Local Administration of Autologous Synovium-Derived Cells Improve the Structural Properties of Anterior Cruciate Ligament Autograft Reconstruction in Sheep

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Background: The structural properties of a tendon autograft deteriorate during the remodeling phase after anterior cruciate ligament (ACL) reconstruction.

Hypothesis: A local application of autologous synovium-derived cells cultured in medium supplemented with transforming growth factor β (TGFβ) may inhibit the deterioration of structural properties of the tendon graft after ACL reconstruction.

Study Design: Controlled laboratory study.

Methods: Fifty-two mature sheep were used. In each animal, the right knee underwent ACL reconstruction using the semitendinosus tendon autograft; then, the animals were divided into 5 groups of 10. No additional treatments were applied in group 1, whereas fibrin sealant was applied around the graft in group 2. In group 3, autologous synovium-derived cells cultured in standard medium were applied around the graft with fibrin sealant, whereas autologous synovium-derived cells cultured in TGFβ-supplemented medium were applied with fibrin sealant in group 4. In group 5, fibrin sealant containing 20 ng of TGFβ was applied around the graft. Each animal was sacrificed at 12 weeks after the surgery. In each group, 7 and 3 sheep were used for biomechanical and histologic evaluations, respectively. The remaining 2 sheep were used to confirm whether the applied cells infiltrated the graft at 1 week after surgery.

Results: Confocal microscope observations showed that the applied cells that were labeled before implantation infiltrated into the superficial portion of the graft at 1 week. Biomechanically, the maximum load and the stiffness of groups 4 and 5 were significantly greater than those of groups 1, 2, and 3. Histologically, necrotic lesions were observed in the core portion of the mid-substance in groups 1 and 2. In groups 3, 4, and 5, no necrotic lesions were found in the mid-substance.

Conclusion: A local application of autologous synovium-derived cells cultured in TGFβ-supplemented medium or a fibrin sealant containing TGFβ significantly inhibits the natural deterioration of the structural properties of the tendon graft after ACL reconstruction.

Clinical Relevance: Administration of autologous synovium-derived cells cultured in TGFβ-supplemented medium or TGFβ and fibrin glue alone can be a potential cell-based therapy to prevent graft deterioration after transplantation or accelerate mechanical restoration of the deteriorated graft.

Keywords: anterior cruciate ligament; cell-based therapy; synovial cells; ligament reconstruction; transforming growth factor β

The structural properties of a tendon autograft deteriorate during the remodeling phase after anterior cruciate ligament (ACL) reconstruction, and the reduced properties are not completely restored even at 12 months after surgery. Therefore, one future goal in ACL reconstruction is to accelerate the healing process of the graft after transplantation and the mechanical restoration of the deteriorated graft. A number of studies have recently shown that the application of various growth factors stimulates wound healing of tendon and ligament tissues. The authors have clarified that the administration of transforming growth factor β1 (TGFβ1) significantly inhibits the deterioration of structural properties of the tendon graft in ACL reconstruction models. However, the intra-articular application of TGFβ1 induces osteoarthritic changes in the knee joint. Therefore, the intra-articular administration of TGFβ1 is considered unsuitable for clinical application during an ACL reconstruction. We must seek another therapeutic method that can accelerate restoration of ACL graft strength without any detrimental effects to the knee joint.
Synovium-derived cells are known as potent cells in intra-articular tissue healing. Therefore, there has been a common expectation that an application of autologous synovium-derived cells around the tendon graft may be able to accelerate restoration of the ACL graft strength. However, our previous study showed that extrinsic fibroblast infiltration reduces the mechanical properties of the in situ frozen-thawed tendon, which is an ideal ACL reconstruction model. As such, we should verify whether the above-described expectation is correct or not. However, our recent study reported that local administration of autologous synovium-derived cells cultured in TGFβ1-supplemented medium significantly inhibits the natural deterioration of mechanical properties of the in situ frozen-thawed ACL. Therefore, a local application of synovium-derived cells cultured in TGFβ1-supplemented medium may be able to accelerate restoration of ACL graft strength, avoiding the detrimental effects of TGFβ1 on the joint cartilage.

