Localized Therapeutic Delivery From Multifunctional Magnetic Nanoparticles for Elastic Matrix Stabilization and Repair

Balakrishnan Sivaraman[1], Gregory Howard[1,2], Anand Ramamurthi[1,2]
Departments of Biomedical Engineering, [1]The Cleveland Clinic, Cleveland, OH and [2]University of Akron, Akron, OH.

Statement of Purpose: Abdominal aortic aneurysms (AAAs) are characterized by progressive degradation of elastin/elastic matrix within the aortic wall by MMPs (matrix metalloproteases) -2 & -9. Doxycycline (DOX) has been shown to be effective in slowing AAA growth in human & animal studies by limiting ECM degradation through inhibition of MMP-2 & -9. Recent studies by our group have examined the functional effects of localized, controlled & sustained DOX delivery from poly(lactic-co-glycolic) acid nanoparticles (PLGA NPs) for regenerative repair in AAAs[1]. Cationic functionalization of NPs demonstrated multifunctionality in enhancing elastic matrix deposition & MMP-inhibition[1]. Although cationic NPs exhibit improved arterial uptake & retention, we hypothesize that incorporation of superparamagnetic iron oxide NPs (SPIONs) within polymeric NPs would further enhance their permeation & localization within the AAA wall, under the application of an external magnetic field. We characterized the physical (size & surface charge) and magnetic characteristics (velocity under applied magnetic field & volume fraction of magnetite within) of NPs containing DOX alone and co-encapsulated DOX & SPIONs, as well as DOX release. Further, we examined their effects on the viability of rat AAA smooth muscle cell (EaRASMCs) cultures *in vitro*. Finally, we propose to evaluate their effects on elastic matrix synthesis & MMP-inhibition over 21 days *in vitro* in EaRASMC cultures.

Methods: PLGA (50:50 lactide:glycolide) NPs were formulated via double-emulsion solvent evaporation method, with didodecyldimethylammonium bromide (DMAB) or polyvinyl alcohol (PVA) as the stabilizer. DMAB imparts NPs with a positive charge, while PVA provides them with a negative surface charge. NPs formulated were blank (no DOX or SPIONs), DOX-loaded, SPION-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 & 0.5 mg/mL NP concentrations) towards EaRASMCs was examined using a Live/Dead assay. Ongoing studies will examine the functional effects of these NPs *in vitro* following 21 days of culture with EaRASMCs. Cell layers will be harvested and biochemically analyzed for cell proliferation (DNA assay), elastic matrix synthesis (Fastin assay) and MMP-production & activity (western blot & gel zymography)[1]. Based on these studies, we also propose to evaluate the *in vivo* uptake and retention of these NPs following their catheter-based delivery to AAA sites in rats in the presence of an applied magnetic field. Functional effects of DOX (and NPs themselves) will also be evaluated in terms of their ability to improve elastic matrix and MMP-inhibitory outcomes at the AAA site.

Results: All NPs formulated exhibited a mean hydrodynamic diameter (NP size) between 300-350 nm, which concurs with our recent studies[1,3]. NPs formulated with DMAB had ζ-potential = +20 mV, with DOX encapsulation efficiency of ~40%, while those formulated with PVA had an average ζ-potential of -30 mV, with a DOX encapsulation efficiency of ~70%. SPION incorporation within the PLGA NPs did not affect their size, surface charge or DOX encapsulation efficiency significantly. DOX release over the first 48 h (ongoing), ranged between 1.5-3.0 µg/mL for DMAB-based NPs, while that for PVA-based NPs was ~8.0 µg/mL (**Fig.1**). This was well below 16-54 µg/mL, which has been shown to limit elastic matrix synthesis by SMCs[4].

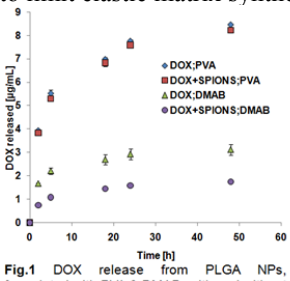

Fig.1 DOX release from PLGA NPs, formulated with PVA & DMAB, with and without co-encapsulated SPIONS. n=3 mean ± SD

Sample	Velocity [µm/s]	Volume fraction of magnetite
SPIONs, PVA	1.97 ± 0.10	0.132 ± 0.004
DOX+SPIONs, PVA	1.42 ± 0.06	0.096 ± 0.004
SPIONs, DMAB	6.04 ± 0.09	0.407 ± 0.006
DOX+SPIONs, DMAB	1.90 ± 0.02	0.128 ± 0.001

Table 1. Magnetic properties of SPION-loaded PLGA NPs, formulated with PVA & DMAB, with and without co-encapsulated DOX. The data represents mean ± SEM for ≥ 500 PLGA NPs from 8 different tracking experiments

Table 1 lists the velocities of SPION-loaded PLGA NPs under an applied magnetic gradient of 0.008 T/ mm. Co-incorporation of DOX along with SPIONs caused a significant decrease in magnetic velocity, as well as the volume fraction of magnetite within the NPs. SPION-loaded DMAB-NPs exhibited higher velocities due to higher volume fraction of magnetite encapsulated within.

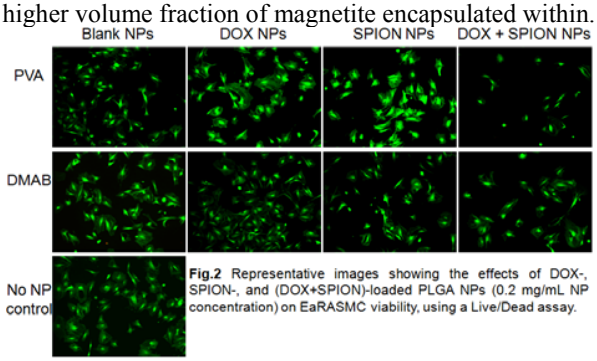

Fig.2 Representative images showing the effects of DOX-, SPION-, and (DOX+SPION)-loaded PLGA NPs (0.2 mg/mL NP concentration) on EaRASMC viability, using a Live/Dead assay.

Viability of EaRASMCs was not different following treatment with 0.2 mg/mL (**Fig. 2**) or 0.5 mg/mL NP concentrations. Ongoing *in vitro* culture experiments utilize NPs at 0.5 mg/mL concentration. Overall, planned *in vitro* & *in vivo* studies will provide insights into the feasibility of this strategy as a modality for enhancing the targeting efficacy of NPs for localized, controlled and sustained therapeutic delivery at the AAA site.

References:

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