Gold Nanoparticle Microgel Composites with Biomimetic and Antimicrobial Properties

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Statement of Purpose: Traumatic hemorrhage remains a significant clinical problem despite decades of research[1]. Subsequent wound repair can be impeded by several complicating factors including infection. Thus, there is a need to develop improved methods for achieving hemostasis and wound healing following trauma. Clot formation is an essential first step for achieving cessation of bleeding and involves the formation of a platelet plug embedded within a fibrin mesh. Overtime, platelets contract the fibrin clot, which stabilizes the network and contributes to enhanced wound healing outcomes. Recently developed platelet-like-particles (PLPs) have been shown to recapitulate key functions of endogenous platelets, including augmentation of clotting of adult plasma *in vitro*, decreased bleeding times *in vivo* in rodent models of traumatic injury, specific homing to injury sites, and clot retraction[2]. These PLPs are created from highly deformable microgels, which are conjugated to fibrin-specific target single domain variable fragment antibodies (sdFvs)[2]. Here we aim to combine PLPs with antimicrobial agents to improve healing outcomes following hemostasis. Previous studies demonstrated that microgel deformability, in conjunction with high fibrin affinity are critical to obtaining PLP-mediated clot retraction. Therefore, the primary objective of these studies was to develop antimicrobial gold nanoparticle-microgel composites that inhibit bacterial growth while maintaining microgel deformability.

Methods: Ultra-low crosslinked microgels (ULCs) were synthesized with N-isopropylacrylamide (NIPAM) containing 5% acrylic acid in a precipitation polymerization reaction. Two methods of gold incorporation were performed to create gold-microgel composites. The first method employed lyophilized ULCs that were rehydrated with a concentrated aqueous suspension containing gold nanospheres (5, 50 or 100 nm diameter). The second method, a modification from Chen et al.[3] synthesized gold-microgel composites through THPC-mediated reduction of $HAuCl_4 \cdot 3H_2O$ to form small Au NPs (size <3 nm) which were then grown in size (~150 nm) using hydroxylamine and Au^{3+} solution. Gold-microgel composite size was analyzed with dynamic light scattering (DLS). To characterize gold nanosphere distribution within the microgels, Transmission Electron Microscopy (TEM) was employed. Antimicrobial assays were performed to assess the ability to inhibit bacterial growth both on agar (Kirby Bauer assay) and in a liquid broth culture. Particle deformability was determined with atomic force microscopy (AFM) by analyzing spreading on a glass surface.

Results: DLS revealed an increase in diameter in microgels containing covalently bound gold and 100 nm gold nanospheres compared to ULCs alone. TEM imaging exhibited a homogenous distribution of gold nanospheres within ULC microgels in both gold incorporation methods (Figure 1). The antimicrobial effect of gold incorporation was demonstrated in the Kirby Bauer assay through a zone of bacterial growth inhibition in response to gold-microgel composites. Also, in liquid broth cultures demonstrated a reduction in E. coli growth compared to ULCs alone. The degree of bacterial growth inhibition is positively correlated with size of incorporated gold nanoparticles, with larger gold nanospheres being the most antimicrobial. AFM characterization of particle deformability demonstrated ULCs retained a high degree of spreading following gold incorporation.

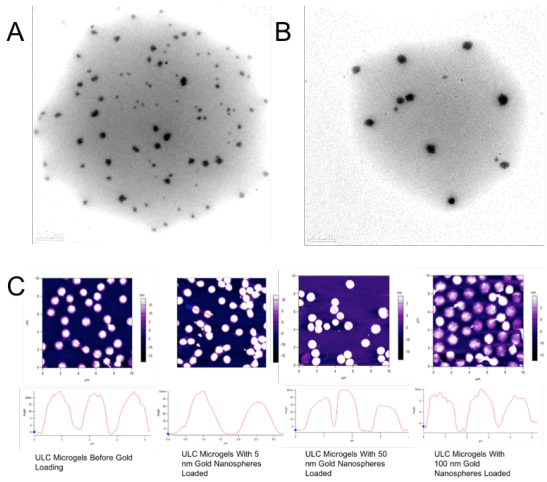

Figure 1. TEM images of gold incorporation into ULC microgels via swelling in aqueous gold nanospheres (A) and via direct growth of gold nanoparticles (B) reveal a homogenous distribution of gold throughout the ULCs (scale bar = 0.5 µm). AFM characterization of particle deformability on a glass surface following gold nanoparticle incorporation demonstrated a similar degree of particle spreading as ULCs alone (C).

Conclusions: Using the swelling method of gold incorporation, we are able to load 5, 50, and 100 nm gold nanospheres into ULC microgels. We also successfully fabricate microgels containing covalently coupled gold nanospheres through THPC-mediated reduction and subsequent gold nanoparticle growth. These gold-microgel composites demonstrate antimicrobial potential and maintain deformability following gold loading, which is essential to PLP-mediated clot retraction. Therefore, gold-microgel composites are a promising material for the development of PLPs to prevent infection and hemorrhage following trauma.

References: [1]Champion HR. J Trauma. 2003; 54: S13-9; [2]Brown AC. Nat Mater 2014; 13: 1108-14; [3]Chen LY. J Am Soc Mass Spectrom. 2014 Nov;25(11):1944-52