Title:

Adipose-Derived Regenerative Cells Promote Tendon-Bone Healing in a Rabbit Model

Abstract

Purpose: To evaluate the therapeutic effect of adipose-derived regenerative cell (ADRC) administration on tendon-bone healing in a rabbit ACL reconstruction model.

Methods: Anterior cruciate ligament (ACL) reconstruction with semitendinosus tendon autograft was performed on the right knees of adult white rabbits. Eighty rabbits were divided into 2 groups: a treatment group, in which the graft was coated with ADRCs mixed in fibrin glue carrier during surgery, and a control group, in which the graft was coated with fibrin glue only. At 2, 4, 6, 8, and 12 weeks postoperatively, 8 rabbits were sacrificed in each group. Three were used for histological evaluation at the tendon-bone interface, and 5 for biomechanical examination.

Results: In histological analysis, chondroid cells appeared more orderly and more regular in size and shape, and Sharpey-like fibers, which connected the tendon graft and bone tissue, appeared earlier in ADRC-treated tissues than in control tissues. In biomechanical analysis, the ultimate failure load in the ADRC-treated group was significantly greater than that in the control group at 2 and 4 weeks (29.5 ± 7.2 N vs. 20.9 ± 2.7 N; $P = .016$ and 32.3 ± 3.9 N vs 22.8 ± 5.4 N; $P = .016$, respectively). Stiffness was significantly higher in the ADRC-treated group than in the control group at 6 weeks (21.7 ± 5.9 N/mm vs. 12.6 ± 4.9 N/mm; $P = .037$). Although the ultimate failure load and stiffness of the ADRC-treated limb was higher than that of the limb in the control group at 8 and 12 weeks, these differences were not significant.

Conclusions: Local administration of ADRCs promoted the early healing process at the tendon-bone junction, both histologically and mechanically, in the rabbit ACL reconstruction model.

Clinical Relevance: ADRCs could be used to enhance graft healing in ACL reconstruction.

Introduction

Autologous tendon grafts are currently popular for anterior cruciate ligament (ACL) reconstruction. Secure healing between tendon and bone is necessary for successful ACL reconstruction. However, tendon-bone healing occurs slowly, which can limit early return to sports activities. Several studies have shown that tendon-bone healing occurs more slowly than bone-to-bone healing,[1-3] which raises concerns regarding the adhesive strength between tendon graft and bone tunnels and the subsequent risk of graft failure. Interventions that can improve and accelerate tendon-bone healing could potentially reduce the rate of graft failure and allow for early aggressive rehabilitation.

Adipose tissue has been gaining attention as a promising source of undifferentiated mesenchymal stem cells. Adipose-derived regenerative cells (ADRCs) have multilineage potential equivalent to bone marrow-derived stem cells and can be easily obtained in large amounts from subcutaneous adipose tissue without the need for culture and expansion.[4,5] Although a number of reports have been published regarding clinical applications of ADRCs,[6-16] we are unaware of any study investigating ADRCs for their potential benefit in enhancing tendon graft healing in a bone tunnel. The aim of this study was to evaluate the therapeutic effect of ADRC administration on tendon-bone healing in a rabbit ACL reconstruction model. We hypothesized that ADRC administration at the tendon-bone interface may promote the healing between tendon graft and bone tunnel.

Methods

Experimental Design

Eighty-two female Japanese white rabbits (age, 15-17 weeks) weighing between 3.0 and 3.5 kg were used in this study. Because of the unified standards, we integrated the sex of the animals. We did not especially mean anything by selecting female rabbits. ACL reconstruction with a semitendinosus tendon autograft was performed on the right knee. Eighty animals were randomly divided into 2 groups, and the remaining 2 were used for tracing of ADRCs. In the treatment group, the graft was coated with ADRCs mixed in fibrin glue carrier during surgery. In the control group, the graft was coated with fibrin glue only. The animals in both groups were divided into five subgroups and killed at 2, 4, 6, 8, and 12 weeks postoperatively; thus, 8 animals per group were sacrificed at each time point after surgery, at which time femur-graft-tibia complexes were harvested for histological and biomechanical evaluations. In all subgroups, 5 of the 8 rabbits were used for biomechanical evaluation, and the remaining 3 were used for histological observation. The animal experiments were conducted with the approval of the Institutional Animal Care and Use Committee and carried out in strict accordance with its regulations.

