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Characterization of nanoparticle delivery in microcirculation using a microfluidic device

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ABSTRACT

This work focuses on the characterization of particle delivery in microcirculation through a microfluidic device. In microvasculature the vessel size is comparable to that of red blood cells (RBCs) and the existence of blood cells largely influences the dispersion and binding distribution of drug loaded particles. The geometry of the microvasculature leads to non-uniform particle distribution and affects the particle binding characteristics. We perform an *in vitro* study in a microfluidic chip with micro vessel mimicking channels having a rectangular cross section. Various factors that influence particle distribution and delivery such as the vessel geometry, shear rate, blood cells, particle size, particle antibody density are considered in this study. Around 10% higher particle binding density is observed at bifurcation regions of the mimetic microvasculature geometry compared to straight regions. Particle binding density is found to decrease with increased shear rates. RBCs enhance particle binding for both 210 nm and 2 μm particles for shear rates between $200-1600\ s^{-1}$ studied. The particle binding density increases about 2 − 3 times and 6 − 10 times when flowing in whole blood at 25% RBC concentration compared to the pure particle case, for 210 nm and 2 μm particles respectively. With RBCs, the binding enhancement is more significant for 2 μm particles than that for 210 nm particles, which indicates an enhanced size dependent exclusion of 2 μm particles from the channel centre to the cell free layer (CFL). Increased particle antibody coating density leads to higher particle binding density for both 210 nm and 2 μm particles.

Introduction

Various techniques in targeted drug delivery have been developed in recent years to reduce side effects, toxicity, and drug dosage (Langer, 1998). The use of particles as drug carrier helps in targeted delivery and release of drugs at disease region, serving the dual role of diagnosis and therapy (Packhaeuser et al., 2004; Singh and Lillard, 2009). Nanopaticles (NPs) in the form of liposomes, dendrimers, micelles and polymers, as well as the more conventional and inorganic carbon, silica, iron and gold NPs are being widely used as drug carriers (Cho et al., 2008). The uptake efficacy of NP based drug carriers is higher compared to their larger micron scale counterparts, which are easily cleared off by the human mononuclear phagocyte system. NPs also have larger surface to volume ratio (Light et al., 2010), which enhances their targeting capabilities. Thus, NP based drug delivery systems have a great potential to achieve efficient targeting of cells and molecules in inflammation and cancer conditions (Suri et al., 2007). In this section, challenges of drug delivery in microcirculation, influence of red blood cells, vessel geometry effect and target selection will be discussed respectively.

Current challenges in the study of drug delivery and distribution

Recent theoretical modelling works demonstrated decreased particle adhesion probability with increased flow rate (Liu et al., 2012; Shah et al., 2011; Tan et al., 2013a). Due to bioethical regulations and complex physiological conditions, it is challenging to quantify the particle delivery process *in vivo*. Most of the current studies are carried out in flow chambers or channels (Haun and Hammer, 2008; Kona et al., 2012) and these result are applicable to large blood vessels but not to microvasculature. Microvasculature refers to part of the circulatory system consisting of capillaries, arterioles, and venules. Microvasculature parameters such as vascular geometry, target-receptor expression levels and flow shear rate must be considered while performing *in vitro* tests. Study on specific receptor mediated binding of nano drug carriers under various physiologically relevant conditions help in understanding the methodologies to enhance targeted delivery efficacy and provides a tool to determine the actual drug bioavailability.

Distribution of drug carriers under the influence of RBC

Blood is a complex bio-fluid consisting of RBCs, monocytes, platelets, proteins etc. Blood flow in microvasculature is a two-phase flow as the vessel diameter becomes comparable to the size of RBCs. *In vitro* studies

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on RBC mediated particle delivery have to consider various *in vivo* microvasculature parameters, such as Fåhraeus–Lindqvist effect (Fåhræus and Lindqvist, 1931), Segre–Silberberg effect (Segré and Silberberg, 1962; Yang et al., 2005), CFL formation (Goldsmith, 1986; Kim et al., 2009; McHedlishvili and Maeda, 2001), vessel geometry/bifurcations (Pries et al., 1996) and blunt velocity profile (Gaehtgens et al., 1970; Reinke et al., 1986; Rosenblum, 1972; Sugii et al., 2002).

RBCs have a biconcave shape of ~8 μm diameter and ~2 μm thickness, and are highly deformable (McHedlishvili and Maeda, 2001; Popel and Johnson, 2005). The flexible RBCs migrate radially towards the centre region in microvessels based on various hemorheology factors such as shear rate, viscosity, hematocrit concentration, RBC aggregation and deformability. This result in a RBC concentrated core region and a cell-free plasma layer near the vascular wall called CFL (Goldsmith, 1986; Ji et al., 2007; Popel and Johnson, 2005). Particles flowing along with RBCs can diffuse towards these CFL and this will influence their distribution and binding dynamics across a channel (Aarts et al., 1984; Goldsmith and Mason, 1962; Nanne et al., 2010).

The deformable RBCs aggregate to form a fast moving core at the centre of the channel while the stiffer cells and particles marginate to the near wall CFL region of the microvessel. This localization of particles closer to the vessel wall would increase the particle density in the CFL region. The targeted binding of drug carriers to diseased cells would be enhanced by this process. In this work we consider the influence of RBCs on 210 nm and 2 μm particle distribution.

