A Study of the Effect of Sample Orientation on Platelet Adhesion Using Negative Charge Density Gradients

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Introduction: Molecular gradients are a popular tool in studying many biological phenomena (Kim, MS. Prog Polym Sci. 2008;33:138-164.). Specifically, gradients have been used to investigate variables such as wettability on platelet-surface interactions (Lee JH. J Biomed Mat Res. 1997;40:180-186.). The high-throughput potential of surface gradients is a useful concept for testing material hemocompatibility, however it is important to consider that local surface effects may be an artifact of upstream surface-platelet interactions. Adhesion is not always irreversible and can be transient (Godo MN. Biomaterials 2000;21:2243-2252.). In this study, the effect of gradient orientation on platelet adhesion was examined, illustrating the extent to which molecular gradients provide information on local adhesion patterns.

Methods:

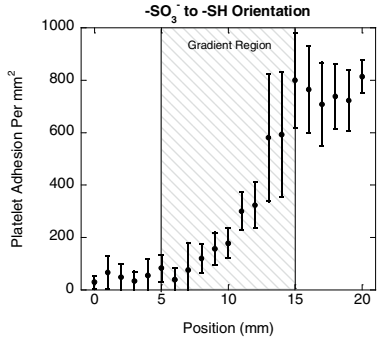
Surface Preparation and Characterization: Thiol monolayers were prepared on clean 3" fused silica slides by incubating samples in a 1% (v/v) 3-mercaptopropyltrimethoxysilane (MTS) solution in toluene. Negative charge gradients were prepared by selective oxidation of the neutral thiol groups to negatively charged sulfonate groups using a custom UV patterning chamber. The presence of the gradient region was confirmed using water contact angle.

Platelet Preparation: Washed platelets were prepared from citrated human blood. Whole blood was centrifuged for 15 minutes at 1500 rpm to separate platelet rich plasma (PRP) from the red blood cells. PRP was centrifuged for 15 minutes at 2100 rpm for platelet isolation. The platelet pellet was gently re-suspended in pre-warmed Tyrodes-HEPES buffer (37 °C, pH 7.4) Platelets were counted using a hemacytometer and concentration was adjusted to $1.5*10^7$ platelets/ml.

Perfusion Studies: A three step perfusion process in a parallel plate flow cell was used (T=37 °C, γ=92 s^{-1}). First 10% human plasma was perfused for 15 minutes to allow protein adsorption to the gradient surfaces.. The protein solution was replaced with a 5 minute washed platelet perfusion (c=10^7 platelets/ml). Finally, a 1% gluteraldehyde solution was injected into the chamber for 15 minutes to fix the sample. All unbound platelets were washed from the sample with 1X PBS prior to counting. Platelet adhesion was quantified (n=10) for 20 positions at 1mm increments including the gradient region. Error bars represent the standard deviation of each sample.

Results: The results for platelet adhesion on negative charge gradients pre-adsorbed with 10% human PFP are shown in figure 1. Platelet adhesion increased with respect to distance from the flow inlet on gradient samples with the upstream sulfonate region (Fig 1a). An increase in adhesion also corresponded with decreasing negative charge density. When the orientation was reversed, adhesion contrast was lost and overall adhesion values on the thiol and sulfonate regions decreased and increased respectively (Fig 1b).

A.



B.

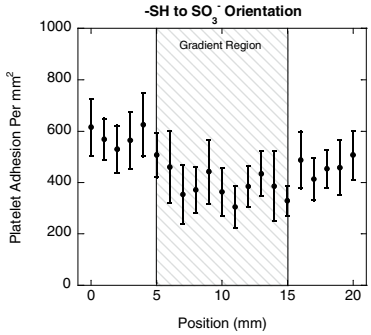


Figure 1: *Results of platelet adhesion on negative charge density gradients with A. The sulfonate region in the upstream position and with B. the thiol rich region in the upstream position of the flow cell. Adhesion values represent a mean standard deviation; n=10.*

Conclusions: The effect of sample orientation on platelet adhesion to negative charge density gradients was investigated. As stated in the results adhesion contrast was lower on gradient samples positioned in the upstream-thiol to downstream-sulfonate orientation. The fact that local adhesion patterns are not reproducible in both orientations supports the conclusion that upstream platelet-surface interactions may exhibit downstream adhesion effects. This also suggests that the history of transient surface contacts must be considered when using gradients as a tool to study various biological phenomena.

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