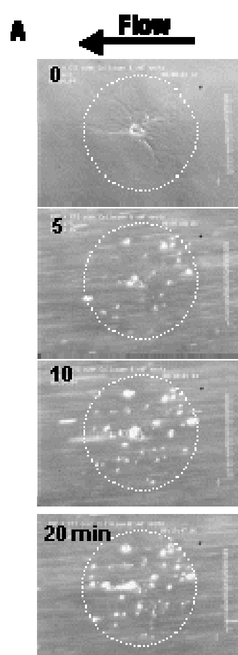
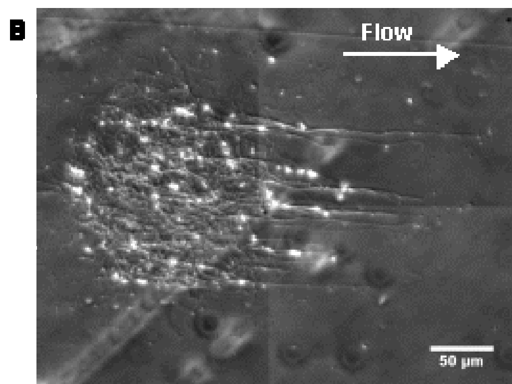
490e Thrombogenic Protein Microarrays for in Vitro Coagulation Studies under Flow
Uzoma M. Okorie and Scott L. Diamond
Currently, in vitro studies of coagulation initiation and propagation are done on a single protein surface or a solution of protein surface. We have developed a protein microarray-based perfusion system that allows for plasma or whole blood to be exposed to a spatially and chemically defined array. Thrombogenic proteins are printed onto cleaned glass slides via robotic contact printing yielding spot sizes of 200 µm. The proteins are printed as well-defined arrays with specific center-to-center distances. Platelet Rich Plasma (PRP) or whole blood is perfused over the slide at a defined shear rate. Adherent platelets and fibrin are fluorescently labeled using immunofluorescence. The slides are then imaged and the intensity of the spots was determined for each fluorochrome. We have demonstrated two potential assays using this method: the effects of serial interactions with collagen spots on platelet adhesion and fibrin deposition and the effects of shear on platelet adhesion on spatially defined collagen and von Willebrand Factor spots. To demonstrate the first assay, collagen Type I spots were printed with a center-to-center distance that varied from 500 µm to 700 µm. PRP was perfused over the slide at a shear rate (γ_w) of 100 s^{-1} for 20 minutes and the slide was stained and imaged as described above. Spot intensity for platelets was constant (relative to the intensity of the first spot upstream) for approximately 1 mm downstream of the first spot. After this distance, there was a marked increase of normalized intensity, to about two-fold at 4 mm downstream from the first spot. These results may indicate that platelet interaction with collagen areas upstream causes increased platelet adhesion and activation downstream. To demonstrate the second assay, von Willebrand Factor spots were printed upstream of collagen spots. There are one, three, or five VWF spots upstream. The last VWF spot has with a varying center-to-center distance of 500 µm or 1000 µm upstream of collagen. The control condition has no VWF spots upstream of collagen. PRP was perfused at a shear rate of either 100s^{-1} or 500 s^{-1} for 20 minutes. For a shear rate of 100 s^{-1}, there was no significant increase in relative spot intensity in comparison to the control. For a shear rate of 500 s^{-1}, there is a significant increase of relative fluorescent intensity for the 3 spots/500 µm and 5 spots/500 µm. The results may mean that VWF is initiating the platelets for subsequent adhesion onto collagen but it only occurs at higher shear. This system can be further extended to study multiple cell and protein interactions in space and time during vascular injury and clot formation.

Image caption: Platelet adhesion to collagen spots. A. Time lapse video microscopy of a collagen spot during perfusion of PRP at γ_w = 100s^{-1}. Circle represents area where the collagen spot is. B. Collagen spot after a 20-minute perfusion of PRP at γ_w = 100s^{-1}.


A
Flow
0
5
10
20 min


B
Flow
50 µm