Adipose-Derived Stem-Cell Treatment of Skeletal Muscle Injury

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Background: The aim of the present study was to investigate whether adipose-derived stem cells could contribute to skeletal muscle-healing.

Methods: Adipose-derived stem cells of male rats were cultured and injected into the soleus muscles of female rats. Two and four weeks after injections, muscles were tested for tetanic force (50 Hz). Histological analysis was performed to evaluate muscle collagen deposition and the number of centronucleated muscle fibers. In order to track donor cells, chimerism was detected with use of real-time polymerase chain reaction targeting the male sex-determining region Y (SRY) gene.

Results: Two weeks after cell injection, tetanus strength and the number of centronucleated regenerating myofibers, as well as the number of centronucleated regenerating myofibers, were higher in the treated group than they were in the control group (mean and standard error of the mean, 79.2 ± 5.0% versus 58.3 ± 8.1%, respectively [p < 0.05]; and 145 ± 36 versus 273 ± 18 per 10³ myofibers, respectively [p < 0.05]). However, there were no significant differences at four weeks. Treatment did not decrease collagen deposition. Male gene was not detected in female host tissue at two and four weeks after engraftment by polymerase chain reaction analysis.

Conclusions: Adipose-derived stem-cell therapy increased muscle repair and force at two weeks, but not four weeks, after injection, suggesting that adipose-derived stem-cell administration may accelerate muscle repair; however, the rapid disappearance of injected cells suggests a paracrine mechanism of action.

Complete functional recovery of skeletal muscle after severe injury remains a challenge. The capacity of skeletal muscle tissue to regenerate resides in a reserve population of mononucleated precursors, which are termed satellite cells. Satellite cells are located beneath the basal lamina that surrounds each myofiber and act as myogenic precursors for repair with stem cell-like characteristics after muscle injury. Although these cells have regenerative potential, complete skeletal muscle recovery is hindered by the development of scar tissue.

Cell therapy techniques with stem cells isolated from adult tissues are a very attractive strategy for the treatment of muscle disorders. Satellite cells represent one choice because of their intrinsic myogenic potential. Nevertheless, they are poorly expandable in vitro, rapidly undergo senescence, and show a poor survival rate of transplanted cells in vivo. Several researchers have reported that bone-marrow-derived mesenchymal stem cells have the potential to differentiate and fuse with myoblasts in vitro and to contribute to muscle-healing and treatment of muscle disorders.

Like bone marrow, adipose tissue is derived from the embryonic mesoderm and contains a heterogenous stromal cell population such as endothelial cells, smooth muscle cells, pericytes, and mesenchymal stem cells. The plastic adherent cell population isolated by collagenase treatment is called adipose-derived stem cells and shares many of the characteristics of bone-marrow-derived mesenchymal stem cells, such as cell surface protein expression and the ability to undergo differentiation along classical mesenchymal lineages: osteogenic, chondrogenic, and adipogenic, even after cryopreservation. Cui et al.

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showed that adipose-derived stem cells are less immunogenic and immunosuppressive. Adipose-derived stem cells have several advantages over bone marrow in clinical trials, such as easy accessibility, abundance, and higher stem-cell proliferation rates than bone-marrow-derived mesenchymal stem cells.\(^{15-16,20}\) Recently, adipose-derived stem cells were shown to secrete multiple angiogenic and anti-apoptotic cytokines, supporting tissue regeneration and minimizing tissue damage.\(^{21-22}\) These cytokines were also classically described to be crucial for satellite cell proliferation and fusion in vitro and in vivo.\(^{24-25}\) The aim of this study was to investigate whether adipose tissue-derived mesenchymal cells could contribute to skeletal muscle-healing after muscle laceration in the rat model.

Materials and Methods

Animals

All procedures were performed in accordance with the Guide for the Care and Use of Laboratory Animals published by the U.S. National Institutes of Health\(^{26}\) (NIH Publication No. 85-23, revised 1996) and were overseen by a competent institutional board. Syngeneic female Wistar rats (twelve to fifteen weeks old; 180 to 210 g) were obtained from the Laboratório de Cardiologia Celular e Molecular (Instituto de Biofísica Carlos Chagas Filho-Rio de Janeiro/Brasil) and Vital Brazil Institute (Niterói, Rio de Janeiro, Brazil). The animals were
housed at a controlled temperature (23°C) on a 12-hour light-and-dark cycle (lights on at 8:00 am) and had free access to both water and standard rat chow.

