Temperature-Controlled Modulation of Fibrin Matrix Properties

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Statement of Purpose: Fibrin is the native provisional protein matrix generated in response to vascular injury, acting to stimulate and support cellular infiltration. Its precursor, fibrinogen, is also used in surgical formulations (e.g. Tisseel, Evicel) as a hemostat or sealant that instantly polymerizes upon mixing with thrombin and factor XIIIa. The fibrin matrix is stabilized by knob:hole affinity interactions and covalent isopeptide crosslinks. In particular, fibrin network morphology (fiber thickness and branching density) is dependent upon the kinetics in which N-terminal cryptic knob peptide sequences on the fibrinogen α and β chains are exposed by the serine protease, thrombin [1]. The slower-acting transglutaminase, factor XIIIa, further contributes to final clot rigidity through crosslinking specific sites on the α and γ chains of the existing network. This close interplay between fibrinogen and its activating enzymes limits the range of fibrin network morphologies generated from this potentially versatile structural protein.

Our previous work has shown the ability to retain therapeutic recombinant proteins displaying fibrin knob sequences (e.g. GPRP) within 3-dimensional fibrin matrices through knob:hole interactions [2]. Additionally, the conjugation of GPRP to flexible polyethylene glycol (PEG) backbone structures facilitates the modulation of fibrin matrix properties by perturbing the native knob:hole interactions that contribute to fibrin network structure (*manuscript in preparation*). The objective of this study is to create structures displaying knob sequences that will modulate fibrin matrix properties in a predictable manner upon application of an external stimulus. Specifically, fibrin knob-displaying elastin-like polypeptides (ELPs) exhibiting reversible phase transitions at specific temperatures can be readily designed and produced using standard protein engineering techniques. The development of such environmentally-responsive fibrin(ogen) engaging elements will facilitate the use of fibrin-based materials in a greater variety of settings, particularly in complex surgical procedures.

Methods: *Production of GPRP-ELP fusions.* ELPs of the desired composition and lengths were cloned using recursive directional ligation. GPRP and desired protein tags were similarly introduced to the expression plasmid pET15b (Novagen) using standard molecular cloning techniques. Proteins were expressed in E. coli, purified using inverse transition cycling, and verified through gel electrophoresis (coomassie stain and Western blot) and mass spectrometry (MALDI-TOF). *Characterization of GPRP-ELP fusions.* Thermo-responsive behavior of the fusions was analyzed using turbidimetry (for transition temperature T_t), dynamic light scattering (for R_h) and multi-angle laser light scattering (for M_W and R_g). *Interaction between GPRP-ELP fusions and fibrin(ogen).* Affinities (K_D, k_{on}, k_{off}) between the fusions and fibrin(ogen) were evaluated using surface plasmon resonance at temperatures below and above T_t. Corresponding rheological characteristics of fibrin(ogen) mixtures with GPRP-ELP fusions were evaluated using a controlled strain rheometer with a cone-and-plate setup.

Results: Fibrin clot turbidity is a known general indicator of fibrin network morphology [1]. The perturbation of clot turbidity readings in the presence of $GPRP_2$-PEG conjugates [Fig. 1A] suggests that constructive interaction with fibrin(ogen) is most pronounced at a 1:1 conjugate-to-fibrinogen molar ratio, as expected from the stoichiometry of fibrin knobs and holes. At this ratio, modulation of the elastic modulus is dependent on conjugate size [Fig. 1B], suggesting that fibrin clot stiffness can be altered in situ through changes in conjugate size upon external stimuli.

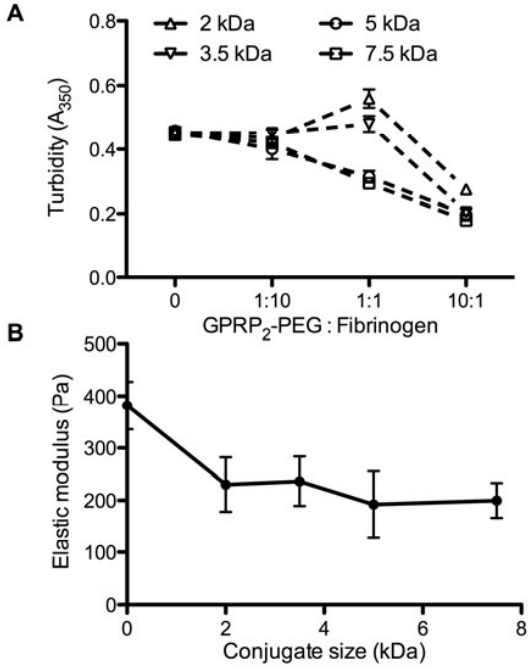

Fig. 1. Characterization of fibrin clots formed in the presence of $GPRP_2$-PEG conjugates through turbidity (A) and rheological characterization (B).

Conclusions: Soluble extended ELP proteins collapse to form β-spirals above T_t. This collapse results in a dramatic contraction of chemically crosslinked ELP hydrogels [3]. In this work, we propose the creation of weak fibrin(ogen)-ELP hybrid hydrogels that shrink to form strong and stable scaffolds upon a temperature stimulus (e.g. when injected into the body). We envision the creation of a flowable fibrin hemostat that will only set in situ, enhancing the utility of current commercial formulations of this native wound repair protein.

References: [1] Weisel JW. Biophys J. 1992;63:111-128.
[2] Soon AS. Biomaterials 2010;31:1944-1954.
[3] Trabbic-Carlson K. Biomacromolecules 2003;4:572-580.