Acta Biomaterialia 52 (2017) 171–186

Contents lists available at ScienceDirect

Acta Biomaterialia

journal homepage: www.elsevier.com/locate/actabiomat

Full length article

Magnetically-responsive, multifunctional drug delivery nanoparticles for elastic matrix regenerative repair ☆

Balakrishnan Sivaraman [a], Ganesh Swaminathan [a,b], Lee Moore [a], Jonathan Fox [a], Dhruv Seshadri [a], Shataakshi Dahal [a], Ivan Stoilov [c,d], Maciej Zborowski [a], Robert Mecham [c,d], Anand Ramamurthi [a,c,d,*]

[a] Department of Biomedical Engineering, Cleveland Clinic, 9500 Euclid Avenue, ND 20, Cleveland, OH 44195, USA
[b] Department of Biology, University of Akron, Akron, OH, USA
[c] Department of Cell Biology and Physiology, Washington University, St. Louis, MO, USA
[d] Department of Molecular Medicine, Cleveland Clinic Lerner College of Medicine of Case Western Reserve University, Cleveland, OH, USA

ARTICLE INFO

Article history:
Received 30 June 2016
Received in revised form 14 November 2016
Accepted 20 November 2016
Available online 21 November 2016

Keywords:
Nanoparticles
Elastic matrix
Regenerative matrix repair
Drug delivery
Aortic aneurysms
Smooth muscle cells
Magnetic targeting

ABSTRACT

Arresting or regressing growth of abdominal aortic aneurysms (AAAs), localized expansions of the abdominal aorta are contingent on inhibiting chronically overexpressed matrix metalloproteases (MMPs)-2 and -9 that disrupt elastic matrix within the aortic wall, concurrent with providing a stimulus to augmenting inherently poor auto-regeneration of these matrix structures. In a recent study we demonstrated that localized, controlled and sustained delivery of doxycycline (DOX; a tetracycline-based antibiotic) from poly(lactic-*co*-glycolic acid) nanoparticles (PLGA NPs), enhances elastic matrix deposition and MMP-inhibition at a fraction of the therapeutically effective oral dose. The surface functionalization of these NPs with cationic amphiphiles, which enhances their arterial uptake, was also shown to have pro-matrix regenerative and anti-MMP effects independent of the DOX. Based on the hypothesis that the incorporation of superparamagnetic iron oxide NPs (SPIONs) within these PLGA NPs would enhance their targetability to the AAA site under an applied external magnetic field, we sought to evaluate the functional effects of NPs co-encapsulating DOX and SPIONs (DOX-SPION NPs) on elastic matrix regeneration and MMP synthesis/activity *in vitro* within aneurysmal smooth muscle cell (EaRASMC) cultures. The DOX-SPION NPs were mobile under an applied external magnetic field, while enhancing elastic matrix deposition 1.5–2-fold and significantly inhibiting MMP-2 synthesis and MMP-2 and -9 activities, compared to NP-untreated control cultures. These results illustrate that the multifunctional benefits of NPs are maintained following SPION co-incorporation. Additionally, preliminary studies carried out demonstrated enhanced targetability of SPION-loaded NPs within proteolytically-disrupted porcine carotid arteries *ex vivo*, under the influence of an applied external magnetic field. Thus, this dual-agent loaded NP system proffers a potential non-surgical option for treating small growing AAAs, via controlled and sustained drug release from multifunctional, targetable nanocarriers.

