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ORIGINAL ARTICLE

Efficacy of Adipose Tissue and Bone Marrow-Derived Mesenchymal Stem Cells for Treatment of Surgically Induced Knee Osteoarthritis in Rats

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Abstract

OBJECTIVE: To evaluate the efficacy of direct intra-articular injection of adipose tissue stem cells (ADSCs) and bone marrow stem cell (BMSC)-derived mesenchymal stem cells to regenerate articular cartilage in surgically induced knee osteoarthritis (OA) in Sprague–Dawley rats.
DESIGN: Randomized controlled study.
ANIMALS: Thirty Sprague–Dawley rats were divided into five groups (six rats in each group).
PROCEDURES: OA was surgically induced by medial collateral ligament transection and medial meniscal tear of the left knee joints to induce joint destabilization of the left knee. Four weeks after the operation, a single dose of ADSCs, BMSCs, and phosphate-buffered saline was delivered to the operated knee by direct aseptic intra-articular injection in treated groups III, IV, and V, respectively. Healing was assessed clinically, grossly, and microscopically at 10 weeks postoperatively.
RESULTS: Rats that received ADSCs grossly showed better efficiency in regenerating the articular surface and close to the normal knee morphology without any noticed defects and showed better cartilage quality and lower degree of cartilage degeneration histopathologically. There was a trend toward higher scores for all parameters in the treated groups, and demonstrated that chondrocytes and chondroid matrix regained their histological architecture on ADSCs more than BMSCs. ADSCs mimic the normal articular cartilage, BMSCs alleviate the inflammatory process and increase the laydown of the chondroid matrix, and superficial cracks remain to some degree. ADSCs exhibited mild immunohistochemical expression of matrix metalloproteinase 9 in comparison with other treated groups; however, the control group showed no expression at all.
CONCLUSION AND CLINICAL RELEVANCE: ADSCs and BMSCs have potent anti-inflammatory properties, strong regenerative capacity for chondrocytes, and increased chondroid matrix deposition, which is considered an effective therapy for OA. Stem therapy has a desirable effect on OA treatment, which should be translated into protocols for the treatment of OA. ADSCs are more effective and easily available than BMSCs, ADSCs are more efficient in the treatment of surgically induced OA than BMSCs.

Keywords: Adipose tissue stem cells, Bone marrow stem cells, Medial collateral ligament, Osteoarthritis, Rat, Surgical

1. Introduction

Osteoarthritis (OA) is the most commonly diagnosed joint disease in both human and veterinary medicine [1]. OA is a chronic degenerative process characterized by progressive cartilage deterioration, subchondral bone remodeling, loss of joint space, marginal osteophytosis, and loss of joint function. Although the etiology of OA may differ across species or among individuals

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within a species, some components of its pathophysiology are consistent [2,3]. OA is not only associated with focal damage of the articular cartilage but also with other intra-articular structures. It is characterized by inflammation, catabolic joint metabolism, and biochemical changes that affect the results of chondrocyte-based treatments [4].

Mesenchymal stem cells (MSCs) have been proposed instead of mature chondrocytes for OA treatment because of their paracrine, anti-inflammatory, and immunomodulatory properties, in addition to their ability to differentiate into cells of chondrocyte lineage within the lesion site [5]. Different cell sources exist, but currently, the most investigated cells for cartilage regeneration and OA treatment are bone marrow stem cell (BMSCs) and adipose tissue stem cells (ADSCs) [6].

ADSCs have numerous advantages, including a high number, easy availability, and the ability to differentiate toward osteoblasts and chondrocytes [7]. Intra-articular injection of ADSCs reduced synovitis, osteophyte formation, and cartilage degeneration [8], and improved cartilage degradation and reduced knee synovitis in an OA rabbit model [9].

BMSCs have multipotent differentiation potential (can differentiate into numerous tissues, such as bone, cartilage, and fat), self-renewal capacity, and immunomodulatory properties and have great potential for use in stem cell-based articular cartilage diseases [10]. BMSCs have also shown desirable effects in the treatment of OA, probably via the secretion of bioactive trophic factors to exert potent anti-inflammatory, immunomodulatory, and antifibrotic effects [11].

Pain and disability are the primary symptoms for patients who suffer from OA. The relationship between a specific molecular, cellular, or pathological event and OA pain requires therapeutic or prophylactic modification of that factor, and the severity of the pain defining the key changes that cause OA pain needs to be investigated in preclinical models where such factors can be therapeutically targeted [12].

This study aimed to evaluate the effectiveness of MSCs derived from ADSCs and BMSCs in the regeneration of surgically induced osteoarthritic knees in Sprague−Dawley (SD) rats.

2. Materials and methods

2.1. Animals, housing, and feeding

This study was carried out on a total number of 30 male SD rats (body weight 200−250 g). Rats were housed at a temperature of 20−25 °C in the animal house of the Medical Experimental Research Center (MERC), Faculty of Medicine, Mansoura University. Animals were maintained under constant conditions and supplied with a standard diet and water ad libitum. The experimental protocol of this study was approved by the Local Ethical Committee, Faculty of Medicine, Mansoura University, in accordance with the Ethics Committee of the National Research Center, Egypt (registration number 09/189).