Thus, based on the above-described fundamental studies, we make the following 3 hypotheses: First, a local application of autologous synovium-derived cells may accelerate extrinsic fibroblast infiltration into the tendon graft but may result in reduction of the mechanical properties of the graft. Second, local administration of autologous synovium-derived cells cultured in TGFβ1-supplemented medium may inhibit the deterioration of structural properties of the tendon graft after ACL reconstruction in sheep. Third, the effectiveness of this cell administration on the graft may be the same as the effect of direct application of TGFβ1. The purpose of this study is to test these 3 hypotheses using the sheep ACL reconstruction model.

MATERIALS AND METHODS

Study Design

A total of 52 skeletally mature female sheep (Suffolk; Sankyo Labo Service Corporation, Inc, Tokyo, Japan) weighing 65 to 85 kg were used in this study. Animal experimentation was carried out at the Institute of Animal Experimentation, Hokkaido University Graduate School of Medicine, under the rules and regulations of the Animal Care and Use Committee. In each animal, the right knee underwent ACL reconstruction using the doubled semitendinosus tendon autograft under general anesthesia and aseptic conditions, according to previous studies. Of the 52 sheep, 50 were randomly divided into 5 groups of 10. In group 1, no treatment was applied around the graft after ACL reconstruction to obtain the control data showing the natural course after ACL reconstruction. In group 2, 2 mL of fibrin sealant (Tisseel L, Baxter, Deerfield, Illinois) was applied as the sham treatment around the graft. In group 3, the synovial tissue was harvested from the left knee at 14 days before ACL reconstruction and cultured for 13 days to obtain cells for grafting, based on the procedure described below. Approximately 5 × 10^7 cells at the first passage were mixed with 2 mL of fibrin sealant and incubated in Dulbecco Modified Eagle Medium (DMEM) for 1 day. The cultured cells contained in fibrin sealant were applied around the graft after ACL reconstruction. In group 4, the same treatment was applied around the graft after ACL reconstruction as in group 3, except for the cell culture method. Namely, in group 4, 10 ng/mL of recombinant human TGFβ1 (R&D Systems, Minneapolis, Minnesota) was supplemented in the 2 mL of medium for the last day of the 14-day culture. This dose of TGFβ1 was chosen according to the study reported by Okuzumi et al. These cells were then washed and rinsed 3 times in phosphate buffered saline (PBS) solution before application of the fibrin glue. In group 5, 20 ng of recombinant human TGFβ1 (R&D Systems) was mixed with 2 mL of fibrin sealant and applied around the graft after ACL reconstruction. This dose of TGFβ1 was chosen on the basis of our previous studies. The total amount of TGFβ1 was same as in group 4. In all the animals, there were no findings indicating infection of the knee joint during the experiment period. At 6 and 8 weeks after surgery, however, 1 animal in groups 1 and 2 died because of nonsurgery-related complications (pneumonia and intestinal obstruction). These 2 animals were excluded from this study. At 12 weeks after surgery, 48 sheep were sacrificed with an overdose of potassium chloride after general anesthesia. In groups 1 and 2, 7 of the 9 animals were used for biomechanical evaluation, and the remaining 2 were used for histologic observation. In groups 3, 4, and 5, 7 of the 10 sheep were used for biomechanical evaluation, and the remaining 3 were used for histologic observation.

In addition, the remaining 2 sheep of the original 52 were used to evaluate cell infiltration from the applied cell sheet into the tendon graft. The 2 animals underwent the same treatment as performed in group 3 and were sacrificed 1 week after surgery.

Preparation of Cells for Grafting

Surgery was performed under anesthesia induced by an intravenous injection of ketamine hydrochloride. After intubation, anesthesia was maintained with halothane. A

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midline longitudinal skin incision was made at the left knee, and the suprapatellar portion was exposed through a lateral parapatellar approach. The synovial tissue was harvested from the suprasynovial bursa of the knee joint.

The synovial tissues were individually placed in DMEM containing 10% fetal bovine serum. The synovial tissues were cut into 2- to 5-mm pieces, which were then placed in dishes. The dishes were incubated at 37°C in a humidified 5% CO₂ atmosphere in a cell-culture incubator. A confluent monolayer formed within 13 days. Confluent synovial cells were removed after trypsin treatment. In group 4 only, 10 ng/mL of recombinant human TGFβ1 (R&D Systems) was supplemented in the 2-mL medium for the last day of the 14-day culture. Then, these cells were washed and rinsed 3 times in PBS solution before application of the fibrin glue.