**Cell Culture**

Adipose-derived stem cells were isolated according to their density and differential adhesion to culture dishes, as described by Zuk et al. Inguinal subcutaneous white adipose tissue from male syngeneic Wistar rats (twelve to fifteen weeks old; 200 to 250 g) was washed with sterile phosphate-buffered saline solution (PBS) to remove contaminating debris and red blood cells. The washed tissue was minced and the cells were isolated by enzymatic digestion (type-II collagenase; Worthington Biochemical, Lakewood, New Jersey) in PBS for forty-five minutes at 37°C with gentle agitation. Enzymatic activity was neutralized with Dulbecco modified Eagle medium (DMEM; Gibco, North Andover, Massachusetts) supplemented with 20% of fetal bovine serum (FBS), and cells were centrifuged and the supernatant containing mature adipocytes was discarded. Pellets were filtered with 80-μm filters, resuspended in DMEM-high glucose (containing 20% FBS, 2-mM/L of glutamine, 100 U/mL of penicillin, and 100 mg/mL of streptomycin), and then plated at a density of 2 x 10^5 cells in 75-cm^2 cell culture flasks. Following twenty-four hours of incubation, nonadherent cells were washed out, and the cultures were maintained at 37°C in normoxic conditions (95% oxygen and 5% carbon dioxide). The medium was changed twice a week, and the cells were replated when 80% confluence was reached. Cells from the third passage were used in this study. Four male syngeneic rats were used as cell donors for the entire experiment.

**Flow Cytometric Characterization of Adipose-Derived Stem Cells**

The cells were removed from culture flasks with trypsin/EDTA (ethylene diaminetetraacetic acid) 0.25% (Sigma-Aldrich, St. Louis, Missouri). Enzyme activity was neutralized with DMEM with 20% of FBS, and the cells were then centrifuged for ten minutes at 200 g at room temperature. The pellets were washed twice with ice-cold PBS. A total of 5 x 10^6 cells in 100 μL PBS with 0.5% bovine serum albumin (BSA) were stained in the dark with the appropriate antibodies for twenty minutes at 4°C. Monoclonal antibodies used in the study were: CD29-phycoerythrin (PE) 0.015 μg/μL, CD45-PerCP-Cy5 (both from BD Biosciences, San Jose, California) 0.006 μg/μL, anti-CD34-PE (Santa Cruz Biotechnology, Santa Cruz, California) 0.006 μg/μL, CD90-fluorescein isothiocyanate (FITC) 0.003 μg/μL, and CD11b-FITC 0.003 μg/μL (both from Caltag Laboratories Inc., Invitrogen, Cordoba, California). Isotype controls for FITC, PE, and PerCP-Cy5 were purchased from Santa Cruz Biotechnology, Caltag Laboratories, and BD Biosciences, respectively. The cells were washed with 2 mL PBS and resuspended in 200 μL PBS for acquisition images. Samples were acquired with use of a flow cytometer (BD Biosciences) and analyzed with use of BD FACS DIVA software, version 6.1.1 (BD Biosciences).

**Muscle Injury Model and Injection of Adipose-Derived Stem Cells**

The animals were anesthetized with a mixture of ketamine (50 mg/kg) and xylazine (5 mg/kg) by intraperitoneal injection. The soleus muscle was exposed and lacerated with a surgical blade (number 11) at its largest diameter, compromising 50% of its width and 100% of its thickness. After the procedure, the superficial muscle and the skin were sutured.

One week after laceration of the soleus muscle of female rats, a suspension of 30 μL of 10^6 adipose-derived stem cells with Matrigel (BD Biosciences) was injected into the damaged area of the muscle belly (twenty-three rats). For the control group, the muscle was lacerated in the same manner but 30 μL of PBS with Matrigel was injected (fourteen rats). This time point of injection was chosen to avoid the first week of intense inflammatory response in an attempt to improve cell survival.