2.2. Study design

All rats were divided into five groups (six rats in each group): group I: (negative control) normal rats without surgical induction of OA; group II: (positive control) rats with surgical induction of OA; group III: osteoarthritic rats treated with BMSCs; group IV: osteoarthritic rats treated with ADSCs; group V: osteoarthritic rats treated with phosphate-buffered saline (PBS). A single dose of a million cells derived from bone marrow and adipose tissue suspended in 100 μl of PBS was delivered to the operated knee by direct aseptic intra-articular injection 4 weeks postoperative [13].

All rats were anesthetized by intraperitoneal injection of a mixture of xylazine hydrochloride at a dose of 10 mg/kg [Xylajet (20 mg/ml); XYLA-JECT, ADWIA Pharmaceuticals Egyptian Company, 5th Settlement New Cairo, Egypt and ketamine HCl at a dose of 75 mg/kg [Ketamax (50 mg/ml); Troikaa Pharmaceuticals Ltd, Gujarat, India), according to Flecknell *et al.* [14].

Skin over the medial aspect of the left knee joint was aseptically prepared. Rats were fixed in dorsal recumbency on a wax plate. A 2−3-cm medial parapatellar incision was made. Blunt dissection was performed to expose the medial collateral ligament, which was then transected to expose the medial meniscus. The medial meniscus was cut across its full thickness to induce destabilization of the left knee. Following transection of the meniscus, the joint capsule and s/c tissues were sutured in a simple continuous pattern using 4/0 Vicryl sutures. The skin was opposed by simple interrupted stitches using 4/0 monofilament Nylon according to Janusz *et al.* [15]. After surgery, the rats were kept in a recovery room for about 2 h under heat lamps to maintain body temperature and to monitor any adverse effects. Each rat was administered amoxicillin (E-MOX; EIPICO, Egypt) at a dose (20 mg/kg intramuscular), and analgesic meloxicam (MOBITIL; MUP, Egypt) at a dose (5 mg/kg subcutaneously) once a day for the first 3 days, and once animals regained complete consciousness, they

were permitted free cage activity until the end of the study.

2.3. Bone marrow stem cell isolation and culture

Rat BMSCs were isolated by flushing morselized femurs and tibia from 4-week-old male, SD rats. The harvested cells were cultured in BMSC culture medium, consisting of low-glucose Dulbecco's modified Eagle's medium containing 10 % fetal bovine serum (Lonza, Verviers, Belgium), 50 μg/ml gentamicin, and 1.5 μg/ml fungizone. All media were renewed twice a week. These primary cells were referred to as passage 0 (P0). Confluent cells were dissociated with 0.25 % trypsin and 0.01 % EDTA and subcultured in new six-well culture dishes at a density of 5×10^4 cells/well. These procedures were repeated four times, and the cultures were referred to as P1, P2, P3, P4, and P5 according to the method of Song *et al.* [16].

2.4. Adipose tissue stem cell isolation and culture

Aseptically perform the steps below in a biosafety cabinet, while wearing appropriate personal protective equipment. Place adipose tissue in a preweighed sterile petri dish and weigh to get a final tissue weight. Mince the fat into small pieces with a sterile scalpel and blade until the tissue is no longer fibrous. Place the tissue into a 15-ml tube with an equal volume of prewarmed PBS and agitate for 45 s. Allow the mixture to separate into phases for 3–5 min and then remove the infranatant. Continue the process until the infranatant is clear for successive four washes. Add an equal volume of collagenase solution to the adipose solution, clean the outside of the tube with 70 % EtOH, cover the top with parafilm, and place in a 37 °C shaking water bath at 75 rpm for 1.5 min or until the tissue becomes homogeneous. Vortex for 15 s to thoroughly mix cells and then centrifuge at 1200 rpm (300 *g*) for 5 min. Vortex the solution for 10 s to get as many cells out into the media as possible. The pellet was centrifuged again at 1200 rpm (300 *g*) for 5 min, and the supernatant consisting of lipids, primary adipocytes, and collagenase solution was carefully removed, leaving the pelleted stromal vascular fraction. The pellet was resuspended in 1 % BSA solution. The solution was transferred to a new 15-ml centrifuge tube, centrifuged at 1200 rpm (300 *g*) for 5 min, and the supernatant was removed. The pellet was resuspended in 1 ml of stromal medium and a 20-μl aliquot was centrifuged in a microcentrifuge tube at 1200 rpm (300 *g*) for 5 min. The supernatant was removed, and the pellet was resuspended in 20 μl of red cell lysis buffer. The mixture was incubated for 5 min at room temperature. Add 20 μl of Trypan blue and count the cells with a hemocytometer. Plate the cells at the appropriate density in complete stromal media and incubate at 37 °C and 5 % CO_2. Change the media after 24 h to remove nonadherant cells. Media were changed every 3 days according to the method described in Koura *et al.* [17].

2.5. Evaluation of osteoarthritis

2.5.1. Clinical assessment

Subjective assessment of pain was performed by observing changes in general locomotor activity (e.g. guarding a specific area or avoiding weight-bearing on an injured limb) and changes in food and water intake and body weight [18].

