A Bioengineered Collagen Meniscus Scaffold

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Introduction: Menisci are crescent shaped fibrocartilages, located between the femoral condyles and tibial plateaus, providing various biomechanical functions within the knee. Once injured, the meniscus has limited capability to self-heal. It is known that removal of a portion or the entire meniscus changes the normal functions of the knee, and frequently results in the development of degenerative osteoarthritis. At present, the search continues for an ideal implant which can fulfil the necessary biomechanical requirements and support tissue regeneration without inducing un-wanted tissue response. To this end, we have engineered a collagen meniscus scaffold (CMS) using bovine meniscus due to its similar structure and function to the human meniscus. Two critical steps are required to reduce the bovine meniscus for human use: the reduction in size and the removal of non-collagenous moieties. We report here the engineering of a CMS that has the composition, structure and properties similar to the human meniscus.

Materials and Methods: The CMS was mechanically engineered, using milling technology, from bovine medial meniscus (3-6 mos.) to the size and shape of the average human meniscus, while preserving the top smooth surface of the material. The shaped CMS were cleaned in H_2O, extracted in 0.2% SDS, 2% Triton X-100, 3M $MgCl_2$, 0.25M NaOH/1M Na_2SO_4, 0.2M HCl/0.5M Na_2SO_4, and isopropanol to remove the non-collagenous moieties. The purified CMS were then freeze dried and sterilized.

SEM: Micrographs were recorded using a scanning electron microscope (JEOL Ltd. JSM 6100).

Composition/Purity Tests: Hydroxyproline, DNA and amino sugar contents in purified CMS were determined by the method of Bergman and Loxley (1), Gendimenico, et al. (2), and Cleland and Sherblom (3), respectively. (n=6)

SDS-PAGE: Analysis was performed for collagen typing according to the method of Ramshaw (4).

Tensile/Suture Retention Strength: Both tests were evaluated using a mechanical tester (Lloyd LF Plus). (n=4, each test)

Hydrothermal Transition Temperature (T_s): Samples were analyzed using Mettler Toledo DSC. (n=4)

Biocompatibility testing: Samples were submitted to Wuxi Apptec (St. Paul, MN) for cytotoxicity, acute systemic toxicity, and intracutaneous reactivity testing according to ISO 10993.

Results and Discussion: Figure 1 shows the mechanically engineered medial CMS. Figure 2 shows SEM micrographs from the top (a), middle cross-section (b), and back (c) of the implant, indicating the porous structure of the CMS. SDS-PAGE analysis showed that the primary component of the CMS is Type I collagen with trace amount of Type III collagen (Figure 3) (lane 1-raw meniscus inner; 2- raw meniscus outer; 3-CMS). The results from hydroxyproline content analysis indicates that purified CMS has minimal amount of non-collagenous moieties (i.e. 98% wt. of collagen). It has a very low DNA content, comparable to the highly purified bovine tendon collagen that is known to be safe for human implantation (5). The hexosamine content (from GAGs) is also very low, as the inner rim of the meniscal tissue was removed during the engineering process. The tensile strengths are high, consistent with the fibers oriented in the physiological stress directions. In addition, the suture retention strength in the radial direction is sufficiently high for stabilizing the implant with the peripheral meniscus rim during surgery. The T_s is 57°C, comparable to the collagen membrane implant from tendon with approximately 6 to 9 months of *in vivo* stability (6). The CMS passed all three biocompatibility tests, providing support that the CMS is biocompatible.

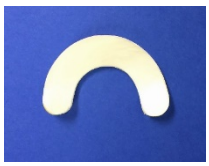

Fig. 1. Medial CMS

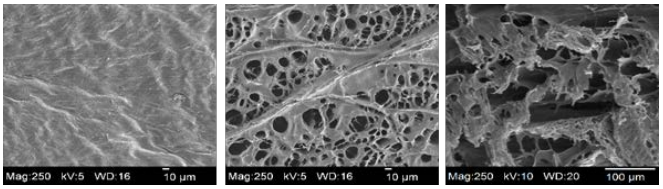

Figure 2. SEMs of CMS (a) Top Surface (b) Middle Cross-section (c) Back (250x)

Table 1. Results of Purity and Characterization Studies

Test	Average ± STD
Hydroxyproline Content (%)	12.8 ± 0.3 (98.5 ± 2.3% of collagen)*
DNA Content (%)	0.0086±0.004
Amino-Sugar Content (%)	0.047±0.014 (0.08±0.02% of GAGs)**
Tensile Strength (kg/cm²)	Circumferential: > 650 Radial: 598 ± 28
Suture Retention Strength (kg)	>3
Hydrothermal Stability (°C)	57±1

*% collagen content was calculated based on 13% by wt. of hydroxyproline in collagen
**GAG content was calculated based on 65% amino sugars in GAGs

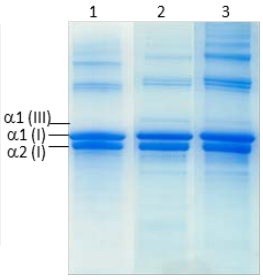

Figure 3. SDS page of CMS

Conclusion: As the native meniscus is a rather dense and non-permeable tissue, it has not been feasible to remove the potential immunogenic materials from the interstitial space of the tissue by conventional chemical and enzymatic treatment procedures. We have engineered a purified CMS that possesses the composition, structure and properties similar to the human meniscus. The resulting porous structure of the engineered CMS can facilitate the cellular ingrowth and subsequent tissue remodeling. We will conduct an *in vivo* study of the CMS for the repair of segmental meniscus defect in a large animal model.

References: (1). Bergman, I and Loxley, R., *Anal. Chem.* 12:1961-1965, 1963. (2). Gendimenico F.J., et al., *Anal. Biochem*. 173: 45-48, 1988. (3). Cleland, R. I. and Sherblom, P., *J. Biol. Chem*. 252: 420-426, 1977. (4). Ramshaw, J. *Con. Tis. Res*. 14:307-314, 1986. (5). Arnoczky A.P., et al., *Arthroscopy*, 33:278-283, 2017. (6). Yuen, D. et al., Society for Biomaterials, 6th World Biomaterials Congress Transaction, 2000.