Spinner Flask Culture and Mechanical Stimulation Enhance Fibrocartilage Regeneration for the Temporomandibular Joint

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Acknowledgments: NSF Grant Number: 0812348.

Introduction: Ten million Americans are affected by TMJ disorders (TMDs), which can be defined as a group of conditions which cause pain and dysfunction in the jaw joint (1). Poly(glycerol sebacate) (PGS) is a biocompatible, biodegradable polymer which has recently shown potential for TMJ tissue engineering applications. PGS is an elastomeric material which may allow seeded cells to observe mechanical forces in a three-dimensional environment. Applying compressive forces to cells seeded on PGS scaffolds may help recapitulate a loaded joint environment and improve collagen production and organization. The goals of this study were twofold: first, to explore spinner flask culture as a way of improving cell seeding efficiency, distribution, and survival in PGS scaffolds and second, to determine the effect of length of mechanical stimulation on fibrochondrocyte ECM production. We hypothesize that allowing scaffolds to mature for six weeks in a spinner flask environment prior to application of mechanical stimulation will result in enhanced collagen organization and greater mechanical properties when compared to the control.

Methods: Scaffolds were cut from a PGS sheet of approximately 1.5 mm thickness using a 4 mm biopsy punch. 50 scaffolds were seeded in chondrogenic media (2) at 75 million goat costal chondrocytes/ml of scaffold. After the 4 weeks of spinner flask culture, mechanical stimulation was applied to scaffolds using the Flexcell® Compression System. A loading regimen of three cycles of 1 hour on and 1 hour off dynamic compression following a sine waveform at 1 Hz was applied. An estimated peak stress of 177 kPa was applied to the scaffolds, corresponding to the lowest possible applied force using the Flexcell system. In the long stimulation group, the dynamic compressive loading regimen was applied 5 days a week for 4 weeks. In the short stimulation group, scaffolds were cultured for an additional 2 weeks exclusively in a spinner flask before undergoing the loading regimen for 2 weeks. Corresponding control scaffolds from each group were also moved to compression plates with platens but were not subjected to mechanical stimulation.

Results: All groups stained positively for both collagen type I and type II (Fig. 1.). In the short and long stimulation groups, there is a collagen type I shell that extends along the entire perimeter of the scaffolds. In the control group, the collagen type I shell is confined to the short end of the scaffold. All groups exhibited collagen type II staining throughout the scaffold with no noticeable differences in patterning between groups. The results from the biomechanical analysis are shown in Fig. 2. There were no significant differences in mechanical properties between mechanically stimulated groups and the control. The control scaffolds exhibited a percent stress relaxation of 96 ± 5% and tangent modulus of 667 ± 270 kPa.

Discussion: The results show that spinner flask culture allowed for high seeding efficiency, homogenous cell distribution, survival at the center of the scaffold, and abundant ECM production. These spinner flask results are in stark contrast to our previous attempts with needle seeding and static culture, which had few cells and matrix deposition in the center of the scaffolds (3). Another major advantage of the spinner flask culture was an increase in scaffold stiffness when compared to our previous results, 667 ± 270 kPa vs.123.6 ±86 kPa (3).

In conclusion, this study demonstrated that the spinner flask provides an ideal environment for both seeding and culturing fibrochondrocytes on PGS scaffolds and mechanical stimulation does have an effect on extracellular matrix organization. PGS demonstrates great potential as a fibrocartilage scaffold material, which may provide TMD patients with a viable graft replacement option in the future.

Significance: Paving the way for in-vitro regeneration of fibrocartilage capable of withstanding the in-vivo loading environment.

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Figure 1: Immunostaining of the middle edge of PGS scaffolds. Row 1: Type I collagen immunostain. Row 2: Type II collagen immunostain. Scale bar is 100 μm.

Figure 2: Simple compression analysis of the scaffolds (n=6) at 8 weeks. (a) Percent stress relaxation (b) Tangent modulus of static control (SC), short mechanical stimulation (SMS), and long mechanical stimulation (LMS) groups. Error bars indicate standard deviation.

ORS 2014 Annual Meeting
Poster No: 0412