Mechanical hyperalgesia was assessed by the Randall–Selitto analgesiometer (Ugo Basil, Varese, Italy). Briefly, rats were maintained in a normal/horizontal position. The left hind paw was placed in an analgesiometer, which was composed of a cone-shaped paw-pressor with a rounded tip that was used to apply a linearly increasing force to test the paw. The withdrawal threshold was defined as the point at which the rat vocalized or struggled vigorously, expressed as the force in grams. The withdrawal threshold (g) was recorded at the first, second, third, and fourth weeks after treatment and compared to that of the control group [19].

All rats in each group were euthanized 10 weeks after surgery by intraperitoneal injection of thiopental sodium at a dose of 120 mg/kg [20]. Assessment of joint stiffness by measurement of the maximum knee extension angle. The left knee of each animal was dissected and the articular cartilage was left intact. After dissection, the maximum extension angle of each knee was measured with zero degrees representing the maximum possible extension, so the lower the angle value, the better the knee function [21].

2.5.2. Gross evaluation

The femoral condyle and tibial plateau were collected, and surfaces of the cartilage were examined macroscopically and photographed using a digital camera. Cartilage lesions were evaluated by two examiners who were blinded to the treatments [22].

2.5.3. Microscopical evaluation

Preparation of knee specimens for microscopic examination at 10 weeks postoperatively. The specimens were fixed in 10 % buffered formalin

and decalcified with EDTA for 8–10 weeks till they became soft. Fixed samples were processed using paraffin embedding, sectioned, and stained with hematoxylin and eosin, Masson's trichrome, and Safranin O. Cartilage changes were graded according to histopathological Mankin scores [23].

2.5.4. Immunohistochemical analysis

Paraffin-embedded sections were rehydrated in graded alcohols, steamed in citrate buffer at pH 6, probed at room temperature for 2 h using matrix metalloproteinase 9 (MMP9) (rabbit polyclonal, 1: 250, Abcam, AB38898, Cambridge, UK), and processed with a polymer-HRP kit (BioGenex) with diaminobenzidine development and Mayer hematoxylin counterstaining according to Qin *et al.* [24].

The immunohistochemical expression of MMP9 for the enzyme MMP displayed marked expression in the articular cartilage in the diseased group in comparison to other groups, and the number of immunopositive cells per 1000 cells.

2.6. Statistical analysis

All data obtained from the experiment are expressed as the mean ± SEM. Statistical analysis of data was carried out using the software SPSS (Statistical Package for the Social Sciences, Chicago, Illinois 60606-6307, USA) program [25] using one-way analysis of variance, followed by post-hoc Tukey's test for significant differences between variables. The results were considered significant only at the level of P value less than or equal to 0.05.

3. Results

3.1. Clinical findings

All rats experienced pain and discomfort in the form of gradually decreased activity, avoidance of weight-bearing on an injured limb, decreased appetite, pale appearance of conjunctiva, and some osteoarthritic rats suffered from weight loss. All these clinical signs gradually diminished.

In mechanical hyperalgesia, the improvement in the decreased withdrawal threshold in the ADSC-treated group was significantly higher than that in all other treated groups, however, this improvement was still significantly lower than that in the control group (Table 1).

Assessment of joint stiffness by measurement of maximum extension angle of the knee, ADSC-treated group showed a significant enhancement of the increased maximum angle of the knee extension found in the diseased group, but was still significantly higher than the control normal group, and less than the PBS-treated group and BMSC-treated group (Table 1).

Table 1. Mean ± SD of assessment of secondary mechanical hyperalgesia using an analgesiometer and assessment of joint stiffness by measurement of maximum extension angle of the knee.

Groups	Withdrawal threshold (g)	Maximum extension angle of the knee
Control group	23.94 ± 0.45^{a}	22.50 ± 2.26^{b}
Diseased group	12.07 ± 3.57^{c}	53.83 ± 3.06^{a}
ADSC-treated group	16.59 ± 1.12^{b}	27.26 ± 2.14^{b}
BMSC-treated group	13.37 ± 0.97^{bc}	39.15 ± 2.26^{a}
PBS group	14.30 ± 1.42^{bc}	51.50 ± 2.26^{a}

ADSC, adipose tissue stem cell; BMSC, bone marrow stem cell; PBS, phosphate-buffered saline.
Means with different superscript letters for each parameter are significantly different at P value less than or equal to 0.05.

3.2. Gross finding

Isolated articular cartilage surfaces from the five groups were grossly observed. There was no gross evidence of any side effects such as infection or tumor formation throughout the observation period. Gross morphological features of the knee joints from the various treatment groups were compared with those of the control group. The osteoarthritic knee joint showed formation of hard, thick, yellowish fibrotic tissue and exhibited severe erosion and fibrillation (granular appearance) (Fig. 1a) over the entire articular surface compared with the gross appearance of the normal group with a smooth, glistering surface with no fibrillation or erosions (Fig. 1b). In addition, macroscopic observations from the femoral condyle in the BMSC group showed intense reddish patches distributed in almost the articular surface resulting from diminishing cartilage thickness, reflecting the color of the red marrow of the sponge bone and grossly evident of less articular cartilage erosion and fibrillation in most regions of the articular knee surface compared with the OA group (Fig. 1c). In the ADSC group, gross observation of the articular surface showed homogeneity of grayish coloration near normal appearance without any noticeable defects, indicating better efficiency in regenerating the articular surface and is closer to the normal knee morphology (Fig. 1d,e). The gross appearance of the PBS group was similar to that of the OA group, and the articular surface showed marked erosion of the trochlear articular surface exposing the underlying vascularized tissue (sponge bone of the distal end of the femur) (Fig. 1f) and partial erosion of the condyloid articular surface with appearance of reddish coloration of sponge bone