Statement of Significance

Proactive screening of high risk elderly patients now enables early detection of abdominal aortic aneurysms (AAAs). There are no established drug-based therapeutic alternatives to surgery for AAAs, which is unsuitable for many elderly patients, and none which can achieve restore disrupted and lost elastic matrix in the AAA wall, which is essential to achieve growth arrest or regression. We have developed a first generation design of polymer nanoparticles (NPs) for AAA tissue localized delivery of doxycycline, a modified tetracycline drug at low micromolar doses at which it provides both pro-elastogenic and anti-proteolytic benefits that can augment elastic matrix regenerative repair. The nanocarriers themselves are also uniquely chemically functionalized on their surface to also provide them pro-elastin-regenerative & anti-matrix degradative properties. To provide an active driving force for efficient uptake of intralumenally infused NPs to the AAA wall, in this work, we have rendered our polymer NPs mobile in an applied magnetic field via co-incorporation of super-paramagnetic iron oxide NPs. We demonstrate that such modifications significantly improve wall uptake of the NPs with no significant changes to their

☆ Part of the Special Issue on Extracellular Matrix Proteins and Mimics, organized by Professor Katja Schenke-Layland.

* Corresponding author at: Department of Biomedical Engineering, Cleveland Clinic, 9500 Euclid Avenue, ND 20, Cleveland, OH 44195, USA.
E-mail address: ramamua@ccf.org (A. Ramamurthi).

http://dx.doi.org/10.1016/j.actbio.2016.11.048

physical properties and regenerative benefits. Such NPs can potentially stimulate structural repair in the AAA wall following one time infusion to delay or prevent AAA growth to rupture. The therapy can provide a non-surgical treatment option for high risk AAA patients.

1. Introduction

Abdominal aortic aneurysms (AAAs) are localized, rupture-prone expansions of the infrarenal abdominal aorta resulting from progressive breakdown of wall elastic matrix by chronically over-expressed matrix metalloproteases (MMPs), specifically MMPs 2 and 9 [1]. AAAs afflict between 1–9% of men and 1–2% of women in developed nations [2], and is the cause of 1–3% of all deaths in men above the age of 65 [3]. Greater than 90% of diagnosed AAAs tend to small with a maximal diameter less than a critical size of 5.5 cm, above which rupture risk greatly increases [4]. These AAAs typically grow slowly (~10% per year) to the critical size [5,6] over ⩾5 years during which period, they are only managed through periodic ultrasound-based growth monitoring. Surgical or minimally invasive interventions are applicable only to larger AAAs and provide no benefit to treating sub-critically sized AAAs. Non-surgical approaches to arrest or regress small AAA growth during the monitoring period are thus warranted.

Restoring elastic matrix homeostasis in the AAA wall is essential to achieving AAA growth arrest or regression to a healthy vessel state. This mandates a) inhibiting chronic proteolysis by elastolytic MMPs-2 and -9 overexpressed by inflammatory cells [7,8] and activated smooth muscle cells (SMCs) within the AAA wall [2,9], and b) augmenting elastic matrix regeneration, which adult [10,11] and aneurysmal [12] SMCs are naturally incapable of achieving. Recent studies in human patients [5,6,13–18] and in animal models [13,16,19,20] suggest that oral dosing of doxycycline (DOX), a modified tetracycline, is useful to inhibit transcription [21–23] and activity [24,25] of elastolytic MMPs in the AAA wall and thus slow their growth. However, drawbacks of oral DOX dosing include systemic side effects [13], and bodywide inhibition of MMPs which can adversely affect healthy tissue remodeling. In addition, elastic matrix synthesis inhibitory effects of the drug at the therapeutically useful oral doses (equivalent to tissue level concentrations of 16–54 μg/mL) [26] is also a concern. The latter aspect has been confirmed in *in vitro* cultures of vascular SMCs as well [26,27]. On this basis, modalities for AAA tissue-localized, predictable, and sustained delivery of DOX must be pursued.

