Title: Role of Surface-Driven Thrombin Generation on Fibrin Clot Structure and Stability

While clot formation is essential for hemostasis, pathologic clot formation is the leading cause of morbidity and mortality in the Western world. Fibrinolytic therapy is a cornerstone for treating pathologic clots associated with heart attack, stroke, and venous thromboembolism. Previous studies have shown that fibrin structure directly modulates the clot's resistance to fibrinolytic and mechanical disruption. However, the impact of cell surface-driven *in situ* thrombin generation on fibrin clot structure and stability has not been evaluated. To determine cellular properties that contribute to fibrin structure, we incubated human dermal fibroblasts with prothrombinase complex components (factors Xa, Va, $CaCl_2$), prothrombin (0.014 or 1.4 M, to modulate the thrombin generation rate, low and high, respectively), and fibrinogen. We found that clots formed during higher thrombin generation rates were composed of a denser fibrin network than clots formed during lower thrombin generation rates. Interestingly, we observed a spatial component to the fibrin structure present in clots formed by *in situ* thrombin generation. The fibrin network was significantly denser proximal *versus* distal to (50 μm above) the cell surface, regardless of the thrombin generation rate. We used a plasmin challenge assay to evaluate fibrin formation and lysis at low and high thrombin generation rates. We observed faster fibrin production and slower lysis during high *versus* low thrombin generation rates, and proximal *versus* distal to the cell surface. These results suggest that the rate of *in situ* thrombin generation on the cell surface can independently modulate the clot's three-dimensional structure and stability. These findings imply that the endogenous procoagulant activity present at the wound site influences thrombus structure and stability, and suggest a mechanism by which lowering the thrombin generation rate may alter clot structure and reduce occlusive thrombus formation.