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Can chondral healing be improved following microfracture? The effect of adipocyte tissue derived stem cell therapy

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ABSTRACT

Background: We aimed to investigate the effect of adipose tissue-derived mesenchymal stem cells (ADSCs) on chondral healing using the microfracture (MF) technique.
Methods: Thirty male rabbits were randomly divided into three groups. Standard cylindrical osteochondral defects (OCDs) were created in the weight-bearing areas of the medial condyles of all the right knees; the defects were four millimeters in diameter and two millimeters in depth. The control group (group A) was restricted to spontaneous healing. For group B, we performed MF with a 1.5-mm drill. For group C, we applied MF using the same method and then applied 3×10^6 ADSCs to the defect area. At eight weeks post-operation, the subjects were sacrificed, and the distal femoral joint surfaces were evaluated histopathologically for chondral healing. The samples were scored according to the International Cartilage Repair Society (ICRS) scale.
Results: The results for group C were significantly better than those for group A in terms of the surface properties ($p = 0.003$). The matrix evaluation was better for group A than for group C ($p = 0.01$). The cell distribution, cell viability and subchondral bone parameters were similar between the groups ($p = 0.198$, $p = 0.387$ and $p = 0.699$). The cartilage mineralization parameter was better for group C than for group A ($p = 0.001$). The signs of healing were better for group C than for group B, but the differences were not significant ($p = 0.185$).
Conclusions: Improvements with additional ADSC treatments were not statistically significant in cases in which ADSC treatment was compared with isolated MF treatment.
Clinical Relevance: Additional ADSCs treatment may have positive effect on chondral healing but it doesn't seem significant.

1. Introduction

Selection of the ideal treatment for articular cartilage pathologies is a challenging issue for orthopedic surgeons [1–4]. The microfracture (MF) technique is frequently used and is based on pluripotent stem cells (SCs) forming fibrocartilage wound tissue after subchondral bone stimulation [1–4]. This technique has been successfully performed, particularly in cartilage lesions that are small, well contained, and Outerbridge 3 to 4 [4].

Although defects can be treated with MF, the newly formed tissue is poor in hyaline cartilage; it is structurally and biomechanically deficient compared with articular cartilage [2,5]. Treatment success is related to the degree of histological and functional similarities of the repaired tissue to the original tissue. Additional studies are being conducted to improve the quality of newly formed cartilage tissue for filling defects [6–11].

The ideal source of SCs is controversial. With the discovery that adult human adipose tissue is a potential source of SCs, a new source of SCs has become available [9,12–15]. Adipose tissue-derived mesenchymal stem cells (ADSCs) appear to be advantageous for the following reasons: they are easily obtained with the least amount of morbidity to the donor site, their reproducibility in a culture environment is simple, they do not lose their multipotency during earlier culture and passaging, and their chondrogenic potential is maintained [8,15–17]. By contrast, obtaining SCs from bone marrow is invasive, difficult and painful. The number of fibroblast colony forming units (CFUs) is three times greater in adipose tissue than in bone marrow, and adipose tissue is more expendable than bone marrow in the culture environment [18].

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Recently, the use of ADSC therapy for chondral pathologies has increased among knee specialists. Most surgeons who use ADSC therapy believe that this procedure affects the healing process and contributes to cartilage regeneration. In this experimental animal study, we aimed to evaluate the contribution of ADSC application to cartilage repair in the MF procedure.

2. Materials and methods

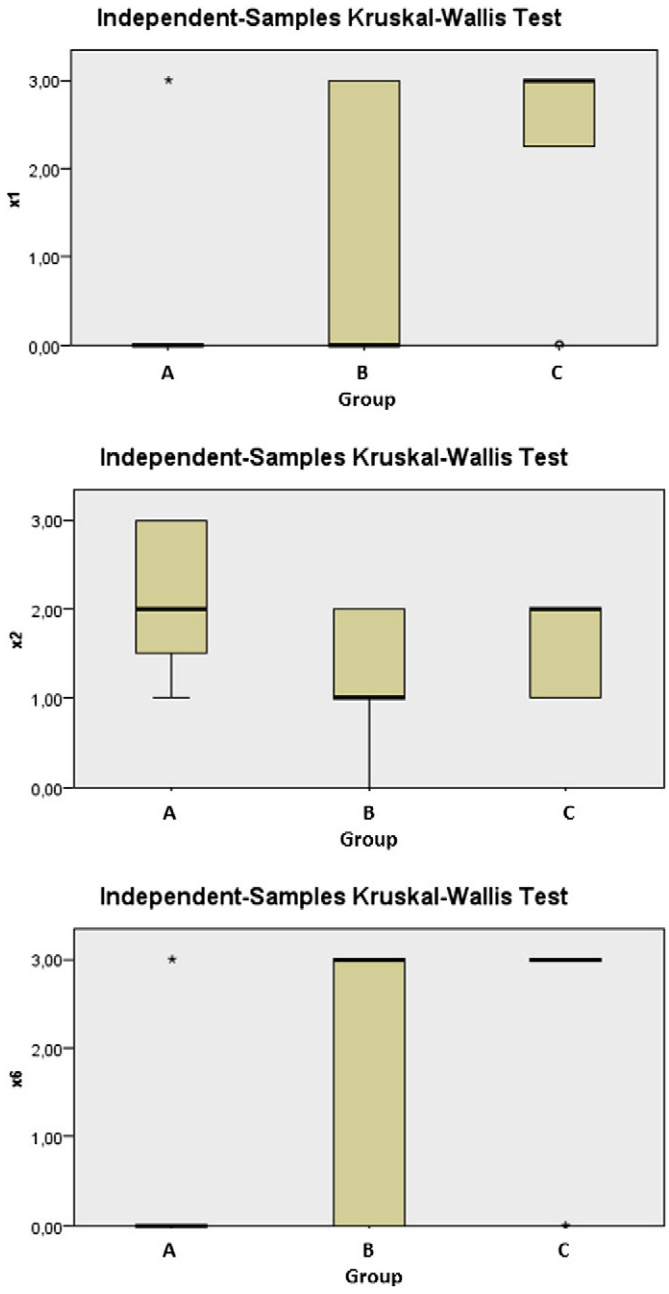
In this study, which was approved by the Institutional Animal Care and Use Committee prior to performing the study, according to the power analysis we used 30 identically bred seven-month-old white male New Zealand rabbits that weighed an average of 3420 ± 230 g (min. 3120 to max. 3580) each and were bred under standard laboratory conditions. Cell culture procedures to acquire the ADSCs were conducted in a cell laboratory using the technique described by Zuk et al. [15].