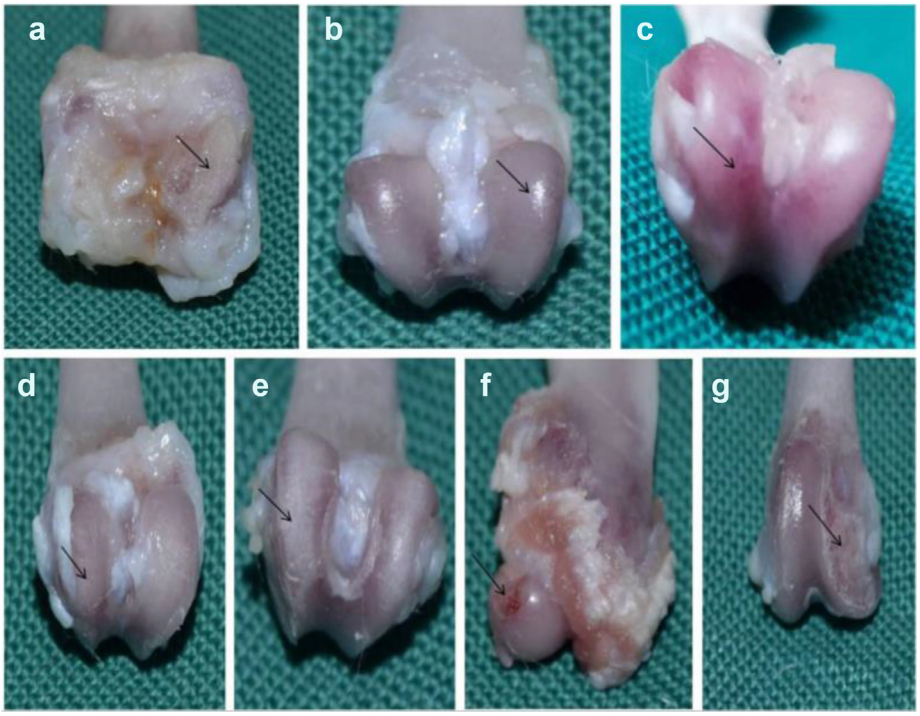

Fig. 1. Gross morphological observations of articular cartilage in the knees of the rat show severe erosion with granular (fibrillation) appearance in joint surface (black arrow; a) in the OA group; smooth and glistering articular surface with no fibrillation or erosions (black arrow; b) in the normal group; congestion and intense red spots distributed in the almost of articular cartilage (black arrow; c) in BMSC group; better efficiency in regenerating of articular surface (black arrow; d, e) in the ADSC group; and marked erosion of cartilage exposing the underlying vascularized tissue (black arrow; f, g) in the PBS group. ADSC, adipose tissue stem cell; BMSC, bone marrow stem cell; OA, osteoarthritis; PBS, phosphate-buffered saline.

(Fig. 1g). In the ADSC group, cartilage regeneration was significantly higher in all treated groups, whereas no significant regeneration was found in the PBS group compared with the other treatment groups.

3.3. Microscopic findings

The histological score for the cartilage structure estimated that the OA group showed significant cartilage destruction toward increasing in the fissures to the deep zone, fissures to the calcified zone, articular degeneration, fibrillation as dentated surface (Fig. 2a, arrow), and articular surface thinning (Fig. 2b, arrow) in comparison to other groups. The OA joint also displayed an articular capsule with exuberant fibrous proliferation protruding from the capsule (Fig. 2c, arrow), hyperplasia in the synovial membrane appearing as multilayer cuboidal epithelium (Fig. 2d, arrow), and congested blood vessels. Meanwhile, the ADSC group showed marked improvement in cartilage structure with only minimal superficial fibrillation, and normal synovial membrane appeared as a monolayer of flattened cuboidal epithelium (Fig. 3b, arrow) with normal underlying stroma nearly similar to the control group. In the BMSC group, the histological view showed that the hyperplastic synovial membrane appeared as a multilayer of cuboidal-to-columnar epithelium (Fig. 3d, arrow), which showed significant improvement in histological score as compared with the OA and PBS groups, but was still less than the control normal (Fig. 2e,f) and ADSC-treated groups (Table 2).

The histological examination of the chondrocytes within the various groups demonstrated that the OA group showed significant chondrocyte pathological alterations, including hypocellularity and destruction of the lacunae and cloning of chondrocytes forming clusters (Fig. 2a, arrow head) in comparison to other groups. In contrast, the ADSC group showed marked significant improvement in chondrocyte histological architecture with normal chondrocytes inside its lacunae and normal basophilic chondroid matrix (Fig. 3a, arrow), which only displayed mild hypercellularity. The histological examination of the chondrocytes in the BMSC group showed focal loss of chondrocytes, and chondroid matrix as fade basophilic devoid of lacunae (Fig. 3c, arrow) showing significant improvement in histological score as compared with the PBS group, which displayed fused lacunae