ADRC Preparation

ADRCs were isolated by modifying a previously established method.[17,18] Adipose tissue (1.5 g) of female rabbits that were not included in the study was harvested from the adipose tissue pouch on the interscapular region located along the dorsomedial line, nearly 5 cm from the skull in the craniocaudal direction, and then washed with phosphate-buffered saline (PBS, Wako, Osaka, Japan). The tissue was cut into strips over a period of 5 min. Collagenase (Wako) was dissolved in PBS for a concentration of 0.12% in 20 ml and used to digest adipose tissue at 37°C for 45 min in a water bath. The mixture was shaken every 15 min during the digestion period. Immediately after the reaction was completed, 20 mL of Dulbecco's modified Eagle's medium (DMEM, Wako) was added, and collagenase activity was neutralized. The resulting solution was filtered. The filtrate

was centrifuged at 1300 rpm for 6 min at 25°C, and the supernatant was removed. The pellet of ADRCs was subsequently administered at the tendon-bone junction. Approximately 1×10^5 cells were included in this pellet.

Surgical Procedure

Surgery was performed in accordance with the Guide for the Care and Use of Laboratory Animals published by the US National Institutes of Health (NIH publication no. 85-23, revised 1996). The animals were operated on under general anesthesia with subcutaneous injection of xylazine (5 mg/kg body weight; Bayer, Tokyo, Japan), and sedation was maintained by intravenous injection of 2.5% sodium phenobarbital (Kyoritsu Pharmaceutical, Tokyo, Japan). Using an aseptic technique, we approached the right knee joint through a medial parapatellar incision. The semitendinosus tendon was identified and transected at its musculotendinous junction, and the graft was prepared by removing the attached muscle and passing the holding sutures through each end of the tendon graft. The original ACL was resected, and then tunnels (2.0 mm in diameter) were drilled in the lateral femoral condyle and the medial aspect of the tibia at the footprint of the original ACL. The graft was routed through the tunnels, and then the previously mentioned materials were injected onto the interface between the grafted tendon and the bone tunnel. In the ADRC group, 0.2 ml of fibrin glue containing ADRCs was injected. On the other hand, in the control group, an equal amount of fibrin glue only was injected onto the interface. In the 2 animals used for tracing of ADRCs, the equal amount of fibrin glue containing ADRCs labeled with 1,1-dioctadecyl-3,3,3,3-tetramethylindocarbocyanine perchlorate (DiI) was injected. After the injection, both graft ends were secured under tension to the neighboring soft tissue with 2-0 Ethibond suture (Ethicon, Somerville, New Jersey). The incision was closed at each layer in a standard fashion. Postoperatively, the limbs were not immobilized, and the animals were allowed normal activity in individual cages. At each time point after surgery, the animals were sacrificed with an overdose of intravenous pentobarbital after general anesthesia.

Histological Evaluation

At the time the animals were killed, the entire joint including the femoral and tibial bone tunnels was harvested. For histological observation, tissues were fixed in 10% formalin, and conventional paraffin-embedded sections were prepared. The samples were cut into 5-μm-thick sections longitudinal to the bone tunnels in the femur and tibia and stained with hematoxylin and eosin (H&E) for the examination of healing at the interface between the tendon graft and bone tunnel under light microscopy. Bone-tendon healing was graded in a semiquantitative manner with a scale from 0 (worst) to 8 (best) using a modified version of the histological scoring system of Nakase et al.[19] based on new tissue formation at the interface and graft remodeling (Table 1). The two sections of each specimen were graded by two investigators (J.N., K.H.) in a blinded fashion, and the mean histologic scores of the two observers were determined as the final results to minimize sampling error and misinterpretation.