For groups 3 and 4, the cells from the first passage were embedded in the fibrin sealant by the previously reported method. Namely, the cells were mixed with the fibrin part; then, this solution was mixed with the thrombin part. The final concentration was approximately 5 × 10⁷ cells/mL. The cell-fibrin sealant mixture of 2 mL was placed in a culture dish (6-cm diameter) and cultured with DMEM in a CO₂ incubator at 37°C for 1 day (see Figure 1). The cell-fibrin sealant mixture was immersed in PBS solution 20 to 30 minutes before application to the ACL graft.

ACL Reconstruction Surgery

Surgery was performed under general anesthesia as described above. A midline longitudinal skin incision was made at the right knee. After a lateral parapatellar arthroscopy was performed, an ACL was excised. The bone tunnels in the femur and the tibia were made at the centers of the insertion sites of the ACL. The tibial tunnel was reamed to 6.5 mm over a guide pin. For the femoral tunnel, a 4.5-mm cannulated drill bit was inserted over a guide pin, and a 6-mm reamer was inserted for 20 mm from the inside of the knee joint. Through a distal part of longitudinal skin incision, the semitendinosus tendon was harvested and prepared (Figure 2A). Then, the graft was doubled over a continuous loop EndoButton CL (Smith & Nephew Endoscopy, Andover, Massachusetts). The length of the EndoButton CL was based on the femoral tunnel length such that 15 mm of graft was placed within the femoral tunnel. Two No. 3 Ethibond sutures were passed through each end of the tendon grafts in an interdigitating whipstitch fashion.

(Figure 2A). Then, the graft was doubled over a continuous loop of the EndoButton CL (Smith & Nephew Endoscopy, Andover, Massachusetts). The length of the EndoButton CL was based on the femoral tunnel length such that 15 mm of graft was placed within the femoral tunnel. Two No. 3 Ethibond sutures were passed through each end of the tendon grafts in an interdigitating whipstitch fashion. In groups 2, 3, and 4, the grafted tendon was wrapped with the prepared fibrin sealant sheet (20 mm long, 20 mm wide, approximately 1 mm thick) with or without cells. These sheets were sutured to proximal and distal sites of the ACL grafts with two 5-0 nylon threads. The gap between the fibrin glue and the ACL was filled with an additional 0.5 mL of fibrin sealant (Figure 4). In group 5, 20 ng of TGFβ1 dissolved in fibrin glue was applied around the graft. The incision was closed at each layer. Bulky cotton dressing was applied after surgery.

Postoperatively, the animals were returned to their cages (2 × 2 m) and allowed to put full weight on their legs.
limbs without restriction of motion. At day 6 after surgery, they were allowed to roam freely in a fenced area (approximately 50 m²) and walk around outdoors for 2 hours per day in a licensed farm (Ichikawa Farm, Shinshinotsu, Japan). At 12 weeks after surgery, the animals were sacrificed with an overdose of potassium chloride after general anesthesia. Immediately after their death, gross assessment of all grafts was performed by a coauthor (R.H.) under a blinded manner. In each group (except for 1 animal in groups 1 and 2), 3 and 7 animals were used for the following histologic and biomechanical evaluations.

Mechanical Testing of the Tendon Graft

At the time of the animal's death, the hind limbs were disarticulated at the hip joint. Each specimen was stored at -32°C until testing. Before mechanical testing, each knee was thawed overnight at 4°C. To determine the anteroposterior (AP) translation of the knee, all the surrounding muscles were carefully removed. The femur and the tibia were separately mounted in cylindrical aluminum tubes, using polymethylmethacrylate resin. During all preparations and testing, specimens were kept moist with saline spray. The knee was mounted on a specially designed testing device with 3 degrees of freedom, which was attached to a tensile tester (RTC-1210, Orientec, Oakabe, Japan). A load cell (UL-260L, Orientec) was attached to the tester. To determine neutral knee position, an AP force of 25 N was applied in both directions. After adjustment, a total of 4 cycles of AP shear loads (50 N) were applied to the knee specimens at 30°, 60°, and 90° of flexion, with a cross-head speed set at 20 mm per minute. The hysteresis curves were drawn with an X-Y recorder (Model 3023, Yokogawa, Tokyo, Japan). The displacement of the femur relative to the tibia with 50-N AP loads was defined as the AP translation of the knee at each angle of flexion.

