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Application of adipose tissue-derived stem cells in a rat rotator cuff repair model

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KEYWORDS

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Stem cell
Adipose tissue
Animal model
Biomechanical

ABSTRACT

Introduction: Healing tissue of the rotator cuff does not regenerate the native enthesis; fibrovascular scar tissue is formed instead and this has less favourable biomechanical properties. The purpose of this study was to determine if the application of adipose tissue-derived stem cells (ASCs) could improve biomechanical and histological properties of the repair.
Material and Methods: Fifty Sprague-Dawley rats underwent detachment and repair of the supraspinatus tendon, 32 for the biomechanical study and 18 for the histological examination. Animals were randomised in two groups to receive either a collagen carrier alone (untreated group) or the carrier plus 2×10^6 ASCs (ASCs group). A control group (suture only) was also included for the histological examination. The animals were sacrificed at 2 and 4 weeks for the biomechanical study and at 24 hours, and 1 and 4 weeks for the histological study. Maximum load failure energy, elastic energy, mechanical deformation, stiffness and absorbed energy were measured. Immunofluorescence testing was conducted to show the presence of ASCs in the repair area.
Results: There were no differences between the untreated group and the ASCs group in any of the biomechanical variables at the 2- and 4-week time points. The mechanical deformation before failure was higher for the ASCs group compared with the untreated group at 2 weeks and 4 weeks ($p=0.09$), as was the absorbed energy ($p=0.06$). Differences in maximum load to failure between 2 and 4 weeks were significant for the untreated group ($p=0.04$) but not for the ASCs group ($p=0.17$). Histological examination showed less acute inflammation with diminished presence of oedema and neutrophils in the ASCs group. There were no differences in the orientation of collagen fibres between groups at either time point. In the ASCs group, collagen was present only at the last time point.
Conclusion: The application of ASCs in a rat rotator cuff repair model did not improve the biomechanical properties of the tendon-to-bone healing. However, the ASCs group showed less inflammation, which may lead to a more elastic repair and less scarred healing.

Introduction

Tears to the rotator cuff are common injuries that often require surgical treatment [1,2]. Operative repair of these tendon tears significantly decreases pain and increases function; however, several studies have demonstrated a high failure rate [3-5]. Whether or not healing of the tear is a prognostic factor for pain and function after rotator cuff repair has been the subject of controversy for the last decade; however, several authors have shown that tear recurrence determines lower functional scores and a decrease in satisfaction [6-8]. The native specialised fibrocartilaginous transition zone between the rotator cuff and the bone does not regenerate after rupture and repair [9,10]. A fibrovascular scar tissue with poorer material properties and diminished mechanical properties has been identified in its place [11]. New materials and surgical techniques to reproduce the anatomical footprint of the rotator cuff have been proposed in an attempt to improve the strength of the surgical repair [12,13]. More recent studies, however, have focused on improving the biological environment around the repair [14-21].

Adipose tissue-derived stem cells (ASCs) are multipotential cells that can differentiate into multiple mesenchymal tissues, such as tenocytes and myocytes [22]. Moreover, they have a paracrine function as they release growth factors and cytokines [23]. Numerous animal studies have shown that ASCs expanded *in vitro* can augment the local repair and regeneration of adipose tissue, muscle, endothelial tissue and bone [22-28]. *In vivo* studies have also shown that ASCs can enhance the healing process in

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Crohn's disease fistulae and chronic ulcers due to radiotherapy [24]. ASCs may enhance tendon-to-bone healing and, hence, the biomechanical properties of the repair.

The purpose of this study was to determine the effects of application of ASCs in an animal model of rotator cuff repair and examine the biomechanical properties of the newly formed tendon-to-bone insertion site.

Material and methods

Study design

This was a controlled laboratory study. Fifty mature Sprague-Dawley rats were used under the guidelines of the Institutional Ethical Committee for Animal Well-being. Thirty-two of the animals were used for biomechanical testing and 18 for histological study. The surgical procedure comprised a detachment and repair of the supraspinatus tendon through an intraosseous suture. The experimental group received 2×10^6 ASCs in a collagen carrier for the tendon-to-bone repair and the second group received only the collagen carrier. A control group with suture only was included for histopathological comparison. In the biomechanical study, the detachment was performed unilaterally and the animals were sacrificed at 2 and 4 weeks. In the histological study, the detachment was performed bilaterally and the animals were sacrificed at 24 hours, 1 week and 4 weeks.

