Fibrin-knob Peptide Variants for Targeting Delivery to Fibrin Matrices

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Statement of Purpose: The coagulation cascade is activated in response to injury or certain pathological conditions. The conversion of fibrinogen to fibrin follows a complex, yet well established chain of enzymatic events that leads to the deposition of a fibrin matrix. While this response aims to enable repair and regeneration, excessive or persistent fibrin deposition can result in altered repair and pathology. This study examines the use of fibrin knob-pocket interactions to develop a delivery system for therapeutic agents that target fibrin matrices. We have characterized the binding affinity constants of alpha fibrin knob derived peptide variants and begun to evaluate the binding of model proteins fused to knob peptides within a fibrin matrix. This study aims to examine the translation of binding constants established through fundamental experimentation to more complex interactions within a pre-formed fibrin matrix. We expect that the variant with strongest binding affinity to fibrin pockets will translate into the most efficient fibrin matrix binding agent.

Methods: This study focuses on both natural and previously reported alpha fibrin knob mutations that alter fibrin polymerization. Binding kinetics of fibrin knob variants were characterized through Surface Plasmon Resonance (SPR) using a Biacore 2000. Self-assembled mono-layer surface chemistry enabled immobilization of either fibrinogen D-domain fragments. Upon fragment D immobilization, kinetic binding experiments were run with the soluble peptide variants as the flow analyates. Under high flow rate conditions, six varying concentrations of peptide variants were flowed over the fibrinogen D domain fragments. SPR sensograms were analyzed with the aid of Scrubber 2 software (Center for Biomolecular Interactions Analysis, Univ of Utah), using a 1:1 Langmuir binding interaction to fit the experimental data. The fitted binding affinity constants (k_a, k_d, and K_D) for each variant were then compared to establish differences in fibrinogen binding interaction. Confocal microscopy and microfluidic perfusion will be employed examine the targeting of fibrin-knob peptide variants to existing fibrin matrices. Fluorescently labeled fibrinogen (Alexa-488nm) will be cast into a microfluidic chamber. Upon gelation, fluorescently labeled fibrin-knob peptide variant fused to a model protein (Alexa-546nm) will be perfused through fibrin gels. Confocal z-stacks acquired at high magnifications will enable co-localization analysis of peptide variants with fibrin matrix.

Variant	k_a ($M^{-1}s^{-1}$)	k_d (s^{-1})	K_D (μM)
GPRP	1410±30	0.00173	1.22±0.02
GPSP	3.8±0.5	0.001	260±5

Table 1 – Kinetic binding constants.

Results: SPR analysis demonstrated the ability to detect alterations in binding affinities between peptide variants. For example, the sequence Gly-Pro-Arg-Pro (GPRP) had a significantly higher binding affinity than that of Gly-Pro-Ser-Pro (GPSP; Figure 1). Modeling the kinetic binding curves assuming a 1:1 Langmuir binding interaction revealed the k_a, k_d, and K_D for the peptide sequences (Table 1). We will in turn use the experimental binding constants to predict the ability for a peptide variant to target and bind to fibrin matrices. We are currently evaluating such binding interactions within a microfluidic perfusion system. This system enables the modeling of an *in vitro* situation whereby our therapeutic agent fused to a fibrin knob peptide sequence encounters an established fibrin matrix. We have confirmed the ability to visualize the fluorescently labeled fibrin matrix within the microfluidic perfusion chamber. Current efforts are focused on examining co-localization of fibrin targeting agents to the fibrin matrices.

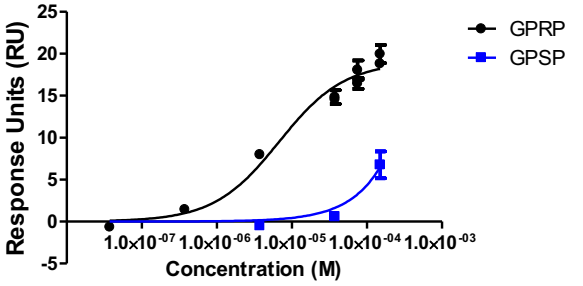

Figure 1 – Binding affinity curve for fibrin knob peptide sequences interacted with immobilized fibrinogen D-domain fragment.

Conclusions: This study examines binding affinities of fibrin knob derived peptide variants in order to develop a targeting strategy for fibrin matrices. Classic binding experiments and an *in vitro* fibrin matrix model will enable us to examine the robustness of the predicted binding interactions. Ultimately, this work will further enhance the ability to augment the provisional matrix in an injured and/or fibrotic environment through fibrin binding motifs.

Acknowledgements: Authors acknowledge the following funding sources: NIH K12 IRACDA Training grant (NIGMS; SES), 1R21EB008463 (NIBIB; THB), and GTF 125-00-0120 (Wallace H. Coulter Foundation; THB). The microfluidics systems were designed and provided by Jelena Vukasinovic and Ari Glezer (Georgia Tech, Mechanical Engineering Department).