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Exploring deformable particles in vascular-targeted drug delivery: Softer is only sometimes better

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ABSTRACT

The ability of vascular-targeted drug carriers (VTCs) to localize and bind to a targeted, diseased endothelium determines their overall clinical utility. Here, we investigate how particle modulus and size determine adhesion of VTCs to the vascular wall under physiological blood flow conditions. In general, deformable microparticles (MPs) outperformed nanoparticles (NPs) in all experimental conditions tested. Our results indicate that MP modulus enhances particle adhesion in a shear-dependent manner. In low shear human blood flow profiles *in vitro*, low modulus particles showed favorable adhesion, while at high shear, rigid particles showed superior adhesion. This was confirmed *in vivo* by studying particle adhesion under venous shear profiles in a mouse model of mesenteric inflammation, where MP adhesion was 127% greater ($p < 0.0001$) for low modulus particles compared to more rigid ones. Mechanistically, we establish that particle collisions with leukocytes drive these trends, rather than differences in particle deformation, localization, or detachment. Overall, this work demonstrates the importance of VTC modulus as a design parameter for enhanced VTC interaction with vascular walls, and thus, contributes important knowledge for development of successful clinical theranostics with applications for many diseases.

1. Introduction

Vascular-targeted drug carriers (VTCs) are particles designed to exploit differential surface protein expression in diseases involving the endothelium, for an increase in drug localization and corresponding decrease in off-target toxicity. The development and clinical approval of VTC systems could revolutionize the treatment of many common but therapeutically challenging diseases, including cancer and cardiovascular diseases. Recent consideration of VTC physical properties, including size, shape, and surface charge [1–5], has improved historically low targeted drug delivery efficiency. More recently, researchers have also explored the impact of particle stiffness, demonstrating that lower modulus particles have increased circulation time *in vivo*, [6–9] attributed to both a slower rate of particle phagocytosis and decreased mechanical filtration in the kidney and spleen [10–12]. The work presented here integrates these two lines of research to present a comprehensive study of the critical role of particle modulus on VTC targeting efficiency.

As VTCs traverse the vasculature, they must navigate complex blood flow to contact the endothelium, otherwise all targeting functionalities are negated. In blood flow, cells segregate into a concentrated red blood cell (RBC) core in the center, while white blood cells (WBCs) and platelets are excluded to the RBC- free layer (RBC-FL) to interact with endothelial cells (ECs). Hydrodynamic, heterogeneous collisions between blood components drive this segregation in flow [13–16]. The differences in cellular size and stiffness are often used to explain WBC and platelet margination,

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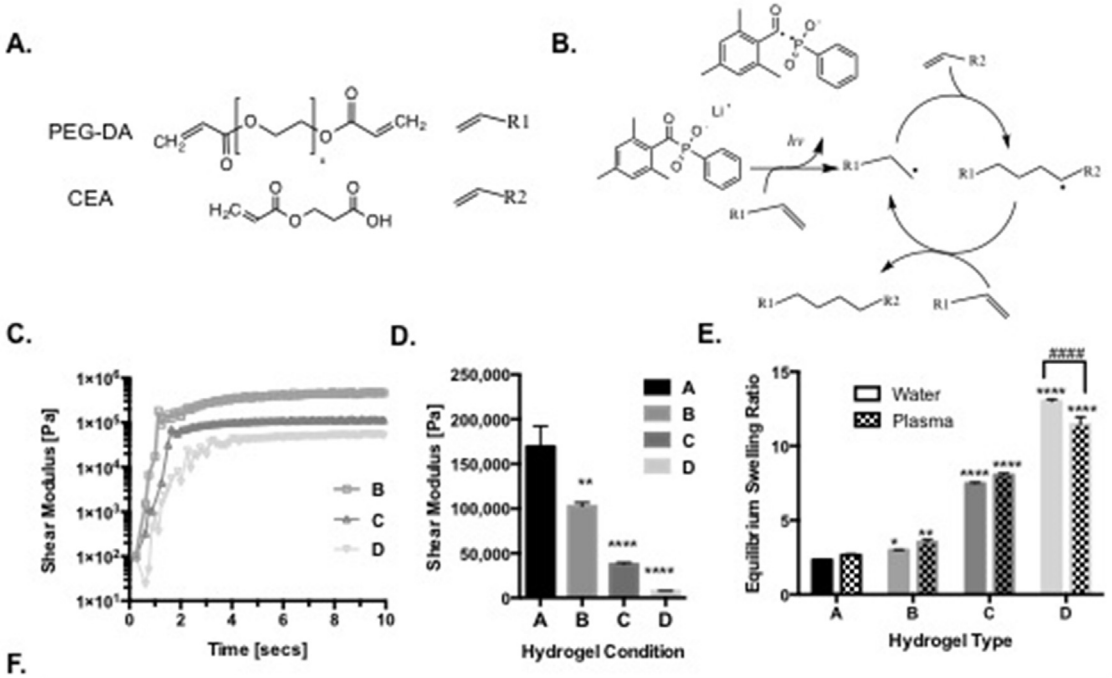
which, by definition, is the localization to ECs from bulk blood flow. RBCs have a Young's modulus of 26 ± 7 kPa; WBCs are an order of magnitude stiffer than RBCs and platelets are 2–5 times more rigid than RBCs [17–20]. It is vital that VTCs mimic this cellular margination process to interact with the designated endothelium and target effectively *in vivo.* One way to achieve this is to control VTC size. Previous work has shown that optimal VTC size depends on the physical blood properties [21,22]; in human blood, 3 μm is the ideal diameter for spherical, rigid particles to maximally marginate to ECs as determined in *in vitro* flow assays [23,24]. Furthermore, rigid nanoparticles (NPs) demonstrate inefficient margination compared to microparticles (MPs) due to decreased localization to the vessel wall, resulting in low adhesion overall to targeted ECs [23,25]. Unfortunately, rigid MP VTCs may cause deleterious capillary occlusions and are impractical for clinical use. Thus, there is strong motivation to explore a modulus range of both MPs and NPs, with interest to circumvent possible capillary occlusions by MPs and to improve the poor margination dynamics of rigid NPs. Recent studies with varying modulus particles focus on the impact of particle modulus on *in vivo* circulation time, which does not necessarily translate to targeted adhesion [7]. There is a lack of research directly comparing an entire range of particle sizes and physiologically relevant VTC moduli for vascular targeting; there is a critical need for well-designed experiments to fill this gap.