Recent studies have demonstrated feasibility of peri-aortic DOX delivery at 1/100th of the oral dose from implanted micropumps [28,29], and shown it to be effective in suppressing AAA formation [28]. While promising, long-term pump implantation is concerning and clinically unacceptable. Accordingly, we recently investigated in aneurysmal SMC cultures, the utility of controlled and sustained DOX delivery from poly(lactic-co-glycolic acid) nanoparticles (PLGA NPs) in attenuating MMPs to preserve the elastic matrix [30]. Surface functionalizing these NPs with a cationic amphiphile (didodecyldimethylammonium bromide or DMAB), which imparts a positive charge to the NP surface and presents a long-chain hydrocarbon, was found to impart the NPs multifunctional properties, independent of the released DOX, that benefited elastic matrix neoassembly and integrity. We showed the NPs to bind elastic fibers via hydrophobic interactions with their surface hydrocarbon chains, and subsequently upregulate protein expression and activity of the elastin crosslinking enzyme lysyl oxidase (LOX), and attenuate MMP-2 synthesis, and MMP-2 and -9 enzyme activities [30]. We attributed these effects to the positive NP surface charge and hydrocarbon groups presented by the pendant DMAB molecules. While the positive charge on the surface of the NPs has been shown to facilitate their uptake into the arterial wall [31], in this study, we have sought to improve such uptake by rendering the NPs responsive to an applied external magnetic field by incorporating superparamagnetic iron oxide nanoparticles (SPIONs) within. Recent studies by other groups have demonstrated the effectiveness of this strategy to deliver drugs to target tissues, particularly deeply situated ones, with reduced systemic side-effects [32,33].

This manuscript represents the first step towards evaluating the utility of these magnetically-responsive PLGA NPs as vehicles for targeted delivery of DOX to AAAs for effecting regenerative elastic matrix repair. We investigated the effects of SPION incorporation on physical properties of the DOX NPs, their loading and release of DOX, and effects on elastic matrix neoassembly and proteolytic activity in cultures of SMCs isolated from elastase-induced rat AAAs (EaRASMCs). We also carried out preliminary *ex vivo* studies to demonstrate benefits of magnetic targeting of the NPs to their uptake and retention in the wall of proteolytically-disrupted vessels in the presence of an external applied magnetic field. The work presented in this manuscript will guide future *in vivo* studies to assess long-term NP retention and therapeutic effect in a preclinical rat AAA model.

2. Methods

2.1. Formulation of DOX and DOX-SPION PLGA NPs

Poly-lactic-co-glycolic acid (PLGA; 50:50 lactide:glycolide; inherent viscosity = 0.95–1.2 dL/g in hexafluoroisopropanol; Durect Corporation, Birmingham, AL) was used for the formulation of a) blank NPs (DOX- and SPION-free), b) DOX NPs (loaded with DOX only) and DOX-SPION NPs (loaded with DOX and SPIONs). All NPs were formulated using a double emulsion solvent evaporation technique, using 1% w/v DMAB (Sigma-Aldrich) as the stabilizer, as described in prior studies by our group [30]. Studies by other groups [32,34] have also utilized this formulation method for the encapsulation of SPIONs, as well as co-encapsulation of a drug along with SPIONs, within PLGA NPs.

Briefly, PLGA was dissolved in chloroform (Fisher Scientific, Fairlawn, NJ) at a concentration of ~2.5–3.0% w/v. For DOX NPs, an aqueous solution of doxycycline hydrate (Sigma-Aldrich) at a loading of 2 wt% ratio of DOX:PLGA was emulsified into the PLGA solution using a probe sonicator (Q500; QSonica LLC, Newtown, CT) for 1 min on ice, at an amplitude setting of 20%. Similarly for DOX-SPION NPs, SPIONs (1.0 mg; fluidMAG amine; chemicell GmbH) along with 2 wt% of DOX in aqueous solution, was emulsified as described above. The water-in-oil emulsion thus obtained was then further emulsified (1 min on ice, 20% amplitude) into an aqueous solution of 1% w/v DMAB to form the water-in-oil-in-water emulsion. This secondary emulsion was stirred for 16 h at room temperature, then desiccated for 1 h under a vacuum to remove any traces of chloroform. NPs formulated were recovered via ultracentrifugation at 35,000 rpm (Beckman L-80, Beckman Instruments, Palo Alto, CA). The NPs were washed twice with nanopure water to remove any traces of DMAB and unencapsulated DOX and SPIONs, then lyophilized for 48 h to obtain a dry