Alterations in fibrin microstructure via competitive knob peptides and the implications for cellular response

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Statement of Purpose: Fibrin is the primary hemostatic agent in response to vascular injury and provides the initial provisional matrix for wound healing. As a result, fibrin has served as the foundation for FDA-approved therapeutic products (e.g. Tisseal®) and *in vitro* 3-D culture systems. However, major limitations of fibrin-based systems include fast rates of polymerization and formulations with high fibrinogen concentrations that inhibit degradation and cell infilatration. High concentrations of short tetrapeptides derived from the N-termini of fibrin Aα-chain (fibrin knobs) will inhibit fibrin polymerization [1] as the knob peptides compete with native fibrin knobs for the polymerization pockets on distal C-termini of fibrin(ogen) molecules. However, at lower peptide:fibrinogen molar ratios, the peptides do not inhibit polymerization but may instead alter the polymerization dynamics and subsequent fiber microstructure. Addtionally, PEGylation of the knob peptides may enhance the efficiency of knob peptides by acting as a bulking agent and stericly hindering the native fibrin knob:pocket interactions. Therefore, the aim of this study was to investigate microstructure alterations that occur when fibrin was polymerized in the presence of low doses of competitive knob-peptide sequences or PEGylated knob-peptides. We hypothesize that the altered polymerization dynamics will translate to modifications in the microstructure without a dramatic effect on the amount of protein incorporated into the fibrin matrix. We evaluated the polymerization rate, fiber stucture, and mechanical properities. Resulting fibrin matrix formulations were then evaluated with a functional cellular assay to assess how the microarchitecture may influence cellular response within the fibrin gel.

Methods: Fibrin gels (1mg/ml or 4mg/ml fibrinogen) were polymerized in the presence of fibrin knob peptides (GPRP) or PEGylated peptide conjugates (GPRP-PEG 5kDa) via 1U/ml thrombin and 1U/ml Factor XIIIa; GPSP was used as the negative control peptide. The rate of polymerization for each varying fibrinogen/peptide formulation was examined with confocal microscopy via fluorescently labeled fibrinogen. Assays on fibrin gels 1hr post thrombin-initiated polymerization included percent clottability, rheological characterization (oscillatory frequency sweep analysis), and fiber structure analysis (scanning electron microscopy on critical point dried fibrin gels). Preliminary cellular analysis involved plating cortical neurons at the interface of two varying fibrin gel formulations; neurite outgrowth was examined at 48hrs.

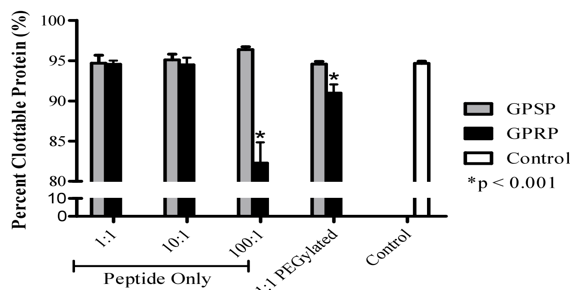

Figure 1. Percent clottable protein

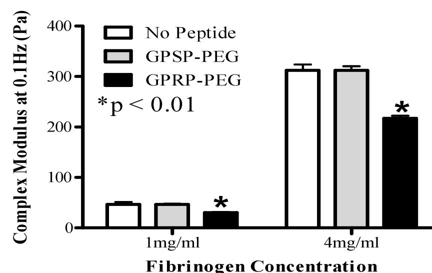

Figure 2. Complex modulus for fibrin gels at 0.1Hz supplemented with equimolar ratios of PEGylated peptides.

Results: In the presence of GPRP at 100-fold molar excess, the rate of polymerization significantly decreased compared to no peptide control and GPSP. Moreover, the amount of protein incorporated into the fibrin gel as measured by percent clottable protein significantly decreased from 95% to 80% with a 100-fold molar excess GPRP, whereas at 10-fold excess or equimolar ratios there was no difference (Figure 1). PEGylated GPRP at equimolar ratio resulted in a modest but significant difference in the percent clottable protein from 95% to 91% (Figure 1). Rheological analysis demonstrated a significant 25-30% decrease in complex modulus with equimolar addition of PEGylated GPRP compared to no peptide control and PEGylated GPSP (Figure 2). SEM analysis of the fibrin fiber structure determined that larger fibers and fiber bundles were formed in the presence of 100-fold molar excess of GPRP; analysis of fibers assembled in the presence of PEGylated peptides is underway. Finally, preliminary studies with cortical neurons and 4mg/ml fibrin formulations indicated neurite extension occurred preferentially in fibrin supplemented with PEGylated GPRP at equimolar ratio.

Conclusions: Collectively, the data indicate that PEGylated fibrin knob peptides present at low doses significantly altered the polymerization dynamics and thus the microstructure and mechanical properties of fibrin gels. Notably, these alterations occurred without a dramatic decrease in protein content (i.e. percent of clottable protein). Functionally, these modifications translated into a moderate increase in neurite outgrowth within the matrix. Future studies will include assessing the susceptibility to plasmin degradation and additional migration-based cell assays with other phenotypes (e.g. fibroblasts and endothelial cells).

References: 1. Laudano et al. PNAS. 1978;75:3085-3089.

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