Multifunctional Drug-Delivery Nanoparticles for Elastic Matrix Stabilization and Repair in Aortic Aneurysms

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Statement of Purpose: Abdominal aortic aneurysms (AAAs) result from progressive elastic matrix degradation by matrix metalloproteases (MMPs) -2 & -9. Orally-delivered doxycycline (DOX) has been shown effective in slowing AAA growth in human & animal studies by limiting ECM degradation within through inhibition of MMP-2 & -9. However, systemic delivery and high circulating doses appear to have adverse side-effects[1]. DOX doses in the delivered µg/mL range also appear to inhibit elastic matrix deposition by vascular cells[2]. Hence, we investigated a nanoparticle (NP)-based approach towards localized, controlled, & sustained DOX delivery to AAAs. We evaluated effects of (a) DOX released from NPs on elastic matrix synthesis & MMP-inhibition in rat AAA smooth muscle cell (EaRASMC) cultures, and (b) surface modification of these NPs with cationic amphiphiles on elastin binding and LOX activity[3], which mediates crosslinking of elastin into a mature matrix.

Methods: Rat AAAs were induced by elastase perfusion & EaRASMCs isolated at 14d post-induction. DOX-loaded PLGA (50:50 lactide:glycolide) NPs formulated via double-emulsion solvent evaporation method, with didodecyldimethylammonium bromide (DMAB) as the stabilizer were incubated for 21d in EaRASMC cultures, with blank NPs (0% w/w DOX) as active-agent controls. Cell layers were harvested and biochemically analyzed for elastic matrix synthesis (Fastin assay) and MMP-production & activity (western blot & gel zymography).

Blank (DOX-free) PLGA NPs (formulated with PVA as stabilizer) were surface-functionalized with cationic amphiphiles dodecylamine hydrochloride (DAH) and dodecyl trimethylammonium bromide (DTAB). BSA-Cy5 was encapsulated within these NPs to enable fluorometric quantification of their binding to bovine aortic elastin. LOX activity (Amplex Red assay) and MMP-2 synthesis & activity in EaRASMC cultures treated with these NPs were compared to that of blank DMAB- and PVA-modified NPs and to determine their potential benefits to elastin crosslinking and preservation. Pilot *in vivo* studies (ongoing) examined uptake of fluorescent PLGA NPs (200 nm, 500 nm) in rat AAA tissues.

Results: DOX-loaded, DMAB-functionalized PLGA NPs exhibited mean size of 350 nm & mean surface charge of +30 mV, with average encapsulation efficiency of 40%. DOX release ranged between 1-7.5 µg/mL over >40 days, well below the 16-54 µg/mL dose range, which has been shown to limit elastic matrix synthesis by SMCs[2].

Overall, NP-treated cultures demonstrated higher matrix elastin production and significantly lower MMP-2 synthesis & activity compared to NP-untreated cultures (Fig.1), while DOX release from NPs led to enhanced effect relative to DOX-free NP controls. This was attributed to surface charge & hydrophobicity of the NPs and DOX released from NPs. The positive surface charge on NPs potentially enables them to mediate recruitment of negatively-charged LOX[3], while repelling positively-charged elastase. Due to two hydrophobic dodecyl chains in DMAB, NPs bind strongly to hydrophobic domains in tropoelastin molecules. In addition to DOX released from NPs, a cationic charge may attenuate MMP-2 production & activity via electrostatic or steric interactions with the MMP-2 active site, which concurs with other studies[4,5].

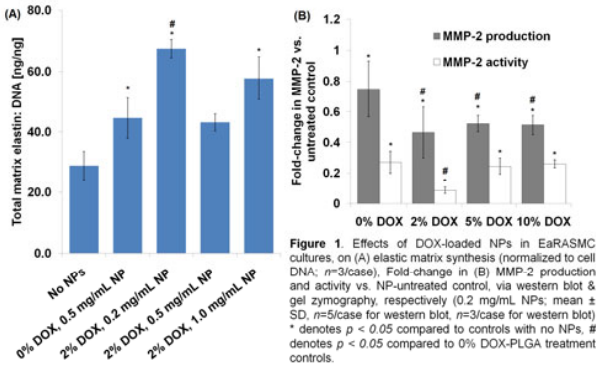

Figure 1. Effects of DOX-loaded NPs in EaRASMC cultures, on (A) elastic matrix synthesis (normalized to cell DNA; *n*=3/case), Fold-change in (B) MMP-2 production and activity vs. NP-untreated control, via western blot & gel zymography, respectively (0.2 mg/mL NPs; mean ± SD, *n*=5/case for western blot, *n*=3/case for western blot) * denotes $p < 0.05$ compared to controls with no NPs, # denotes $p < 0.05$ compared to 0% DOX-PLGA treatment controls.

While blank DMAB-, DTAB- & DAH-NPs increased LOX activity compared to negatively-charged PVA-NPs (mean ζ-potential=-35 mV), DMAB-NPs increased elastin binding (Fig.2A), due to increased cationic surface charge (mean ζ-potential for DTAB & DAH-NPs were +1.3 & +2.5 mV, respectively), in addition to enhanced hydrophobicity. DMAB-, DTAB- and DAH-NPs caused a significant inhibition of MMP-2 production & activity (Fig.2B) even in absence of DOX, confirming the role of cationic functionalization of NPs in attenuating MMPs.

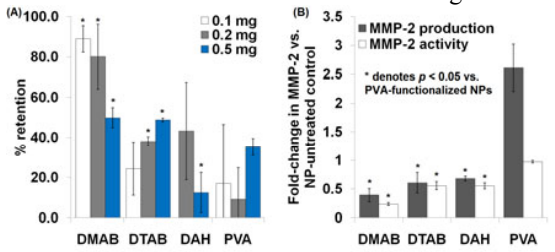

Figure 2. (A) Percentage of amphiphile-functionalized PLGA NPs bound to elastin. (B) Effect of these NPs on MMP-2 synthesis & activity. (*n*=3/case)

NPs of 200 & 500 nm size were taken up and retained in rat AAA tissue *in vivo*, with the latter localized in the media (Fig.3B,C). Future studies will assess *in vivo* uptake & retention of amphiphile-modified NPs functionalized, and benefits to elastic matrix synthesis & MMP-inhibition towards slowed AAA growth.

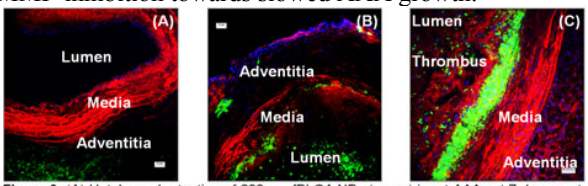

Figure 3. (A) Uptake and retention of 200 nm fPLGA NPs (green) in rat AAAs at 7 days post-infusion (Magnification 20X). (B,C) fPLGA NP uptake (500 nm) in rat AAA in the presence of a thrombus (Magnifications: 20x (A), 40X (B)). Elastic matrix structures in the medial layer autofluoresce red following pontamine sky blue pretreatment of tissue cryosections.

References: 1. Baxter *et al.* J Vasc Surg. 2002;36:1-12; 2. Franco *et al.* Am J Pathol. 2006;168:1697-709; 3. Kagan *et al.* J Biol Chem.1981;256:5417-21; 4. Mendis *et al*. Bioorg Med Chem Lett. 2009; 19:2755-9; 5. Tezvergil-Mutluay *et al.* J Dent Res. 2011; 90:535-40.