ADRC Labeling

In the 2 animals used for tracing of ADRCs, cells were labeled with DiI (Vybrant® DiI Cell Labeling Solution; Life Technologies, Carlsbad, CA, USA) and transplanted to confirm the survival potential and location of transplanted ADRCs. DiI binds to cellular thiols and has long-term stability, which enables the tracing of DiI-labeled transplanted cells in the host tissue. Two weeks after injection of labeled cells, the rabbits were euthanized, and frozen sections were prepared in the longitudinal plane. The presence of Dil-labeled cells in the bone tunnel was then observed using a fluorescence microscope.

Biomechanical Evaluation

At the time of each animal's death, the femur and tibia were sectioned 5 cm from the knee joint. In addition, 5 knees of normal rabbits were evaluated to obtain the structural biomechanical properties with intact ACLs. All soft tissues around the knee were carefully removed except for the tendon graft connecting the 2 bones. The suture material used to secure the grafts during surgery

was also disturbed. Each specimen was stored at -80°C until testing. Before mechanical testing, each knee was thawed overnight at 4°C. The femur and tibia were separately mounted in cylindrical aluminum tubes using polymethylmethacrylate resin. During all preparations and testing, specimens were kept moist with saline spray. These samples were mounted on an electromechanical testing machine (Legacy 4482, Instron, Kanagawa, Japan), and all mechanical testing was conducted by one investigator (M.K.). Each femur-graft-tibia complex was mounted in a custom jig to ensure that the tensile load could be applied along the longitudinal axis of both the femoral and tibial tunnels. The femur-graft-tibia complex was applied with a preload of 1 N for 30 seconds, and the specimen was cycled 5 times between elongation limits of 0 and 0.75 mm at a rate of 2 mm/min. A tensile force was applied at a constant elongation rate of 20 mm/min. The ultimate load at the point of failure was recorded. The stiffness was calculated from the slope of the linear region of the load-displacement curve. Furthermore, the site of failure, either by pullout of the tunnel or midsubstance graft rupture, was determined by gross examination.

Statistical Analysis

The semiquantitative histological scores, mean ultimate failure load, and stiffness between treatment and control groups were compared using the Mann-Whitney U test. The inter-observer reliability was assessed by kappa (κ) statistic, and agreement in percentage was calculated. The Mann-Whitney U test was also used for the comparison of biomechanical properties at different time points within each group. The failure patterns of the femur-graft-tibia complex shown by the biomechanical testing were analyzed with the Fisher exact test. Differences were considered statistically significant at $P < .05$.

Results

In this study, except minor redness and swelling at the reconstructed knee, there were no premature deaths, nor were there any serious joint infections on postmortem examination of the knees. All of the animals were euthanized at the planned times.

Histological Analysis

The adhesion of tendon to bone was analyzed histologically during the 12 weeks after treatment. At 2 weeks after surgery, in the control group, light microscopic examination revealed inflammatory response around the autografts and a highly cellular, vascular, and fibrous tissue infiltrating the interface (Fig 1A). Poorly organized and sparse collagen fibers appeared at the interface of the bone and the tendon graft. At 2 weeks in the ADRC-treated group, fibrovascular tissue appeared better organized, and cartilaginous tissue had appeared (Fig 1B). At 4 weeks, loose connective tissue characterized by directionally arranged collagen fibers was seen in fibrovascular tissue, and large areas of fairly disorganized cartilaginous tissue were observed around the tendon-bone interface in the control group (Fig 1C). In the ADRC-treated group, the fibrovascular interface tissue became denser and better organized, and occasionally Sharpey-like fibers, which connected the tendon graft and bone tissue, appeared. In addition, the chondroid cells appeared more regular in size and shape, with more orderly laying down of chondro-osteoid matrix (Fig 1D). At 6 weeks, the area between tendon and bone was seen to become more mature, with the fibrous connective tissue and narrowing of the distance between the tendon and bone in both groups (Fig 1E,F). The chondroid cells were closely associated with surrounding tendon and bone, and fibrocartilaginous tissue was seen to gradually blend into the tendon substance especially in the ADRC-treated group (Fig 1F). At 8 weeks, the interface area between tendon and bone was still a distinct entity, although it was definitely narrower in both groups (Fig 1G,H). The interface was more organized and mature in the ADRC-treated group (Fig 1H). At 12 weeks, gradual blending of fibrocartilaginous tissue into the tendon substance and occasionally mineralized fibrocartilaginous tissue appeared in the control

