Tissue Factor Bearing Microparticles and Biomaterial Thrombogenesis

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Statement of Purpose: Thrombosis on materials implanted in contact blood remains an important concern. Recent evidence suggests a role for the extrinsic pathway of coagulation not previously considered in biomaterial thrombogenesis. Blood-borne tissue factor (TF) bearing microparticles adhere to artificial surfaces from a flowing system and impart TF activity to the surface. The effects of flow dynamics and the presence of red blood cells and platelets on MP adhesion to biomaterials were examined using experimental and computational models.

Methods: The adhesion of MPs to artificial surfaces under applied flow was examined experimentally and using a computational fluid dynamics model. Microparticles were generated from the THP-1 monocytic cell line by calcium ionophore (A23187) activation. Microparticle (MP) suspensions supported TF-dependent activation of FX to FXa in the presence of FVIIa and calcium. MP suspensions were exposed to plasma-coated polystyrene (PS) at wall shear rates of 100, 400 and 1200 sec^{-1} for one hour in the presence and absence of red blood cells or platelets. The resulting TF activity of the surface was evaluated by measuring the activation of FX and the adherence of TF bearing microparticles and platelets confirmed by fluorescence microscopy.

Results/Discussion: The increase in adherent activity from the presence of RBCs observed at the moderate wall

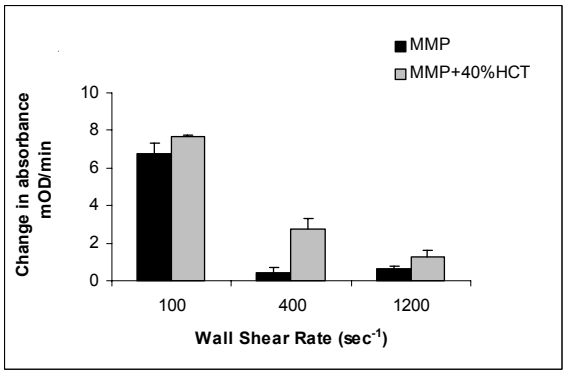

Figure 1 TF activity imparted to polystyrene from a flowing suspension of MPs compared to MPs and red blood cells. The presence of red blood cells did not significantly alter the extent of procoagulant activity imparted to the surface.

shear rate of 400 sec^{-1} was not significant at 1200 sec^{-1} (Figure 1). The effects of MP removal from shear stress have yet to be determined, however, the presence of RBCs increases the wall shear stress by a factor of 3-3.5 and this may lead to a net decrease in adherent TF activity.

Fluorescence microscopy was used to visualize adherent TF bearing microparticles on PS following a one hour perfusion at a wall shear rate of 100 sec^{-1} (Figure 2).

In vivo, PSGL-1 bearing monocyte derived microparticles bind to P-selectin on activated platelets and incorporate TF into growing thrombi. Therefore, the adhesion of THP-1 microparticles to PS from a platelet

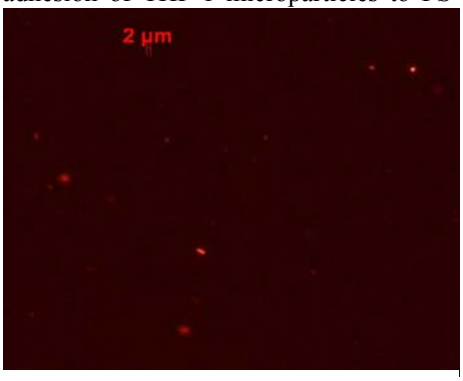

Figure 2 TF bearing microparticles adherent to plasma coated polystyrene from a re-circulating flow system.

suspension was examined using fluorescence microscopy. Microparticles and platelets adhered to PS and co-localized. Figure 3 shows platelets in green (Panel A), TF labeled MPs in red (Panel B) and their co-localization on a polystyrene surface (Panels C and D). The specific role of P-selectin and PSGL-1 remains to be confirmed on biomaterial.

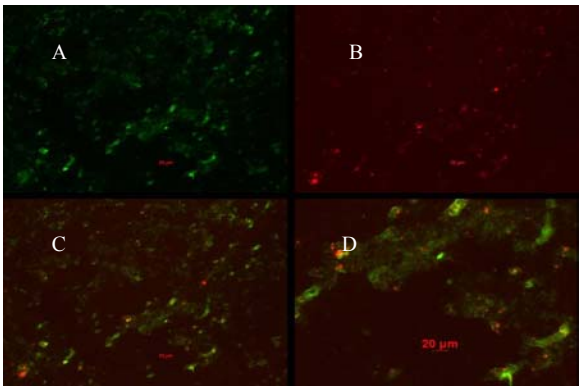

Figure 3 Adhesion of platelets (Panel A); TF bearing MPs (Panel B) and co-localization (Panels C, D)

Conclusions: TF bearing microparticles from the monocytic cell line, THP-1 adhere to artificial surfaces under a range of shear conditions and impart TF activity to the surface. The MPs also adhere and co-localize with adherent platelets. Further investigation is required to confirm the putative role of PSGL-1 and P-selectin as well as to evaluate the effect of shear removal in the presence of RBCs.