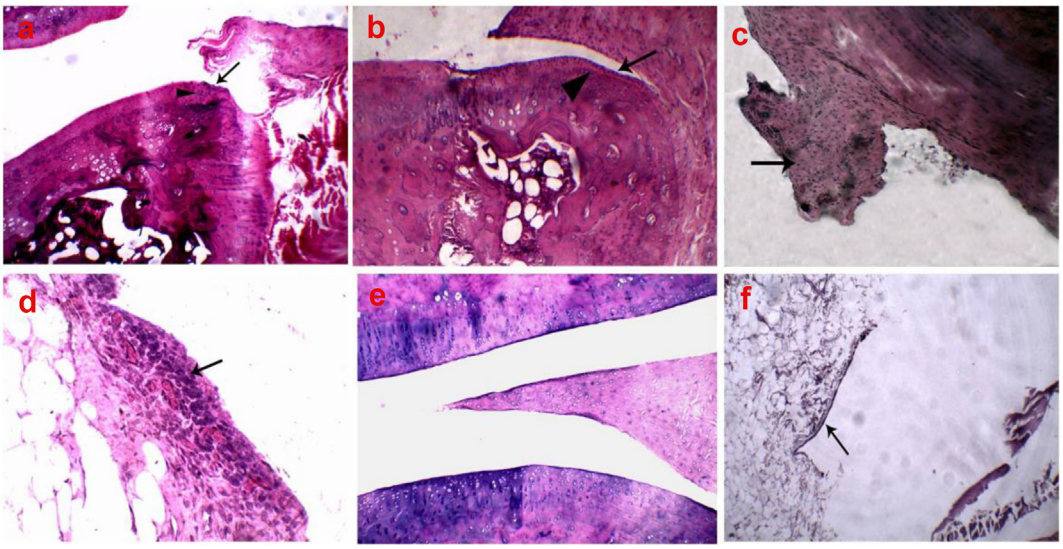

Fig. 2. Histological view of femoral condyles at week 10 after surgery. H&E staining. The OA group at week 10 a, b, c (H&E, ×100), d (H&E, ×400); the control group, e (H&E, ×100), f (H&E, ×100). H&E, hematoxylin and eosin; OA, osteoarthritis.

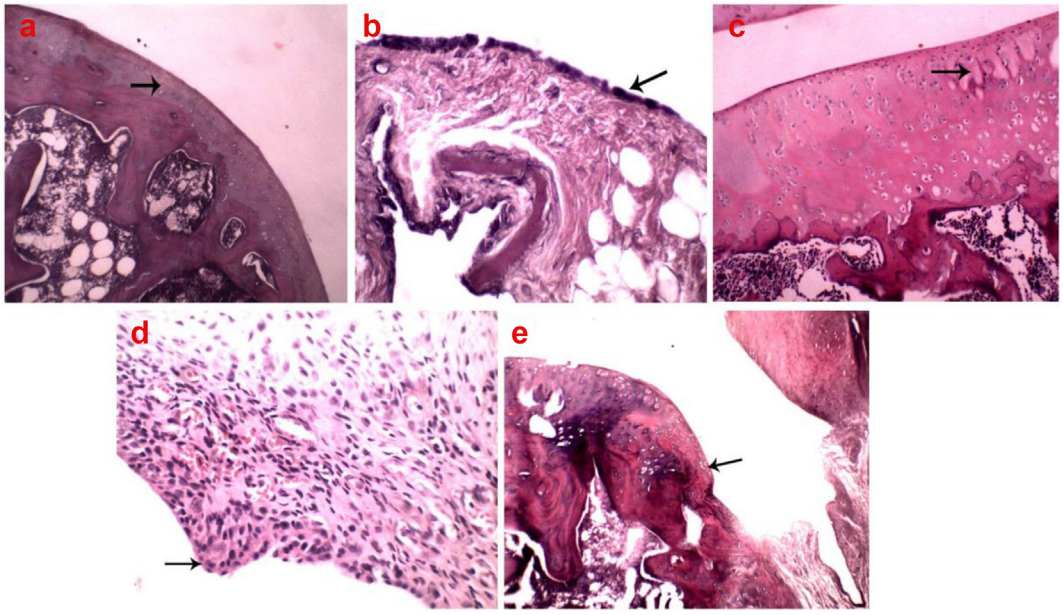

Fig. 3. Histological view of femoral condyles at week 10 after surgery. H&E staining the ADSC group at week 10, a (H&E, ×100), b (H&E, ×400); the BMSC group at week 10, c (H&E, ×100), d (H&E, ×400); the PBS group, e (H&E, ×100). ADSC, adipose tissue stem cell; BMSC, bone marrow stem cell; H&E, hematoxylin and eosin; PBS, phosphate-buffered saline.

Table 2. Median and range of histopathological evaluation of articular cartilage in the knees of the rat using histopathological Mankin score.

