Tissue Engineering of Growth Plate using IGF-I Releasing PLGA Scaffolds

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Statement of purpose: Damage in the growth plate can result in retarded growth and unequal limb length. Unfortunately there is no current treatment available for complete regeneration of the damaged growth plate. This research focused on regeneration of growth plate cartilage by delivery of IGF-I from porous PLGA scaffolds at the site of injury.

Methods: Porous PLGA scaffolds were fabricated by mixing IGF-I-encapsulated microspheres with NaCl (60 wt %) followed by "sintering" and salt-leaching to create pores. T o assess the degradation, scaffolds were incubated in phosphate-buffered saline, pH 7.4 (PBS), cell culture medium (DMEM) and cell culture medium along with macrophages seeded on top of the scaffold. Samples were incubated at 37ºC with shaking. Supernatant was collected on consecutive days for first one week followed by every three days for the rest of the study. The amount of IGF-I released was determined by measuring fluorescence from the labeled protein encapsulated in the microspheres.

In vitro studies involved seeding of bone marrow stromal cells on the porous scaffolds to determine bioactivity of released IGF-I. Cells were seeded at a density of 20,000,000 cells/ml. Cells seeded on the blank scaffolds were used as controls. IGF-I was added (15 µl every two days at the concentration of 100µg/ml) to another set of blank scaffolds seeded with cells to compare the effect of IGF-I added to the medium and encapsulated IGF-I on cell proliferation and matrix synthesis. The medium was replaced every two days to maintain the growth of cells.

The in vitro studies were followed by in vivo studies, which included implantation of blank and IGF-I encapsulated scaffolds in New Zealand white rabbits after inducing growth plate injury in the proximal medial tibia growth plate. Radiographic images were obtained at various stages of the study to enable anatomical measurements. At the end animals were euthanized and histology was conducted for further analysis of regenerated tissue.

Results and Discussion: The release profile for IGF-I encapsulated in PLGA scaffolds and degraded in PBS, cell culture medium and cell culture medium with macrophages were compared (Figure 1). Release consisted of a large initial burst (approximately 65-70% of the release occurred within the first 10 days) followed by a gradual reduction in the amount of protein released. IGF-I was released in a more sustained manner in the case of scaffolds degraded in DMEM and DMEM with macrophages. The slopes of the release curves were found to be significantly different between the scaffolds degraded in PBS, α-MEM and α-MEM with macrophages ($p = 0.0039$). Results from the GAG assay showed that the amount of matrix produced by cells seeded on the IGF-I added and encapsulated scaffolds was greater when compared to that of cells seeded on blank scaffolds.

Radiographic images showed there was no significant difference in the bone angles between animals with control, blank, and IGF-I encapsulated scaffolds. The histological results (Figure 2) showed that cartilage was regenerated at the site of scaffold implantation, even though structure of the cartilage was disorganized and not identical to that of native growth plate cartilage.

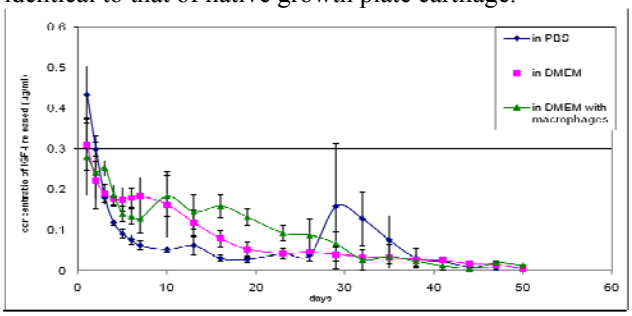

Figure 1. Comparison of release profiles for IGF-I encapsulated scaffolds incubated in PBS, DMEM, and DMEM with macrophages.

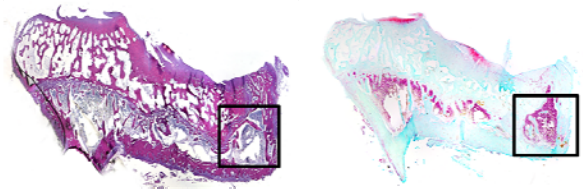

Figure 2. Proximal tibia 8 wk after implantation of IGF-I releasing scaffold, the square shows the site of implantation and cartilage regeneration. Staining: H&E (left), safranin O (right).

Conclusion: Protein release from IGF-I encapsulated PLGA scaffold was more sustained in cell culture medium, indicating an effect of the degradation environment on swelling and hydrolysis of the polymer. The amount Of GAG produced by mesenchymal cells was more on IGF-I added and encapsulated scaffolds confirming the effect of IGF-I on cells. Tissue sections obtained from in vivo studies showed regeneration of cartilage, albeit with disorganized structure, at the site of implantation.

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