Multifunctional Magnetic Nanoparticles to Restore Elastic Matrix Homeostasis in Abdominal Aortic Aneurysms

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Statement of Purpose: Abdominal aortic aneurysms (AAAs) are characterized by progressive degradation of elastic matrix within the aortic wall by MMPs (matrix metalloproteases) -2 & -9. Oral doxycycline (DOX) therapy has shown promise in slowing AAA growth in pre-clinical models and some clinical studies by attenuating MMPs-2 & -9 but has systemic effects and inhibits elastin synthesis within AAAs at the high delivered doses. Since DOX provides a pro-elastogenic stimulus at low micromolar doses, we investigated localized, controlled & sustained low dose DOX delivery from poly(lactic-co-glycolic) acid nanoparticles (PLGA NPs) for regenerative elastic matrix repair within AAAs[1]. Surface functionalization of NPs with cationic amphiphiles was shown to impart them pro-elastogenic and anti-proteolytic properties independent of delivered DOX[1] and improve their arterial uptake & retention. To provide improved targeting of the DOX NPs to AAA tissue and enhance efficiency of uptake, we have now sought to incorporate superparamagnetic iron oxide NPs (SPIONs) within polymeric NPs and direct them to the AAA wall under an applied external magnetic field.

Methods: PLGA (50:50 lactide:glycolide) NPs were formulated via double-emulsion solvent evaporation method, with didodecyldimethylammonium bromide (DMAB) as the stabilizer, which imparts NPs with a positive charge. NPs formulated were blank (no DOX or SPIONs), DOX-loaded and (DOX+SPION)-loaded. The size & surface charge (ζ-potential) were determined via phase analysis light scattering. UV spectrophotometry (λ=270 nm) was used to determine DOX release from the NPs. Velocity of NPs under an applied magnetic field (0.105 T; magnetic gradient 0.008 T/mm) was determined using cell tracking velocimetry[2]. Cytotoxicity of NPs (0.2 mg/mL NP concentrations) towards EaRASMCs was examined (Live/Dead assay). Functional effects of the NPs in vitro were evaluated in terms of cell proliferation (DNA assay), elastic matrix deposition (Fastin assay) and MMP-production & activity (western blot & gel zymography)[1] following 21d of culture with EaRASMCs. Preliminary ex vivo studies were carried out to visualize NP uptake & retention (whole-tissue imaging; Bruker), following their catheter-based delivery to the proteolytically-disrupted wall of rat coronary arteries (30 min) in the presence of an applied magnetic field.

Results: All NPs exhibited a mean hydrodynamic diameter (NP size) between 300-350 nm, which concurs with our recent studies[1]. NPs formulated with DMAB had ζ-potential = +50 mV, with DOX encapsulation efficiency of ~42%. SPION co-incorporation with DOX did not affect the NP size, surface charge or DOX encapsulation efficiency significantly. Cumulative DOX release over 18 days was ~4.5 µg/mL for DOX NPs, while that for (DOX+SPION) NPs was ~3.5 µg/mL; well below 16-54 µg/mL, which has been shown to limit elastic matrix synthesis by SMCs[3]. (DOX+SPION) NPs demonstrated a magnetic velocity of 1.90 ± 0.02 µm/s under the influence of the applied magnetic field. Compared to NP-untreated controls, EaRASMC cultures treated with NPs showed enhanced elastic matrix deposition (Figure 1), as well as increased inhibition of MMP-2 synthesis, and MMP-2 & -9 activities (vs. NP-untreated controls; Figures 2A, B). MMP-inhibition due to DOX released from NPs was more pronounced versus that observed for blank NPs.

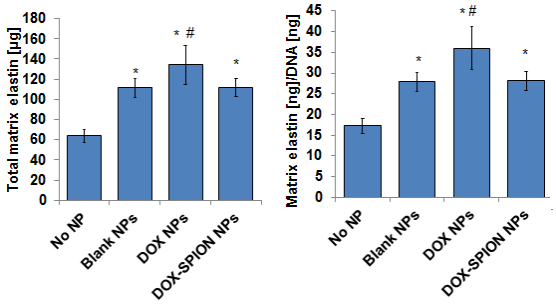

Figure 1. Effects of DOX released from NPs on elastic matrix deposition by EaRASMCs on (A) an absolute basis (left) and (B) DNA-normalized basis (right). (n=6, mean±S.D. * denotes $p < 0.05$ vs. NP-untreated controls, # denotes $p < 0.05$ vs. blank NP control)

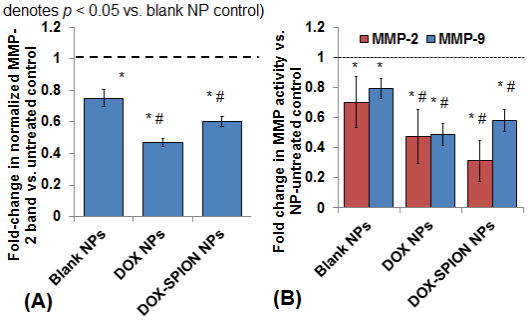

Figure 2. Effects of DOX released from NPs on MMP synthesis (left; western blot) and activity (right; gel zymography) by EaRASMCs (n=3, mean±S.D. * denotes $p < 0.05$ vs. NP-untreated EaRASMC control, # denotes $p < 0.05$ vs. blank NP control. MMP-9 synthesis could not be quantified reliably.

Ongoing ex vivo studies with SPION-loaded NPs show enhanced uptake & retention in the arterial wall in the presence of an external magnetic field (Figure 3).

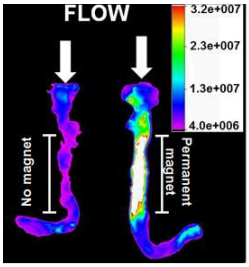

Figure 3. Uptake & retention of AlexaFluor633-tagged SPION-loaded NPs within the wall of matrix-disrupted porcine coronary arteries ex vivo in the absence (left) and presence (right) of an external magnetic field. Scale indicates fluorescent intensity (white indicates saturation)

Conclusion: Our in vitro & ongoing ex vivo studies demonstrate feasibility of magnetic guidance of SPION-DOX-NPs to enhance their targeting to, and uptake by AAA tissue with no adverse impact on their pro-elastogenic & anti-proteolytic effects. Future studies will further optimize NP surface properties to improve their functional effect and proceed to in vivo testing for efficacy of regenerative matrix repair in a rat AAA model.

References: 1. Sivaraman et al. Acta Biomater. 2013;9: 6511-25. **2.** McCloskey et al. Anal Chem. 2003;75: 6868-74. **3.** Franco et al. Am J Pathol. 2006;168:1697-709.