group (Fig 1I). In the ADRC-treated group, a gradual and smooth transition was seen from bone to mineralized fibrocartilaginous tissue, fibrocartilaginous tissue, and finally tendon (Fig 1J). Bone-tendon healing was graded in a previously described manner, and the score of both groups increased with time. For example, in one case histological appearance was scored 2 (1 based on graft remodeling and 1 based on new tissue formation at the interface) at 2 weeks (Fig 1A), and scored 6 (2 based on graft remodeling and 4 based on new tissue formation at the interface) at 8 weeks (Fig 1G). The semiquantitative score of tendon-bone healing quality was significantly higher in the ADRC-treated group than in the control group at 2, 4, and 6 weeks postoperatively (all $P < .05$) (Table 2). The percentage of agreement and κ for inter-observer reliability were 78.3 and 0.737, respectively.

ADRC Labeling

The distribution of DiI-positive (red) areas was detected at 2 weeks after transplantation (Fig. 2). DiI labeling suggested that the transplanted cells had survived and were localized to the site where they were transplanted at 2 weeks post-transplantation. The transplanted cells survived up to at least 2 weeks after transplantation, with some cells being focally distributed in the bone tunnel.

Biomechanical Analysis

Fifty limbs were analyzed after ACL reconstruction. The ultimate failure load of the 2 groups increased with time. The ADRC-treated limb generally had a greater failure load than the limb in the control group. There were significant increases between the ADRC-treated group and the control group at 2 weeks (29.5 ± 7.2 N and 20.9 ± 2.7 N, respectively; $P = .016$) and 4 weeks (32.3 ± 3.9 N and 22.8 ± 5.4 N, respectively; $P = .016$). Although the ultimate failure load of the ADRC-treated limb was higher than that of the limb in the control group at 6 weeks (34.2 ± 5.3 N and 26.6 ± 5.9 N, respectively; $P = .076$), 8 weeks (43.7 ± 17.5 N and 36.5 ± 15.1 N, respectively; $P = .076$), and 12 weeks (61.0 ± 18.5 N and 57.2 ± 12.5 N, respectively; $P = .754$), the difference was

not significant (Fig 3). The within-group analysis showed significant differences between 2 weeks and 12 weeks, 4 weeks and 12 weeks, and 6 weeks and 12 weeks in the ADRC-treated group, and between 2 weeks and 8 weeks, 2 weeks and 12 weeks, 4 weeks and 8 weeks, 4 weeks and 12 weeks, 6 weeks and 12 weeks, and 8 weeks and 12 weeks in the control group ($P = .016$, $P = .016$, $P = .016$, $P = .009$, $P = .009$, $P = .016$, $P = .009$, $P = .009$ and $P = .047$, respectively). The ultimate failure loads of the limbs in both ACL reconstructed groups were much weaker than those of intact ACLs (273.1 ± 27.3 N) ($P < .001$).