Influence of vessel geometry in drug carrier distribution

Human circulatory system consists of large blood vessels such as arteries and veins (~15-0.5 mm), and smaller vessels such as arterioles, venules (100–500 μm) and capillaries (~10 μm). The distribution of drug particles in a real vascular network having hierarchical geometry will depend on local shear rate, flow velocity, pressure and volume (Mayrovitz et al., 1977). Our study considers the distribution of nano and micron sized particles in a branching channel that mimics the geometry and flow conditions of a dividing vascular network. A comparison of particle binding density between the branching and the straight channel geometry is performed to examine non-uniform distribution of particles at vessel bifurcation with and without RBCs. In order to study the effect of a difference in flow velocity distribution between daughter channels on particle binding, we conducted flow tests on channel geometries that would produce asymmetric flow rate distribution in the two daughter channels.

Specificity in drug carrier targeting

Specificity in targeting is introduced by applying ligand-receptor chemistry in the microfluidic platform. The biomimetic chip is coated with intercellular adhesion molecule 1 (ICAM-1) protein, a cell-surface glycoprotein member of the Ig super-family. Under inflammatory conditions, interaction between endothelial cells and blood constituents occur by the up-regulation of intercellular adhesion molecules such as ICAM-1 on the surface of endothelial cells and leukocyte (Gimbrone et al., 1997; Zhai et al., 1998). This mediates the targeted migration of leukocytes into specific areas of inflammation (Min et al., 2005). Various ICAM-1 based therapeutic agents for cancer immunotherapy and other modes of treatment are used nowadays (Kanwar et al., 2003; Tanaka et al., 2002). Here anti-ICAM-1 coated 210 nm and 2 μm particles are used as a model system to study the influence of RBCs and vascular geometry on particle delivery.

This work studies binding distribution of anti-ICAM-1 coated particles on ICAM-1 protein coated microfluidic platform. Influence of particle size (210 nm and 2 μm), shear rates, vessel geometries (straight and branched channels) and RBCs are examined. Microfluidic device fabrication, protein coating (on channels and particles) and their characterization, and testing are described in methods section. Results and discussions under aforementioned conditions are given thereafter. Finally, the conclusion and future work are presented.

Materials and methods

Materials

Human ICAM-1/CD54 MAb (Clone BBIG-I1) Mouse IgG1, ICAM-1/human IgG1 Fc chimera, biotinylated anti-human ICAM-1 (clone BBIG, mouse IgG1 κ) and Normal Goat IgG biotinylated control was purchased from R&D Systems, Minneapolis, MN). Protein G was bought from Biovision, Milpitas, CA. Horseradish peroxidase (HRP)-conjugated rat anti-mouse κ-light chain monoclonal antibody, neutrAvidin coated fluorescent yellow-green polystyrene 210 nm particles, Block-Aid, Amplex Ultra Red reagent and biotinylated-HRP was purchased from Invitrogen Carlsbad, CA. Mouse anti-human ICAM-1 monoclonal IgG1 antibody (clone 15.2) was got from Ancell, Bayport, MN and HRP-conjugated rat anti-mouse IgG1 monoclonal antibody from BD Biosciences San Jose, CA. Bovine serum albumin (BSA), 3-aminopropyltrimethoxysilane was bought from Sigma Aldrich, St Louis, MO. Streptavidin coated 2 μm fluorescent green polystyrene particles was purchased from Bangs Laboratories Inc., IN and polydimethylsiloxane from Dow Corning, Midland MI. Single donor human whole blood and plasma was bought from Innovative Research Inc.

Fabrication of microfluidic device

The required microfluidic design is photo-lithographically patterned on a silicon wafer using SU-8 2050 photoresist. Microfluidic devices are fabricated using Sylgard 184 PDMS. PDMS base is mixed with its cure at 1:10 (v/v) ratio following the basic techniques in soft lithography (McDonald and Whitesides, 2002). The PDMS mixture is poured on the silicon wafer, which acts as the master template. The hard PDMS layer is peeled out after baking and the features are inspected. The microfluidic device is made by binding the PDMS pattern on a clean glass slide after exposing them to oxygen (O_2) plasma. The flow channels are 100 μm wide and 100 μm in height. Both straight and bifurcating features are included in the design. The bifurcating channels have a branching angle of 60°.

ICAM-1 functionalized PDMS substrate

PDMS devices were coated with ICAM-1 as previously reported (Haun and Hammer, 2008). After binding with glass the PDMS microfluidic devices were silanized with 3-aminopropyltrimethoxysilane to improve protein adsorption. After washing the device with an adsorption buffer (0.1 M NaHCO3, pH 9.2), the devices were incubated for 2 hrs at room temperature with saturating concentration of protein G in adsorption buffer (100 μg/ml). ICAM-1 Fc chimera protein solution in PBS (100 nM) was introduced after washing the substrates three times with PBS to remove any excess protein G. The devices were incubated with ICAM-1 for 1 h at room temperature. The microfluidic chips were purged with 1% BSA containing 0.05% Tween20 one hour prior to testing.

Substrate ICAM-1 density characterization

In order to quantify the ICAM-1 protein density on the PDMS surface, ELISA was performed using an HRP-conjugated anti mouse IgG1 antibody. ELISA reaction was carried out in a 12-well plate and PDMS coated 22 mm circular glasses were used. The technique for substrate ICAM-1 characterization was performed as reported in literature (Haun and Hammer, 2008).

Silicone isolators (Grace Bio Labs) were used to assure that the same volume of reagents were applied on PDMS coated glass as in the microfluidic device, to maintain the same surface area to volume ratio.