Histopathological Mankin score				
Groups	Cartilage structure	Chondrocytes	Safranin-O staining	Tidemark
Control group	0.17^{d} (0.00–1.00)	0.00^{c} (0.00–0.00)	0.00^{c} (0.00–0.00)	0.11^{b} (0.00–0.00)
Diseased group	3.50^{a} (3.00–4.00)	2.50^{a} (2.00–3.00)	3.33^{a} (3.00–4.00)	1.00^{a} (1.00–1.00)
ADSC-treated group	1.33^{c} (1.00–2.00)	1.00^{b} (1.00–1.00)	1.00^{b} (1.00–1.00)	0.00^{b} (0.00–0.00)
BMSC-treated group	2.33^{b} (2.00–3.00)	1.67^{ab} (1.00–3.00)	1.33^{b} (1.00–2.00)	0.00^{b} (0.00–0.00)
PBS-treated group	3.33^{a} (3.00–4.00)	2.33^{a} (2.00–3.00)	3.17^{a} (3.00–4.00)	0.67^{a} (0.00–1.00)

ADSC, adipose tissue stem cell; BMSC, bone marrow stem cell; PBS, phosphate-buffered saline.
The median and range with different superscript letters for each parameter are significantly different at *P* value less than or equal to 0.05.

(Fig. 3e, arrow) and loss of chondroid matrix on the articular surface, which appeared pale eosinophilic (Table 2).

The histological examination of cartilage stained with safranin-O stain revealed that the OA group showed a severe reduction in the staining of chondroid matrix, which appears faint from red safranin staining, indicating marked loss of chondrocytes and loss of proteoglycan in the chondroid matrix (Fig. 4a, arrow) in comparison to other groups; in contrast, the ADSC group showed a slight loss of proteoglycan in the chondroid matrix (Fig. 4b, arrow), while the remaining articular surface appeared normal red-stained against safranin stain in comparison to other treated groups. Histological examination of cartilage in the BMSC-treated group showed articular degeneration represented by a fissured surface and uneven distribution of safranin-O stain with loss of chondrocytes (Fig. 4c, arrow), and the histological score for cartilage stained with safranin-O stain in the BMSC-treated OA group and PBS-treated group (Fig. 4d, arrow), but was still significantly higher than that in the normal group (Fig. 4e, arrow) and no significant improvement in histological score as compared with the ADSC-treated group (Table 2).

The histological findings in the OA group showed a decreased length of the articular surface with tidemark near the articular surface (Fig. 2b, arrow head), it was significantly destroyed and penetrated blood vessels, more significantly in the OA group compared with all other groups (Table 2).

Masson's trichrome staining, which is a specific stain for collagen fibers in the chondroid matrix, revealed loss in the OA group (Fig. 5a) and PBS group (Fig. 5d), while the ADSCs (Fig. 5b) and BMSCs (Fig. 5c) displayed marked restoration. The normal group showed a normal histological appearance (Fig. 5e).

The immunohistochemical expression of MMP9 for the enzyme MMP displayed strong marked expression in the articular cartilage in the OA group (Fig. 6a) compared with other groups. The ADSC-treated group exhibited mild expression (Fig. 6b) in comparison with other treated groups at the time the control normal group showed no expression at all (Fig. 6e), while the BMSC group displayed moderate expression in the articular cartilage (Fig. 6c), which showed a significant increase in expression in the articular cartilage as compared with the control group and ADSC-treated group. However, the PBS-treated group showed a significant increase in expression in the articular cartilage (Fig. 6d) compared with all treated groups, but showed no significant improvement in histological score in expression in the articular cartilage compared with the OA group (Table 3).

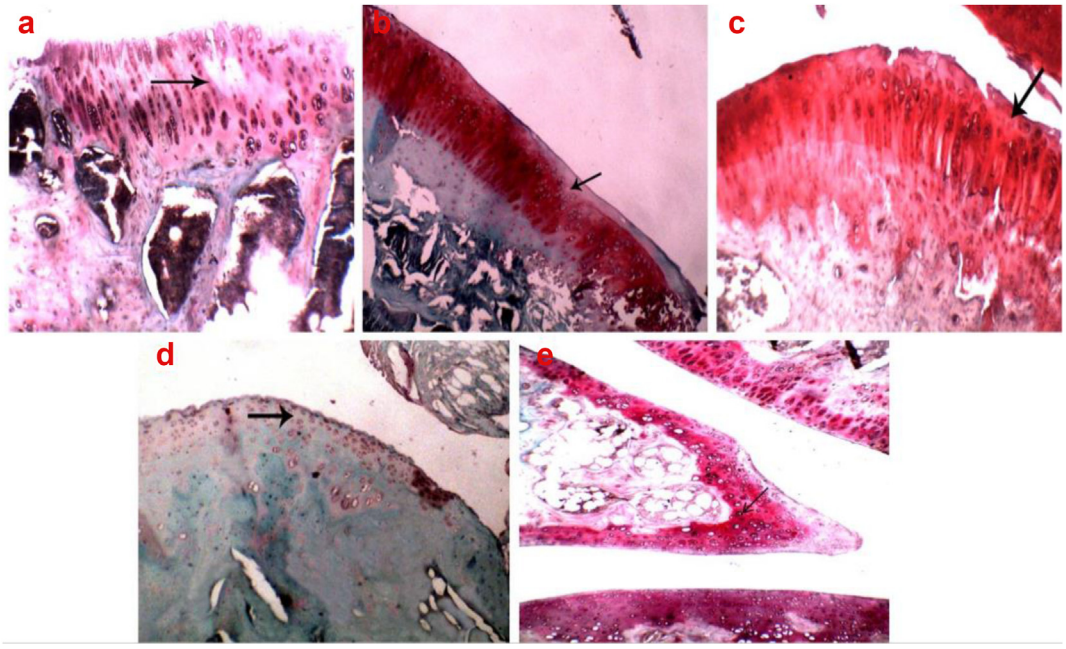

Fig. 4. Histological view of femoral condyles at week 10 after surgery. The OA group at week 10, a (safranin-O stain, ×100); the ADSC group at week 10, b (safranin-O stain, ×100); the BMSC group at week 10, c (safranin-O stain, ×100); the PBS group, d (safranin-O stain, ×100); the control group, e (safranin-O stain, ×100). ADSC, adipose tissue stem cell; BMSC, bone marrow stem cell; OA, osteoarthritis; PBS, phosphate-buffered saline.