After AP drawer testing, the joint capsule and all ligaments except for the ACL graft were carefully dissected in each specimen. The femur was clamped with an alligator jaw attached to a steel stand, and the tibia was suspended from the femur by the ACL graft. The length of the ACL graft was measured with a vernier caliper (Mitutoyo, Kanagawa, Japan) at the anterior, posterior, medial, and lateral aspects. The mean of the 4 values was defined as the length of the ACL graft. The cross-sectional area of the ACL graft was measured under the same condition as the optical noncontact method using a change-coupled device camera (WV-BD400, Panasonic, Osaka, Japan) and a video dimension analyzer (HTVC1170, Hamamatsu Photonics, Tokyo, Japan). The femur was attached to the stepping motor, and a constant tensile load of 1 N was applied to the ACL graft by suspending a weight to the tibia. The femur was rotated with the stepping motor set at angular increments of 5° (through 360°), and the corresponding profile width of the ACL graft was recorded with the video dimension analyzer. The cross-sectional shape of the ACL graft was reconstructed using a computer algorithm. The measurement was made at the middle of the graft to quantify a part of the gross observation on the thickness of the whole ACL graft.

For tensile testing, the femoral-graft-tibial complex specimen was mounted on a tensile tester (RTC-1210, Orientec) using a set of specially designed grips. The knee was rotated approximately 90° internally to remove the normal distortion of the ACL. The specimens were tested with the axis of loading along the anatomic axis of the ACL (ligament axis) at 45° of knee flexion. Before the tensile test, the specimen was preconditioned with a static preload of 5 N for 10 minutes, followed by 10 cycles of loading and unloading (3% strain) with a cross-head speed of 20 mm per minute. Then, each specimen was stretched to failure under the same conditions with preconditioning at a cross-head speed of 50 mm per minute. The structural
properties of maximum load, stiffness, and elongation at failure were determined directly from the load-elongation curves. The maximum load was defined as the load at the point of failure of the specimen. The stiffness was defined by the slope of the load-elongation curve, which was determined by applying a least squares linear regression analysis to the data curve between the endpoint of the toe region and the point that starts to bend before failure.

Histologic Examination of the Tendon Graft

Tissue samples were taken from the intra-articular portion of the graft. Samples were fixed in 5% buffered formalin at room temperature for 48 hours. Thereafter, specimens were dehydrated and embedded in paraffin. The longitudinal sections (4 μm thick) were cut and mounted on slides coated with 3% saline (Sigma Chemical, St Louis, Missouri). The sections were stained with hematoxylin and eosin. For each graft, 3 sections with hematoxylin and eosin were evaluated by 2 authors (H.T., K.Y.) under a blinded manner. Cellularity, shape of the nuclei, and collagen striations of the graft were observed with light and polarized light microscopy.

Evaluation of Cell Infiltration From the Applied Cell Sheet Into the Graft

To confirm that the cultured cells from the applied cell sheet infiltrated the graft, we used a technique described by Ohlendorf et al. Two sheep were treated in the same manner as in group 3. Before immediate implantation of the cultured cells in the fibrin sealant sheet, nuclei of the cells were labeled with 4 μL of calcein-acetoxy methyl ester (Molecular Probes, Eugene, Oregon). At 1 week after ACL reconstruction, the animals were sacrificed, and the implanted grafts were harvested from the knees. The grafts were washed 3 times with PBS, then frozen and sliced. Afterward, nuclei of cells in the harvested grafts were labeled with 5 μL of propidium iodide (Molecular Probes). They were then stained by incubation for 30 minutes at room temperature in a 10-mL PBS solution containing fluorescein diacetate. The cells in the harvested grafts were observed with a confocal laser microscope (MRC-1024, BIO-MED Laboratories, Tokyo, Japan). Live cells that originated from the cultured cells implanted with fibrin sealant metabolized calcein and generated green fluorescence. Yet, live cells with other origins were penetrated by propidium iodide and so generated red fluorescence. In a micrograph, implanted cells that migrated from the fibrin sealant sheet to the graft therefore appeared yellow because green fluorescence and red fluorescence were overlaid. In contrast, live cells with other origins appeared red.

