A New Type I Collagen-based Meniscus Implant

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Introduction

Menisci are two semilunar shaped wedges anatomically located between the femoral condyles and the tibia plateau serving important biomechanical functions of the knee. Damage to the meniscus is prelude to the development of osteoarthritis. Due to the complexity of the structure and function of the meniscus, there has yet to be a breakthrough in developing an ideal implant for the repair of torn meniscus. Removal of the torn tissue is still the preferred modality of treatment of meniscal tear. Thus, our goal is to develop an innovative type I collagen-based tissue engineered meniscus implant to meet the unmet needs for the treatment of meniscus injury.

In the first part of development we present the design, engineering and characterization of a type I collagen-based scaffold. Subsequent development will include: (1) the evaluation of scaffold with bioactive elements (i.e. platelet-rich plasma and Adipose-derived MSCs) in vitro under static and dynamic culture systems; (2) a pilot animal model study to validate the various design parameters of the scaffold and the chondrogenic differentiation of MSCs; (3) a full-scale animal study to evaluate the efficacy of a tissue engineered implant; and (4) the validation of the tissue engineered implant in the clinical set up.

Materials

- Type I collagen fibers were purified from bovine deep flexile tendon following the procedure of Li and Stone [1].
- Hyaluronic acid (HA, MW ~1.5 MDa) was obtained from LifeCore.

Methods

Two key parameters were incorporated into the design: (1) orientating collagen fibers to improve mechanical properties and (2) impregnating HA to reduce the surface friction for minimizing shear induced damage. These design parameters were not considered in the first-generation implant [2].

Engineer Type I collagen meniscus scaffold with circumferentially orientated collagen fibers

A fixed weight of collagen fibers was suspended in a fixed volume of 0.07M lactic acid overnight. The swollen fibers were homogenized (Silverson) to obtain a uniform dispersion. An aliquot of dispersion was titrated with 1M NH_4OH to coacervate collagen fibrils. The coacervated fibers were aligned circumferentially using a rotating mandrel, partially dehydrated and molded. The molded fibers were freeze dried, crosslinked with formaldehyde vapor, rinsed with saline, and air dried. 4mg of HA was impregnated into the scaffold and air dried. The scaffold was packaged and sterilized with ethylene oxide.

Engineer Type I collagen meniscus scaffold with randomly orientated collagen fibers

The coacervated fibers were partially dehydrated and placed directly into the molds to produce first-generation scaffolds with randomly oriented fibers.

Characterizations

- **SEM**: SEM (Auriga) micrographs were taken to examine the pore structure, the pore size, and the fiber orientation.
- **Percent pore volume**: Percent pore volume was calculated by using empty volume (total volume of water) divided by total volume (total volume of water plus total volume of collagen).
- **Fiber orientation**: Fiber orientation shown on the SEM micrographs was measured by ImageJ (NIH), where a radial summation of the pixel intensities between 0 and 360° was obtained by an oval projection of fast Fourier transform (FFT) frequency.
- **Mechanical properties**: Tensile strength and suture retention strength were determined using a mechanical tester (Chatillon).
- **Hydrothermal stability (T_s)**: The hydrothermal transition temperature was defined as the endothermic peak of the melting curve from a DSC (Mettler Toledo).
- **Surface friction**: Surface Friction was measured and calculated using an in-house designed pendulum apparatus based on Crisco, et al. [3].
- ***In vitro* studies**: MEM cytotoxicity test protocol was followed to evaluate the cytocompatibility of the scaffold. Osteoblasts were used for the test. Cell proliferation study was conducted by injecting osteoblasts into the scaffold and cultured according to standard techniques. Samples were collected at day 1 and day 4, and analyzed with CyQUANT Cell Proliferation Assays.

Results

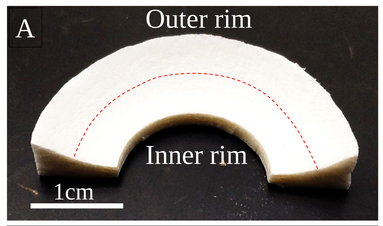

Fig. 1 Prototype of type I collagen-based human size meniscus scaffold.

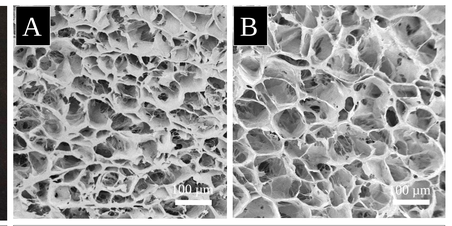

Fig. 2 Cross sectional pore structure taken by SEM: (A) the inner rim and (B) the outer rim.