The stiffness in the ADRC-treated group was significantly higher than that in the control group at 6 weeks (21.7 ± 5.9 N/mm and 12.6 ± 4.9 N/mm, respectively; $P = .037$). There were no significant differences in stiffness between the groups at 2 weeks (15.4 ± 8.1 N/mm and 11.5 ± 2.9 N/mm, respectively; $P = .465$), 4 weeks (17.2 ± 8.0 N/mm and 13.9 ± 5.6 N/mm, respectively; $P = .917$), 8 weeks (23.3 ± 13.6 N/mm and 17.3 ± 12.3 N/mm, respectively; $P = .076$), and 12 weeks (30.2 ± 12.7 N/mm and 26.8 ± 11.1 N/mm, respectively; $P = .917$) (Fig 4). The within-group analysis showed significant differences between 2 weeks and 12 weeks in the ADRC-treated group, and between 2 weeks and 8 weeks, 2 weeks and 12 weeks, and 6 weeks and 12 weeks in the control group ($P = .047$, $P = .046$, $P = .047$ and $P = .047$, respectively).

At 2 and 4 weeks, all tendons were pulled away from the bone tunnel at the failure point. At 6 weeks, 1 of the 5 ADRC-treated limbs failed by rupture in the midsubstance of the tendon, while all of the control limbs failed by tendon pullout from the bone tunnel. At 8 weeks, 1 each of the 5 ADRC-treated limbs and control limbs failed by rupture in the midsubstance. At 12 weeks, 3 of 5 ADRC-treated limbs and 2 of 5 control limbs failed by rupture in the midsubstance. Fisher exact test showed no significant difference between the rates of pullout in the ADRC-treated and control limbs in each time point and overall.

Discussion

This study demonstrated that local administration of ADRCs has the potential to promote healing at the tendon-bone interface, both histologically and mechanically, in a rabbit model of ACL reconstruction. Histological maturation occurred earlier, and the semiquantitative score of tendon-bone healing quality at 2, 4, and 6 weeks postoperatively was significantly higher in ADRC-treated tissues than in control tissues. Biomechanical properties were significantly better in the ADRC-treated group than the control group at 2 and 4 weeks after surgery in terms of the ultimate failure load.

Several investigators have demonstrated positive effects of stem cells on tendon-bone healing. Lim et al.[20] reported that coating of tendon grafts with mesenchymal stem cells (MSCs) in ACL reconstruction promoted healing by the formation of an intervening zone of cartilage resembling the chondral enthesis of the normal ACL insertion. They also found that MSC-enhanced reconstructions demonstrated significantly higher failure load and stiffness than controls on biomechanical tests in rabbits. Similarly, Soon et al.[21] reported that MSCs applied at the tendon-bone interface during ACL reconstruction resulted in the development of an intervening zone of fibrocartilage and improvement in load-to-failure rates. Li et al.[22] reported that bone marrow mesenchymal stem cell (BMSC) transplantation to the tendon-bone interface was shown to enhance its mechanical properties by promoting tendon-bone tunnel healing at early time points 4–8 weeks after ACL reconstruction. Mifune et al.[23] reported that ACL-derived stem cells contributed to the tendon-bone healing after ACL reconstruction by enhancing angiogenesis and osteogenesis, which in turn contributed to increasing biomechanical strength. Lui et al.[24] reported that wrapping the ACL graft with a sheet of tendon-derived stem cells before graft insertion promoted graft healing in the early stage after ACL reconstruction radiographically, histologically, and biomechanically in a rat model.

These studies suggest that many types of stem cells can improve the tendon-bone healing process and result in better mechanical properties. However, these agents also have drawbacks to clinical use because of concerns such as donor site morbidity, limitation of the cell source, difficulty of isolating stem cells, time and effort required for ex vivo culture of stem cells and formation of a cell sheet if needed, possible side effects after transplantation in humans, and ethical considerations.

