Rat Rotator Cuff Repair Using A Cell Sheet Composed Human Rotator Cuff Derived Cells

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Disclosures:

Introduction: Biological regeneration of tendon-bone junction is ultimate goal of rotator cuff (RC) tissue engineering. We have already reported the cells from torn rotator cuff have a potential of self-proliferation and multilineage differentiation. Recently, alternative methods of cell delivery, such as cell sheet technology, have been developed, and may represent an approach to deliver adult stem cells to the tendon and tendon bone interface (1). The engineered cell sheets from this method showed preserved cellular communication, junctions, endogenous extracellular matrix, and integrative adhesive agents (2). We developed a cell sheet composed of human torn rotator cuff derived cells and examined its therapeutic effects using a rat RC injured model.

Methods: Preparation of human rotator cuff derived cells
Human rotator cuff derived cells were isolated from torn edges of human supraspinatus tendons, which were obtained during arthroscopic rotator cuff repair with informed consents from the patients (2 males aged 66, 55, and 1 female aged 75). The tissues (weighing about 0.3 g each) were cut into small pieces under sterile conditions, followed by a digestion in Dulbecco’s modification of Eagle’s Medium (DMEM) supplemented with collagenase II. After digestion, the cells were pelleted, washed in phosphate-buffered saline (PBS), and subsequently cultured in 75-cm² cell culture flasks with DMEM supplemented with 10% fetal bovine serum (FBS) and 1% penicillin-streptomycin (PS) (regular medium). All experiments were performed with 1 or 2 passaged cells, and the same passage of cells was used for each experiment.

Cell sheets preparation
After washing, the 5x10⁵ cells per well were incubated on 24-well temperature-responsive culture plates (UpCell) in regular medium at 37°C. After 17 h, the plate was incubated at room temperature for 20 min. During that time, the cell sheets detach from the well as free-floating monolayer cell sheets.

Animal model of RC repair
All operations were performed under general anesthesia. Infraspinatous tendons in 12 immunodeficiency rats were resected bilaterally. In the right shoulders, tendons were repaired by transosseous technique using 4-0 Nylon and covered with the cell sheet (sheet group) (Fig.1). In the left shoulders, the tendons were repaired in the same way without coverage of the cell sheets (control).

Histological examination
The scapula-humeral complex with infraspinatus muscle in the Optimal Cutting Temperature blocks was sectioned serially with a thickness of 6 mm, mounted on a silane-coated glass slide, and air-dried for 1 h before fixation with 4% paraformaldehyde at 4 °C for 5 min. After fixation, tissue sections were stained with toluidine blue to identify fibrocartilage and collagen fibers at week 4 (n=6 in each group), according to standard protocols.

Immunofluorescence staining
We visualized the regenerated capillaries and/or neovascularity via fluorescence microscopy with using immunofluorescence staining for isolectin B4-FITC conjugate, a rat-specific endothelial marker at week 4 (n=6 in each group). Fibrocartilage appearance was visualized via immunofluorescence staining with rat type II collagen (Col2) antigen conjugated with FITC at week 4 (n=6 in each group).

Biomechanical test
8 weeks after surgery, 6 shoulders from each group were biomechanically tested. Before the biomechanical tests, all soft tissue except the ISP muscle was carefully removed from the scapula-humeral complex. The prepared scapula-humeral complex was mounted in a conventional tensile tester (model AGIS 5kN Shimadzu).

Statistical analysis
All data are expressed as mean values ± standard deviation. The Mann-Whitney U test was used to compare two groups. Results were considered significant with p-value < 0.05.
**Results:** Histological examination
In the sheet group at week 4, numerous chondrocytes at the repaired sites were detected by toluidine blue staining, while few chondrocytes were confirmed in the control group.

**Immunofluorescence staining**
Angiogenesis at the RC repaired site were confirmed by immunofluorescence staining for endothelial markers. Using tissue samples collected 4 weeks after surgery, Vascular staining with isoelectin B4 demonstrated an enhancement of intrinsic neovascularization around the RC repaired site in the sheet group (Fig.2). Capillary density measured by immunofluorescence staining of isoelectin B4 was significantly greater in the sheet group compared with the control group (sheet, 354.29±111.78; control, 174.29±78.92/mm². P<0.05 for sheet vs. control) (Fig.2). Immunofluorescence staining of Col2 demonstrated an enhanced healing process of bone-tendon junction in rats treated with the cell sheet (Fig.2). The number of Col2 positive cells was significantly greater in the sheet group compared with the control group (sheet, 217.14±94.81; control, 102.86±39.04/mm². P<0.05 for sheet vs. control) (Fig.2).

**Biomechanical test**
In the mechanical testing at week 8, the sheet group showed higher tensile strength than the control group with significant differences (sheet, 26.8N±4.4; control, 16.6N±8.6 respectively. P<0.05 for sheet vs. control) (Fig.3).

**Discussion:** Retear after arthroscopic RC repair was often reported in clinical studies, and it was caused by the weakness of the initial strength at the repair site (3). A number of reinforcing materials for RC repair, such as a platelet-leukocyte membrane (4), suture methods in clinical studies and PLGA sheet (5) in in vivo study, had been reported. Among these materials, the tissue-engineered contractile cell sheet has been recently considered as a very promising method for enhancing angiogenesis (6-8). Our results indicated that the cell sheet could promote fibrocartilage regeneration and angiogenesis around tendon-bone junction, resulting in superior mechanical strength compared to the control. In conclusion, the use of cell sheet of RC derived cells has a potential to enhance biological healing of the tendon-bone junction after RC repair.

**Significance:** The RC derived cell sheet could promote fibro-cartilage regeneration and angiogenesis at tendon-bone junction, with superior mechanical strength compared to control. Rotator cuff repair using cell sheet could be a promising strategy for rotator cuff tissue engineering.

**Acknowledgments:**

**References:**
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Infraspinatus tendons in 11 immunodeficiency rats were resected. In the right shoulders, tendons were repaired by transosseous repair using 4-0 Nylon and covered with the cell sheet.
Figure 2
Immunofluorescence staining for IsoB4 and Col2 demonstrated greater than control. There was significant difference compared with the control (P<0.01).
Fig. 3 Tensile strength

* P<0.05

In the mechanical testing at week 3, the sheet group showed significantly higher tensile strength than the control group (sheet, 26.8N±4.4; control, 16.6N±3.6 respectively. P<0.05 for sheet vs. control).