To address this deficiency in the research field, we explore two sizes of four polyethylene glycol (PEG)-based VTCs with a range of Young's moduli, spanning an order and a half of magnitude, including one modulus similar to that of WBCs and one to that of RBCs. We explore how particle modulus and size collectively dictate VTC efficacy based on a range of physiological blood flow conditions, by using particles fabricated with well-controlled surface ligand properties. Our data demonstrate that VTCs cannot be designed as "one size fits all", but rather, require the deliberate selection of VTC modulus based on known hemodynamics of the disease state. Importantly, this is the first study to report how VTC modulus directly affects the final, targeted adhesion of particles both *in vitro* and *in vivo,* providing an exciting and unique perspective on VTC engineering with potential to influence future VTC system development.

2. Results

2.1. Characterization of PEG-based hydrogel particles

We systemically varied fabrication conditions to synthesize hydrogel particles of varying modulus, as detailed in Fig. 1. Reaction conditions for the PEG hydrogels are detailed in the Supplemental Information; we decreased the moduli from A to D by decreasing



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Type	% Solids	% MeOH	% PEGDA	% CEA	% PI	Density (kg/m^3)	Pore Size (nm)[27]	Young's Modulus (Pa)
A	50 %	50 %	89	10	1	1051	0.376	508,300
B	40 %	60 %	89	10	1	1039	0.527	307,700
C	20 %	80 %	87	10	3	1017	1.318	113,000
D	15 %	85 %	86	10	4	1007	3.539	23,000

Fig. 1. Hydrogel material properties. (A) Designation of chemical moieties for synthetic scheme. (B) Synthetic scheme for lithium phenyl-2,4,6-trimethylbenzoylphosphinate photoinitiated polymerization of (poly)ethylene glycol diacrylate (PEGDA) and 2-carboxylethyl acrylate (CEA) as described in more detail in the SI. (C) *In situ* rheometry of particle conditions B, C, and D. Condition A too rigid to be tested *in situ*. (D) Swollen shear moduli of particle conditions A-D, statistics displayed represent comparison to A. (E) Equilibrium swelling ratios of particle conditions A-D, where (*s) indicate significance within hydrogel types to A and (#s) indicate difference between water and plasma. (F) Synthesis compositions and calculated bulk material properties of hydrogels [27]. Statistical analyses were performed using one- and two-way ANOVA with Fisher's LSD test, where (*) indicates $p < 0.05$, (**) indicates $p < 0.01$, and (***) indicates $p < 0.001$, (****) indicates $p < 0.0–01$ and ($^{\#\#\#\#}$) indicates $p < 0.0001$. Error bars represent standard error.