Photo-Carbon Monoxide Releasing Molecules within Electrospun Scaffolds for Modulating Vascular Cells

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Introduction: The therapeutic potential of carbon monoxide (CO) in small doses has been studied and applied, including in a clinical trial using inhaled CO to treat pulmonary fibrosis. Cardioprotective properties of CO include enhancing re-endothelialization and improving graft survival in mice and rats [1]. In this study, we use visible light activated CO releasing materials (CORMs; unsaturated cyclic α-diketones) with electrospun scaffolds as a carrier. These CORMs allow controlled release of CO by activation with visible light, and provide a simple, nondestructive method to track the extent of photoreaction and CO release through fluorescence. This is due to generation of the fluorophore anthracene. The electrospun scaffolds can provide a good hydrophobic carrier for the CORM, which is required for high CO yield. The goals of this study are to verify the release profile (e.g., maximum duration and rate) from CORMs in physiological conditions, and determine the corresponding impacts on vascular smooth muscle cells (SMCs) and endothelial cells.

Materials and Methods: We produced electrospun poly (ε-caprolactone) (PCL) scaffolds with and without CORMs using a 90% v/v chloroform / DMF solution, and characterized the scaffolds with SEM. We modified a previously developed protocol to determine CO release for use with CORM-loaded electrospun meshes. The meshes were sterilized with ethylene oxide and then incubated in DMEM with 10% FBS for different length of time. Measurements were taken before activation, and after 0.5, 3, 30, and 60 min of activation with 470 nm light. To verify the cell viability in response to the CORM itself, we seeded rat SMCs onto coverslips and added up to 100 μM of CORM to cell culture media and incubated for 3 days. We also seeded SMCs and endothelial cells on CORM loaded meshes, and activated them for 30 min (5 min on and 5 min off) to verify the impacts of CO.

Results and Discussion: We obtained a similar average fiber diameter of 1.5±0.1 for pure PCL and 1.3±0.4 for PCL with CORM. We verified that we can control CO release with irradiation time (470 nm light for activation) by measuring an increase in fluorescent intensity of the CORM, using 350 nm excitation for detection. The scaffolds can be activated in for up to 1 h when in cell culture conditions, although longer irradiation times are needed compared to dry meshes. We have also confirmed that both the CORM material itself and the CO released with CORM activation are not toxic to vascular SMCs. A preliminary study with endothelial cells seeded-scaffolds, with and without CORMs, showed that the cells attached, spread, and express endothelial cell specific markers (i.e., eNOS and CD31, immunofluorescence). A study to investigate the activation profile and endothelial cell response with different concentrations of CORMs is ongoing.

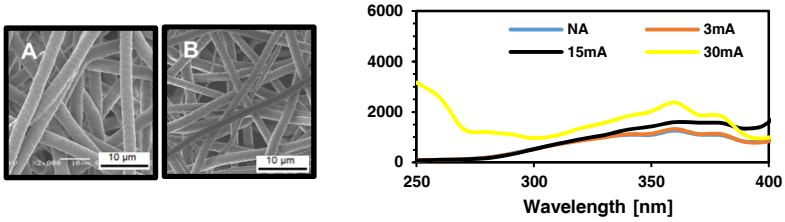

Figure 1. Electrospun meshes, pure PCL and PCL+CORM, and emission spectra of PCL+CORM scaffolds incubated for 30 min and activated for various time points.

Conclusions: We demonstrated that we can control CO release from CORM-incorporated PCL scaffolds. The CORM-loaded scaffolds provide the hydrophobic carrier needed for activation in cell culture conditions. The CORM is not toxic to both SMCs and endothelial cells at reasonable levels. Further modification of scaffold properties would aid in providing higher CO levels and improve vascular cell response (e.g., promoting vascular graft endothelialization). One approach, the effect of varying CORM-loading in the fibers, is ongoing.

References: [1] Sato, K. et al. J. Immunol., 166, 4185-4194 (2001).