Surface Modified Poly(vinyl alcohol) Vascular Grafts Maintain Hemocompatibility

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Statement of Purpose: The biomaterials available for vascular grafts have not changed significantly in over 30 years. While the clinical standard, ePTFE, is suitable for vascular bypasses of large vessels (>6mm in diameter), small ePTFE grafts fail due to thrombosis and intimal ingrowth, which lead to graft narrowing and eventual occlusion. Compliant biomaterials such as poly(vinyl alcohol), PVA, have the potential to reduce intimal ingrowth, while maintaining a non-thrombogenic surface. Yet to encourage *in vivo* cellularization for long term implantation, the surfaces of PVA must be modified to support cell growth. Biochemical coatings and surface patterning of biomaterials have the potential to encourage endothelial cell growth and support endothelial cell functions. However, the effect of these modifications on the hemocompatibility of PVA is unknown. To evaluate the hemocompatibility of two surface patterned PVA vascular grafts (Figure 1) with and without cyclic RGD (cRGD) covalent coatings, we used an *ex vivo* shunt model of non-anti-coagulated blood flow to quantify platelet adhesion and fibrin incorporation.

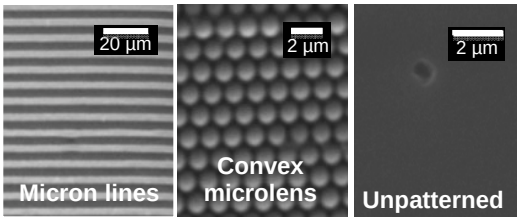

Figure 1. SEM images of PVA surface patterns.

Methods:

PVA biomaterial grafts: An aqueous solution of crosslinker sodium trimetaphosphate (STMP) and sodium hydroxide were added to aqueous PVA solution (10% w/v). This PVA-STMP solution was cast on 4 mm diameter cylindrical molds, which were either smooth or patterned (Figure 1). The patterns consisted of longitudinal micron lines or convex microlens. The cRGD covalent coating was attained by mixing the peptide sequence of Cys-Cys-Arg-Arg-Gly-Asp-Try-Leu-Cys into the PVA-STMP solution prior to casting.

Hemocompatibility testing: Femoral AV shunts in non-human primates were used to quantify hemocompatibility of the PVA grafts. Briefly, the shunt was surgically implanted between the femoral artery and vein. Autologous platelets were labeled with ^{111}In and infused with ^{125}I-fibrinogen prior to study. The shunt was elongated with silicone tubing and the PVA grafts were inserted centrally over a gamma scintillation camera. As circulating blood contacted the graft, radiolabeled platelets accumulated, which was measured dynamically at 5 min intervals for the 60 min duration. We quantified total platelet deposition, rate of platelet deposition, and total fibrin accumulated on the PVA grafts. Fibrinogen accumulation was determined after the ^{111}In decayed. The clinical standard ePTFE graft was used as a negative control and collagen-coated ePTFE grafts were used as positive controls.

Statistics: For all experiments, ANOVA with Tukey's post-hoc was performed, with differences considered to be significant if $p<0.05$ based on the *post hoc* analysis.

Results: All grafts remained patent for the duration of the study. As expected, the collagen coated ePTFE graft had significant platelet attachment within 15 minutes of the onset of flow, which steadily increased until a final average of $1.18 \pm 0.60 \times 10^9$ platelets (Figure 2). Platelet attachment to the ePTFE graft began at 25 min, plateaued at 45 min and had a final average of $0.35 \pm 0.13 \times 10^9$ platelets. The unpatterned-cRGD-coated PVA grafts and the 2µm longitudinally lined-cRGD coated grafts had $0.96 \pm 1.19 \times 10^9$ and $0.29 \pm 0.41 \times 10^9$ platelets, respectively, at 60 min, which was statistically equivalent to ePTFE. All other PVA grafts had significantly less platelet attachment (Figure 2). All PVA grafts had minimal fibrogen incorporation, which was significantly less than the ePTFE and collagen controls.

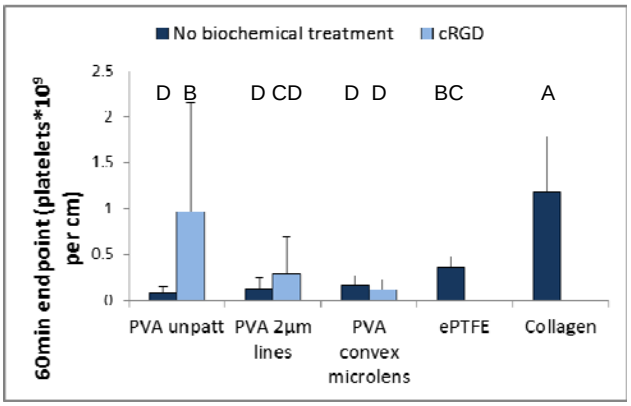

Figure 2. Platelet attachment at 60 minutes. Groups separated by different letters are significantly different.

Conclusions: The unmodified PVA grafts have excellent hemocompatibility properties. In an *ex vivo* shunt model in the absence of any anti-coagulation these PVA biomaterials had minimal platelet adhesion. The surface patterned PVA grafts also had no significant platelet attachment. Platelet adhesion did increase on the biochemically modified PVA surfaces, but in a pattern dependent manner. Previous studies indicated improved endothelial cell attachment on the cRGD-coated PVA. The cRGD peptide, which is a modified derivative of the RGD binding moiety enhanced with steric hindrance, improved cell attachment by binding $\alpha_v\beta_3$ integrins on endothelial cells. These hemocompatibility studies suggest the potential that PVA grafts hold for synthetic small diameter vascular graft replacement. Future work will explore *in vitro* and *in vivo* cellularization and longer-term performance for preventing tissue ingrowth *in vivo*.