Growth Plate Regeneration Using IGF-I Plasmid Releasing PLGA Scaffolds

Nirmal Ravi[1], Todd A Milbrandt[1,2], David A Puleo[1].
[1]University of Kentucky, Lexington, KY, USA
[2]Shriners Hospital for Children, Lexington, KY, USA

Statement of Purpose: Approximately 18.5% of fractures in children involve the growth plate. Physeal fractures often heal by formation of a bony bar, leading to angular deformity and limb-length inequality. Current treatments involving replacing the bony bar with fat, silicone, bone cement, etc., are often ineffective, with up to 43% of such patients having growth problems (Clin Orthop Relat Res 405:242, 2002). Our goal is to develop a polymeric system that can direct native pluripotent mesenchymal cells to appropriate lineage allocation by controlled delivery of plasmid encoding IGF-I.

Methods: Plasmid DNA was complexed with PEI at ratio 14 using previously described methods. PEI:DNA-encapsulated in 50:50 PLGA (acid-terminated) microspheres were prepared by a standard w/o/w double emulsion technique. PLGA was mixed with 60 wt%-NaCl overnight and compression-molded into 6mm diameter discs. These discs were then "sintered" at 43ºC for 2 days, and salt was leached out in deionized water for 12 hours. Scaffolds for release experiments were incubated at 37ºC in PBS, and DNA release measured using the Picogreen assay. *In vitro* transfection was determined by culturing pluripotent mesenchymal cells on PEI:DNA encapsulated scaffolds and examining them by confocal microscopy. *In vitro* production of cartilage was determined by culturing rat bone marrow cells on PLGA scaffolds for four weeks with the addition of 250ng/ml IGF-I, and analyzing the amount of GAG produced using the Blyscan® (Biocolor Ltd, Northern Ireland, UK) assay. A rabbit model of growth plate injury was created by removing the medial half of the proximal tibial growth plate. At 3 weeks, the bony bar was replaced with blank scaffolds and the animals were euthanized 8 weeks after implantation for radiographic and histologic analysis of the implantation site.

Results and Discussion: Encapsulated PEI:DNA complexes were released in a typical biphasic profile, with the first phase due to the release of complexes at the scaffolds' surfaces, and the second phase after swelling of the scaffolds. The initial phase of release lasted 2 weeks, followed by the second phase, which was completed in 4 weeks. These scaffolds supported the adhesion and growth of mesenchymal cells, which were also successfully transfected by released PEI:DNA complexes. Bone marrow stromal cells grown on blank scaffolds with the addition of rhIGF-I protein produced matrix with 120% higher GAG content compared to controls (Figure 1). Figure 2 shows a representative H & E stained section of rabbit tibia that had a PLGA scaffold implanted after removal of its bony bar. Remnant of the native growth plate can be seen growing into the scaffold 2 weeks after implantation. Figure 3 shows a radiograph illustrating the greater angular deformity in the control leg (right tibia) compared to the contralateral leg, that had a blank scaffold implanted after growth plate injury. Varus, as indicated by the medial proximal tibial angle, was 60º in the untreated leg compared to 86º in the treated leg.

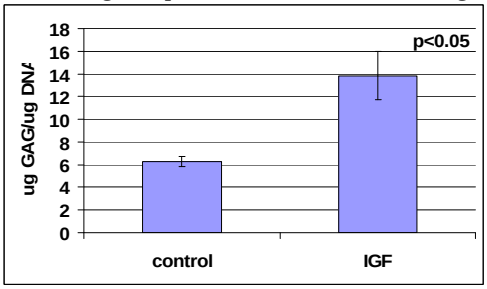

Figure 1. Normalized GAG content on scaffolds seeded with BMCs and IGF-I added for 4 weeks.

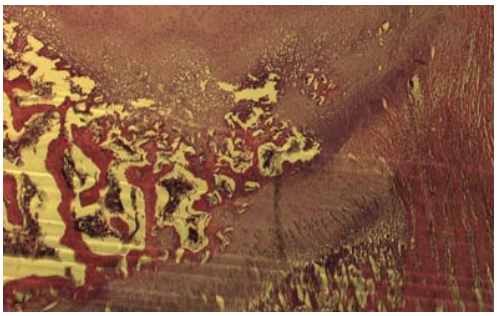

Figure 2. H&E stained section of rabbit tibia 2 weeks after implantation with blank PLGA scaffold.

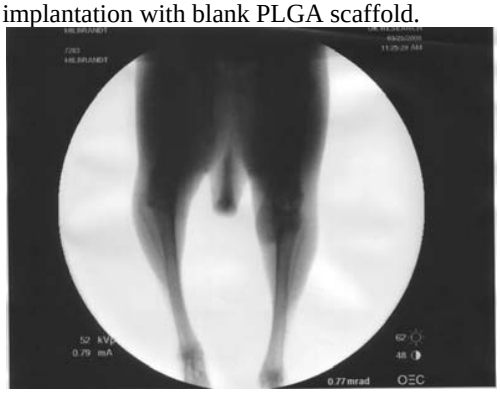

Figure 3. Radiograph of rabbit hind legs 2 weeks after scaffold implantation.

Conclusions: Controlled release of plasmid was achieved from porous PLGA scaffolds. Culture of bone marrow cells on these scaffolds resulted in transfection from released PEI:DNA particles, and resulted in cartilage formation with the addition of IGF-I protein. Histological evaluation showed that implantation of scaffold in the animal model of growth plate injury resulted in some regeneration of cartilage in the treated leg, with less angular deformity compared to untreated control leg.

Acknowledgement: This work was supported in part by Kosair Charities, Inc.