Statistical Analyses

All data were shown as means with standard deviations. For each parameter, 1-way analysis of variance (ANOVA) was performed among the groups. When a significant result was obtained, a post hoc test with a Fisher protected least significant difference test was made for multiple comparisons. The significance level was set at $P = .05$.

RESULTS

Anteroposterior Translation of the Knee

The ANOVA showed no significant differences in the AP translation of the knee among the groups at 30° and 60° of knee flexion (Table 1). However, it did indicate a significant difference among the groups at 90° of knee flexion ($P = .005$). The post hoc test demonstrated that group 4 had significantly less translation than group 1 ($P = .0042$). There were no significant differences among groups 2, 3, 4, and 5, although group 4 had a tendency to demonstrate less than the other groups.

Gross Observation

During the specimen preparation for mechanical testing, we observed the morphologic structures of the knee joint for each specimen. The grafted tendons were enveloped by synovium-like tissues in the intercondylar notch in each group. These tissues were generally thicker and more abundantly vascularized in groups 3, 4, and 5 than in groups 1 and 2. There were no obvious degenerative changes on the articular cartilage and no tear of the menisci in any animal at 12 weeks after ACL reconstruction.

Tissue Dimension of the ACL Graft

The ANOVA showed no significant difference in the length of the ACL graft among the groups (Table 1). Regarding the whole cross-sectional area of the ACL graft, the analysis indicated no significant differences among the groups.

Structural Properties of the Femur-Graft-Tibia Complex

Regarding failure modes, all specimens failed at the mid-substance in all groups. Figure 5 shows the averaged load-elongation curves. In tensile testing, the maximum load was 301, 211, 216, 498, and 572 N and the stiffness was 92, 77, 57, 148, and 152 N/mm in groups 1, 2, 3, 4, and 5, respectively. The ANOVA demonstrated a significant difference in the maximum load ($P = .0003$) and stiffness ($P = .0018$) among the 5 groups (Table 1). The post hoc test showed that, first, there were no significant differences in the maximum load and the stiffness among groups 1, 2 and 3, although the stiffness of group 3 tended to be less than that of the other groups. Second, the maximum load and stiffness of group 4 were significantly greater than those of group 1 ($P < .0485$), group 2 ($P < .0133$), and group 3 ($P < .0029$). Third, the maximum load and
In group 3, in the periphery of the graft, the thick synovial tissue was covered around the graft. The central area of the graft was hypercellular compared with groups 1 and 2 (Figure 6). The fibroblast-like cell nuclei in the central area were oval shaped and found within an area of randomly oriented collagen. With polarized light, the collagen bundles throughout the graft showed random orientation without a uniform sinusoidal clump pattern.

In groups 4 and 5, collagen fibers were well organized, and cell nuclei appeared to be more spindle shaped than in groups 1 and 2 (Figure 6). There were no obvious differences between groups 4 and 5. We could not find any necrotic lesions in the midsubstance in groups 3, 4, and 5, but we did observe some necrotic lesions in groups 1 and 2. The cell density was significantly higher in groups 3 and 4 than in group 1 (P < .0001), group 2 (P < .0002), and group 5 (P < .0049) (Table 1) and significantly higher in group 3 than in group 4 (P = .0087).

Evaluation of Cell Infiltration to the Grafts

In the confocal laser microscope observation, cells having yellow fluorescence (originating from the cultured cells implanted with fibrin sealant) were seen in a superficial layer of the tendon graft at 1 week (Figure 7), although cells having red fluorescence (other origins) existed in the superficial and core portion of the graft.

DISCUSSION

This study, using the sheep ACL reconstruction model, clearly demonstrated the following 2 facts: First, local administration of autologous synovium-derived cells accelerated extrinsic fibroblast infiltration into the tendon graft but reduced its stiffness. Second, local administration of autologous synovium-derived cells cultured in TGFβ-supplemented medium or fibrin sealant containing TGFβ inhibited the natural deterioration of the structural properties of the tendon graft after ACL reconstruction.