**Contractile Properties**

Two weeks (six rats from the control group and six rats from the adipose-derived stem-cell group) or four weeks (eight rats from the control group and eight rats from the adipose-derived stem-cell group) after treatment, the animals were killed for functional evaluation as described previously. Soleus muscles were removed, mounted in a double-jacketed tissue organ bath containing 15 mL of Krebs-Ringer solution at 37°C (116.8 mM NaCl; 25 mM NaHCO₃, 5.9 mM KCl; 2.2 mM MgSO₄, 1.25 mM CaCl₂; 5 mM glucose), and constantly agitated by bubbling with a mixture of 95% oxygen and 5% carbon dioxide. Isometric contractions were measured with strain-gauge transducers (model FT03; Grass Instrument, West Warwick, Rhode Island) coupled with a CyberAmp strain-gauge amplifier (Molecular Devices, Sunnyvale, California), decoded by a Digitdata 1440A low-noise data acquisition system (Molecular Devices) and recorded on a computer with use of a data-acquisition program (AttoScope version 11.0 for Microsoft Windows; Molecular Devices). During twenty minutes of equilibration with 0.2-Hz stimulation, electrical field stimuli were applied with use of the Grass S88 stimulator (Grass Instrument) through two silver wire electrodes positioned on the top and bottom of the organ bath and separated by a distance of 2 cm. The muscles were stimulated with 2 ms of square wave pulses under maximum voltage (50 V). First, 1-Hz stimulation was applied, and the muscle twitches were recorded for twenty seconds, followed by the application of one 50-Hz tetanic stimulation until muscle fatigue was reached. Muscle strength was reported as a percent of that of the contralateral, uninjured muscle.

**Muscle Regeneration and Fibrosis**

After functional assessment, soleus muscles were fixed in 4% paraformaldehyde in phosphate buffer and the middle portion of each muscle was mounted in paraffin embedding medium with the fibers oriented perpendicularly to the plane of sectioning in order to permit a cross-sectional view. Muscle samples were sectioned (5 μm) and stained with hematoxylin and eosin and sirius red according to standard protocols.

Muscle regeneration was assessed by counting the number of centrally nucleated myofibers two weeks (five specimens from the control group and three specimens from the stem-cell study group) or four weeks (four specimens from the control group and five specimens from the stem-cell study group) after treatment. At least ten fields from a single cross-section...
section area were analyzed for each muscle in order to analyze all of the muscle cross-sectional area. The average number of regenerating myofibers per 10³ total fibers was compared between groups. Nuclei with no discernible surrounding cytoplasm were excluded.

For collagen analysis, muscle sections stained with sirius red were used. Ten random fields were selected for each sample. Morphometry was performed with use of an imaging system constituted by a digital Q-Color5 camera (Olympus, Tokyo, Japan) coupled to an epifluorescence Axiovert 100 microscope (Carl Zeiss, Thornwood, New York). Ten randomly picked fields of sirius-red sections were captured from each animal with use of an objective lens at X4 magnification. Quantification was estimated by assessing the percentage of stained area in comparison with the total area of fields examined, with use of Image-Pro Plus, version 5.0 (Media Cybernetics, Bethesda, Maryland) image analysis software. A blinded observer performed all analyses.

Chimerism Detection with Use of Real-Time Polymerase Chain Reaction

In order to track adipose-derived stem-cell male donor cells in the host tissue through sex-determining region Y (SRY) gene detection, we treated lacerated female muscle as described above. Soleus muscles were harvested three hours (three specimens), twenty-four hours (three specimens) and seventy-two hours (three specimens), fourteen days (three specimens), and twenty-eight days (three specimens) after treatment. Only the muscle specimens from fourteen and twenty-eight days were submitted to functional assessment, as described above.

DNA was extracted from entire soleus muscle homogenates with use of the proteinase K protocol adapted from Stefani et al. DNA was diluted in Tris-EDTA (TE) (5 mM Tris-HCl, pH 7.4; 0.1 mM EDTA), quantified on a NanoDrop spectrophotometer (ND-1000; Thermo Fisher Scientific, Wilmington, Delaware) and then stored at −20°C.

