Identification of Fibrin-Specific Binding Motifs for Development of Fibrin-Targeted Therapeutics

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Statement of Purpose: The coagulation cascade is activated in response to injury or pathological conditions and leads to localized 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. Therefore, the ability to identify and target regions of fibrin deposition may facilitate more improved treatment of internal injuries and cardiovascular diseases. Previously, we have investigated the use of synthetic peptide sequences derived from the N-terminal fibrin knobs to target and incorporate non-fibrin binding proteins to fibrin. However, a limitation of this targeting sequence is that the fibrin knob peptides also bind fibrinogen. Therefore, in this study we aimed to identify binding motifs that binds specifically to mature fibrin fibers, but not to fibrinogen. By using a combination of phage display and microfluidics, we are able to identify and then thoroughly test and characterize the specificity of identified fibrin-binding motifs.

Methods: We have coupled confocal microscopy and microfluidic perfusion to specifically examine the targeting of fibrin-binding motifs to existing fibrin matrices. Fibrin gels with fluorescently labeled fibrinogen (Alexa-555nm) were cast into a microfluidic chamber. Upon gelation, fluorescently labeled fibrin-knob peptide sequences (Alexa-633nm) were perfused through fibrin gels. Confocal z-stacks acquired at high magnifications enable co-localization analysis of binding motifs with fibrin matrix. To further extend the search for binding motifs, we have employed phage display techniques with two different phage libraries. First, we used a phage library displaying a 6-residue peptide sequence on the minor protein coat, pIII (Fuse5 library; gift from Dr. George Smith at University of Missouri). The second library is a phagemid complex that enables the display of a single chain variable antibody fragment on pIII (scFv Tomlinson I + J library; Geneservices). For each of these systems, multiple negative screens are performed against immobilized fibrinogen prior to positive screens for immobilized fibrin fibers. Upon identifying the peptide binding sequence and the antibody fragment, competitive binding experiments will be performed to confirm specificity to fibrin. Microfluidic experiments with the newly identified binding sequences will be performed to examine the targeting capabilities in a more complex environment.

Results: We have developed a technique that enables the real-time monitoring of fibrin binding interaction within mature fibrin matrices (Figure 1). Perfusion of fluorescently labeled fibrin knob peptide variants through a microfluidic chamber containing fibrin demonstrated increased co-localization over the duration of the experiment (Figure 1). After 15min of perfusion of the active knob peptide variant at 10μL/min, moderate co-localization was observed (Figure 1 A-C). More pronounced co-localization was observed after 55min of perfusion (Figure 1 D-F). In contrast, only trace amounts of non-specific binding were observed with the negative control peptide variant (data not shown). While the fibrin knob peptide-based targeting strategy showed promise, we determined that the capability of binding to both fibrin and fibrinogen was a disadvantage for targeting fibrin *in vivo*. Therefore, we have begun to identify binding motifs with the two different phage libraries to identify peptide and antibody. We have established negative screening protocols against fibrinogen in effort to deplete the phage population that non-specifically and specifically bind to fibrinogen covalently immobilized to a glass surface. Additionally, we have begun our positive screens for thin fibrin layers polymerized on top of immobilized fibrinogen. Positive phage binding sequences along with binding affinities and colocalization with fibrin matrices will be presented.

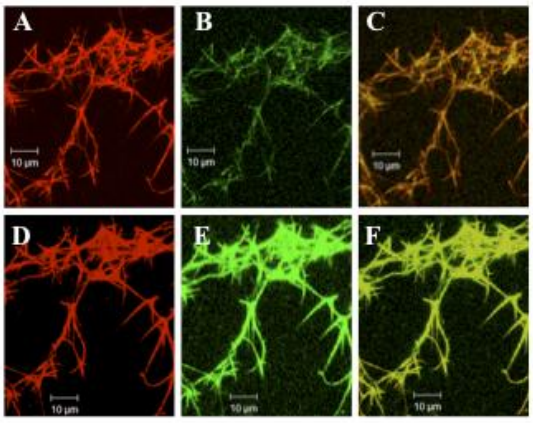

Figure 1. Microfluidic perfusion of fibrin knob peptide variants (green; B,C, E, F) through fibrin matrix (red; A, C, D, F). Confocal micrographs at 15min (A-C) and 55min (D-F) post-perfusion.

Conclusions: In conclusion, we are using phage display technology to identify motifs (i.e. short peptides and antibodies) that specifically bind mature fibrin matrices. We have developed a microfluidic perfusion system to thoroughly test our phage-identified binding domains within 3D environment mimicking *in vivo* conditions. Ultimately, we aim to couple the fibrin-binding domains with contrast agents for imaging purposes and also therapeutic agents for localized targeted drug delivery.

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