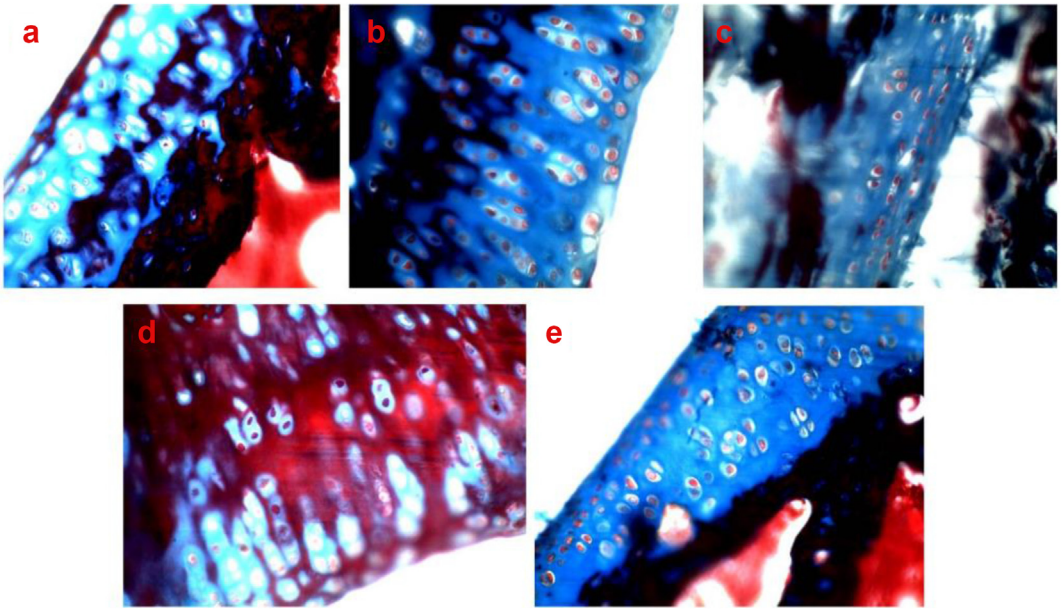

Fig. 5. Histological view of femoral condyles at week 10 after surgery. The OA group at week 10, a (Masson trichrome, ×400); the ADSC group at week 10, b (Masson trichrome, ×400); the BMSC group at week 10, c (Masson trichrome, ×400); the PBS group, d (Masson trichrome, ×400); the control group, e (Masson trichrome, ×400). ADSC, adipose tissue stem cell; BMSC, bone marrow stem cell; OA, osteoarthritis; PBS, phosphate-buffered saline.

4. Discussion

OA is the most common degenerative joint disorder, characterized by progressive articular cartilage degradation, subchondral bone thickening, osteophyte formation, synovial inflammation, and calcification of ligaments [26]. Its treatment remains a great challenge; therefore, in this study, we attempted to find an effective use of ADSCs and

