A Novel Repair Method for Meniscal Radial Tear in vitro Using Aligned Electrospun Nanofibrous Scaffold

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Disclosures:

Introduction: The meniscus plays important roles in the knee joint, including force transmission, shock absorption and provision of joint stability (1,2). Importantly, meniscal tears are the most common injury of the knee regardless of age, and loss of the meniscus is recognized to predispose the knee joint to degenerative changes (3,4). A critical anatomical feature of the meniscus is that the mostly circumferential arrangement of collagen fibers (5,6) that resist hoop stresses. Radial tears of the meniscus, which separate the circumferential meniscal fibers, cause loss of biomechanical meniscal function (7). Radial tears have been mostly commonly treated by partial meniscectomy (8). Recently, meniscal repair is being considered as an alternative treatment for radial tears that involve the red-red or red-white zone (avascular lesion) to preserve the important functions of the meniscus. However, meniscal repair using several suture techniques for radial tears of the meniscus is seldom reported (7), and satisfactory results have not been obtained. In our previous studies, we have observed that adult meniscal stem cells (MSCs) cultured on aligned poly(e-caprolactone) (PCL) nanofiber scaffolds organize and deposit collagen along the fiber direction, producing cartilage-like engineered constructs (9,10). With these regenerative tools, our goal is to develop a functional cell-seeded scaffold and a new surgical method to enhance meniscal radial repair.

Methods: Cell isolation, fabrication of electrospun scaffold and production of cell-seeded scaffold: Meniscal fibrochondrocytes (MFCs) were isolated using sterile techniques from the inner avascular part of adult bovine meniscus by first mincing the tissue into small pieces and then allowing cells to migrate out onto tissue culture plates to establish primary cultures. To fabricate aligned nanofibrous scaffolds, a custom-designed electrospinning device was utilized to generate a 40:60 composite scaffold consisting of 14.3% w/v poly(e-caprolactone) (PCL, 80 kDa) and 10% polyethylene oxide (PEO, 200 kDa) fibers. After PEO removal with phosphate buffered saline, each scaffold was cut into a rectangular shape (5 mm x 8 mm) of approximately 500 μm in thickness, and 100,000 MFCs at passage 3 were seeded onto per side of the scaffold. Formation of meniscus and scaffold constructs: Menisci from adult skeletally mature cow were aseptically dissected and cylinder-shaped explants (5 mm diameter x 8 mm height) were excised from the inner avascular part. Two or three explants were harvested from one meniscus, with the long axis aligned along the direction of the meniscal circumferential main fibers. A tear of half depth was created in the center of the explant, and radial tear was mimicked by cutting perpendicular to the meniscal main fibers. The torn site was wrapped with either the scaffold alone or MFC-seeded scaffold (scaffold group and cell group, respectively), with the scaffold fiber direction matching that of the meniscal main fibers. The scaffold was fixed to the meniscal explant with sutures to prevent detachment from its surface. A control group was prepared as explants without scaffolds or cells. The composite constructs in each group were cultured in DMEM growth medium supplemented with 50 μl/ml ascorbic acid 2-phosphate and 10 ng/ml TGF-β3 for 4 and 8 weeks, and were then assessed histologically (n=2 per group) and biomechanically (n=5-7 per group). Histology was performed with Picrosirius red for collagen, Safranin O/Fast green for sulfated glycosaminoglycans (GAGs), and DAPI to identify cell nuclei. Tensile testing was performed to calculate load to failure, stiffness and Young’s modulus of the repaired site.

Results: DAPI staining showed that cells were recruited into the gap of radial tear in each group. By Picrosirius red staining, the radial tear wrapped with the scaffold alone or cell-seeded scaffold exhibited a partial repair, but complete repair was not observed in either group, although the cell-seeded group showed overall improved healing compared to the scaffold group. On the other hand, the gap remained until 8 weeks in the control group. (Fig.1a) The scaffold, regardless of cell-seeding, was observed to adhere closely to the native meniscal tissue at 4 and 8 weeks. Also, the scaffold exhibited positive Picrosirius red staining in both the scaffold and cell groups, while only the cell-seeded scaffold showed positive Safranin O staining. (Fig.1b) Mechanical testing of repaired meniscus showed that the load-to-failure and stiffness values in the cell group at 8 weeks was significantly higher than the control group at 4 and 8 weeks. The Young’s modulus in the cell group at 8 weeks was significantly higher than that of the control group at 4 weeks. Also, these parameters of the cell group at 8 weeks were approximately 40% of those of a normal meniscus. (Fig.2)

Discussion: In this study, we have demonstrated the feasibility of the MFC-seeded scaffold to repair the meniscal radial tear based on both histological and mechanical analyses. In particular, the highly adhesive property of the cell-seeded scaffold to the meniscal tissue should be beneficial to stabilizing the circumferential meniscal fibers and help to preserve the function of hoop structure and prevent the development of OA in clinical practice. Our results also show that the in vitro model will be useful for evaluating the reparative efficacy of an engineered constructs or therapeutics, such as growth factors and pharmacological agents prior to in vivo testing. The in vitro model can also accommodate the inclusion and the testing of biologics, such as fibrin clot or...
platelet-rich plasma, that have been introduced into the gap of meniscal tear or injected into the joint in clinical practice. In this manner, the in vitro model will contribute towards both the analysis of the underlying mechanisms as well as the efficacy of potential therapeutic methods of meniscal repair.

An obvious limitation of the present study is that in vitro culture is fundamentally different from the intra-articular environment, which includes the synovium, cartilage, ligaments, and blood vessels. Nevertheless, we believe that the in vitro model may serve as an initial proof-of-concept platform that will contribute towards experimental design of subsequent in vivo animal studies and clinical trials.

**Significance:** Our study shows that a MFC-seeded nanofibrous scaffold sutured over a meniscal tear enhances the repair of the meniscus by strengthening the interface between the appositional edges of the wound and itself recruiting cells and promoting their differentiation into fibrocartilaginous tissue. Together, these affects increase the mechanical strength of the meniscus at the site of the tear. This will increase the functionality of the repaired meniscus and improving clinical outcomes.

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**References:**
Fig. 1a. The gap of radial tear at 4 or 8 weeks culture in each group with DAPI and Picrosirius red staining.

Fig. 1b. The scaffold and adjacent meniscal tissue at 4 or 8 weeks culture with DAPI and Safranin O/Fast green staining.
Fig. 2. Mechanical testing of repaired meniscus at 4 or 8 weeks culture in each group.

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