Introduction: The development of vascular-targeted carriers (VTC) for the localized delivery of therapeutics could greatly improve the treatment of many human diseases. In order for VTCs to be effective, they must be able to **marginate** (localize to the vessel wall) and adhere to diseased endothelium from blood flow. Recent research has shown that particle size and shape affect particle margination in the presence of red blood cells (RBCs).[1,2,3,4] Microspheres with diameter ≥ 2 μm have been shown to undergo a forced localization to the cell free layer (CFL); analogous to the mechanism by which RBCs force leukocytes and platelets to the CFL, allowing them to effectively interact with the endothelium. Sub-micron sized spherical particles (200-1000 nm ESD) which are neutrally buoyant do not benefit from this effect, therefore making them suboptimal for use as VTCs. However, sub-micron sized particles are still of interest as potential VTCs for their ease in navigating the vasculature while still carrying a significantly higher drug load compared to nanoparticles. One area of this field which has yet to be explored is how **particle density** affects the **margination and adhesion** of targeted sub-micron particles to the endothelium in the presence of blood flow.

The purpose of this study is to examine how the density of submicron-sized particles affects their margination to the CFL and adhesion to inflamed endothelium *in vitro* from human blood flow.

Materials and Methods: Spherical sub-micron sized particles of three different materials (Polystyrene-PS, Silica-Si, Titania-TiO2) with different densities (1.05, 2.0, 3.9 g/cm^3, respectively) but equivalent diameters (500 nm) were conjugated with targeting ligands (sialyl Lewis a, sLe^a) which bind to selectins at a fixed surface density. Particles were also conjugated with a fluorescent tag for imaging purposes. An *in vitro* blood flow assay using a parallel plate flow chamber (PPFC) was used to quantify the **adhesion efficiency** of these particles to inflamed endothelium from blood flow at varied shear rates and flow profiles. Also, a microchannel setup was used to test the **margination efficiency** of particles with different densities to the CFL in blood flow.

Selectin-targeted spheres at a fixed concentration were added to a mixture of RBCs from human donors and phosphate buffer (with viscosity adjusted to that of human plasma). The blood/particle mixture was allowed to flow over a layer of IL-1β activated human umbilical vein endothelial cells (HUVEC) lining one wall of a parallel plate flow chamber. Adhesion of VTCs from laminar, steady blood flow from a range of wall shear rates (200-1000 s^{-1}) was investigated. Channel orientation was either upright (gravity acts towards the HUVEC) or inverted (gravity acts away from HUVEC), in order to investigate the effect that gravity has on adhesion of particles with densities greater than the fluid. Particle adhesion in "recirculation" blood flow was also investigated, to see if an induced eddy in the flow chamber altered the adhesion profile of particles with different densities. This is done using a modified PPFC, in which a sudden expansion in the PPFC causes a region of recirculating flow to occur in the channel immediately downstream of the expansion. The number of adherent particles under various flow conditions was imaged/quantified using brightfield and fluorescence microscopy.

The margination efficiency of particles with different densities was investigated using confocal microscopy. The particle/blood mixture was flowed through a microchannel, which was placed

References:
1. Thompson et al. **Biomaterials** (2013).
2. Namdee et al. **Langmuir** (2013) *29* (8), 2530-2535.
3. Charoenphol et al. **Atherosclerosis** (2011) vol 217, 364-370.
4. Charoenphol et al. **Biomaterials** (2010) vol 31, 1392-1402.

under 20x magnification on an upright confocal microscope. The focal plane was set in the CFL at the top of the microchannel, and number of fluorescent particles present in the CFL was imaged during blood flow.

Results and Discussion: Results from *in vitro* laminar flow assays show that when the chamber is in the upright orientation (HUVEC on the bottom of the chamber), spheres with higher density (Si and TiO2) adhere at roughly 3 times higher levels than PS spheres; despite having the same number of targeting ligands attached on their surface. When the chamber is inverted, Si adhere roughly 3 times higher than PS spheres, while TiO2 adhere at equal or slightly higher levels than PS spheres. PS and Si spheres show no dependence on chamber orientation, while TiO2 spheres (the most dense) show a decrease in adhesion when the chamber is inverted.

Initial results from margination experiments using only PS and Si spheres suggest that even when the particles are marginating against the direction of gravity, Si spheres marginate at higher levels than PS spheres. This result is interesting because although gravity effects would act to bring Si particles downward (away from the CFL), we actually find more Si particles in the CFL – suggesting a potential effect due to a density-related separation of particles from RBCs. Ongoing work in this area will include margination experiments with the TiO2 microspheres.

In recirculation flow, initial results show that at a shear rate of 200 s^{-1}, Si microparticles adhere at higher levels than PS microparticles whether the chamber is oriented with the HUVEC monolayer at the top or the bottom. It is possible that centrifugal force caused by the recirculation region helps Si redistribute to the CFL in this disturbed flow, resulting in an improved adhesion in this area. This finding could have implication in targeting atherosclerosis, which is known to occur in areas of the vasculature where this type of disturbed flow is found. Ongoing work in this area will include recirculation experiments with the TiO2 microspheres and at higher shear rates.

Ongoing work in this project includes investigating the margination smaller (20-200 nm) and more dense (FeO, Au, etc) particles with the goal of identifying particle characteristics which would make them suitable for targeting specific areas of the vasculature.

Conclusions: Our study shows that particle density can affect the margination/adhesion of vascular-targeted carriers, and thus should be considered when designing carriers to target specific diseased areas via the vasculature. This particle density effect could potentially have importance in targeting atherosclerosis, which occurs in areas of the vasculature that exhibit unique and complex hemodynamics.

References:
1. Thompson et al. **Biomaterials** (2013).
2. Namdee et al. **Langmuir** (2013) *29* (8), 2530-2535.
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