The stromal vascular fraction of adipose tissue contains a mixed, multipotent population of cells, and a number of investigations have described the potential applications of adipose-derived stem cells (ADSCs).[4,5,17,18,25] ADRCs are the nonbuoyant cellular fraction containing several types of stem and regenerative cells, including ADSCs, vessel-forming cells such as endothelial and smooth muscle cells and their progenitors, and preadipocytes.[26] ADSCs and ADRCs could be used in regenerative medicine in various conditions.[6-16] According to an in vitro study, ADSCs, similar to BMSCs, can differentiate into various cell types, including adipocytes, chondroblasts, endothelial cells, fibroblasts, myoblasts, and osteoblasts.[4,5,17,25,27] On the basis of both in vitro experiments and preclinical studies, multiple reports have already been published regarding clinical applications of ADSCs and ADRCs, including beneficial results of their use in breast reconstruction.[6,7] There are also reports of their use to treat ischemic cardiomyopathy,[8] calvarial bone defects,[9] enterocutaneous fistulas in patients with Crohn's disease,[10] and chronic ulcers caused by radiotherapy.[11] Recently, various basic and applied studies on the use of ADSCs in the orthopaedic field also have been conducted.[12-16] In addition, ADSCs have immunosuppressive properties that can be used to control graft-versus-host disease.[28]

ADRCs have several advantages as a source of tissue stem cells that led us to focus on them in this study. First, autologous ADRCs can be easily isolated in large amounts from abundant and accessible subcutaneous adipose tissue. Furthermore, harvesting of ADRCs is less invasive than that of BMSCs and other stem cells, and many more stem cells can be harvested at one time.[29] Adipose tissue yields approximately 500-fold more stem cells than the same amount of adult bone

marrow.[4,27] In addition, as mentioned above, ADRCs have already been applied in various clinical fields. For the above reasons, we have considered ADRCs a potentially efficient source for clinical applications in promoting tendon-bone healing.

ADSCs have the ability not only to directly differentiate into some types of topical cells but also to indirectly facilitate the healing process by promoting the secretion of various humoral factors also called paracrine effects.[30,31] In secretory protein analysis, ADSCs secrete significantly larger amounts of growth factors and inflammatory cytokines, such as vascular endothelial growth factor, hepatocyte growth factor, and interleukin 6, than BMSCs.[30] ADRCs contain ADSCs, vessel-forming cells such as endothelial and smooth muscle cells and progenitors, and preadipocytes.[26]

Limitations

There are several limitations to this study. First, we chose to use a rabbit model of ACL reconstruction. This model has been validated in previous reports in the literature.[3,20] However, studies using small-animal models of ACL reconstruction have inherent problems such as a wide range in biomechanical results, which was also seen in the present study.[20] In addition, the healing potential of small animals is different from that of humans. Therefore, the results obtained from this animal model cannot be assumed to be directly applicable to clinical settings. More accurate and reliable results may have been achieved with the use of a larger animal model. Second, we did not conduct a histological analysis of the intra-articular portion of the graft. Although this evaluation was not included as an aim of this study, remodeling of the graft midsubstance is also an important part of graft healing and could affect the results of biomechanical analysis. The failure patterns of the femur-graft-tibia complex shown by biomechanical testing changed from pullout from the bone tunnel to rupture in the midsubstance with time. This shift could be the result of changes in the histological features of the intra-articular graft. Third, the sample size in each group was relatively small, while we studied multiple time points and thus had information on changes over time. Although the blinded nature of this study avoids biases in the assessments, the small

sample size limits the statistical rigor of the findings and efficacy conclusions of the study. Future investigations with a larger sample size at specific time periods would increase the strength of this study for a more accurate evaluation of the effects of ADRCs on tendon-bone healing. Fourth, we did not identify the proper cell concentration in the pellet and its true contents. We calculated the number of cells in the pellet using a microscope, and our result was similar to the concentration assumed from previous studies. Additionally, ADRCs are known to contain ADSCs and several other types of cells and their progenitors, as mentioned above. However, for a stricter analysis, a rigorous analysis of the pellet is necessary using indirect methods, such as flow cytometry and indirect immunofluorescence of the pellet. In addition, we did not trace labeled ADRCs over a longer period, although we confirmed that the cells implanted with the fibrin glue infiltrated into the bone-tendon interface at 2 weeks after ACL reconstruction. We are thus unable to comment on whether the healing tissue at the tendon-bone junction originated from the ADRCs themselves or from cells recruited locally. Analysis of the fate of the ADRCs is the subject of a subsequent study by our group. Fifth, we did not have a group without fibrin glue. Shoemaker et al.[32] had previously examined the effects of fibrin glue on the healing of tendons to bone tunnels in the proximal tibia in dogs. In the first 2 weeks after surgery, fibrin glue appeared to speed up the organization of the fibrovascular interface, but no histological differences were visible between the two groups with or without fibrin glue at 28 days. Although biomechanical evaluation was not performed in that experiment, we believe that using a fibrin glue carrier in both the treatment and control reconstructions did not appreciably influence the outcome of our results. Sixth, the histological findings presented in this study were mainly subjective and preliminary. More objective and precise quantitative methods are needed for more accurate evaluations. Molecular biological data or immunohistological examination may also be valuable for showing the underlying mechanisms by which ADRCs aid ACL reconstruction.

