IGF-I-Releasing Scaffolds for Growth Plate Regeneration

Sharath kumar C. Sundararaj[1], Todd A. Milbrandt[2,3], and David A. Puleo[1]
[1] Center for Biomedical Engineering, University of Kentucky, Lexington, KY, USA
[2] Department of Orthopedic Surgery, University of Kentucky, Lexington, KY, USA
[3] Shriners Hospital for Children, Lexington, KY, USA

Statement of Purpose: Lack of the ability of growth plate to regenerate after injury can result in angular deformity of the limbs or limbs of unequal length. This research focuses on regeneration of the growth plate by controlled delivery of insulin-like growth factor I (IGF-I) from biodegradable poly(lactic-co-glycolic acid) (PLGA) scaffolds.

Methods: IGF-I labeled with fluorescent dye (Alexa 488) was microencapsulated within PLGA (50:50, acid terminated) by a double emulsion method. These IGF-I loaded microspheres were mixed with NaCl (60 wt%), compacted, sintered, and salt leached to create porous scaffolds. Scaffolds were degraded in phosphate-buffered saline (PBS), pH 7.4, or cell culture medium (α-MEM). 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.

To examine the effect of microencapsulation of protein within PLGA, release profiles were determined for scaffolds either encapsulated with the model protein lysozyme as described above or simply adsorbed with the protein.

Bone marrow cells (BMCs) obtained from rat femora were seeded on the PLGA scaffolds encapsulated with unlabeled IGF-I. The cells were seeded at a density of 300,000 cells/scaffold. Control groups included BMCs seeded on blank scaffolds and BMCs cultured on blank scaffolds in the presence of 250 ng/ml soluble IGF-I. Medium was replaced every two days, with replenishment of IGF-I in the soluble growth factor controls. After three weeks, GAG contents were measured.

Results and Discussion: The release profile for IGF-I encapsulated in the PLGA scaffolds that were degraded in PBS consisted of a large initial burst (84% of the release occurred within the first 10 days) followed by a gradual reduction in the amount of protein released (Figure 1). There was no significant amount of protein released after one month, and it took around 50 days for the scaffolds to degrade completely. The study conducted by degrading scaffolds in cell culture medium (α-MEM) showed a different kind of a release profile; the release of IGF-I occurred in a more sustained manner (Figure 1). The cumulative release of encapsulated IGF-I from scaffolds in PBS and α-MEM differed by only 18% after 25 days.

The release profile of lysozyme adsorbed on the scaffold surface showed a steeper release (74% of protein released within first five days) when compared to that of the scaffolds with encapsulated protein. As shown previously for IGF-I, a significant amount of encapsulated lysozyme was released initially, followed by a gradual decrease in the amount delivered.

BMCs cultured on scaffolds in the presence of IGF-I produced extracellular matrix containing more GAG than did cells grown on blank (protein-free) scaffolds. Similar GAG contents were measured in the matrix for both soluble and encapsulated IGF-I, even though a significantly smaller amount of protein was delivered directly from the scaffolds.

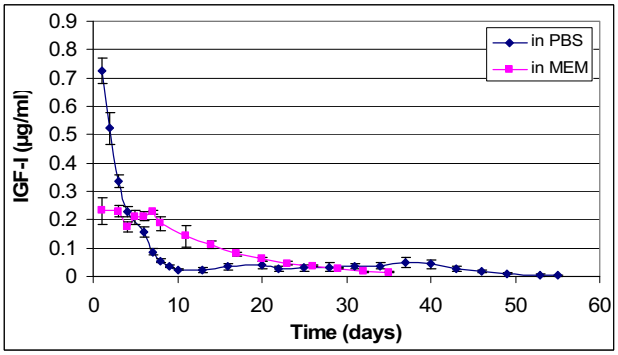

Figure1. Release profiles for IGF-I encapsulated scaffolds incubated in PBS or α-MEM.

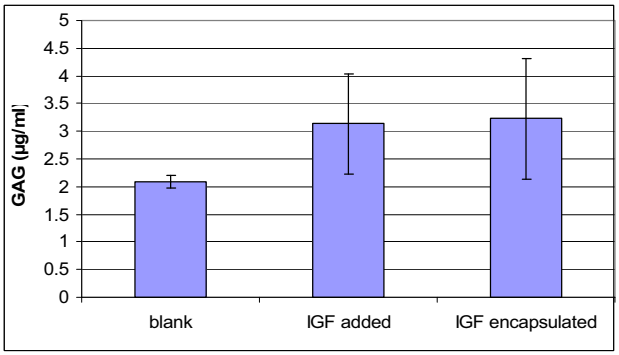

Figure2. GAG content for BMCs cultured on PLGA scaffolds for 21 days.

Conclusions: As desired, microencapsulation of protein in the scaffolds resulted in a more sustained release when compared to protein adsorbed on the scaffold surface. The release profile for IGF-I encapsulated scaffolds degraded in cell culture medium was significantly different from that for scaffolds degraded in PBS. BMCs cultured with IGF-I, even with smaller amounts of encapsulated growth factor, produced more GAG-containing extracellular matrix compared to cells on protein-free scaffolds. These IGF-I loaded scaffolds may be useful for enhancing regeneration of damaged growth plate.

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