Adipose tissue-derived mesenchymal stem cell harvest and culture

ASCs were obtained from subcutaneous fat tissue of two rats according to a previously described protocol in humans, but with minor modifications [24]. Briefly, the lipoaspirate was washed with phosphate-buffered saline (PBS; Gibco, Invitrogen, Paisley, UK) and digested at 37°C for 30 minutes with collagenase (Type I; Gibco, Invitrogen). Enzymatic activity was neutralised by the addition of 10% foetal bovine serum (FBS; Gibco) and the mixture was centrifuged at 300 g for 10 minutes. The cell pellet was treated with 160 mM ammonium chloride for 10 minutes to lyse any remaining erythrocytes; cells were washed and suspended in Dulbecco's modified Eagle's medium (DMEM) plus 10% FBS. The resulting cell suspension from a further centrifugation cycle was filtered through a 70 μm nylon mesh. Lastly, the material obtained was resuspended in DMEM with glucose and pyruvate, 10% FBS, 2 mM glutamine, 1% streptomycin 10 μm/ml and penicillin 1 UI/ml. This product was named Stromal Vascular Fraction (SVF). The cells were then plated in 100-mm tissue-culture dishes at 10 to 15×10^3 cells/ml and cultured at 37°C in a humid atmosphere with 5% carbon dioxide in DMEM containing 10% FBS and 1% penicillin/streptomycin (Gibco, BRL). The medium was changed to remove non-adherent cells 24 hours after seeding, and every 4 days thereafter. For subculturing, cells were detached with 0.05% (v/v) trypsin in PBS when 70-80% confluence had been reached. Characterisation studies were carried out by flow cytometry; the expression of the surface markers CD90, CD29, CD45 and CD11b was analysed to confirm the ASCs phenotype of the cultured cells. ASCs in two of the specimens were treated with CM-Dil for immunofluorescence cellular tracking.

Surgical technique

Animals were anaesthetised with isoflurane in high flow oxygen. All animals underwent detachment and repair of the tendon. The side the procedure was conducted was randomised in the cases in which the detachment was unilateral. The surgery was performed in the lateral decubitus. A longitudinal incision was made on the anterolateral aspect of the shoulder and the deltoid was split to expose the supraspinatus tendon. Then, an intraosseous tunnel was created in the most proximal part of the humerus. The supraspinatus tendon was sharply detached from its insertion in the greater tuberosity. A modified Masson stitch with a non-absorbable 5/0 suture was placed into the tendon. The posterior end of the suture was then passed through the tunnel and both were tied over the bone bridge in the lateral aspect of the humerus. At this point the animals were randomised to one of the groups (suture only; suture and carrier; or suture, carrier and ASCs; depending on the allocation to the biomechanical or histological study groups). The deltoid was repaired with non-absorbable sutures and skin closure was performed with 3-0 braided silk sutures. Tramadol (5.10 mg/kg) was administered subcutaneously for analgesia at the immediate postoperative period and 24 hours after surgery. Free cage activity was permitted postoperatively.

Histological analysis

At the predetermined time points, euthanasia was performed with intracardiac potassium chloride (KCl) injection under anaesthesia and the complete glenohumeral joint, including deltoid muscle, was obtained. The specimens were fixed in 4% neutral-buffered formalin, decalcified with EDTA for 14-21 days and embedded in paraffin. Sections of 4 μm were cut in the coronal plane. The slides were stained with haematoxylin and eosin, Masson's trichrome, Verhoff-Van Gieson stain, Orcein and Sirius Red stain. All the samples were examined using a polarised light microscope. Samples designated to the immunofluorescence study and marked with cellular tracker CM-Dil were analysed in an inverted microscope Leica DMI 6000. Data were recorded on the presence of oedema, vessels, inflammatory cells, fibroblasts, collagen fibres and fibrocartilage. An independent pathologist with experience in musculoskeletal pathology examined the specimens.

Biomechanical testing

1. Preparation of the sample

There were two groups of 16 specimens. The groups were comparable in weight at the preoperative setting and at each sacrifice time. At the predetermined time points, euthanasia was performed with intracardiac KCl injection under anaesthesia and the specimens obtained. Each shoulder was dissected to isolate the osseous components of the glenohumeral joint and the supraspinatus tendon. The humerus was embedded in polymethylmethacrylate. The tendon attached to the humerus insertion was protected by saline solution at room temperature to avoid thermal necrosis during the exothermic cement-curing process. The specimens were then kept frozen at -80°C and thawed at room temperature for testing.

2. Biomechanical test

The biomechanical study was performed with a linear encoder with a sensor for position and two load cells of 20 N and 200 N, calibrated for detection of small loads of even 1 N (Figure 1). Data were registered with universal software, PCD-2K, designated for Windows 7 and Vista, to obtain a tension-deformation curve. The scapula was secured to the load cell attached to a linear bearing that allowed alignment of the tendon in the direction of its pull. The specimen was preloaded to 0.10 N and then loaded to failure at a rate of 14 μm/sec. The following biomechanical data were recorded: maximum load to failure (N), elastic load (N), deformation (mm), stiffness (N/mm^2) and absorbed energy (J).