Harnessing the Relaxation of Fibrin-microgel Assemblies for Control of Cell Spreading and Motility

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Statement of Purpose: Approaches for controlling bleeding, binding tissues, and inducing tissue regeneration are significant needs in surgery, trauma, and emergency response medicine. Current fibrin matrices used for wound healing applications utilize high concentrations of fibrinogen and thrombin, forming a dense matrix in order to form a stable clot. These tend to hinder cell infiltration *in vitro,* [1] and *in vivo* studies of fibrin-based sealants have demonstrated that dense and low porosity structures impede cell invasion and delay wound healing [2]. The overall goal of this work is to design materials to be incorporated into fibrin scaffolds that will create a mechanically robust clot, but allow for increased cell infiltration and regeneration. We investigated the use of ultra-low crosslinked (ULC) poly(N-isopropylacrylamide) pNIPAm microgels (μgels), which are hydrogel microparticles on the order of 1 μm in diameter [3]. Through the incorporation of ULC μgels into fibrin matrices, we hypothesized that we could alter network architecture, mechanical properties, cell spreading (Figure 1) and cell motility allowing for greater cell infiltration. Our preliminary data demonstrates the ability for μgels to partition into discrete domains within the fibrin gel, forming pores or μgel 'pockets'. Additionally, the storage modulus (elasticity) of fibrin is maintained by recapitulating the characteristic fibrin mesh length scale within the pockets through the use of μgels of a similar size. In developing these materials, we have exploited properties of ULC μgels, namely softness or deformability.

Methods: Fibrin gels were formed with fibrinogen in the presence of $CaCl_2$, thrombin, and μgels at varying concentrations. Laser scanning confocal microscopy was used to visualize fibrin matrices using 5% labeled fibrinogen and fluorescent μgels. Oscillatory rheology experiments were performed on the various constructs to quantify the viscoelastic properties, such as the storage and loss moduli of the matrices. Fibroblasts were embedded within the constructs and allowed to spread overnight before being fixed and stained with phalloidin for visualization of the actin cytoskeleton. For motility experiments, cells were monitored in real time taking measurements every 10 minutes for 12 hours after overnight initial plating in the various constructs. Quantification of cell migration velocity and directionality was performed with using manual tracking ImageJ plugins.

Results: ULC μgels serve as space filling gels that modify the fibrin network architecture by forming pockets within the fibrin gel. Additionally, cell shape is affected within these 3D gel constructs in a dose-dependent manner depending on μgel concentration. Figure 1 shows maximum intensity projections of confocal z-stacks of actin-stained fibroblasts within high concentration fibrin clots displaying altered cell spread area and shape with increasing μgel concentration. Cell area increases in a statistically significant manner with μgel concentration as cell circularity decreases due to the cells becoming more elongated. The presence of μgels also increases the ability of cells to migrate within the dense fibrin matrix while not affecting the mechanics of the network (data not shown).

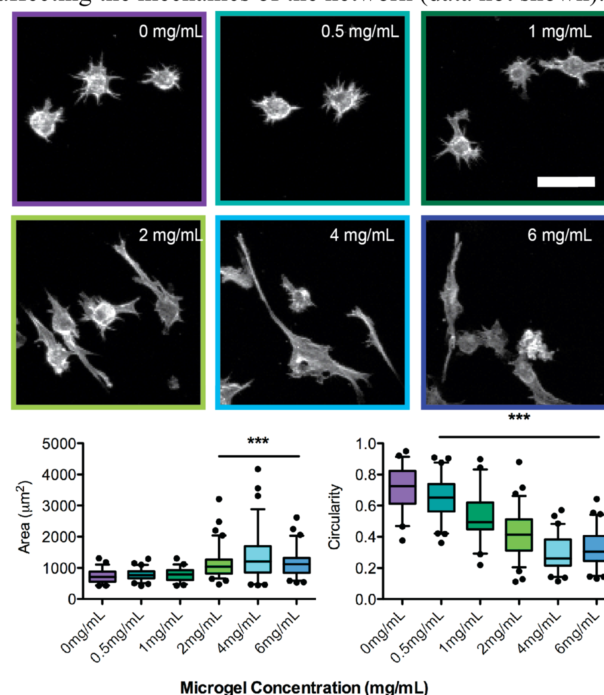

Figure 1: Cell shape and spreading is modified by the presence of μgels in a dose-dependent manner. Laser scanning confocal microscopy was used to acquire maximum intensity projections of fibroblasts within 8 mg/mL fibrin gels with increasing concentrations of μgels. Measurements of cell area and circularity display statistical significance with increasing μgel concentration. ($p<0.0001$, ANOVA with Tukey's multiple comparison post-hoc test).

Conclusions: We have found that incorporating μgels into fibrin matrices alters the network architecture. We have also seen that μgels increase the ability for fibroblasts to migrate throughout the 3D matrix more efficiently than in unmodified fibrin only controls. Investigating polymers that can modulate the bulk and local properties of a network will provide a better understanding of design parameters for use in controlling cells and motility within biomaterial constructs. Future investigations are geared towards understanding the structural relaxation of the colloidal assemblies and how they permit these cell phenotypes.

References:

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