In addition to the experimental animals, one male rabbit with identical characteristics was used as the source of the adipose tissue. For sedation and analgesia, 15 mg/kg of xylazine (Rompun, Bayer, Leverkusen, Germany) was administered intramuscularly (IM). For anesthesia, 45 mg/kg of ketamine hydrochloride (Ketalar, Zentiva, Kirklareli, Turkey) was administered intramuscular (IM) 10 min later. A two-centimeter bikini incision was made to the inguinal region, from which a 2 × 2-cm subcutaneous adipose tissue sample was obtained.

2.1. Cell culture procedure

Approximately 100 mg of adipose tissue that was acquired from the subject was delivered to the laboratory under sterile conditions and washed with a physiological saline solution (PSS) three times. After the PSS was discarded, the adipose tissue samples were incubated at 37 °C for two hours in three milliliter 0.075% phosphate buffered saline (PBS) solution with type 2 collagenase. After three hours of centrifugation in an 800-g falcon tube, separation of the tissue from the stem cells occurred. The acquired cells were cultured in DMEM-LG in a T-150 flask with one percent antibiotic and 10% fetal bovine serum (FBS). After the cells in the primary culture covered ≥70% of the flask bottom, the cells were passaged. The cells that were adherent to the flask bottom were lifted with trypsin and washed with PBS. Using identical medium and environmental conditions, the cells were re-cultured on a wider surface area. Throughout the passage, the cells were observed once every three days under a microscope; the medium was changed, and the cells in the flask were expected to reach 70% confluence. After the primary culture stage was completed and ≥70% confluence was reached, the cells were lifted with trypsin; after washing with PBS, the cells were placed in identical medium (DMEM-LG containing one percent penicillin and 10% FBS) for further incubation. The cells collected with trypsinization after the first passage were used for the quality verification stage.

The desired outcomes from the quality verification stage were as follows: 1) survival at the end of production of >80% of the number before production with 2×10^4 of at least 2×10^7 cells; 2) current cytometric analysis performed on a sample taken at the end of the second passage showing that the mesenchymal SCs are CD73, CD105 and CD90 positive and CD34, CD45 and HLA-DR negative (purity); 3) maintenance of the sterility of the culture area throughout the study and the absence of mycoplasma; 4) apyrogenicity; 5) fixation of the collagen synthesis of the activity analysis at five micrograms per milliliter per 1×10^7 cells per day; 6) maintenance of RTA (relative telomere enzyme activity) at <1.2; and 7) viability >70% before application (stability). The MSCs were produced and resuspended in 0.1 cm^3 PBS (contains one percent



Graphic 1. The graphic shows the surface morphology (top), matrix composition (middle) and cartilage mineralization (bottom) of each group.

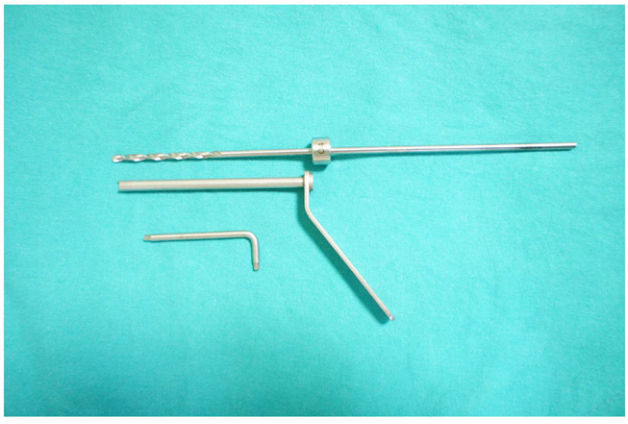


Fig. 1. To obtain the standard defects, we used a four-millimeter-diameter drill that allowed us to create a controlled two-millimeter-depth defect in all of the objectives.