A New Biomechanically Enhanced Type I Collagen-based Meniscus Implant

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Visual Abstract

Biomechanical Enhanced Scaffold

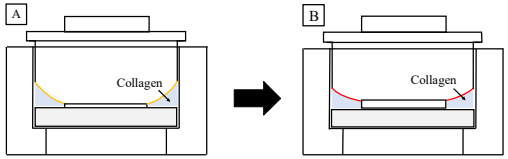

Fig. 1. 2-Step Molding Procedure: Use (A) Shaping Mold first, followed by (B) Collagen Redistribution Mold.

Introduction

Menisci provide important biomechanical functions for the knee joint. Injury of the meniscus, such as meniscal tear, frequently results in partial or total meniscectomy. Loss of meniscal tissue increases focal stress on the articular cartilage, which subsequently leads to the development of knee osteoarthritis. A long-term clinical study of the previous type I collagen meniscus implant [1] showed that the implant supported meniscal tissue regeneration. However, the regenerated tissue was smaller than the initial implant, indicating that biomechanical insufficiency of the implant occurred during the period of healing of the implant/new tissue complex. Due to the complexity of meniscal structure and force distribution, development of an ideal implant to support meniscal regeneration is still an unmet clinical need. In our initial report [2], we successfully enhanced the tensile strength along the circumferential direction of the collagen meniscus implant. The goal of this study is to improve mechanical strength along the radial direction to enhance the suture retention strength and minimize the potential post-surgery tear of the implant from suture related injuries. In order to strengthen the radial strength, a 2-step molding process was developed, using an initial shaping mold followed by a collagen redistribution mold. Fig. 1 illustrates the molding procedure involved in the current new prototype development. A 1-step molded implant with randomly oriented fibers, which is similar to the previous collagen meniscus implant [3], was also fabricated for comparison. The results showed that this 2-step molding process significantly improved both the radial and longitudinal strengths which could minimize the potential post-surgery tear of the implant.

Materials

Type I collagen fibers were purified from bovine deep flexile tendon following the procedure of Li and Stone [4].

Methods

Scaffold Preparation: First, the collagen dispersion was coacervated to form the fibers [2]. Next, the coacervated fibers were aligned circumferentially. A shaping mold was placed on top of collagen fibers to dehydrate and shape the fibers into a meniscus-like implant. After the shaping mold reached the bottom, it was removed and replaced by a second mold (collagen redistribution mold) to further compress the outer rim (see Fig. 1). Once the second mold reached the bottom, the molded implant was lyophilized, formaldehyde crosslinked, and rinsed with pure water. Finally, a meniscus implant was packaged and sterilized with ethylene oxide. Implants with randomly oriented fibers engineered by the 1-step molding method were prepared as a control group.

Weight Ratio: The weight ratio of the inner and outer rims was measured to evaluate the collagen weight distribution.

Density: Density was calculated by using implant weight divided by total implant volume.

Pore Volume: Pore volume was measured by using empty volume divided by total implant volume.

SEM: SEM images were used to examine the pore sizes and fiber orientation. Pore size was measured using ImageJ (NIH).

Tensile strength, compression, and suture retention: Strengths were determined using a mechanical tester (Chatillon).

In vitro study: The experiment was conducted by injecting fibroblasts into implants to evaluate the cell activity. Cell seeded implants were stained with DAPI on day 1 and day 7 to locate and visualize the cells. Each testing had at least 3 samples in duplicates and was repeated once.

Statistical Analysis: The experimental data were analyzed using Analysis of Variance (ANOVA). Statistical significance was set to value ≤ 0.05.

Results

The cross-sectional view of prototypes shown in Fig. 2 revealed that a 2-step molding method successfully lowered the outer rim profile and increased suture retention strength without significantly affecting the pore structure (Table 1 and Fig. 2). After aligning fibers along the circumferential orientation and increasing the density, the overall mechanical properties improved to a level (Table 1) which could correct many of the deficiencies of the original implant that was marketed in the 2000s.

Table 1. Summary of Characterization Results

	1-Step Molded Implant		2-Step Molded Implant	
	Inner rim	Outer rim	Inner rim	Outer rim
Weight Ratio	1	2.1±0.1	1	1.5±0.2
Density (g/cm³)	0.19±0.02	0.12±0.01	0.26±0.01*	0.19±0.01*
Pore size (μm)	56.3±15.1	76.3±31.5	55.6±24.3	63.6±21.3
Pore Volume (%)	90.8±0.5*		86.1±1.1	
Tensile Strength (N/cm²)	75.3±20.4	56.6±8.8	546.6±106*	214.5±33.2*
50% Compression (N/cm²)	N/A	7.3±1.3	31.9±4*	18.2±2.2*
Suture Retention (N)	7±0.7		11.1±0.4*	

Data are presented as Mean±1 SD. * indicates significant difference ($P<0.05$).

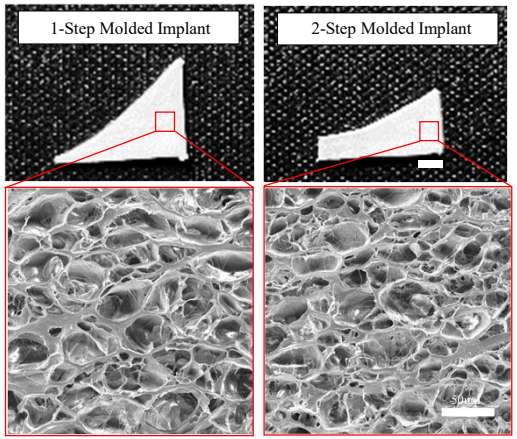

Fig. 2. Cross-sectional views of each prototype and SEM images.

The *in vitro* study confirmed that a 2-step molded implant is not only biocompatible but also supportive of cell proliferation within the implant space without limiting cell migration through the interconnected pores (Fig. 3). It is important to note that aligned fibers along the circumferential orientation was not disrupted by a 2-step molding method (data not shown). Overall, a new biomechanically enhanced Type I collagen meniscus implant showed significant improvements in all aspects as compared to the previous 1-step molded implant with randomly oriented fibers [2].

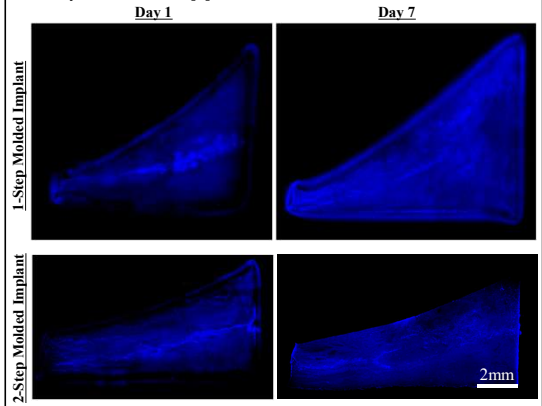

Fig. 3. Fluorescent images showed both groups promoted cell growth and migration.

Discussion

This report is a continuation of our effort to develop a collagen-based implant to treat a meniscus injury. As stated in our initial report [2], our overall goal is to develop a tissue engineered implant that will not only provide a means to promote the body to regenerate the host meniscal tissue, but also to provide an adequate temporally meniscus substitute during the post-surgery rehab period. Towards that goal, a biomechanically competent extracellular matrix implant is a prerequisite to the development strategy. Our next phase of research will be the inclusion of bioactive components to promote the cell growth and differentiation in a dynamic bioreactor system.

Significance

The goal of this research is to advance meniscus repair from a scaffold alone to a tissue engineered implant. A tissue engineered implant can meet the needs of a broader population of meniscus repair.

Acknowledgements

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