Polymerase chain reaction (PCR)-specific primers for the rat sex-determining region Y gene (SRY) 5'-CGT GAA GTT GCC TCA ACA AA-3' (forward) and 5'-TCT GGT TCT TGG AGG ACT GG-3' (reverse) were used to identify injected mesenchymal cells. A control primer was designed that detected both the male and female constitutively expressed glyceraldehyde 3-phosphate dehydrogenase (GAPDH) gene (5'-ACATGCGCGCCTGGAAAGAAA-3' (forward) and 5'-AAGGGGACGAAAGGATGAA-3' (reverse). An entire soleus female muscle was mixed with 10⁶ male adipose-derived stem cells to reproduce host tissue injected with adipose-derived stem cells. Five nanograms of DNA extracted from this mixture were used as a positive control for SRY detection. Results of engrafted female muscles are shown as a percentage of the positive control.

DNA was amplified on an ABI 7000 sequence detection system using SYBR Green PCR Master Mix according to the manufacturer’s instructions (Applied Biosystems, Foster City, California) in a final volume of 20 μL. Thermal cycling parameters included ten minutes at 95°C followed by forty cycles of fifteen seconds at 95°C and seventy-five seconds at 55°C.

Statistical Analysis

All data are expressed as the mean plus the standard error of the mean. Two-way analysis of variance was used to compare the effects of treatment and time on muscle force, fiber regeneration, and muscle collagen deposition, followed by a Bonferroni post test. One-way analysis of variance was performance to analyze...
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Results

Adipose-Derived Stem Cells Express Cell Surface Antigens That Reflect Bone-Marrow-Derived Mesenchymal Stem Cells

The adipose-tissue-derived mesenchymal cells were analyzed by flow cytometry to obtain a cell surface marker profile. According to the International Society for Cellular Therapy (ISCT), the criteria for defining mesenchymal stem cells are adherence to plastic, specific surface antigen expression, and multipotent differentiation potential. More than 95% adipose-derived stem cells expressed high levels of CD90 and CD29 and were negative for CD34, CD45, and CD11b (Fig. 1), which is consistent with the well-characterized profile of bone-marrow-derived mesenchymal stem cells. In addition, adipose-derived stem cells differentiate into adipogenic and osteogenic lineages.

Influence of Adipose-Derived Stem Cells on Muscle Contractile Properties

All damaged muscles exhibited impaired function compared with muscle function in the contralateral leg (p < 0.001). Tetanus strength was higher in the cell-treated group compared with the vehicle-treated group (79.2 ± 5.0% versus 58.3 ± 8.1%, respectively; p < 0.05) two weeks after cell injection, although no significant difference was observed in tetanus strength between the two groups four weeks after injection (Fig. 2).

Muscle repair accelerated, whereas fibrosis did not decrease in adipose-derived stem-cell-treated muscle.

We observed a higher number of centronucleated regenerating myofibers in the adipose-derived stem-cell-treated group (273 ± 18 per 10³ myofibers) than in the vehicle-treated group (145 ± 36 per 10³ myofibers) two weeks after treatment (p < 0.05) (Fig. 3). However, no difference was detected at four weeks in the adipose-derived stem-cell-treated group (282 ± 58 per 10³ myofibers) compared with the vehicle-treated group (278 ± 25 per 10³ myofibers).

Sirius-red staining (collagen staining) revealed a large area covered by collagen in all injured muscles as compared with normal uninjured muscles either at two weeks (normal muscle, 7.5 ± 1.2%; vehicle-treated group, 27.4 ± 7.1%; and adipose-derived stem-cell group, 30.3 ± 1.77%) or at four weeks (normal muscle, 8.0 ± 0.9%; vehicle-treated group, 25.4 ± 3.6%; and adipose-derived stem-cell group, 23.1 ± 3.0%) after cell injection (Fig. 4-A). Figures 4-B, 4-C, and 4-D show representative sirius-red-stained samples from the three groups at four weeks.

SRY-positive cell signal gradually decreased over time as shown by real-time PCR.

