Ectopic Bone Formation from Hydrogel Carriers Loaded with BMP-transduced Cells

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Introduction: Recombinant adenoviral vectors encoding bone morphogenetic protein type 2 (BMP2) have been shown to induce bone formation after intramuscular injection of *ex vivo* transduced cells.[1] However, this process can result in targeted immune reactions towards the cells as well as spatially uncontrolled gene expression and bone formation. Therefore, we have designed biocompatible, immunoprotective hydrogels to act as cell carriers. Human diploid lung fibroblasts (MRC-5) transduced with a chimeric adenovirus type 5 (Ad5) with a fiber gene of adenovirus type 35 encoding BMP2 (Ad5F35-BMP2) have been encapsulated within poly(ethylene glycol) diacrylate (PEG-DA) hydrogels. These hydrogels have permeability sufficient to allow diffusion of BMP2 protein out of the hydrogels but are impermeable to antibodies and immune cells.

Methods: PEG-DA (6 kDa, 10 kDa, 20 kDa) was synthesized as previously described. The hydrogel disks (11.5 mm × 0.5 mm) were photopolymerized with 0.1 g/mL PEG-DA in 1.5 % (v/v) triethanolamine/Hepes buffered saline (HBS, pH 7.4), 10 mM eosin Y, 37 mM 1-vinyl-2-pyrrolidinone, and varying amounts of MRC-5 or transduced MRC-5 (tMRC-5) cells. Hydrogels were cultured up to 15 d and the conditioned media were then assayed for BMP2 protein with ELISA. The BMP2 activity was assayed with an alkaline phosphatase assay. Cell viability was determined with a Molecular Probes Live/Dead Staining kit after 1d and 7d. Mechanical strength of the hydrogels was determined on an Instron 3342 under compression with a crosshead speed of 1 mm/min after 1d. To assess *in vivo* efficacy, PEG-DA hydrogel beads that were polymerized on suture to enable retrievability were surgically implanted into the hindlimb muscles of both legs in NOD/SCID mice. After 3 wk, the limbs were excised and the volume of mineralized ectopic tissue in the muscles was determined with a commercial micro-CT system (GE Locus SP, GE Heathcare, London, Ontario) using the eXplore MicroView, v. 2.0, GE Healthcare software.

Results/Discussion: The ELISA data for encapsulation of 1, 5, and 10 million tMRC-5 cells within PEG-DA hydrogels of varying molecular weight showed a biphasic response to BMP2 expression in which both molecular weight as well as cell number can affect BMP production. PEG-DA 10 kDa and 20 kDa favored higher BMP release in comparison with the 6 kDa possibly due to the larger pore size that may facilitate better diffusion of nutrients and oxygen throughout the hydrogel as well as BMP diffusion out of the hydrogel. In addition, 10 million cells per hydrogel provided highest BMP production on day 5 with continued expression up to at least 15 d. Subsequent analyses were performed with 10 million cells within PEG-DA 10 kDa hydrogels. BMP2 activity was determined with an alkaline phosphatase assay which showed that the secreted BMP2 protein from the hydrogels had comparable levels of activity as the BMP2 secreted from the plated cell control. Cell viability for MRC-5 and tMRC-5 cells encapsulated within the hydrogels showed that ~70 % of the MRC-5 cells were alive after 24 h and that was maintained at day 7 and ~60 % of tMRC-5 cells were alive and also maintained at day 7. In addition, transduction of cells with the adenovirus also reduced cell viability compared with the control. The compression modulus for the hydrogels with cells was 236 ± 48 kPa compared to 395 ± 55 kPa. Analysis of the micro-CT data (Figure 1) showed that the mice injected

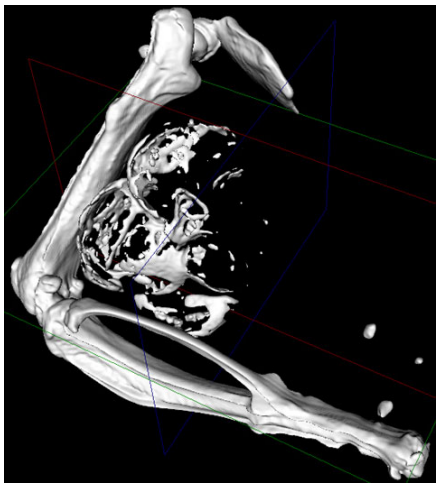

Figure 1 Micro-CT analysis of ectopic bone formation in NOD/SCID mice implanted with hydrogel constructs containing adenovirus-transduced after 3 weeks.

with tMRC-5 cells had an average of 31.8 ± 7.8 mm^3 of mineralized tissue in the muscle compared with 39.5 ± 25.0 mm^3 for the mice with the constructs ($p>0.05$). These data suggest that encapsulation of transduced cells may be a viable strategy for induction of ectopic bone formation.

Conclusions: Immunoprotective cell carriers in combination with gene therapy represent a promising strategy for the formation of bone. This data indicates that hydrogels may be able to protect cells from the immune system and control spatial gene expression. Future studies will involve the design of biodegradable scaffolds that would obviate surgical removal of the constructs.

References: 1. Olmsted-Davis et. al. (2002) *Human Gene Ther* **13**, 1337-1347.