Enhancing Mechanical Properties of Fibrin Matrices for Wound Healing Applications through Optimized B-knob Engagement

Kelly C. Clause[1], Ashley C. Brown[1], Alison Douglas[1], Martha Alvarez[2], Elliot Botvinick[2], Thomas H. Barker[1].
[1]Wallace H. Coulter Department of Biomedical Engineering, Georgia Institute of Technology and Emory University School of Medicine, Atlanta, GA
[2]Departments of Surgery and Biomedical Engineering, University of California Irvine, Irvine, CA

Statement of Purpose: Fibrin, the crux of the coagulation cascade, is a biological material that has been well studied and heavily utilized for commercial use. However, there is a need to control the final macroscopic properties of the fibrin matrix to enhance cell infiltration and matrix degradation while maintaining mechanical integrity necessary for commercial use. During fibrin polymerization, thrombin binds to fibrinogen at the centrally located aminoterminus and cleaves a portion of the Aα-chains, known as fibrinopeptide A, and the b-chains, known as fibrinopeptide B, thus exposing fibrin knobs 'A' and 'B', respectively. These knob domains are then free to interact with neighboring fibrin/fibrinogen monomers at the carboxyl-termini of the g- and b-chains referred to as the polymerization holes 'a' and 'b', respectively. We have previously shown that synthetic fibrin knob peptide mimics that perturb either the A:a or B:b interactions significantly influence the dynamics of fibrin monomer and fiber assembly; specifically engagement of hole 'b' with PEGylated 'B' knob conjugates during polymerization significantly enhances the porosity and subsequent diffusivity through fibrin polymers. Furthermore, incorporation of PEGylated 'B' knob intro fibrin gels results in increased viscoelastic properties and decreased susceptibility to degradation[1]. However these changes were effected with very low concentrations of conjugates (1:1 molar ratio). Thus to optimize B-knob engagement, we investigated in depth the influences of peptide conjugate concentration on B-knob/fibrinogen binding kinetics and resulting fibrin network structure and mechanical properties.

Methods:
A 'B' knob and a "nonbinding" peptide, AHRPYAAC (B) and GPSPFPAC (-), respectively were synthesized and PEGylated via maleimide-sulfhydryl chemistry. The binding affinity of the fibrin knob mimics were evaluated with surface plasmon resonance (SPR). Based on previous SPR protocols developed in our lab[2], using a Biacore 2000, a thin layer of fibrinogen fragment D was immobilized on the surface of a gold SPR chip using EDC/NHS chemistry. A range of concentrations of fibrin knob mimics were flowed across the surface and the dissociation constant (K_d) of knob mimics was calculated. Knob mimics were then incorporated into fibrin matrices at concentrations above and below this calculated K_d and perturbations to the mechanical and structural properties of the resulting matrices were analyzed. Mechanical properties within fibrin gels were measured by active microrheology (AMR) as previously described[3]. For these experiments, silica beads of 2 μm diameter were mixed into 1mg/mL fibrinogen solutions with and without PEGylated knob mimics prior to polymerization. To analyze clot structure, fibrin clots with and without PEGylated knob mimics were examined using confocal microscopy (Zeiss 510 VIS). To allow for visualization of the fibrin matrix, Alexa Fluor 555-labeled fibrinogen was utilized. Clots were formed directly on a glass slide, overlaid with a coverslip and allowed to polymerize for an hour prior to imaging.

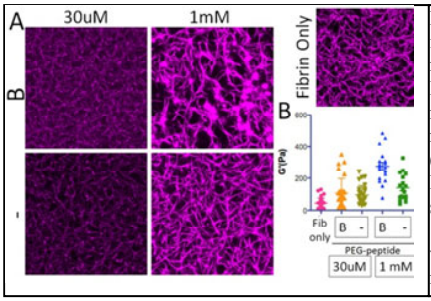

Figure 1. (A) Confocal images of fibrin matrices with PEGylated 'B' knob mimic (top panels) and nonbinding control (bottom panels). (B) Microrheological storage modulus for 1mg/mL fibrinogen with or without peptide mimics.

Results:
To rationally determine relevant concentrations of peptide conjugates we calculated the binding kinetics of the fibrin knob mimics using SPR. The calculated dissociation constant of the PEGYlated 'B' knob peptide was 5.72 $x10^{-5}$, an order of magnitude lower than the nonbinding control of 2.9 x 10^{-4}. Fibrin clots were polymerized in the presence of the PEGylated 'B' knob peptide or the non-binding control and examined using confocal microscopy. Inclusion of low concentrations (below the dissociation constant) of either peptide did not alter fibrin network structure. However, inclusion of higher concentrations (above the dissociation constant) of the PEGylated 'B' knob peptide, greatly altered the network structure, resulting in thicker fibers and an increased porosity (Figure 1A). Inclusion of higher concentrations of the PEGylated 'B' knob peptide also significantly increased the local storage modulus (Figure 1B) as assessed by AMR and the bulk compression modulus compared with the nonbinding and fibrin only controls.

Conclusions:
The observed changes in fibrin network structure and mechanical properties are specific to inclusion of the PEGylated 'B' knob peptide mimic and is concentration dependent, with significant differences observed when concentrations are above the dissociation constant. Future studies will characterize cellular responses to fibrin clots modified with high concentrations of PEGylated 'B' knob peptides through *in vitro* angiogenesis assays.

References:
1. Stabenfeldt SE. Biomaterials. 2012;33:535-544.
2. Stabenfeldt SE. Blood. 2010;116:1352-9.
3. Kotlarchyk MA. PLoS One. 2011;6(5):e20201.