**TABLE 1**

Anteroposterior Translation of the Knee, Tissue Dimensions, Structural Properties, and Cell Density of the Graft

<table>
<thead>
<tr>
<th></th>
<th>Group 1</th>
<th>Group 2</th>
<th>Group 3</th>
<th>Group 4</th>
<th>Group 5</th>
</tr>
</thead>
<tbody>
<tr>
<td>AP translation, mm</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>30°</td>
<td>8.5 ± 1.7</td>
<td>7.7 ± 1.5</td>
<td>7.8 ± 3.2</td>
<td>6.0 ± 1.9</td>
<td>7.8 ± 2.1</td>
</tr>
<tr>
<td>60°</td>
<td>9.3 ± 2.2</td>
<td>8.1 ± 1.0</td>
<td>7.7 ± 3.1</td>
<td>6.5 ± 1.7</td>
<td>8.5 ± 0.3</td>
</tr>
<tr>
<td>90°</td>
<td>8.3 ± 2.4</td>
<td>5.9 ± 1.9</td>
<td>6.2 ± 2.4</td>
<td>5.2 ± 0.7</td>
<td>6.6 ± 1.4</td>
</tr>
<tr>
<td>Length, mm</td>
<td>19.1 ± 2.9</td>
<td>19.8 ± 1.1</td>
<td>20.2 ± 1.3</td>
<td>20.0 ± 1.3</td>
<td>20.3 ± 1.0</td>
</tr>
<tr>
<td>Cross-sectional area, mm²</td>
<td>26.7 ± 9.4</td>
<td>32.0 ± 6.1</td>
<td>26.5 ± 8.5</td>
<td>27.7 ± 6.1</td>
<td>26.5 ± 6.6</td>
</tr>
<tr>
<td>Maximum load, N</td>
<td>300.7 ± 145.6</td>
<td>211.4 ± 140.5</td>
<td>125.7 ± 175.1</td>
<td>492.8 ± 181.5</td>
<td>572.1 ± 161.5</td>
</tr>
<tr>
<td>Stiffness, N/mm²</td>
<td>91.9 ± 55.1</td>
<td>77.2 ± 48.2</td>
<td>75.1 ± 42.2</td>
<td>143.1 ± 38.3</td>
<td>154.4 ± 49.9</td>
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<tr>
<td>Elongation, mm</td>
<td>6.3 ± 3.5</td>
<td>4.1 ± 0.3</td>
<td>5.2 ± 0.7</td>
<td>4.2 ± 1.1</td>
<td>5.0 ± 1.0</td>
</tr>
<tr>
<td>Cell density, per mm²</td>
<td>921 ± 380</td>
<td>931 ± 129</td>
<td>2188 ± 291</td>
<td>1874 ± 269</td>
<td>1169 ± 172</td>
</tr>
</tbody>
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*Significantly different from the control, group 1 (P < .05).
*Significantly different from group 2 (P < .05).
*Significantly different from group 3 (P < .05).
*Significantly different from group 4 (P < .05).

**Figure 5.** Mean load-elongation curves of the femoral-ACL graft-tibial complexes. Error bar, SD.
First, we speculated why local administration of autologous synovium-derived cells accelerated extrinsic fibroblast infiltration into the tendon graft but reduced the mechanical properties of the graft. De Bari et al.\(^8\) reported that mesenchymal stem cells can be isolated from the synovial membrane. These stem cells have the ability to extensively proliferate in culture and maintain their multilineage differentiation potential in vitro. In addition, Sakaguchi et al.\(^21\) and Ju et al.\(^8\) showed that mesenchymal stem cells derived from the synovium have higher potential of proliferation and differentiation than do mesenchymal stem cells derived from the other tissues. Therefore, we speculated that the autologous synovium-derived cells grafted in the present study might include mesenchymal stem cells and that they might accelerate extrinsic fibroblast infiltration into the tendon graft. Yet, infiltration of a number of cells into the tendon graft, with or without blood vessel regeneration, is accompanied by formation of a number of "flaws" in the grafted tendon (i.e., microinjury or disorganization of the matrix). Shrive et al.\(^20\) reported that the flaw formation in the ligament tissue reduces the material properties of the tissue. In the present study, we therefore speculated that the enhanced infiltration of autologous synovium-derived cells resulted in reduction of the mechanical properties of the graft due to enhancement of the flaw formation. The present study indicated that the conventional expectation is not always achieved—namely, that acceleration of cell infiltration into the necrotic tendon graft may inhibit the natural reduction of the mechanical properties of the ACL graft.