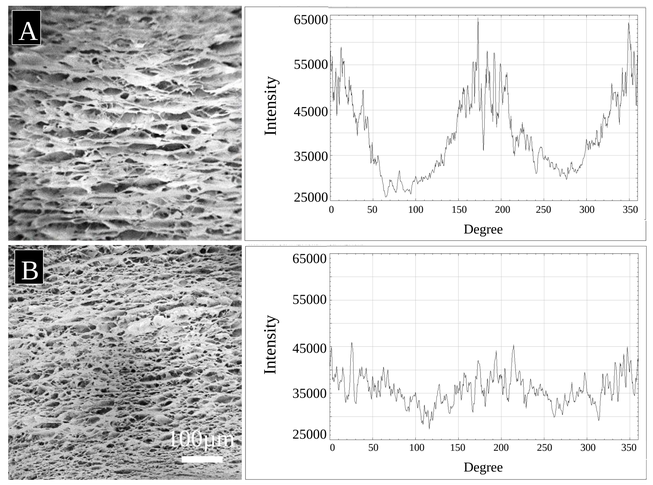

Fig. 3. Fiber orientation analyzed by SEM and FFT: (A) the aligned group and (B) the random group.

Table 1. Summary of Characterization Studies

	Random		Aligned	
	Inner rim	**Outer rim**	**Inner rim**	**Outer rim**
Pore size (μm)	59±20	100±30	61±28	130±43
Pore Volume (%)	89±1		89±1	
Tensile Strength (N)	31±5	30±5	62±5*	50±6*
Suture Retention (N)	4.6±0.8		5.1±0.4	
Hydrothermal Stability (˚C)	72±1		72±1	
			No HA	**HA**
Friction coefficient (μ)			0.25±0.04	0.13±0.01

Data are presented as Mean±1 SD. * indicates highly significant difference (P<0.01).

- Fig. 1 shows the human size meniscus scaffold.
- Fig. 2 and Table 1 show that the range of pore sizes (both the inner rim and the outer rim) and percent pore volume are adequate for cell and tissue ingrowth.
- Analysis of FFT from SEM micrographs (Fig. 3) shows that in the aligned group fibers were circumferentially oriented, whereas in the random group there was no preferred fiber orientation.
- Table 1 shows that oriented fibers significantly improved the tensile strength along the circumferential direction while maintaining the suture retention strength of the scaffold.
- T_S data indicates that the total resorption time of the scaffold *in vivo* will be similar to the previous studies [2, 4].
- The friction study demonstrated that defused HA from the scaffolds reduced the surface friction and could potentially minimize the risk of shear induced surface damage post-surgery.

Overall, the scaffold with circumferentially oriented fibers significantly improved the physical and the mechanical properties over the scaffold with randomly oriented fibers.

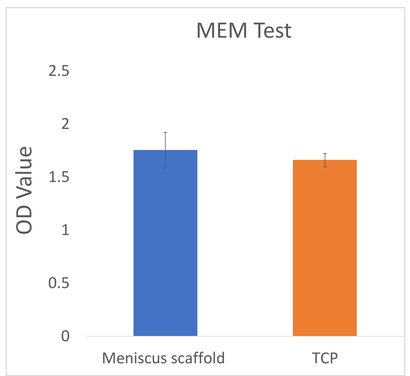

Fig. 4 MEM Test - cytotoxicity

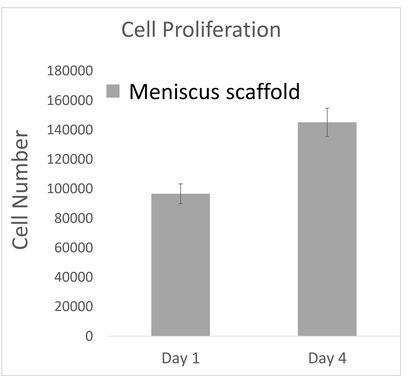

Fig. 5 Cell proliferation study.

- Fig. 4 and Fig 5. demonstrate that the engineered type I collagen-based meniscus scaffold was cytocompatible and supported cell proliferation using osteoblasts as a tested cell line.

Discussions

The first-generation type I collagen-based meniscus scaffold implant demonstrated the utility of this implant for supporting meniscal tissue regeneration [4,5]. However, the long rehabilitation time and the risk of re-tear post-surgery has made this implant less acceptable for individuals active in physical activities.

One objective of this research is to improve mechanical properties for *in vivo* stability and to potentially minimize the shear induced re-tear post-surgery. The other objective is to incorporate autologous bioactive elements at the point-of-surgery to enhance healing, shorten the rehab time and facilitate the translational process. The characteristics of the scaffold in combination with bioactive elements would provide a more acceptable tissue engineered implant for the repair of meniscal tear for all ages with different levels of physical activity.

Significance and Clinical Relevance

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

Acknowledgements

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References

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