Tuning Mechanical and Structural Properties of Fibrin with Fibrin Knob Peptides

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Statement of Purpose: Fibrin is the primary haemostatic agent in response to vascular injury and thereby serves as the initial provisional extracellular matrix during wound healing. A multitude of FDA-approved products utilize fibrin as a base hemostatic/sealant matrix. Yet, fibrin assembly and polymerization in these formulations occurs rapidly and is highly sensitive to surrounding conditions (e.g. ionic strength and zymogen, protein, and calcium concentrations). Additionally, formulations with high fibrinogen concentrations ultimately inhibit cell infiltration and remodeling. To address this major concern, we are interested in developing peptide-based additives to tune the mechanical properties of fibrin without altering fibrinogen concentrations. Fibrin assembly occurs via non-covalent interactions between thrombin-exposed 'knob' sequences on the N-termini of fibrin Aα- and Bβ-chains (A- and B-fibrin knobs) and two polymerization holes on the distal C-termini of fibrin(ogen) molecules ('a' and 'b' polymerization holes). A-knob mimetic peptides (e.g. GPRxxx) have been shown to compete for both fibrin holes, potentially disrupting bulk polymerization rate. Meanwhile, certain variants B-knob peptides (i.e. AHRPxxx) bind only the 'b' polymerization hole that is thought to mediate lateral protofibril interactions. We have recently shown that PEGylation of knob peptides enhances their efficiency presumably by steric hindrance of the native fibrin knob:pocket interactions. At low peptide-PEG:fibrinogen 1:1 molar ratios, bulk fibrin polymerization is not inhibited but fibrin assembly dynamics are instead altered. Therefore, the aim of this study was to investigate potential alterations in fibrin material properties elicited by low doses of competitive PEGylated A- and B-knob-peptides. We evaluated clottability, plasmin degradation, mechanical properties, diffusion, and fiber structure.

Methods: Fibrin gels (1mg/ml or 4mg/ml fibrinogen) were polymerized in the presence of PEGylated fibrin knob peptide conjugates (5kDa PEG; A-knob = GPRPFPAC-PEG and B-knob = AHRPYAAC-PEG) via 1U/ml thrombin and 1U/ml Factor XIIIa; GPSPFPAC-PEG served as the negative control. All experiments were performed at an equimolar ratio of peptide-PEG conjugate to fibrinogen. Assays on fibrin gels 1hr post thrombin-initiated polymerization included percent clottable protein, rheological characterization (oscillatory frequency sweep analysis), diffusion analysis, plasmin degradation and fiber structure analysis (confocal and scanning electron microscopy).

Results: Fibrin clots polymerized in the presence of GPRPFPAC-PEG and AHRPYAAC-PEG resulted in a modest but significant difference in the percent clottable protein (91% and 92%, respectively) compared to no peptide control fibrin (95%). SEM analysis demonstrated a significant decrease in fiber bundle density with GPRPFPAC-PEG and AHRPYAAC-PEG compared to control gels, indicating a more porous network. Direct comparison of the A- and B-knob peptide conjugates revealed stark differences in single fiber and bundle structure between the two groups. Consequently, the measured effective diffusion coefficient significantly increased for both GPRPFPAC-PEG and AHRPYAAC-PEG (720 and 650 $\mu m^2/s$, respectively) compared to fibrin control (450 $\mu m^2/s$). Rheological analysis demonstrated a significant 25-30% decrease in complex modulus with equimolar addition of GPRPFPAC-PEG (~200 Pa) compared to no peptide and GPSPFPAC-PEG controls (~300 Pa; Figure 1). In contrast, the complex moduli of AHRPYAAC-PEG clots significantly increased by 30% to ~425 Pa compared to the control groups (Figure 1). Plasmin degradation, measured via turbidity and soluble protein, indicated a significant rate decrease with AHRPYAAC-PEG compared to all groups (Figure 2).

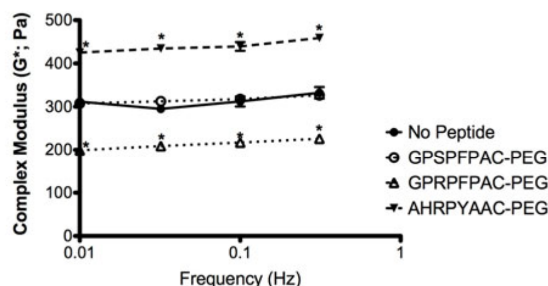

Figure 1 – Complex moduli of fibrin gels (4mg/mL fibrinogen)

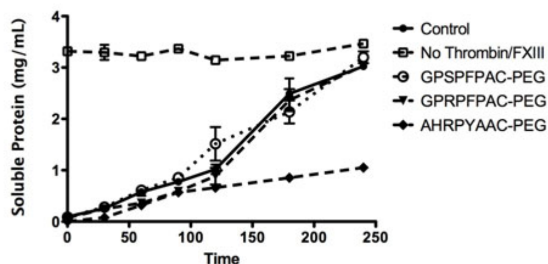

Figure 2 – Plasmin degradation as measured via soluble protein in the clot liquor.

Conclusions: Collectively, the data indicate that PEGylated fibrin knob peptides present at low doses significantly altered the dynamics of fibrin assembly and thus the mechanical, structural, diffusive, and degradation properties of fibrin gels. Additional, we demonstrated the type of knob mimetic (A- versus B-knob) played a significant role in resulting material properties, particularly mechanical strength and plasmin degradation. We speculate the protofibril assembly at the molecular level is contributing to this phenomenon. Ultimately, such peptide conjugates may be utilized to tune the material properties of fibrin-based clinical products.

Acknowledgements: Authors acknowledge the following funding sources: NIH (1R01EB011566) to T.H.B. and the NIH FIRST Fellowship (K12 GM000680) to S.E.S.

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