As such, we speculated on the mechanisms of the effect of local administration of synovium-derived cells cultured in TGFβ-supplemented medium. The cell density was significantly higher in group 4 than in groups 1, 2, and 5 (Table 1). Although the high cell density might have induced deterioration of the mechanical properties in group 3, the same phenomenon did not occur in group 4. This fact suggests that functions of the synovium-derived cells cultured in TGFβ-supplemented medium may be different from those of the simply cultured synovium-derived cells. Another possibility is that the TGFβ was responsible for the change in biomechanical performance and that it may not have been the TGFβ-supplemented medium with synovium-derived cells. We should therefore consider that both the cells and the TGFβ inhibited the flaw effect, not just the cells themselves. TGFβ is a multifunctional growth factor. TGFβ promotes extracellular matrix deposition through several distinct mechanisms: enhancement of matrix macromolecule production and protease inhibitor synthesis as well as inhibition of extracellular matrix protease. For example, TGFβ stimulates type I collagen synthesis in fibroblasts in vitro\(^27\) and in vivo.\(^30\) Second, TGFβ stimulates the production of a variety of other extracellular matrix macromolecules, including fibronecin,\(^16\) thrombospondin,\(^16\) osteopontin,\(^12\) tenascin,\(^15\) elastin,\(^9\) and decorin.\(^30\) Third, TGFβ inhibits degradation of the extracellular matrix by inhibiting production of several proteases\(^4,16\) and stimulating the production of specific protease inhibitors.\(^4\) We therefore speculated that it may have been the synovium-derived cells cultured in TGFβ or TGFβ alone that not only inhibited the flaw effect by stimulating production of specific protease inhibitors but enhanced organization of the graft matrix by stimulating type I collagen synthesis and producing extracellular matrix macromolecules. However, TGFβ is capable of regulating the expression of various different growth factors, such as vascular endothelial growth factor and platelet-derived growth factor.\(^24\) Therefore, we should conduct a study to determine which is the main mechanism.

In this study, the results from group 4 (autologous synovium-derived cells cultured in TGFβ supplemented medium) and group 5 (TGFβ with fibrin sealant) were
Nearly identical as far as, not only the maintenance of cellularity within the central portion of the graft histologically, but also the biomechanical performance. With the exception of elongation in the biomechanical analysis, there was no difference between groups 4 and 5 throughout the study. The elongation at failure in group 4 was significantly lower than that in groups 1 and 5. A possible reason for this is that group 4 was wrapped in a graft with sutures, which can add resistance to elongation, as compared with groups 1 and 5. In group 5, we reported mixing the TGFβ in a fibrin glue scaffold to apply to the ACL graft, according to our previous in vivo studies.11,22,23 This appears to be a reasonable carrier of TGFβ and may decrease the amount of TGFβ present within the synovial fluid, therefore potentially decreasing the incidence of osteoarthritis associated with TGFβ administration. At the 12-week point, there was no evidence of osteoarthritis in groups 4 and 5, although this was an early time point after surgery, and we did not perform an objectively histologic evaluation. Further studies—including long-term results, quantitative evaluations of osteoarthritis, and elution studies—to demonstrate how long the growth factors are present—are needed to establish the clinical utility of an ideal carrier for TGFβ to prevent the osteoarthritic occurrence.

There are several limitations in the present study. The first is that we evaluated only the 12-week follow-up results in the sheep ACL reconstruction model. The second is that we evaluated the effect of cells cultured in only an ex vivo condition. Therefore, we could not clarify the effective range of the condition, including that of TGFβ concentration in the medium, that of culture period, and so on. The third limitation is that we did not perform molecular biological examinations of cells that infiltrated into the grafted tendon. The fourth is that we did not completely determine the origin of the cells in the tendon graft, although we confirmed that the implanted cells with the fibrin sealant infiltrated into the superficial layer of the graft at 1 week after ACL reconstruction. The fifth limitation is that we did not perform histologic evaluation of the joint cartilage. This study showed that there was no evidence of osteoarthritis in each group. However, this was at an early time point, and further studies are needed to determine the ideal carriers for TGFβ to prevent the occurrence of osteoarthritis. In spite of these limitations, we believe that the present study has provided useful information concerning the basic science of ACL reconstruction.

