Nitric Oxide-releasing, but not Nitric Oxide-generating, Polymer Coating Preserves Platelet Count and Function in Extracorporeal Circulations

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Statement of Purpose: Hemocompatibility of polymeric tubing used in extracorporeal circulation is highly dependent on quiescence of circulating blood platelets. Nitric oxide (NO), an endogenous inhibitor of platelet adhesion and activation, can be either catalytically generated from plasma S-nitrosothiols (RSNOs) by copper[1] or directly released from NO donor compounds[2]. A comparison between NO releasing (NORel) and NO-generating (NOGen) polymeric coatings were evaluated in a rabbit thrombogenicity model.

Methods: A 4 hour rabbit thrombogenicity model was utilized to evaluate NORel- and NOGen-coated ECC. NORel was composed of plasticized polyvinyl chloride (PVC) blended with a lipophilic *N*-diazeniumdiolate while NOGen was a plasticized PVC doped with a lipophilic Cu(II)-cyclen complex. Platelet count was obtained using a Coulter Z1 particle counter and function measured via optical aggregometry (Chrono-Log). Platelet P-selectin was quantified by flow cytometry and expressed as mean fluorescence intensity (MFI).

Results: Both the NORel and NOGen polymers significantly ($p<0.05$) reduced ECC thrombus formation compared to their respective controls after 4 hrs blood exposure (6.7 $\pm$ 0.4 NORel control vs 2.8 $\pm$ 0.7 pixels/cm^2 NORel; 6.4 $\pm$ 0.2 NOGen control vs 3.7 $\pm$ 1.1 pixels/cm^2 NOGen). Platelet count and function after 4 hrs on the NORel ECC was significantly increased over the control for count (3.4 $\pm$ 0.3 vs 2.3 $\pm$ 0.3 x 10^8 /ml, respectively) and was 83% of baseline platelet count while platelets remained functionally viable (baseline 74 $\pm$ 2 vs 4 hr NORel 71 $\pm$ 2% aggregation, NS). However, platelet count and function after 4 hrs on the NOGen-coated ECC were preserved only 66% of the baseline count and function was already 44 $\pm$ 15% aggregation after just 3 hrs compared to baseline (74 $\pm$ 2% aggregation). Plasma fibrinogen levels significantly decreased in all polymer-coated ECC for both the NORel and NOGen groups. Platelet P-selectin, a marker of activated platelets, was attenuated after 4 hours on the NORel ECC to ex vivo collagen stimulation (27 $\pm$ 1 MFI) compared to the NORel control (40 $\pm$ 2 MFI).

Conclusions: These results suggest that the NORel polymer coating preserved platelet count and function after 4 hours on ECC as compared to the NOGen coating. NORel-coated ECC attenuated the collagen-induced platelet P-selectin whereas the NOGen ECC enhanced the collagen-induced P-selectin expression. These NO-mediated platelet changes indicate that the NORel-polymer-coated ECCs, and not the NOGen-coated ECC, could improve thromboresistance for future biomedical devices.

References: [1]Oh BK. *J Am Chem Soc*. 2003;125:9552-9553. [2]Reynolds MM. *Free Radic Biol Med.* 2004;37:926-936.