Glyceraldehyde 3-phosphate dehydrogenase (GAPDH) was amplified in all samples, and negative controls presented no amplification. Primer sets were then tested for sensitivity by performing PCR with use of a mixture of DNA containing decreasing amounts (10 ng to 0.005 ng) of male DNA and 5 ng...
cells in the regeneration of several disease models, including twenty-eight days after adipose-derived stem-cell engraftment. Results represent the mean of three independent biological replicates (B). *p < 0.05; **p <

Fig. 5
Detection of donor cells. Sensitivity of the primer set used for detection of the SRY gene in a DNA mixture containing decreasing amounts of male DNA (1.0 to 0.005 ng) and 5 ng of female DNA (A). PCR was partially inhibited by male DNA concentrations above 5 ng (e.g., 10 ng). Real-time PCR for the detection of the SRY gene as an estimation of male cell chimerism in rat female skeletal muscle at three hours, twenty-four hours, seventy-two hours, fourteen days, and twenty-eight days after adipose-derived stem-cell engraftment. Results represent the mean of three independent biological replicates (B). *p < 0.05; **p < 0.01; and ***p < 0.001 compared with positive control.

of female DNA. The SRY primer set generated PCR products of expected sizes from as low as 5 pg of male DNA or approximately a one-cell genome of murine DNA (Fig. 5-A).

We performed real-time PCR with genomic DNA to detect the presence of the SRY gene from the Y chromosome of male rat cells that were engrafted in female rat tissues. The SRY gene was only detected in the treated groups at early time points after injection (Fig. 5-B). The SRY gene signal was 70.9% ± 25% of the positive control three hours after engraftment (Fig. 5-B). Detection of the SRY gene decreased at twenty-four hours (4.4 ± 0.3%) and seventy-two hours (2.2 ± 0.2%) after engraftment. Furthermore, no signs of SRY-positive cells were found at two or four weeks in either adipose-derived stem-cell treated muscles or vehicle-treated muscles.

Discussion
The present study revealed that adipose-derived stem-cell therapy accelerates skeletal muscle repair in rats by increasing both tetanic force and the number of regenerating myofibers two weeks after engraftment, but not at four weeks. We observed a large amount of scar tissue in lacerated muscles two and four weeks after injury, and adipose-derived stem-cell treatment did not decrease collagen deposition. We did not find the grafted cells in the host tissue analyzed with use of real-time PCR at two or four weeks after treatment. It is reasonable to speculate that a mechanism other than differentiation and fusion of transplanted cells might be involved in transient acceleration of skeletal muscle repair.

The participation of bone-marrow-derived mesenchymal cells in the regeneration of several disease models, including skeletal muscle regeneration, has been studied extensively. Nevertheless, the investigation of the potential of adipose-derived stem cells in skeletal muscle-healing is scarce, although myogenic differentiation from adipose-derived stem cells has been described in vitro by many researchers. Indeed, the culture of cells from adipose tissue in the presence of dexamethasone and hydrocortisone results in a time-dependent pattern of expression of muscle-related genes that is consistent with normal myogenesis. In humans, there should be some benefits of using adipose-derived stem cells, as follows: easily harvested donor abundance, autologous source of mesenchymal stem cells, less immunogenic and immunosuppressive effects, and reduced morbidity and high rate of proliferation, which facilitates ex vivo expansion. Adipose-tissue-derived cells have been used to treat other disorders such as liver failure, cartilage regeneration, spinal cord injury, and cardiovascular disease.

Although the differentiation potential into myocytes has been demonstrated in vitro, little is known about adipose-derived stem-cell transplantation after skeletal muscle injury. Bacou et al. were the first to test the potential of cells from adipose tissue in muscle-healing. They used the nonphysiological cardiotoxin model to induce muscle damage in rabbits, and, three days after injury, they transplanted cells cultured from the stromal vascular fraction (SVF). Bacou and coworkers cultured SVF for three (SVF-1) or seven (SVF-2) days. SVF-1 slightly increased maximum tetanic force and the number of centronucleated fibers. Two weeks after treatment, transplanted cells incorporated to regenerating muscle fibers with a low efficiency and some cells expressed skeletal muscle markers such as merosin, myogenin, and multiple isoforms of myosin heavy chain, suggesting myogenic differentiation. Conversely, in the present study, we isolated SVF and cultured the adherent cells until the third passage. Our cells exhibited undifferentiated mesenchymal cell phenotype since they were positive to CD90 and CD29 and were negative or low positive for CD34, CD45, and CD11b and were able to form adipocyte and osteogenic lineages. In contrast to Bacou's work, long-term culture of SVF in the present study improved...
muscle force and the number of muscle fibers in the process of regeneration and participated in muscle-healing following ischemia, as shown by others. It is difficult to compare our data with those of Bacou et al. because they did not perform any phenotype analysis and because the source of adipose-derived stem cells (i.e., perirenal adipose tissue) was different.

