Towards Thromboresistive Artificial Lungs: The Role of Copper-Doped Nitric Oxide-Generating Silicone for Blood-Contacting Surfaces

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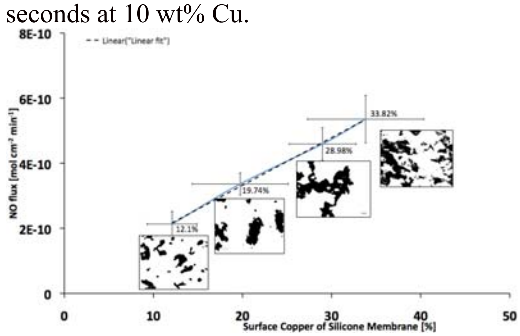
Statement of Purpose: The only treatment for end-stage chronic lung disease is lung transplantation. Currently, 1300 lung transplants are performed every year in the US but unfortunately due to the limited pool of available donor lung, about 2500 end-stage lung disease patients die every 5 years on the lung-transplant waitlist. Respiratory support with artificial lungs may work to reduce the mortality rate of end-stage lung disease patients. However, their gas-exchange membranes cause blood to clot, and systemic anticoagulation given to prevent clot formation can cause bleeding. An alternative solution is the imposition of endothelial-like properties, e.g. nitric oxide generation, on biomaterials.

Methods: An *in vitro* test system was created by coating Hemochron tubes (P214 flip-top, ITC, Edison, NJ) with silicone elastomer (Nusil Silicone Technology,CA) blended with 3 micron copper (Cu) particles (Sigma Aldrich Chemical Co. ST. Louis, MO) at 3 to 10 weight percent (wt%) Cu. All cured coatings (70 µm thick) were bathed in a 10mM phosphate buffer saline (PBS) at pH 7.4 inside an Innova 4000 incubator shaker at 37ºC for 24 hours to oxidized Cu^0 to Cu^{2+} by spontaneous corrosion of metallic copper [1]. The samples were then characterized for the spacing between Cu dendrites exposed on their surfaces (Philips XL30 FEG SEM). To determine the percentage of Cu on the surfaces of coatings, energy dispersive x-ray spectroscopy (EDAX Inc. Mahwah NJ) and image processing (Image J) was used. Next, NO generation from 0, 3, 5, 8, and 10 wt% Cu coatings (n = 6 each) was measured by chemiluminescence. Specifically, 0.5 – 4.5 cm^2 coated pieces were introduced into a 1 µM S-nitrosoglutathione (Sigma Aldrich Chemical Co. ST. Louis, MO) to activate NO generation. Also the activated clotting times (ACTs) of sheep blood exposed to each Cu wt% coating (n = 6 each) was measured.To determine the nature of platelet adhesion and aggregation, 0 (control) and 10 wt% Cu-doped silicone coatings were exposed to blood for various times. The surfaces were then rinsed gently with saline and fixed with 2% gluteraldehyde (Sigma Aldrich).

Results: Dendrite spacing as a function of wt% Cu varied from about 3µm at 10 wt% Cu doping to 100 µm at 3 wt% Cu doping. Spacing shortened with increasing wt% Cu. Chemical mapping and spectral outputs of energy dispersive x-ray spectroscopy verified the presence of surface Cu. The percentage of surface-Cu on coatings was determined to be 12.10 ± 2.80%, 19.74 ± 5.38%, 28.98 ± 3.75% and 33.82 ± 6.53% at 3, 5, 8 and 10 wt% Cu doping respectively (n=5 each). Peak NO flux increased with wt% Cu from $2.13 \times 10^{-10} \pm 0.38 \times 10^{-10}$ mol cm^{-2} min^{-1} at 3 wt% to $5.35 \times 10^{-10} \pm 0.73 \times 10^{-10}$ mol cm^{-2} min^{-1} at 10 wt% Cu (see figure). These fluxes overlap published human endothelial NO flux range of (1-4) x10-10 mol cm^{-2} min^{-1} [1]. The ACT of sheep blood increased with wt% Cu from 80 ± 13 seconds at 0 wt% Cu to 339 ± 44 seconds at 10 wt% Cu.



Micrographs of control and Cu-doped surfaces exposed to blood for 30 seconds showed smooth, disc-shaped,seemingly unactivated, yet adhered platelets. Areas around visible Cu dendrites showed no platelet adhesion and aggregation. However after 480 seconds, pseudopodal and seemingly activated platelets were visible on both control and Cu-doped surfaces. Platelets were uniformly spread over control surfaces and aggregated into colonies interconnected by fibrin on the NO-generating surfaces. Regions with exposed Cu dendrites remained free of platelet aggregates.

Conclusion: The Cu-doped silicone polymer matrix composite (PMC) was characterized for its ability to generate a tunable amount of NO, prolong ACT of sheep blood, and inhibit platelet aggregation towards a potential application to hollow fiber oxygenators. It was determined that exposure of Cu dendrites on the surface of PMC was important for NO generation. However the oxygen transfer rates of a Cu-doped silicone fiber oxygenator may be reduced, as transmembraneous gas diffusion is directly proportional to surface area. The extent of this reduction may be influenced by the structures of the embedded Cu and the extent to which the diffusional resistance of the fiber affects gas transfer. Also, the amount NO generated was tunable with the PMC's Cu-content. Sheep blood ACT did increase by about 300% and platelet aggregation was observed to localize away from exposed NO-generating Cu-dendrites. Copper-doped PMCs inhibited coagulation in a dose-dependent fashion related to the extent of Copper exposure on the surface. It is widely accepted that clinical applications lasting for anywhere from hours (cardiopulmonary bypass) to years (stents, vascular grafts) allow biomaterial-induced clotting of blood. Therefore imposing nitric oxide generation properties to their surfaces by this approach may be clinically beneficial in preventing bleeding and in maintaining device patency.

Reference: Wu Y. Sensors and Actuators B: Chemical. 2007;121:36-46.