Conclusions

Local administration of ADRCs promoted the early healing process at the tendon-bone junction,

339 both histologically and mechanically, in the rabbit ACL reconstruction model.

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Figure Legends

Fig 1. The healing process between tendon graft and bone tissue after anterior cruciate ligament reconstruction. Representative photomicrographs from the control limbs (upper row: A-E) and the adipose-derived regenerative cell (ADRC)-treated limbs (lower row: F-J) at 2, 4, 6, 8, and 12 weeks after surgery (hematoxylin and eosin, original magnification ×100). The histological maturation at the tendon-bone interface occurred earlier and was more improved in the ADRC-treated tissues than that in the control tissues, especially in the early period after surgery. (B, bone; IF, interface; T, tendon graft.)

Fig 2. Serial sections of an adipose-derived regenerative cell-treated limb showing (A) histology of the tendon-bone interface at 2 weeks after surgery stained with hematoxylin and eosin (original magnification ×40) and (B) fluorescence microscopy for DiI. Dil-labeled cells at the tendon-bone interface were observed under a fluorescent microscope using frozen tissue sections. Red fluorescence indicating Dil-labeled cells was clearly visible on the tendon-bone interface at 2 weeks after surgery (original magnification ×40). (B, bone; IF, interface; T, tendon graft)

Fig 3. Biomechanical properties of the femur-graft-tibia complex after anterior cruciate ligament (ACL) reconstruction. Bar charts showing the ultimate load at the point of failure of the femur-graft-tibia complex at each time frame after ACL reconstruction in the control and adipose-derived regenerative cell (ADRC)-treated groups (*$P < .05$). The ultimate failure load in the ADRC-treated group was significantly greater than that in the control group at 2 and 4 weeks after surgery ($P = .016$ and $P = .016$, respectively).

Fig 4. Bar charts showing the stiffness of the femur-graft-tibia complex at each time frame after anterior cruciate ligament reconstruction in the control and adipose-derived regenerative cell (ADRC)-treated groups (*$P < .05$). The stiffness in the ADRC-treated group was significantly higher

than that in the control group at 6 weeks after surgery (P = .037).

453 **Table 1.** Scoring System for Histological Examination

Histological features	Points
Graft remodeling	
None (0% of graft)	0
Slight (<10% of graft)	1
Fair (<25% of graft)	2
Moderate (<50% of graft)	3
Abundant (≥50% of graft)	4
Interface connection / integration	
None	0
No directivity of collagen fiber	1
Appearance of directivity of collagen fiber	2
Appearance of fibrocartilaginous tissue	3
Appearance of mineralized cartilaginous tissue	4

454 NOTE. The maximum possible score is 8 points.

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456 **Table 2.** Histological Scoring and Comparison between Groups

Time Point	ADRC-treated	Control	*P* value
2 wk	3.8 ± 0.7	2.3 ± 0.4	.005
4 wk	4.7 ± 0.4	3.1 ± 0.6	.005
6 wk	5.8 ± 0.4	3.6 ± 0.8	.005
8 wk	6.5 ± 0.5	5.8 ± 0.8	.093
12 wk	6.9 ± 0.7	6.3 ± 0.5	.149

457 NOTE. Data are given as mean ± standard deviation.

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