Catalyzed Nitric Oxide Release Via Cu Nanoparticles Leads to Increase in Antimicrobial Effects and Hemocompatibility for Short Term Extracorporeal Circulation

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Statement of Purpose: Devices used for extracorporeal circulation (ECC) are met with two major medical concerns: thrombosis and infection. A device that allows for anticoagulant-free circulation while exhibiting bactericidal activity has yet to be developed. Current ECC procedure requires a careful balance between over- and under-administration of anticoagulants in order to prevent thrombosis while simultaneously avoiding uncontrolled bleeding. In addition, antibiotic-resistant bacteria have emerged in hospital settings. Due to the essential role of the nitric oxide (NO) in the immune and cardiovascular systems, NO donors have been incorporated into device coatings. However, the effectiveness of NO is dependent on the NO flux during the relevant application period. In this work, we report the effects of using copper nanoparticles (Cu NP) as an antimicrobial and catalyst on the endogenous NO donor S-nitrosoglutathione (GSNO) in a coating applied to ECC loops to reduce viability of adhered bacteria and overall clot formation. The presence of Cu NPs enhances the NO surface flux, increasing *in vitro* antibacterial and *in vivo* antiplatelet activity and resulting in superior hemocompatibility and antifouling capabilities, making it an attractive option for biomedical applications.

Methods: A multilayered system was employed for the preparation of ECC loops. Two coats of a GSNO- and Cu-CarboSil solution were applied each followed by a final CarboSil topcoat to coat the loop surface. Loops consisting of only GSNO, Cu NPs, or CarboSil were also prepared. SEM/EDS was performed to confirm presence of GSNO and Cu NPs in their respective layers. NO release was measured using a chemiluminescence nitric oxide analyzer for 4 h at 37°C in PBS. Viable bacterial adhesion was analyzed by incubating loops with *Staphylococcus aureus* and *Pseudomonas aeruginosa* for 24 h at 37°C. Loops were then washed and homogenized in PBS buffer, and resulting solutions were serially diluted and plated to assess adhered bacterial viability. Cytotoxicity towards 3T3 mouse fibroblast cells was measured with a Cell Counting Kit-8 (CCK-8). Platelet count (% of baseline) was assessed in a 4 h ECC rabbit model to determine hemocompatibility.

Results: The combination of Cu NPs and GSNO showed a number of positive effects such as increased NO release, decreased bacterial viability, best maintenance of platelet counts, and no cytotoxic affect towards mammalian cells. SEM/EDS confirmed the presence of GSNO and Cu NPs in their respective layers. NO release measurements indicated Cu-GSNO coated devices sustained an average flux of 8.14514 ± 0.667665 x10^{-10} mol cm^{-2} min^{-1} during the 4 h period, while the flux of GSNO loops without Cu NPs only measured 1.97125 ± 0.22769 x10^{-10} mol cm^{-2} min^{-1}. Adhered bacterial viability after 24 h of exposure showed Cu-GSNO had the greatest bactericidal activity, reducing *S. aureus* and *P. aeruginosa* by 99.94% and 99.68%, respectively (Figure 1). All loops were found to be non-cytotoxic towards mammalian cells.

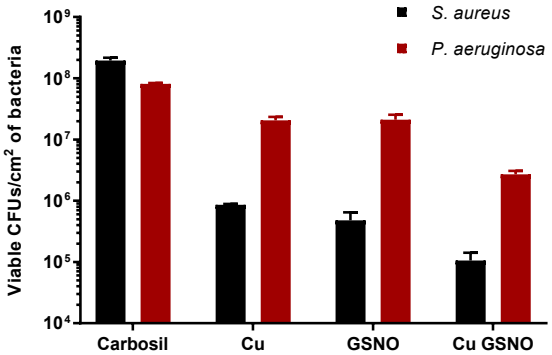

Figure 1 – Reduction in viable adhered *S. aureus* (99.94%) and *P. aeruginosa* (99.68%) compared to control surfaces.

For the first time, this study examined the combination of Cu NPs with a NO donor in a 4 h ECC rabbit model. Cu GSNO loops best maintained the baseline platelet count, retaining 89.29% ± 8.48% of platelets, while GSNO, Cu, and CarboSil loops dropped to 56.71% ± 9.09%, 76.69%, and 67.63 ± 20.42%, respectively (Figure 2).

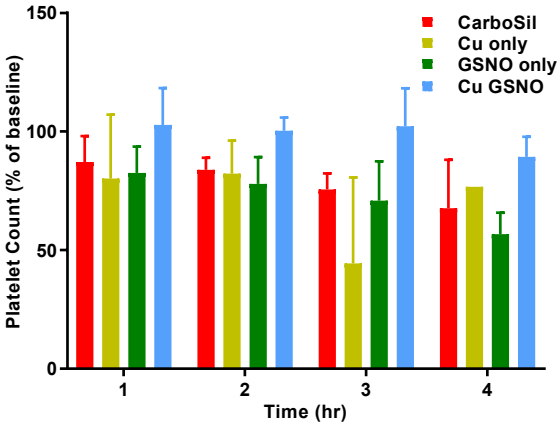

Figure 2 – Platelet count analysis of 4 h ECC rabbit model. Cu GSNO loops best maintained baseline platelet count.

Conclusions: Comparison of devices with and without Cu NPs demonstrates the superior NO release, antibacterial, and antiplatelet activity when Cu and GSNO are combined. Cu NPs catalyzed NO release up to four times higher while presenting no cytotoxic effect towards mammalian cells. The increased NO flux combined with the oligodynamic role of Cu NPs led to the reduction of viable *S. aureus* and *P. aeruginosa* by 99.94% and 99.68%, respectively. Cu GSNO loops best maintained the baseline platelet count in the *in vivo* rabbit model due to the increased NO release. Hence, this study demonstrates the strength in combining Cu NPs with GSNO for device coatings, resulting in superior hemocompatible and antibacterial activity.