

## CELLULAR REPAIR OF MENISCAL TEARS IN THE AVASCULAR REGION

+\*Gill T J; \*Weinand C; \*\*Peretti G M; \*Adams S B; \*Randolph M A

+\* Massachusetts General Hospital, Harvard Medical School, Boston, MA,

\*\* Ospedale San Raffaele, Milan, Italy

**INTRODUCTION:** Spontaneous healing of traumatic meniscal tears occurs only in the vascularized outer third of the meniscus. Current repair techniques for tears in the avascular inner third of the meniscus, however, are prone to failure. Recent tissue engineering techniques using cell-based therapies have shown promise in attempts to heal meniscus tears, but complete healing has not been achieved thus far. To minimize donor-site morbidity, only very low numbers of autologous chondrocytes are often available for autologous chondrocyte transplantation. Therefore, efficient seeding of the cells onto scaffolds is essential for engineering new cartilage tissue. Previous research has shown that fluid flow in seeding conditions positively influences tissue engineered cartilage qualities. As an alternative to sutures or resorbable devices, we evaluated the healing capacity of a cell-seeded construct as an implant in artificially created tears in this avascular zone of the medial meniscus in swine as a large animal model. This two-part study addresses the issue of applying tissue-engineering techniques to repair meniscus injuries. Part I evaluates the efficiency of different seeding techniques using low numbers of cells. Part II investigates the potential for a cell-seeded scaffold to repair meniscal lesions in a large animal model—swine.

**METHODS:** All studies using animals, cells, or tissues were approved by the Institutional Animal Care and Use Committee (IACUC).

**Part I: Scaffold Seeding.** In the first part of the study, we evaluated two different chondrocyte sources at three cell concentrations on two types of scaffolds, Vicryl mesh® and a solid scaffold prepared from devitalized meniscus. Articular and auricular chondrocytes were harvested from three-month-old Yorkshire swine and seeded onto these scaffolds at cell concentrations of 1, 2, and 5 million/ml. The three seeding techniques were evaluated a) *Static*, in which the chondrocytes were allowed to attach to the scaffolds by gravity only, b) *modified centrifugal cell immobilization (CCI)*, in which the chondrocytes were seeded using centrifugal force and turning the scaffold, and c) *Dynamic Oscillating Seeding*, in which a continuous oscillating movement was applied to the cell-scaffold suspension in co-culture. The constructs were harvested after 7 days of culture and histologically examined for cell phenotype, cell distribution and cellular adhesion. Quantification of cellular attachment was evaluated biochemically by DNA count for each cell type on the mesh scaffold. Each parameter was statistically analyzed by the two-way ANOVA.

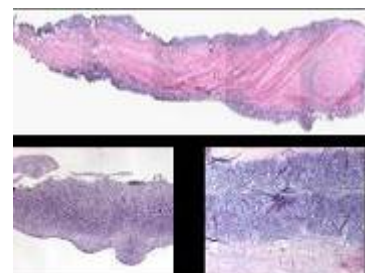
**Part II: Meniscus Repair in Swine.** In the second part of the study, we evaluated the healing capacity of a seeded implant inserted into a surgically made lesion in the avascular region of swine meniscus. Auricular and articular chondrocytes were harvested from three-month-old Yorkshire swine for seeding of the Vicryl mesh® scaffolds under the *Dynamic Oscillation* conditions for 8 days. Before implantation, the constructs were histologically analyzed for cellular attachment and homogenous cell distribution. Sixteen swine had a one-centimeter bucket-handle tear created at the medial border of the middle and inner avascular zone of their menisci. The controls consisted of 4 swine with no repair and 4 swine having suture repair only. The experimental groups were 4 swine receiving an implant with auricular cells, and 4 swine with articular cells. The implanted constructs were secured with 2 vertical mattress sutures to allow full weight bearing immediately after the operation. Menisci were harvested after 12 weeks and examined macroscopically, mechanically, and histologically for healing.

**RESULTS: Part I: Scaffold Seeding.** Auricular chondrocytes, in general, revealed better cellular attachment on both scaffolds when using any of the three seeding techniques. Examination of the *Static* seeding technique showed only few cells attached onto the different scaffolds over a period of 7 days. The greatest amount of cellular attachment was found on the solid scaffold, whereas nearly no cells were present on the Vicryl mesh scaffold. The same results were found using the *modified CCI* technique. The largest number of cells was found on the solid scaffolds using these two techniques, although the cells were attached only in defined small areas on the scaffolds. The most homogeneous cell distribution was found using the *Dynamic Oscillating Technique* using auricular chondrocytes. Although the articular chondrocytes covered all matrices completely, these

cells were found to concentrate at points along the scaffolds. Cell-cell interdigitations were found throughout the Vicryl mesh scaffold, independent of the type of chondrocyte used. The devitalized meniscus chip was thoroughly covered with chondrocytes. Biochemical analysis of the chondrocytes attached to the PLGA mesh scaffold revealed that the *Dynamic Oscillating* technique resulted in up to 50% higher cellular attachment (**Figure 1**) than the *Static* technique and up to 150% higher than the *modified CCI* seeding.

**Part II: Meniscus Repair in Swine.** All 8 of the control samples failed to heal or show any new tissue formation inside the lesion. Closure of the meniscal lesions was observed grossly in all experimental samples after 12 weeks. Gross mechanical testing of the menisci samples with two Adson forceps demonstrated bonding of the meniscus tear. Histological analysis showed formation of new fibrocartilagenous tissue in all 8 experimental samples with integration into the native meniscus tissue. With the exception of one meniscus treated with auricular chondrocytes and one with articular chondrocytes, where partial healing was achieved, complete tear closure with newly formed fibrocartilagenous tissue was observed in all experimental samples.

**Figure 1.** Auricular chondrocytes seeded dynamically onto meniscal cartilage (top) and Vicryl scaffold (lower left). Integration of Vicryl scaffold seeded dynamically with auricular chondrocytes between meniscal tissue (lower right).



**Figure 2.** View of meniscal repair using auricular chondrocytes on Vicryl scaffold indicated by black arrow on left. Photomicrograph of healed meniscal lesion on right. Black arrows indicate region of the lesion.

**DISCUSSION:** This study demonstrates improved cell seeding of scaffolds under *Dynamic Oscillating* conditions. There was a clear trend towards a higher cellular content and a more evenly distributed cellular coverage over the entire surface of both types of scaffolds using *Dynamic Oscillating* seeding conditions. We conclude the *Dynamic Oscillating* seeding technique is an efficient technique for seeding low numbers of cells onto various scaffolds, which can be useful implants for meniscus tears.

Current repair techniques for tears in the inner avascular zone of the meniscus, such as sutures or resorbable devices, fail to effect healing. Lacking a suitable means for repair of the inner third of the meniscus, partial or total meniscectomy results in higher contact pressure of approximately fifty percent resulting in early onset of osteoarthritis after resection, even in young patients. Therefore, new meniscus repair techniques are needed. In previous studies, we demonstrated that cell-based implants in artificially created medial meniscus bucket-handle tears inserted into the subcutaneous pouch of nude mice developed a newly formed tissue integrating into the torn meniscus. The data from this present study extend those findings to a large animal model of meniscus repair. The results from this study provide evidence of the efficacy of this cell-based approach for treating meniscus tears and demonstrate the potential of tissue-engineered, cellular repair to provide successful healing of tears in the avascular zone in a large animal model.

Funded by a grant from the AO-ASIF Foundation, Switzerland.