are targeted to acute inflammation for relevance to many inflammatory diseases, including atherosclerosis and arthritis. We investigate how the gravity effects change the adhesion profile of targeted spheres by orienting the chamber such that the direction of margination is either toward or away from gravity. Using a channel with a step expansion, we examine whether centrifugal force due to an induced recirculating flow region allows particles with higher densities to better target the endothelium compared to neutrally-buoyant particles.

2. Materials and methods

2.1. Preparation of vascular-targeted spheres

Fluorescent carboxylated polystyrene spheres were purchased from Polysciences, Inc (Warrington PA). Fluorescent carboxylated silica spheres were purchased from Corpuscular, Inc (Cold Spring, NY). Amine-terminated titania spheres were purchased from EPRUI Nanoparticles & Microparticles Co Ltd (Nanjing, China). All particles had an approximate diameter of 500 nm. The size and zeta potential of each of the particle types used are given in Table S1. Biotinylated sialyl Lewis A was purchased from Glycotech, Inc (Gaithersburg, MD). Biotin-phycoerythrin conjugate was purchased from Life Technologies (Grand Island, NY). Anti-cutaneous lymphocyte antigen – phycoerythrin conjugate was purchased from Miltenyi Biotec (San Diego, CA).

Carboxylated particles were conjugated first with Neutravidin Biotin Binding protein (Thermo Scientific) via a carbodiimide intermediate using EDAC (Thermo Scientific) as previously described [52]. Aminated particles were first conjugated with NHS-biotin, followed by conjugation with Neutravidin Biotin Binding protein overnight as with carboxylated particles. On the day of an adhesion experiment, Neutravidin conjugated particles were then conjugated with biotinylated sialyl Lewis A (sLe^a) so that the surface density of approximately 7000 sites μm^{-2} (Table S1). Particles were then soaked for an additional 45 mins with biotin FITC to aid in post-experiment imaging. For localization experiments, particles were saturated with biotin-phycoerythrin in order to aide with imaging and prevent non-specific protein adhesion to the microchannel. Conjugation of targeting ligand to the particle surface was confirmed and quantified using an Attune® Acoustic Focusing Flow Cytometer after staining with anti-cutaneous lymphocyte antigen – phycoerythrin.

2.2. HUVEC culture

Primary HUVEC were isolated from fresh umbilical cords following a modified Jaffe protocol and cultured in tissue culture flasks as previously described by Huang et al. [53]. Umbilical cords were generously donated by Mott Children's Hospital following an Internal Review Board exempt protocol. Briefly, the umbilical veins were filled with a collagenase solution for 30 min, degrading the extracellular matrix to release the HUVEC. For adhesion assays, HUVEC were cultured onto glass coverslips, which were coated with porcine gelatin and cross-linked with glutaraldehyde before seeding.

2.3. Blood collection and treatment

Blood was obtained from healthy human donors via a 60 mL syringe containing citrate anticoagulant (acetate-citrate-dextrose, ACD) according to a protocol approved via the University of Michigan IRB and in line with standards set by the Helsinki