CONTROLLED DELIVERY OF IGF-I FROM PLG MICROSPHERES TO CHONDROCYTES FOR CARTILAGE TISSUE ENGINEERING

Nichole R. Mercier[1], Rick Costantino[2], Mark A. Tracy[2], Lawrence J. Bonassar[1]

[1]Center for Tissue Engineering
University of Massachusetts Medical School
Worcester, MA

[2]Alkermes, Inc.
Cambridge, MA

INTRODUCTION

Polylactic acid (PLA), polyglycolic acid (PGA), or their copolymers (PLGA) are commonly used in cartilage tissue engineering. Often these polymers are constructed as fibers [1] or sponges [2], which can be seeded with cells and implanted into the recipient. One drawback to this approach, however, is the invasive procedure of implantation, as these fibers and sponges must be surgically implanted into the recipient.

Polylactide co-glycolide (PLG) microspheres are small, spherical polymers that offer and alternative to these more invasive tissue engineering scaffolds. PLG microspheres have proven biocompatibility and injectability, making them appealing for cartilage tissue engineering applications. Although microspheres are predominately used for drug delivery systems, Elisseeff et al made a first attempt at using microspheres for tissue engineering applications. This group loaded microspheres with IGF-I, which were then mixed in a subsequent hydrogel polymer and seeded with chondrocytes [3]. Recent reports demonstrate the use of PLG microspheres as a single scaffold for cartilage tissue engineering [4].

IGF-I has been a prominent growth factor for cartilage tissue engineering designs, as it is known to promote chondrocyte proliferation, differentiation and stimulate extracellular matrix production. Some cartilage tissue engineering studies have looked at supplementing construct growth with IGF-I media [5], while others have looked at combining IGF-I and cells together in the scaffold [6]. However, the limiting factor in each of the designs remains protein half-life.

To circumvent the issue of protein half-life, microspheres have been used to deliver growth factors and proteins over an extended period of time. Recent studies have demonstrated the feasibility of encapsulating and delivering IGF-I from PLG microspheres up to 4 weeks [7]. This study examines the controlled delivery of IGF-I to growing cartilage tissue, using only PLG microspheres as a polymer scaffold.

METHODS

PLG microspheres were fabricated by a non-aqueous, cryogenic process [8] using 50:50 lactide:glycolide polymer with a molecular weight of 50kDa and a 10 g IGF-I/ mg polymer, with an average diameter of 70 µm. To obtain the appropriate release of IGF-I, IGF-I microspheres (loaded) were mixed with empty placebo microspheres (unloaded). The following IGF-I concentrations were tested: 0.07%, 0.02%, 0.007%, 0%, and 0% supplemented with exogenous hIGF-I (table 1).

For *in vitro* cartilage disc formation, the wells of a 48 well plate were coated with 0.5% agarose to prevent cell adhesion. Chondrocytes were isolated from the articular cartilage of calf gleno-humeral joints and incubated for 4 hours with PLG microspheres. 500µL of the cell-sphere suspensions were delivered to each well. Discs were cultured using serum-free F-12 media, supplemented with 1x antibiotics and 50 g/mL ascorbic acid. At media changes, media was completely removed and 4mL media was added to the disc. External IGF cultures were supplemented with 30ng/mL hIGF-I. Media was changed three times a week. Cartilage discs were harvested at 2, 4 and 8 weeks.

Sample Condition	Total Protein released over 8 weeks	Average [IGF-I] in culture media
no serum	0	0
ext IGF	0	ext. 30 ng/mL
0.07% IGF	10.6 ug	100 ng/mL
0.02% IGF	3.2	30 ng/mL
0.007% IGF	1.06 ug	10 ng/mL

Table 1

Upon harvest, sample mass was recorded, and samples were divided for multiple analysis. One portion of the sample was digested with papain and used to determine proteoglycan content. Another part of the sample was fixed and sectioned. Slides were stained with safranin-O, hematoxylin and eosin, and immunostained for collagen type I and collagen type II.

RESULTS

In vitro cultured cartilage discs maintained their circular shape and grew in thickness over the 8 weeks of culture. Further, tissue samples receiving IGF-I maintained their structure integrity throughout the 8 weeks of culture, while the structure of samples that

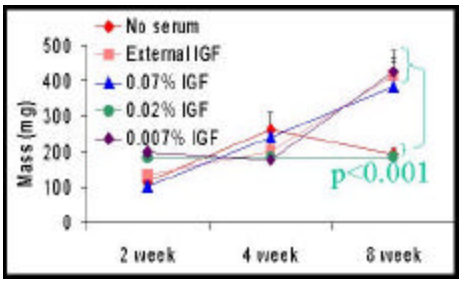

Figure 1

did not receive IGF-I was fragile. All tissue specimens showed homogenous safranin-O staining, however, tissue in the no serum conditions was sparse and non-contiguous as early as two weeks. Samples that received IGF-I supplementation exhibited positive staining for proteoglycans that surrounded the microspheres, which was clear even after 8 weeks (figure 1). Tissue mass increased in samples receiving 0.07%, 0.007%, or exogenous IGF-I and after 8 weeks showed a significant difference ($p<0.001$) in mass from samples cultured without any IGF (figure 2).