As for clinical relevance, the present study suggests that local administration of autologous synovium-derived cells cultured in TGFβ-supplemented medium or TGFβ and fibrin glue alone can be a potential cell-based therapy to prevent the graft deterioration after transplantation or accelerate mechanical restoration of the deteriorated graft. This cell-based therapy includes a few advantages: A sufficient amount of the synovium-derived cells can be more easily, safely, and noninvasively harvested from the suprapatellar pouch of the knee joint, in comparison with bone marrow-derived stem cells. However, further studies should be conducted before applying this potential therapeutic method to the clinical field.

CONCLUSION

This study, using the sheep ACL reconstruction model, clearly demonstrated the following two facts: First, local administration of autologous synovium-derived cells accelerates extrinsic cellular infiltration into the tendon graft but reduces its stiffness. Second, local administration of autologous synovium-derived cells cultured in TGFβ1-supplemented medium or fibrin sealant containing TGFβ1 inhibits the natural deterioration of the structural properties of the tendon graft after ACL reconstruction.

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REFERENCES

Greater Than 10-Year Results of Red-White Longitudinal Meniscal Repairs in Patients 20 Years of Age or Younger

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**Background:** A prospective longitudinal investigation was conducted to determine the long-term outcome of single longitudinal meniscal repairs extending into the central avascular region in patients aged 20 years or younger.

**Purpose:** To determine the long-term success rate and reoperation rate of meniscal repairs extending into the avascular zone.

**Study Design:** Case series; Level of evidence, 4.

**Methods:** Thirty-three meniscal repairs were performed using an inside-out multiple vertical divergent suture technique. A concomitant anterior cruciate ligament reconstruction was done in 18 patients. The mean follow-up was 16.8 years (range, 10.1-21.9 years). The long-term success rate was determined in 29 repairs (88%) by the presence of normal or nearly normal parameters from 2 validated knee rating systems, assessment of magnetic resonance imaging and weightbearing posteroanterior radiographs by independent physicians, and follow-up arthroscopy when required. A 3 Tesla magnetic resonance imaging scanner with cartilage-sensitive pulse sequences was used, and T2 mapping was performed. A comparison was made between the short-term (mean, 4 years) and long-term outcomes.

**Results:** Eighteen (62%) of the meniscal repairs had normal or nearly normal characteristics in all of the parameters assessed. Six repairs (21%) required partial arthroscopic resection, 2 had loss of joint space on radiographs, and 3 that were asymptomatic failed according to magnetic resonance imaging criteria, for a total of 11 documented failures (38%). There was no significant difference in the mean articular cartilage T2 scores in the healed menisci between the involved and contralateral tibiofemoral compartments in the same knee. There were no significant differences between short- and long-term evaluations for pain, swelling, jumping, patient knee condition rating, or the overall Cincinnati rating score.

**Conclusions:** A chondroprotective joint effect was demonstrated in the healed meniscal repairs, which warrants the procedure in select patients. The long-term evaluation of the anterior cruciate ligament-reconstructed knees with concurrent successful meniscal repairs demonstrated a low rate of radiographic arthritis.

**Keywords:** meniscus; red-white repair; longitudinal

Preservation of meniscal function is paramount for long-term knee function, with poor clinical results documented after partial and total meniscectomy. Many meniscal repair procedures have been described, including outside-in, inside-out, and all-inside suture techniques with meniscus fixator devices. Failure rates of suture repairs for tears that extend into the central avascular region vary from 0% to 40%.

To our knowledge, only 2 long-term (minimum 10-year follow-up) clinical studies reported results of repair of meniscal tears extending into the central region. The number of tears totaled only 20 in these studies. Neither investigation used 3 Tesla magnetic resonance imaging (MRI) with cartilage-sensitive pulse sequences and T2 mapping and weightbearing posteroanterior (PA) radiographs to evaluate the chondroprotective effects of surgery. T2 mapping provides noninvasive insight into the ultrastructure of hyaline cartilage to detect early degenerative changes before discernible loss of cartilage thickness is visible on conventional MRI. The ability of meniscal repairs for tears extending into the avascular region to provide a chondroprotective effect to the knee joint is unknown.

The purpose of this investigation was to prospectively determine the long-term outcome of repair of single longitudinal meniscal tears that extended into the central avascular region in a group of patients age 20 years or younger. To our knowledge, this is the first report in the English-language literature to investigate the findings of this