Leukocyte Response to Elastin-Like Polypeptides as Blood-Contacting Devices

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Introduction:

While there has been success with large diameter vascular grafts, small diameter vascular grafts (≤6mm) generally fail due to thrombosis and intimal hyperplasia. In 2008, there were over 1.5 million procedures that required small diameter vascular grafts, including diseases like peripheral arterial disease and coronary artery disease (Lith & Ameer, 2011). Although the common practice is to use autologous blood vessels to treat such conditions, there is a population of 20-30% to which this method is inapplicable due to body geometry or previous surgery (Darling & Linton, 1971), exemplifying the need for a synthetic small diameter vascular graft.

Previous work in the Woodhouse laboratory has shown that elastin-like polypeptides (ELPs) physically adsorbed to Mylar may hold promise in vascular graft applications. It has been shown by (Srokowski et al., 2010) that using the ELP-coated Mylar reduces the platelet response in whole blood as compared to uncoated surfaces during the shear rate of $300s^{-1}$. However, platelets alone do not contribute to thrombosis. Leukocytes and complement also work intricately in this trio of factors to form a thrombus. The goal of this work is to determine if the ELPs' impact the leukocyte response, possibly impacting either coagulation or thrombus formation.

Materials and Methods:

Two ELPs, varying by sequence length and molecular weight, were used in this study: ELP1 (9.8kD) and ELP4 (30.8kD). These were passively adsorbed for 1 hour to Mylar films at $0.3mg/cm^2$. Surfaces were subjected to human whole blood from 2x10 drug-free and healthy volunteers under constant shear in a cone and plate device for 2 hours at 37^oC. Blood collection was done in accordance with ethics approval (#CHEM-003-11). Controls were resting whole blood, low density polyethylene (LDPE), 5ug/mL endotoxin and 0.25U/mL thrombin activated whole blood. Cells on the surface were evaluated at shear rates of $100s^{-1}$, $200s^{-1}$, and $300s^{-1}$ for platelet-leukocyte aggregation on uncoated Mylar and LDPE. These samples were fixed overnight with 2.5% gluteraldehyde, run through a series of ethanol dehydrations and then chemically dried in a series of increasing HDMS-ethanol dilutions before analysis on a scanning electron microscope. Cells in the bulk under shear rate of $300s^{-1}$ were analyzed for leukocyte activation and platelet-leukocyte aggregation. After incubation, 100uL samples were taken from each sample surface and labeled with AF488 anti-CD142 and PE anti-CD11b (for leukocyte activation) as well as FITC anti-CD61 (for platelet-leukocyte aggregation gated in the leukocyte population). Samples were fixed in 1% paraformaldehyde for 30 minutes, and then diluted in a HEPES Tyrode's buffer at 4^oC until analysis on a Cytomics FC500 flow cytometer.

Results:

From Figure 1, it can be seen that there appears to be no leukocytes present on the LDPE (A-B) or on the uncoated Mylar (D-F) controls, regardless of shear rate. In all images there seems to be what looks like a protein layer under platelets expressing morphology consistent with activation. At the higher shear rates on LDPE (B and C), the platelets have begun to aggregate.

Flow cytometry analysis is presented in Figure 2. Mouse anti-human fluorochromes (CD142-AF488, CD11b-PE, CD61-FITC) were used against 100uL samples from ELP1-coated Mylar, ELP4-coated Mylar, uncoated Mylar and negative control LDPE. Cell expression is reported in percentage.

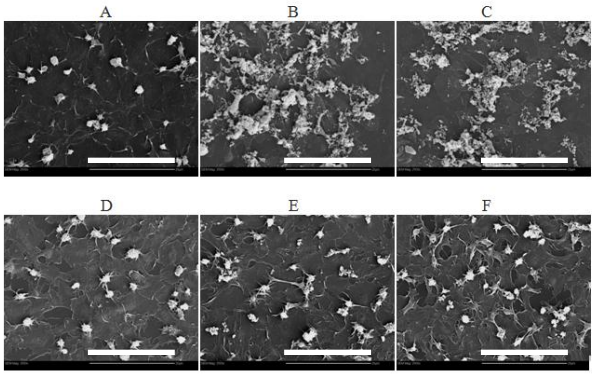

Figure 1: Scanning electron micrographs at 2500X magnification of whole blood under shear at 37°C for 2 hours in a cone and plate device. A-C: Uncoated low density polyethylene (shear rate of 100s^{-1} (A), 200s^{-1} (B) and 300s^{-1} (C)). D-F: Uncoated Mylar (shear rate of 100s^{-1} (D), 200s^{-1} (E) and 300s^{-1} (F)). Scale bars are 20µm, N=2.

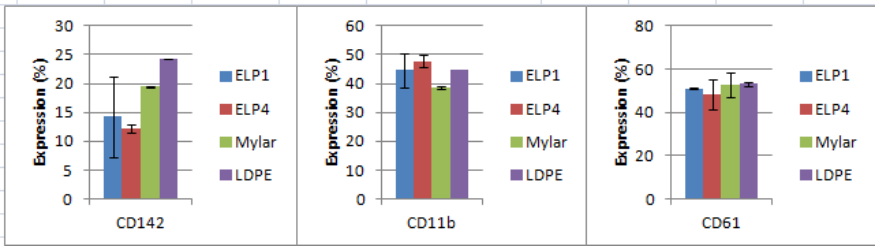

Figure 2: Preliminary flow cytometry analysis of leukocyte activation (CD142 and CD11b) and platelet-leukocyte aggregation (CD61) of whole blood under 300s^{-1} shear at 37°C for 2 hours. Mean +/- SD, N=10, n=2.

Discussion:

Low shear rates allow leukocytes to adhere to the surface and the shear rates tested for surface evaluation in this work correspond to vessel diameters of approximately 25mm (100s^{-1}), 10mm (200s^{-1}) and 5mm (300s^{-1}). Figure 1 indicates that leukocytes have not adhered to the surface at shear rates of 300s^{-1} and lower, therefore it is not likely that they will adhere at higher shear rates corresponding to smaller vessel diameters. Since the interest of this work is in vessel diameter smaller than 6mm, it is unlikely that there will be any leukocyte activity on the surface of coated Mylar, as there was no activity seen on the uncoated Mylar and positive control LDPE.

Flow cytometry results indicate that the expression of surface protein CD142 has been reduced in the bulk with the ELP coatings at 300s^{-1}. However it also appears that the CD11b and CD61 expression remained relatively unchanged. This suggests that leukocyte activation and platelet-leukocyte aggregation is inconclusive until a greater sample size is used.

These findings suggest that the ELP coatings may reduce leukocyte activation and platelet-leukocyte aggregation in the bulk and on the surface. Further testing will be undertaken to explore the complement reaction to the coatings.

References

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