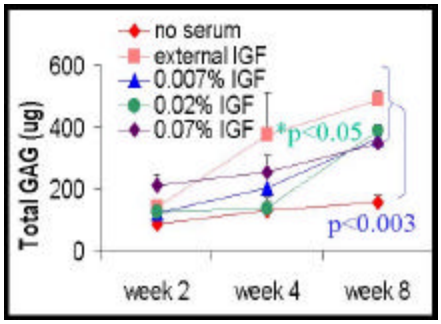

Figure 2

Proteoglycan content was measured using DMB dye method on papain digested samples. In each IGF condition, proteoglycan content had a steady increase of gag accumulation. Cartilage grown in the absence of IGF-I showed stagnant GAG accumulation even after 8 weeks. After 8 weeks, samples receiving IGF-I amassed significantly more proteoglycans than samples that did not receive IGF-I ($p<0.003$, figure 3).

Collagen immunostaining demonstrated that cartilage samples exhibited a positive collagen type II phenotype and stained negative for collagen type I (figure 4).

DISCUSSION

This study demonstrates the long-term effects of IGF-I released to growing cartilage tissue. Cartilage receiving IGF-I accumulated higher GAG content and had positive staining for proteoglycans and type II collagen. Samples without IGF-I supplement had significantly smaller mass and accumulated fewer proteoglycans after 8 weeks in culture.

Many alternatives exist for including IGF-I effects in tissue engineered constructs. Commonly, IGF-I is added supplementally to media during culture of the construct to increase matrix content. Pei et al. added IGF-I to the culture media of dedifferentiated chondrocytes to induce re-differentiation [9]. Although the constructs expressed increased GAG accumulation and collagen type II, the supplemental IGF-I must be added with each media change. Another alternative to this method of growth factor delivery is to induce cellular overexpression of IGF-I by adenoviral infection [10] or direct transfection of chondrocytes [11]. However, the present work demonstrates that both exogenous addition of IGF-I to the culture media and controlled delivery of IGF-I to the growing construct increase matrix accumulation to the same extent.

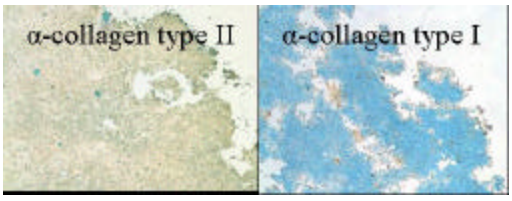

Figure 3

Microsphere research has centered on controlled drug delivery of growth factors and proteins, but few studies have looked at microspheres as a scaffold for tissue engineering studies. Elisseeff et al. released IGF-I from PLG microspheres embedded with cells in a photopolymerizable hydrogel [3]. Increases in GAG and collagen production were compared to empty microspheres samples, but only out to two weeks. The present study describes PLG microspheres as a single polymer scaffold for the use in cartilage tissue engineering and microsphere release of IGF-I over an 8 week culture period. Elisseeff showed that after 2 weeks IGF-I delivery from microspheres increased GAG accumulation [3]. The results of this study mirror those of Elisseeff, specifically that release of IGF-I to cartilage tissue significantly increases ECM accumulation out to 8 weeks.

Figure 4

It is interesting that the effects of IGF-I that we see are carried out to 8 weeks. Typically, microsphere delivery pattern has an initial burst-release of growth factor or protein, but subsequent content release coincides with microsphere degradation. The polymer used in this study generally degrades by 4-6 weeks, with complete release of IGF-I by this time, based on typical microsphere degradation patterns [8]. One explanation for the extended effects of IGF-I could be due to IGFBP sequestering of the IGF-I protein. Cartilage tissue is known to express IGFBPs, which binds IGF to create an IGF-I reserve. Another possible explanation could be due to the pH buffering conditions of the growing cartilage tissue. In this study we have seeded microspheres with chondrocytes, which produce ECM proteins. Therefore, it is possible that the ECM could buffer microsphere degradation since it helps to maintain total tissue pH.

Overall, this work demonstrates that controlled delivery of IGF-I from PLG microspheres significantly increases matrix accumulation and tissue mass when compared to unloaded microspheres samples.

REFERENCES

1. Freed, LE., JC. Marquis, A. Nohria, J. Emmanual, AG. Mikos, R. Langer. J Biomed Mater Res. 27:11-23. 1993.
2. Mooney, DJ., K. Sano, PM. Kaufmann, K. Majahod, B. Schloo, JP. Vacanti, R. Langer. J Biomed Mater Res. 37: 413-420. 1997.
3. Elisseeff, J., W McIntosh, K Fu, BT Blunck, R Langer. J Ortho Res 19:1098-1104. 2001.
4. Mercier, NR, Costantino, HR, Tracy MA, Bonassar, LJ. Trans ORS 27:470. 2002.
5. Blunk T, AL Sieminski, KJ Gooch, DL Courter, AP Hollander, AM Nahir, R Langer, G Vunjak-Novakovic, LE Freed. Tissue Eng 8:73-84. 2002.
6. Fortier FA, HO Mohammed, G Lust, AJ Nixon. J. Bone Joint Surg Br. 84:276-288. 2002.
7. Singh M., B Shirley, K Bajwa, E Samara, M Hora, D O'Hagan. J Control Release 70:21-8. 2001.
8. Tracy, MA., KL. Ward, L Firouzabadian, Y. Wang, N. Dong, Y. Zhang. Biomat 20:1057-1062. 1999.
9. Pei, M, J Seidel, G Vunjak-Novakovic, LE Freed. Biochem Biophys Res Commun. 294:149-54. 2001.
10. Brower-Toland BD, RA Saxer, LR Goodrich, Z Mi, PD Robbins, CH Evans, AJ Nixon.
11. Madry H, R Padera, J Seidel, R Langer, LE Freed, SB Trippel, G Vunjak-Novakovic. Hum Gene Ther. 13:1621-30. 2002.