Scaffolds with Incorporated Photo-Carbon Monoxide Releasing Materials for Engineering Vascular Grafts

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Statement of Purpose:
Carbon monoxide (CO) has been shown to have therapeutic benefits in small doses (e.g., in a clinical trial using inhaled CO to treat idiopathic pulmonary fibrosis). Cardioprotective properties of CO include enhancing re-endothelialization [1] and improving graft survival in mice and rats [2]. A promising strategy for local release of CO involves using CO releasing materials (CORMs). In this study we use visible light activated CORMs (i.e., 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 nondestructive method to track the extent of photoreaction and CO release through fluorescence. This is possible because the fluorophore anthracene is produced when CO molecules are released. The electrospun scaffolds provide a good hydrophobic carrier for the CORM, which is required for high CO yield. The goals of this study are to determine the activation profile of CORM-loaded meshes under culture conditions and characterize the response with smooth muscle cells.

Methods: We produced electrospun poly (ε-caprolactone) (PCL) scaffolds with and without CORMs using a 90% v/v chloroform / dimethylformamide solution, and characterized the scaffolds with SEM. To initially verify the release of CO, light for activation was provided for 15 s at a time up to a total of 10 min, with measurements taken in between. We modified a previously developed protocol for use with electrospun meshes [3]. To determine if meshes loaded with CORM release CO in cell culture conditions, we first sterilized them with ethylene oxide and then incubated them in DMEM with 10% fetal bovine serum for different length of time. Measurements were taken before activation and after 3, 15, 30, and 60 min of activation with 470 nm light. To verify the cell viability in response to the CORM, we seeded cells into coverslips and added up to 100 μM of CORM to cell culture media and incubated for 3 days. We also seeded CORM loaded meshes and activated them for 30 min (5 min on and 5 min off) to verify impacts of CO. Statistics was calculated with one-way ANOVA and Tukey multiple comparisons (p<0.05)

Results: We found that the presence of the CORMs (2% w/w CORM / PCL) reduced the average fiber diameter from 1.75±0.41 to 1.54±0.11 for a 20% w/v electrospinning solution. We verified the release of CO by measuring an increase in fluorescent intensity of the CORM, using 350 nm excitation, after activation with 470 nm light. We also determined that we can control the CO release through irradiation time. A study determined that CORM-loaded meshes can be activated after incubation in cell culture media for 1 h. In preliminary results, we determined the cell viability on glass coverslips was not effected by 100 μM exogenous CORM. Cell viability was also not affected on CORM-loaded meshes incubated in serum-containing media for 1 h and activated for 30 min, compared to CORM mesh controls. A detailed analysis of SMC phenotype in response to CO is ongoing.

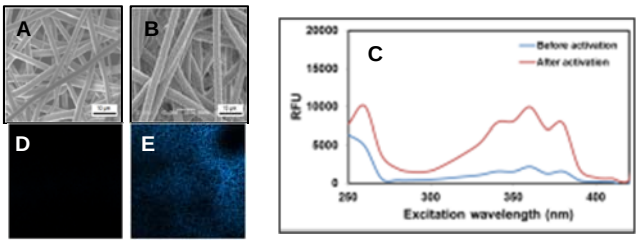

Fig.1. PCL (A) and CORM (B) meshes. Fluorescence spectra (C) 470 nm with 5 min activation, and images Pre- (D) and Post (E) activation fluorescence.

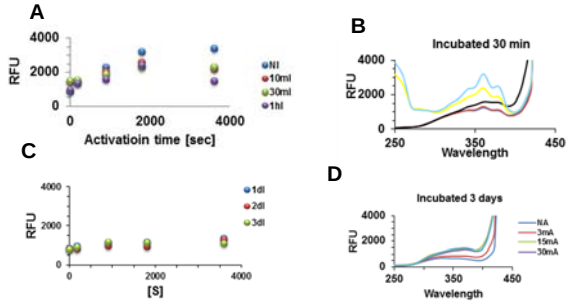

Fig.2. Activation and release with short (A, B) and long (C, D) incubation times. Excitation spectra (B, D). (n=6)

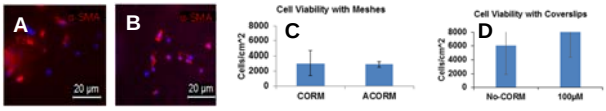

Fig.3. Cellular Response of SMCs in PCL (A) and CORM (B) meshes. DNA assay for cell viability on Meshes (C) and Coverslips (D) (n = 3)

Conclusions: We demonstrated that we can produce functional PCL meshes with incorporated CORMs and we can track CO release by an increase in fluorescence. Further, we can also control the CO release through irradiation time. An increase in fluorescence was observed with light exposure through 1 h, unlike within micelles in a previous study where all activation occurred within 5 min [3]. This may be related to the 3-D nature of electrospun meshes. We also demonstrated that our CORM-loaded meshes can still be activated after incubation in cell culture conditions. Finally we have shown that both the CORM material itself and CORM activation is not toxic to vascular SMCs.

References: [1] Wegiel, B. et al. Circulation 121, 537-548 (2010). [2] Sato, K. et al. 166, 4185-4194 (2001). [3] Ping, P. et al. Organic and biomolecular Chemistry 11, 6661-6864 (2013).