Introduction: Meniscectomy is the most common orthopaedic surgical procedure performed in the United States.1 The meniscus plays a critical role in knee joint biomechanics.2 Loss of meniscus function can lead to articular cartilage degeneration and osteoarthritis.3,4 Current treatment options for meniscal injury are meniscal repair when possible, partial meniscectomy, total meniscectomy, or fresh frozen meniscal allograft transplantation. The goal of this research is to improve meniscus transplantation by providing an alternative that is better than fresh-frozen allografts. By increasing porosity and decreasing cellular content in the meniscal scaffold, the potential for cellular ingrowth and reduced immunologic response respectively can be realized. The objective of this study was to develop and evaluate an acellular, three-dimensional collagen meniscal scaffold, and thereby, provide an alternative to total meniscectomy or meniscal allograft transplantation.

Materials and Methods: Twelve ovine medial menisci were harvested, n=6 were used to create scaffolds and n=6 were left as intact. All menisci were stored at -20°C in saline soaked gauze until further processing. The scaffold group was decellularized and oxidatively etched using aqueous Triton X-100 and peracetic acid.5 Tissue was equilibrated in phosphate buffered saline. The central 1/3 body of each meniscus was fixed in 10% formalin and paraffin processed. For both groups (intact, scaffold) sections were cut in the coronal plane and stained with hematoxylin and eosin (H&E) for routine cellular assessment and 4',6-diamidino-2-phenylindole (DAPI) to identify residual nuclear components. Samples were assessed using light microscopy. DNA was then isolated using a commercially available kit (DNEasy, Qiagen, Valencia, CA). The DNA concentration in the resulting volume was used to calculate total DNA content which is standardized using the initial dry weight of the sample. These values were averaged, a standard deviation was determined, and a comparison made using a Student’s unpaired t-test with a p-value <0.05 accepted for statistical significance. The architecture of the sheep intact meniscus and the meniscus scaffold was examined using scanning electron microscopy (SEM). Specimens were examined in the coronal plane. Porosity was qualitatively compared between the intact meniscus and meniscus scaffold.

Results: Upon gross inspection, the decellularization and oxidative etching did not change the general shape and architecture of the meniscus. The histological findings clearly illustrate a decrease in cellular and nuclear content. The H&E slides also suggest an increase in porosity. DAPI nuclear staining also showed a decrease in nuclear content in the scaffold compared to the intact fresh frozen meniscus. DNA content analysis revealed approximately a 70% decrease in DNA content in the scaffold compared to the intact meniscus (Figure 1). SEM images illustrate an increased porosity in the scaffold compared to the intact meniscus (Figure 2). The SEM also showed that the architecture of the meniscus is intact.

Discussion: This study indicates that decellularization and chemical oxidation decreases DNA content in a meniscus scaffold. Similar to previous studies in our laboratory 5, these data illustrate that the extracellular matrix architecture of the meniscus remains intact through the decellularization process. This process was able to increase porosity and decrease nuclear content thus bringing us one step closer to an ideal scaffold that minimizes immunologic response and encourages cellular ingrowth.

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