In another study, Di Rocco and colleagues showed that both freshly harvested uncultured SVF and adipose-derived stem cells were able to differentiate into myoblasts when co-cultured with myoblasts. Adipose-derived stem cells, cultured until passage 8 to 12, accounted for 1% of newly formed myoblasts, while only 0.2% of SVF differentiated into myoblasts when co-cultured with muscle cells. However, soluble factors from myoblasts only induced differentiation in SVF cells, suggesting the presence of distinct populations capable of muscle formation. In addition, they injected SVF cells in mice submitted to hind-limb ischemia and dystrophic mice. A wide region of muscle fibers was covered by donor cells (20%) in ischemic muscle and 10% of the dystrophic muscles. Unfortunately, they neither performed any muscle contractile analysis nor tested adipose-derived stem cells in vivo. The present study made use of adipose-derived stem cells injected into a classical laceration model, one week after injury. In contrast to previous results described above, we were not able to find donor cells in the host tissue by PCR analysis, which suggests a possible paracrine effect of adipose-derived stem cells (see discussion below).

The healing process in injured muscle usually comprises three phases: (1) degeneration and inflammation; (2) regeneration and fibrosis; and (3) remodeling. The regenerative capacity of skeletal muscle is due to the presence of a tissue-specific population of myogenic stem cells, termed satellite cells. The satellite cell was first described by Mauro and is so called due to its peripheral location on the skeletal muscle myofiber, where it lies between the sarcolemma of the myofiber cell and its surrounding basal lamina. During inflammation and regeneration phases, there is an intense production of cytokines and growth factors released by inflammatory cells and injured muscle, suggesting a crucial role for these signaling molecules in muscle-healing. Hayashi et al. observed that hepatocyte growth factor (HGF) and insulin-like growth factor-1 (IGF-1) are mainly expressed during the regeneration process. Furthermore, histochemical analysis showed that HGF is expressed mainly during early stages of regeneration, and may, therefore, be available to bind to c-met receptors on satellite cells, leading to their activation. IGF-1 was strongly expressed by macrophages and myoblasts during myotube formation and the differentiation process. IGF-1 is also important as a survival signal for satellite cells during regeneration. Since adipose-derived stem cells secrete a variety of growth factors, such as IGF-1, vascular endothelial growth factor (VEGF), and HGF, and we demonstrated here that the number of regenerating myoblasts augmented after cell treatment, it is possible that paracrine-induced satellite cell activation, proliferation, and differentiation occurred. Also, we observed that injected cells disappeared rapidly from the host tissue; as a result, only 2% of cells were still present seventy-two hours after injection. There were no adipose-derived stem cells two or four weeks after injection. We cannot exclude the possibility that some fusion and/or differentiation of injected cells occurred in our model; however, this phenomenon seems to be rare.

In an attempt to clarify whether adipose-derived stem cells were able to prevent the formation of scar tissue, we quantified the area of fibrosis through histological examination and did not observe any difference between the adipose-derived stem-cell treated group and the vehicle-treated group. Several researchers have accepted that transforming growth factor beta-1 (TGF-β1) is a potent stimulator of collagen deposition and fibrosis formation in various tissues, including skeletal muscle. Huard's group previously reported that antifibrotic therapies such as suramin, decorin, losartan, halofuginone, interferon gamma and relaxin improve muscle regeneration functionally and histologically by directly or indirectly blocking TGF-β1 activity. The event that triggers TGF-β1 induction is the muscle injury, and the triggering effect depends on the severity of the damage. Therefore, it is reasonable to think that adipose-derived stem cells do not modulate muscle fibrosis induced by injury. Additionally, adipose-derived stem cells were injected one week after muscle laceration.

