Localized, Sustained Non-viral Gene Delivery from Alginate Hydrogels for Osteogenesis

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Statement of Purpose: Bone regeneration by tissue engineering approaches has great potential for healing bone defects. For promoting osteogenesis, the delivery of recombinant bone morphogenetic proteins has been approved for clinical use but still has limitations such as rapid degradation of the proteins *in vivo* and difficulties retaining protein at the site of injury. Localized gene delivery is a promising alternative therapy, as it would allow sustained expression of specific osteoinductive growth factors by cells near the damaged site. Here we show a system capable of localized, sustained non-viral gene delivery from alginate hydrogels containing preosteoblast cells and calcium phosphate – DNA nanoparticles.

Methods: Two types of Calcium-Phosphate DNA Nanoparticles (CaP NPs) were created: CaP core with DNA coating, and CaP – DNA core with BSA coating. Both types of particles were prepared by modifications to previously described methods[1,2]. The DNA incorporation efficiency of each particle type was determined by centrifugation of the particles followed by measurement of DNA in the supernatant by PicoGreen®. The size of the particles and their stability over time was determined by transmission electron microscopy (TEM). These particles were tested for their ability to transfect preosteoblastic cells (MC3T3-E1, subclone 4) *in vitro* by β-gal expression. Next, particles or naked DNA were incorporated into purified alginate, which was then crosslinked with calcium to form a hydrogel. The release rate of particles or DNA was measured over time. Finally, MC3T3s and particles or naked DNA encoding for lacZ or BMP-2 were incorporated into alginate covalently modified with a cell adhesive amino acid sequence (GRGDSP) and injected subcutaneously into the backs of SCID mice. The constructs were recovered after 2.5 and 6 weeks and examined histologically for evidence of bone formation.

Results: CaP NPs were sized at 0, 1, and 2 weeks, and their size did not increase during this time. At time zero, CaP core – DNA coat NPs (Figure 1) were 75 +/- 73 nm in diameter, while CaP-DNA core – BSA coat NPs were 161 +/- 179 nm in diameter. The DNA encapsulation efficiency of the CaP core – DNA coat NPs was 66.5% +/- 3.5%, while for the CaP-DNA core – BSA coat NPs it was 79.5% +/- 16.2%. The ability of CaP NPs to transfect preosteoblast cells *in vitro* was examined (Figure 2). Although the efficiency was low, we demonstrated these particles can be used to transfect cells. The release of these particles from alginate hydrogels was compared to that of naked DNA. We show sustained release from the hydrogels containing particles or naked DNA for more than two months (Figure 3). A pilot study to examine the ability of this system to form bone *in vivo* was conducted. Histology of samples containing the CaP core – DNA coat NPs 2.5 and 6 weeks post-injection exhibited evidence of bone-like tissue formation (Figure 4). Bone was not formed in any of the controls.

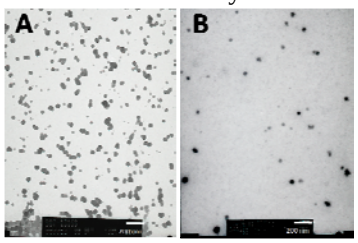

Figure 1. *TEM images (A) CaP core – DNA coat (B) CaP-DNA core – BSA coat. Scale bar represents 200 nm.*

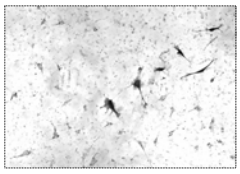

Figure 2. *Transfection of MC3T3s with CaP-DNA core – BSA coat NPs, visualized by X-Gal staining of β-gal expression.*

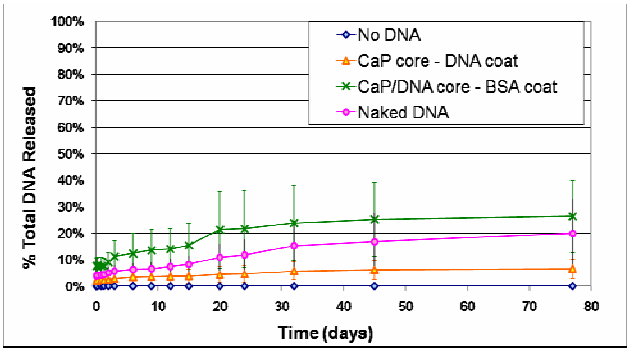

Figure 3. *Release of DNA from alginate hydrogels.*

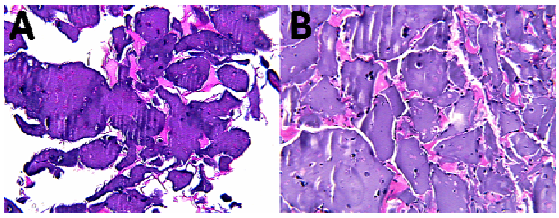

Figure 4. *H&E staining of alginate / CaP core – DNA coat NPs / MC3T3 injections at (A) 2.5 weeks and (B) 6 weeks. (Purple = alginate; pink = bone)*

Conclusions: We have shown the ability to create CaP NPs that remain stable over time and that have a high incorporation efficiency of DNA. We integrated these NPs into alginate hydrogels for localized, sustained release. This system shows much promise for non-viral gene delivery to transplanted or host osteoblasts or progenitor cells for promotion of osteogenesis. Future work will include optimizing transfection efficiency and further characterization of bone tissue formed *in vivo*.

References: [1]Sokolova VV. Biomaterials. 2006;27:3147-3153. [2]Li Y. Int J Pharm. 2004;269:61-70.

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