EDTA loaded BSA nanoparticles for targeted therapy to reverse vascular calcification in chronic kidney disease animal model

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Statement of Purpose: Vascular calcification can occur at two different sites in the arteries – the intima and the media. Intimal calcification is normally associated with atherosclerosis while medial arterial calcification (MAC) occurs as deposits of calcium along the elastic lamellae in patients with chronic kidney disease (CKD). Vascular calcification is a major predictor of cardiovascular disease morbidity and mortality. There are currently no non-invasive treatments available for reversal of vascular calcification. Surgical treatments, like directional atherectomy and stent grafts are used for intimal calcification that occlude lumen of the vessel. However, there are no such treatments available for elastin-specific MAC. One possible approach is the use of chelating agents. However, chelation therapy is not yet accepted in the United States and not approved by FDA as there have not been sufficient clinical studies to show that it is effective in reversing vascular calcification and improving cardiovascular function. Previous studies in our lab have shown that EDTA can be a useful chelating agent to reverse elastin calcification both *in vitro* and *in vivo* when delivered at the site of calcification[1]. We aim to develop a nanoparticle-based chelation therapy to target the vascular calcification sites and test the reversal of calcification. Towards this aim, we prepare BSA nanoparticles loaded with chelating agents and propose to test their efficacy in an *in vivo* calcification model. Single administration of adenine injection leads to kidney failure in rats and leads to elastin specific medial arterial calcification as observed in patients with chronic renal failure [2].

Methods Bovine serum albumin (BSA) nanoparticles loaded with EDTA were prepared using an ethanol-desolvation method. Nanoparticles were obtained by dissolving 100 mg of EDTA and 200mg of BSA (BSA; Seracare, MA, USA) in 4 mL DI water. The pH was adjusted to ~8.5 and with 4% glutaraldehyde (0.5µg-1µg of glutaraldehyde/mg of protein) was added as a crosslinker. This aqueous solution was added drop-wise to 16 mL ethanol under probe sonication for 30 minutes (20 Watts, Omni Ruptor 400 Ultrasonic Homogenizer, Omni International Inc, Kennesaw, GA) to prepare NPs. NPs were characterized for their size, surface charge, drug loading, drug release, cytotoxicity etc. We will test the optimized nanoparticles for reversal of vascular calcification induced in rats by dietary adenine model. Calcification will be induced by feeding the rats with a synthetic diet with 0.75% adenine for 2-4 weeks. Rats will then be injected with NPs loaded with chelating agents like EDTA and delivered to the sites of mineralization.

Results: EDTA-loaded albumin NPs with an optimized size of approximately 200 nm, negative zeta potential (~ −20mV), and EDTA loading efficiency of ~25% were prepared. Characterization of NPs are shown below in Table 1.

Table 1. Nanoparticle characterization

Particle Size	ζ potential	Loading Efficiency
~200nm	-20mV	~25%

Figure 1 shows sustained release profile of EDTA from the NPs. Sustained release of EDTA up to 4-5 days could be achieved. Such release is desirable to remove calcium from arteries.

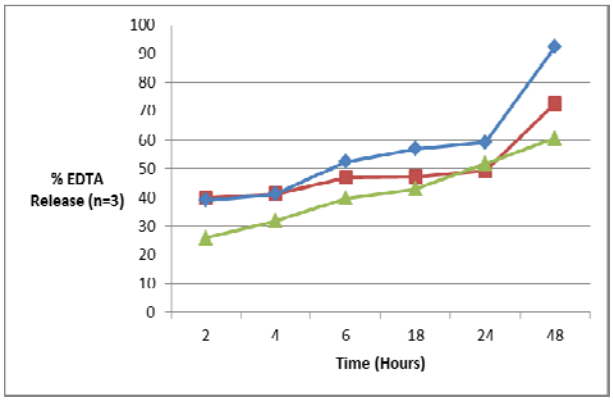

Figure 1: Release profile of EDTA from 3 independent batches of NPs showing consistency.

Conclusions:
We were able to produce BSA nanoparticles loaded with chelating agent EDTA. These nanoparticles showed a sustained release of the EDTA in 4-5 days as desirable. We aim to use these nanoparticles to target vascular calcification induced in uremic rats by adenine injection and study the reversal of calcification. These studies are underway and results will be presented.

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References:

1. Lei, Y., et al (January 01, 2014). Targeted chelation therapy with EDTA-loaded albumin nanoparticles regresses arterial calcification without causing systemic side effects. *Journal of Controlled Release.*

2. Price, P. A., et al (January 01, 2006). Artery calcification in uremic rats is increased by a low protein diet and prevented by treatment with ibandronate. *Kidney International, 70,* 9, 1577-83.