In Vitro and *In Vivo* Release of Doxycycline from *In situ* Cured, Biodegradable Polymer to Treat Aortic Aneurysms

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Statement of Purpose: Abdominal aortic aneurysms (AAA) are the 13th leading cause of death in the United States with 15,000 people dying from aortic ruptures each year. To treat this condition, a biodegradable polymer coating has been proposed that will deliver doxycycline and mechanically restrain the aneurysm. Doxycycline addresses the biological mechanism of AAA formation by decreasing matrix metalloproteinase (MMPs) activity, which promote elastin degradation [1]. Biodegradable poly(β-amino ester)s (PBAE) networks have been chosen because they have a wide range of mechanical properties and can be photopolymerized, which allows for *in situ* delivery. The objectives of this study are (1) to determine release profiles of doxycycline from PBAE and (2) assess the effect of doxycycline on MMP activity.

Methods: PBAE macromers were formed from mixing neopentyl glycol diacrylate and 3-methoxypropylamine in a molar ratio of 1.2:1. Further addition of 2-hydroxyethyl acrylate, isobornyl acrylate, and doxycycline in varying concentrations followed [2] prior to photopolymerization at 405nm to form PBAE networks. Doxycycline elution was measured by UV-Vis of absorbance peak at 355nm (Biotek Synergy H4) from 1 mm thick samples soaking in phosphate buffered saline at 37°C. *In vitro*: 10 ng/mL of tumor necrosis factor-alpha (TNF-α) was added to adult human aortic smooth muscle cells (SMC) (ScienCell) to induce MMP-9 expression [3]. 10 mm discs of PBAE+10mg/ml doxycycline were added to SMC and cultured for 72 hours, then media supernatants were collected for use in MMP-9 ELISA assay (R&D Systems). ANOVA with individual group comparisons including Bonferroni adjustment was performed on ELISA data. *In vivo*: all procedures were performed in accordance with IACUC of the University of Georgia. AAA were created in 12 male swine, where half were control animals with AAA creation and the remainder had AAA creation and delivery of PBAE+doxycycline coating to adventitia. At 3 and 12 weeks, animals were euthanized. Abdominal aortas were harvested, divided, and then half were fixed in 10% formalin and the remainder was frozen. Tissue was preserved fresh in saline and embedded in O.C.T. (Tissue-Tek) and frozen sections were cut at 6 µm for *in situ* zymography.

Results: Figure 1 shows the cumulative release profile of PBAE networks with varying concentrations of doxycycline. The drug release increased as drug loading concentration increased. The release profile shows an initial burst, which is followed by a decrease in release rate. Figure 2 shows the MMP-9 concentration from SMC. There was a significant increase in MMP-9 when TNF-α was delivered, and there was a significant decrease in MMP-9 when the SMCs+TNF-α were exposed to the PBAE+doxycycline. Figure 3 shows the presence of MMPs (green) in the sections of the AAA tissue at 3 weeks. The control aorta has no appreciable amount of MMP activity. The aorta with induced AAA shows MMP activity at the lumen and in the adventitia. The treated aorta (AAA+PBAE+doxycycline) shows less MMP activity than induced AAA without doxycycline.

Conclusions: Due to use of hydrophilic monomers and degradable PBAE, the doxycycline release is controlled by a combination of swelling of the polymer and diffusion of the drug from the polymer. *In vitro* testing confirmed that the polymer is able to release doxycycline and decrease MMP activity with SMCs. *In vivo* tests confirmed decrease in presence of MMP in aorta with use of extravascularly applied PBAE+doxycycline coating. Further studies will include further AAA model development, quantification of MMP, and refinement of delivery device.

References: [1] Manning M. Arterio, Thromb, Vasc Biol. 2003; 23:483-488. [2] Safranski D. Polymer. 2011;52:4920-4927.[3] Lee CW. Am J Physiol Lung Cell Mol Physiol. 2007; 292:799-812.

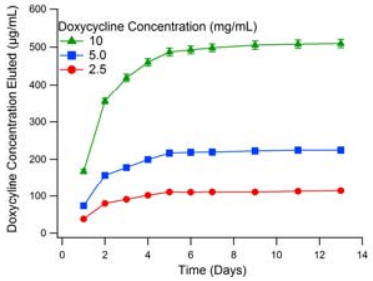

Figure 1. *In vitro* doxycycline release profile.

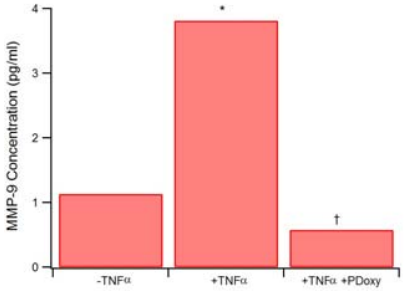

Figure 2. MMP-9 concentration of SMC –TNF-α, +TNF-α, and +TNF-α+PBAE+doxycycline at 72 hours.

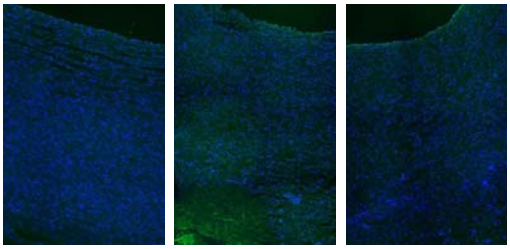

Figure 3. *In situ* zymography of (left) non-aneurysmal aorta, (middle) aneurysmal aorta, (right) aneurysmal aorta treated with PBAE+drug coating. MMPs show as green.