Meniscus Allograft Transplantation Using Chemically Sterilized Menisci: Biochemical, Histological and Biomechanical Evaluation in an Ovine Model

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**Introduction:** Meniscus allograft transplantation (MAT) has gained popularity in reducing pain and restoring normal joint function following meniscectomy1. Similarly to other allograft tissues, meniscal allografts may carry the risk of disease transmission if the tissue cannot be sterilized. The BioCleanse® sterilization process is a low-temperature chemical sterilization process that has been successfully applied to both bone and tendon while preserving native tissue biomechanical properties2, 3. Recently, we have also shown that BioCleanse® did not significantly alter material properties of meniscus allograft4. In the present study we further looked at in vivo remodeling of BioCleanse® processed meniscus allograft and compared it with standard, aseptically processed meniscus in a functional ovine model.

**Materials and Methods:** Meniscal allograft transplantation. Medial menisci were harvested from skeletally mature male Dorset sheep and were either: aseptically processed and frozen within 48 hours of death (Aseptic), or exposed to four controlled freeze/thaw cycles and processed through BioCleanse (BioCleanse). On the day of surgery, recipient sheep were meniscectomized and donor menisci were implanted by suturing the menisci horns in tunnels drilled at the native horn insertion sites. The sheep were euthanized at 8 weeks (Aseptic n=3, BioCleanse n=2) and 16 weeks (Aseptic n=6, BioCleanse n=6). All implanted menisci, together with the menisci from contralateral knees (Control), were split into anterior, central and posterior regions and analyzed. Unimplanted Aseptic (n=6) and BioCleanse (n=6) meniscus allografts were used for time 0 evaluation. All animal procedures were approved through Rush IACUC.

**Inflammation:** The inflammatory response induced by the MAT was assessed by subjective scoring of H&E stained sections of synovial tissue taken from each joint.

**Cell viability:** Upon sacrifice, menisci were immediately assessed for cell viability using a confocal microscope and live/dead calcein/ethidium bromide staining.

**Histological evaluation:** Meniscal sections were stained with H&E, Safranin-O and fast green, or Picrosirius Red (PSR).

**Biochemical evaluation:** Meniscal sections were analyzed for water, proteoglycan, and collagen content using standard protocols as reported previously4.

**Biomechanical evaluation:** Discs measuring 3 mm in diameter and ~1.5 mm in height were harvested from the mid-depth of each region of the excised menisci. Stress relaxation tests were performed on the specimens in unconfined compression at 15% final strain and Young’s modulus was determined from the ratio of stress and strain at equilibrium.

**Cartilage evaluation:** Gross evaluation of cartilage at the time of necropsy was determined using India Ink staining and Outerbridge scoring. In addition, histological sections were scored using the Mankin scale.

**Statistical analysis:** Differences among groups where evaluated using one or two-way ANOVA where appropriate, and α=0.05 was taken to be significant.

**Results:** During the 16 week course of study the synovium of operated joints showed slight inflammation at 8 week time point (score less than 1 on the scale of 0 to 3) which subsided by 16 weeks and reached the level of unoperated joints. No statistical differences were observed between BioCleanse and Aseptic group at either time point. At 8 and 16 weeks, the Aseptic group had 51.1%±7.5% viable cells and the BioCleanse group had 36.4%±16% and 61.4%±7.5% viable cells, respectively. There were no statistically significant differences between groups at either time point. H&E staining showed no apparent differences among Aseptic, BioCleanse and Control. A closer look at PSR stained sections under polarized light also revealed no apparent differences among groups in collagen structural organization. While the Safranin-O proteoglycan staining showed no differences among Aseptic and BioCleanse group at either 8 or 16 weeks, both MAT groups showed reduced signal when compared to the Control group (Fig. 1). Biochemical evaluation showed that there were no statistically significant differences between the Aseptic and BioCleanse groups with respect to GAG, water and collagen content during 16 weeks duration of study. On the other hand both MAT groups showed significantly reduced content of GAG, and increased water content when compared to Control (Fig. 2). Similarly, stiffness in compression showed no significant differences between the BioCleanse and Aseptic groups neither before or during implantation; however, both MAT groups showed a decrease in stiffness after 8 and 16 weeks when compared to Control knees (Fig. 2). Finally, we observed no significant differences between the Aseptic and BioCleanse groups with respect to India ink staining, Outerbridge or Mankin scoring.

**Discussion:** The major finding of this study is that during 16 weeks of implantation in the ovine model, BioCleanse processed menisci underwent the same extent of remodeling as aseptically processed menisci based on histological, biochemical and biomechanical evaluation. Furthermore, BioCleanse and aseptically prepared menisci were the same with respect to viability of incorporated cells. Joint capsule inflammation, which normally occurs with meniscal transplantation, subsided over time and was no different between groups. As expected in the early phases of remodeling, menisci showed a decrease in GAG content with a corresponding increase in water content, and a decrease in elastic stiffness, similar to the meniscus remodeling observed previously in the goat model5. We conclude from this study that 16 weeks after implantation, the BioCleanse process will render sterile, biocompatible and functional meniscus allograft comparable to the traditional aseptically prepared meniscus.


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