In conclusion, data from our study lead us to suggest that treatment with adipose-derived stem cells may accelerate the process of muscle repair, probably by paracrine mechanisms, since the number of regenerating muscle fibers and muscle developed force only increased two weeks after treatment (but not at four weeks) and adipose-derived stem cells were not present in host tissue at these time points. Furthermore, the therapy seems to be unable to block the fibrotic signaling cascade induced by skeletal muscle injury.
ADIPOSE-DERIVED STEM-CELL TREATMENT OF SKELETAL MUSCLE INJURY

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References


ADIPOSE-DERIVED STEM-CELL TREATMENT

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References


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Anatomical and Functional Results After Arthroscopic Hill-Sachs Remplissage

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Background: Large osseous defects of the posterosuperior aspect of the humeral head can engage the glenoid rim and cause recurrent instability after arthroscopic Bankart repair for glenohumeral dislocation. Filling of the humeral head defect with the posterior aspect of the capsule and the infraspinatus tendon (i.e., Hill-Sachs remplissage) has recently been proposed as an additional arthroscopic procedure. Our hypothesis is that the capsulotenodesis heals in the humeral bone defect without a severe adverse effect on shoulder mobility, allowing return to preinjury sports activity.

Methods: Of 459 patients operated on for recurrent traumatic anterior shoulder instability, forty-seven (10.2%) underwent arthroscopic Bankart repair combined with Hill-Sachs remplissage with use of suture anchors. All had a large Hill-Sachs lesion (Calandra grade III), engaging over the glenoid rim, without substantial glenoid bone loss. Nine patients had had prior unsuccessful surgery to address glenohumeral instability (three Bankart and six Bristow-Latarjet procedures). The average age at the time of surgery (and standard deviation) was 29 ± 5.4 years. Postoperatively, comparative shoulder motion was precisely measured with use of digital photographic images. Capsulotenodesis healing was assessed on a computed tomography (CT) arthrogram (n = 38) or magnetic resonance image (MRI) (n = 4). The mean duration of follow-up was twenty-four months.

Results: Healing of the posterior aspect of the capsule and the infraspinatus tendon into the humeral defect was observed in all forty-two patients who underwent postoperative imaging, and thirty-one (74%) had a remplissage of >75%. Compared with the normal (contralateral) side, the mean deficit in external rotation was 8° ± 7° with the arm at the side of the trunk and 9° ± 7° in abduction at the time of the last follow-up. Of forty-one patients involved in sports, thirty-seven (90%) were able to return postoperatively and twenty-eight (68%) returned to the same level of sports, including those involving overhead activities. Ninety-eight percent (forty-six) of the forty-seven patients had a stable shoulder at the time of the last follow-up.

Conclusions: Arthroscopic Hill-Sachs remplissage, performed in combination with a Bankart repair, is a potential solution for patients with a large engaging humeral head bone defect but no substantial glenoid bone loss. The posterior capsulotenodesis heals predictably in the humeral defect. The slight restriction in external rotation (approximately 10°) does not significantly affect return to sports, including those involving overhead activities. The procedure, which may also be useful for revision of previous failed glenohumeral instability surgery, is not indicated for patients with glenoid bone deficiency.

Level of Evidence: Therapeutic Level IV. See Instructions for Authors for a complete description of levels of evidence.

Posterosuperior humeral head bone defects, commonly known as Hill-Sachs lesions, have been reported to occur in 47% of individuals with a first-time glenohumeral dislocation and in up to 90% of those with recurrent antero-inferior glenohumeral instability. With recurrent episodes of glenohumeral instability, the lesions become larger and deeper, increasing the risk of further instability. The term “engaging Hill-Sachs lesion” has been used by Burkhart and De Beer to describe a compression fracture of the humeral head that is large enough for the edge of the humeral head to drop over the glenoid rim as the arm is abducted and externally rotated. Such large and engaging defects of the posterosuperior aspect

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