Altering Fibrin Matrix Properties with pNIPAm Microgels for Wound Healing Applications

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Statement of Purpose: 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. We have designed materials to be incorporated into fibrin scaffolds that will create a mechanically robust clot, but allow for increased cell infiltration and regeneration [1]. The overall goals of this research are to promote mesenchymal stem cell (MSC) homing and migration within engineered fibrin networks. Through the incorporation of ultra low cross-linked poly(N-isopropylacrylamide) pNIPAm microgels into fibrin matrices, we hypothesize that we can alter network architecture (Figure 1), mechanical properties, and cell motility making it more amenable for cell infiltration and maintenance. In developing these materials, we have exploited the attractive properties of ultra low cross-linked pNIPAm microgels, which include the ability to self-associate, interpenetrate, and swell extensively upon assembly. Our preliminary data demonstrates their ability to soften a fibrin matrix at the microscale. Furthermore, in this context the microgels generate a space-filling colloidal gel, as opposed to an association of local aggregates [2], which alters the mechanics of the network.

Methods: Fibrin gels were formed using human fibrinogen in the presence of $CaCl_2$, thrombin, FXIIIa, and microgels at varying concentrations. Laser scanning confocal microscopy was used to visualize fibrin matrices using 5% labeled fibrinogen and fluorescent microgels. Oscillatory rheology experiments were performed on the various constructs to quantify the viscoelastic properties, such as the storage and loss moduli of the matrices. Platelets isolated from whole blood were incorporated into polymerizing microgel containing fibrin matrices and imaged with confocal microscopy for assurance that microgels would be retained in a contracted clot. For motility experiments within the various constructs, mesenchymal 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 in house MATLAB cell migration algorithms.

Results: Ultra low cross-linked pNIPAm microgels serve as space filling gels that modify the fibrin network architecture. Figure 1B,C show confocal slices clearly displaying altered fiber density and fiber properties compared to the control, 1A. Microgels are maintained within platelet-contracted clots as shown in 1D and are thus resistant to expulsion out of the network. They additionally resist platelet contraction compared to control clots without microgels as seen through differences in resulting overall clot volume. Incorporation of microgels into fibrin networks also alters the mechanical properties of the matrices, making them softer at the microscale. The presence of microgels also increases the ability of cells to migrate within the dense fibrin matrix.

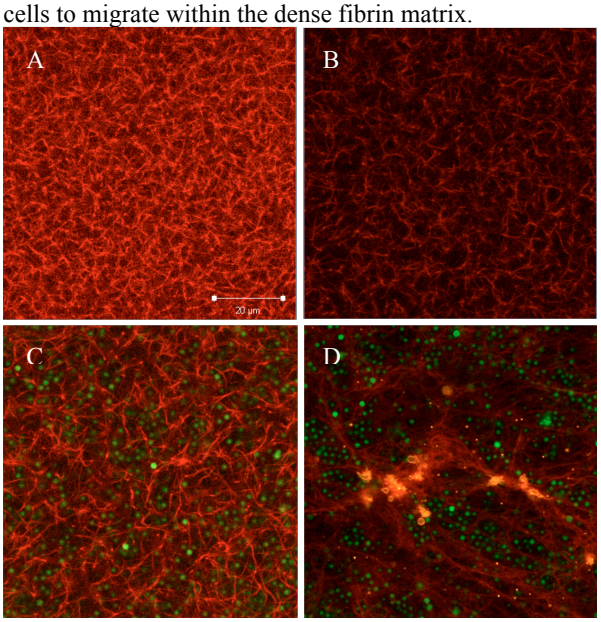

Figure 1: Fibrin network architecture is altered by the incorporation of pNIPAm microgels. Laser scanning confocal microscopy was used to acquire single 0.5um slices through fibrin gels with and without pNIPAm microgels. A) Fibrin only 2.5mg/mL, B) Fibrin (2.5mg/mL) with 2mg/mL pNIPAm microgels, C) Fibrin (2.5mg/mL) with 2mg/mL fluorescein-pNIPAm microgels D) Platelet contraction of microgel containing clot, Fibrin (2.5mg/mL) with 2mg/mL fluorescein-pNIPAm microgels (Fibrin=red, Microgels=green, Platelets=orange, Scale bar=20um).

Conclusions: We have found that incorporating microgels into fibrin matrices alters the network architecture. We have also seen that microgels increase the ability for mesenchymal cells 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 stem cells and cell motility.

References:

1. Stabenfeldt SE, et al. Biomaterials. 2012; 33:535-544.
2. Meng ZY, et al. J Phys Chem B. 2007; 111 (25):6992-6997.