Stiffness-dependent toxicity of platelet-like particles

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Statement of Purpose: Transfusion of human platelets remains the gold standard of treatment for patients with thrombocytopenia, but regular administration of human platelets is plagued by limited availability, contamination, and immunogenicity concerns, demonstrating the unmet need for a synthetic platelet product.[1] We have previously reported on the development of a platelet-like particle (PLP) capable of recapitulating several platelet behaviors, such as fibrin specificity and clot contraction. The particle consists of a poly(N-isopropylacrylamide-co-acrylic acid) (pNIPAm-AAc) microgel core functionalized with a fibrin-specific single-domain antibody fragment (sdFv). PLPs have been shown to significantly reduce bleeding time in an *in vivo* rat femoral vein injury model, as well as enhance clot formation and decrease clot degradation in several *in vitro* systems.[2] Notably, the pNIPAm-AAc core can be synthesized in a crosslinker-free reaction, yielding ultra-low crosslinked (ULC) microgels, or microgels can be crosslinked with N,N'-Methylenebisacrylamide (BIS), resulting in stiffer microgels. Preliminary experiments testing varying crosslinking conditions in animal models revealed apparent lung toxicity for microgels of high crosslink density, motivating the hypothesis that crosslinked microgels induce endothelial damage and pulmonary inflammation.

Methods: Microgel stiffness was measured using atomic force microscopy (AFM) nanoindentation on single microgels swollen in deionized water, phosphate-buffered saline (PBS), or human blood plasma. For microfluidic experiments, branching microfluidic devices were generated with PDMS to model the architecture of lung microvasculature. Depending on the experiment, some devices were coated with endothelial cells and cultured to form a monolayer inside the channels prior to running samples. Microgel particles of varying crosslink density were labeled with Cy3b NHS Ester (GE LifeSciences, UK) and mixed with whole blood at 1 mg/mL, then flowed through the device at physiologic shear using a syringe pump (Harvard Apparatus, MA). For static aggregation studies, microgels were labeled with Cy3b NHS Ester, incubated with either PBS or human blood plasma, and spotted on glass coverslips for confocal imaging and analysis. For animal studies, microgels were delivered to wild type mice via tail vein injection at a dose of 50 mg/kg. At a specified time point, mice were sacrificed and lungs were harvested, sectioned, and stained with H&E, Martius Scarlet Blue, or IHC for macrophages (F4/80) or neutrophils (Ly-6G).

Results: As expected, increasing crosslink density increases the Young's modulus of pNIPAM-AAc microgels. In human plasma, ULC microgels exhibited a modulus of 5.63±1.40 kPa, as compared with 8.26±1.99 kPa for 2% BIS-crosslinked microgels and 22.64±10.74 kPa for 8% BIS-crosslinked microgels. In static experiments, BIS-crosslinked microgels swollen with plasma were found to form large aggregates (greater than 100 μm^2 in cross sectional area) more frequently than ULC microgels. Interestingly, this effect was mitigated when microgels were swollen with PBS (Fig. 1).

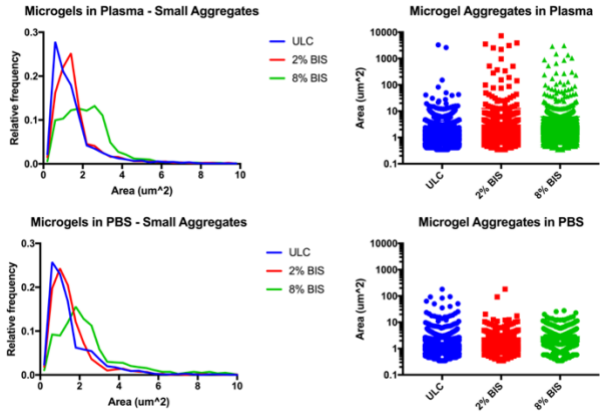

Figure 1: Microgel aggregates in PBS and blood plasma

Microfluidic experiments modeling lung microcirculation with physiologic shear demonstrated that these aggregates are large and durable enough to occlude small blood vessels. In animal studies, higher crosslink density was associated with denser lung tissue (Fig. 2), greater inflammation, and increased neutrophil infiltration.

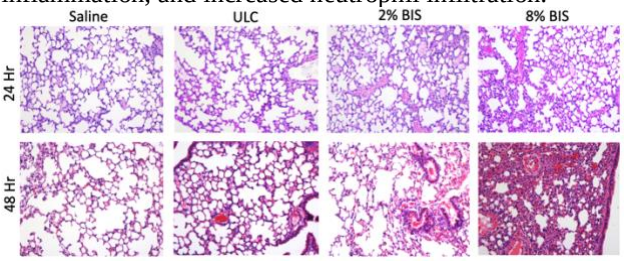

Figure 2: H&E staining of mouse lung sections, 20X

Conclusions: Crosslink density of deformable pNIPAM-AAc microgels appears to correlate with the formation of aggregates in the presence of blood plasma, suggesting that aggregate formation is dependent on changes in the protein corona around microgels. These aggregates can occlude microvessels in the lung and lead to lung inflammation and toxicity, perhaps through endothelial disruption. Future work will elucidate the immunological processes by which microgel aggregates cause lung toxicity, and clarify the changes in protein corona that lead to aggregate formation. This work has broader impacts to those designing deformable microparticle systems for drug delivery or biosensor applications.

References:

1. Kiefel V et al. Transfus Med Hemother. 2008; 35:354-358.
2. Brown AC et al. Nat Mater. 2014; 13:1108-1114.