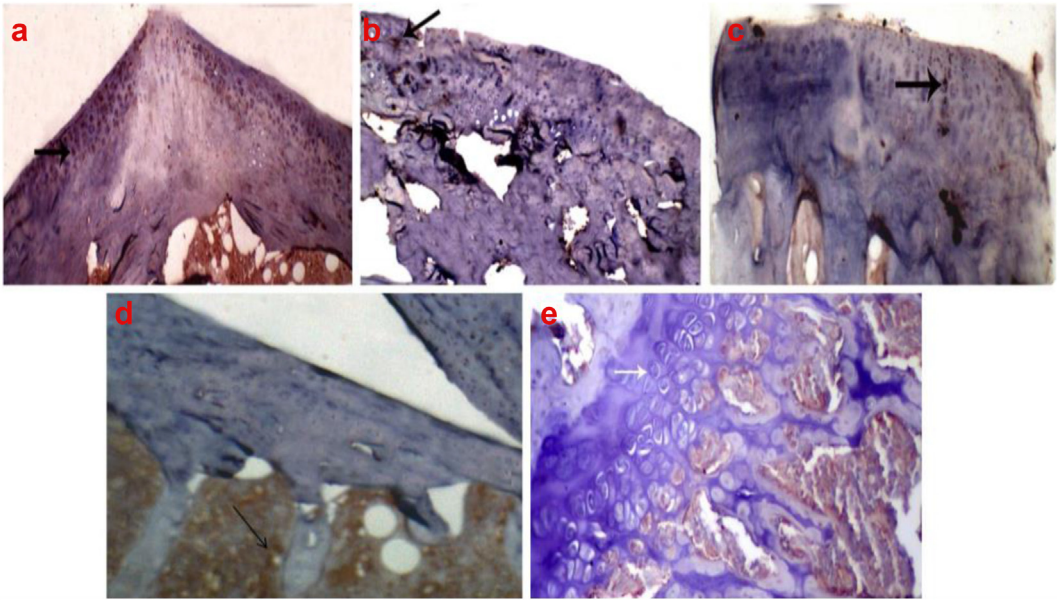

Fig. 6. Histological view of femoral condyles at week 10 after surgery. The OA group at week 10, a (IHC, DAB immunostain, and hematoxylin as counter stain, ×100); the ADSCs group at week 10, b (IHC, DAB immunostain, and hematoxylin as counter stain, ×100); the BMSC group at week 10, c (IHC, DAB immunostain, and hematoxylin as counter stain, ×100); the PBS group, d (IHC, DAB immunostain, and hematoxylin as counter stain, ×100); the control group, e (IHC, DAB immunostain, and hematoxylin as counter stain, ×100). ADSC, adipose tissue stem cell; BMSC, bone marrow stem cell; OA, osteoarthritis; PBS, phosphate-buffered saline.

Table 3. Median and range of the immunohistochemical expression against matrix metalloproteinase 9 for the enzyme matrix metalloproteinase.

Groups	The MMP9 relative expression
Control group	0.00[d] (0.00–0.00)
Diseased group	111.20[a] (105.00–116.00)
ADSC-treated group	23.00[c] (18.00–29.00)
BMSC-treated group	45.00[b] (43.00–47.00)
PBS-treated group	105.60[a] (102.00–112.00)

ADSC, adipose tissue stem cell; BMSC, bone marrow stem cell; MMP9, matrix metalloproteinase 9; PBS, phosphate-buffered saline.
The median and range with different superscript letters for each parameter are significantly different at *P* value less than or equal to 0.05.

BMSCs for treating surgically induced OA that may be promising for future clinical applications.

OA treatment is usually symptomatic and noncurative, with the aim of pain reduction and symptom control [27], using analgesics and NSAIDs [28]. Even surgical interventions, such as total knee or hip arthroplasty, can relieve pain and deformity but also induce postoperative complications [29]. Therefore, the main target of this study is the therapeutic effect of reconstitution of the articular surface and alleviation of inflammation and pain to an optimal state that mimics the normal condition. In this study, the ADSC-treated and BMSC-treated rats showed a significant increase in clinical parameters, while OA-induced rats demonstrated a marked decrease in mechanical hyperanalgesia and increased joint maximum extension. This indicates decreased pain sensation and improved state of the joint to a higher degree. It was noticed that ADSCs induced the best improvement and were time-dependent, where rats after 4 weeks of treatment greatly resembled the others in the normal group, which may be due to their paracrine actions by secreting various soluble and insoluble cytokines and chemokines that alleviate the pain sensation [30,31].

Gross lesions show a remarkable normal cartilage covering in ADSCs and BMSCs and absence of fibrillation or minute erosion, despite the lesion being somewhat more pronounced in BMSCs, as stated by Sato *et al.* [32], who confirmed that mesenchymal cells differentiate into chondrocytes and fill cartilage lesions. Additionally, they found that direct transplantation of human MSCs into the knee joints of Hartley strain guinea pigs with spontaneous OA could differentiate into chondrocytes, which were found to be located in new cartilage.

In addition, Jacer *et al.* [22] and Mei *et al.* [33] agreed with this study in observing the femoral condyle in the ADSC-treated group, finding that it was grossly evident of less articular cartilage erosion and fibrillation in most regions of the articular knee surface compared with the OA group.

Although there are many differing opinions on disease pathogenesis, the gross lesion remains the same. OA has a multifactorial etiology, including joint injury, aging, obesity, and heredity [34]. The present OA-induced group displayed progressive articular erosion and fibrillation with multiple noticed cracks, which are considered identical signs of OA, in agreement with previous results [35,36].

The gold standard for evaluation and comparison between the different groups in this study was histological examination. Improvements in various histopathological parameters in the adipose tissue-treated group in comparison with bone marrow indicate greater efficiency of adipose tissue-derived stem cells. The adipose tissue-treated group demonstrated minimal superficial fibrillation and normal chondrocytes inside the lacuna, which is compatible with the results of Reich *et al.* [37], who investigated the potential role of adipose tissue-derived cells in the alleviation of OA.

In contrast, BMSC-treated group showed enhancement in the histological parameters and restoration of the chondroid matrix, as previously mentioned [38–40].

This study estimated that ADSC-treated group was more efficient in restoring the chondroid matrix. The inflammatory process was markedly attenuated in the adipose tissue group with nearly normal tidemarks and normal vascularization without invasion of the articular surface, which may be attributed to the paracrine anti-inflammatory response initiated by adipose tissue-derived cells [41–43].

The chondrocytes were actively divided and surrounded by normal lacunae and well-organized patterns in ADSCs and BMSCs, rather than necrosis and depletion in the PBS-treated group. This indicates the differentiation ability and extremely regenerative role of MSCs, which possess multilineage differentiation potential and can be used in cell therapy for OA [44,45]. Similarly, Jacer *et al.* [22] agreed with this result and suggested that co-cultures using ADSCs and chondrocytes possessing a pericellular matrix can produce cartilaginous tissue *in vivo*.

In addition, the chondrocytes in ADSCs were apparently more normal than those in BMSCs, which were approved by the most-studied MSCs that were derived from bone marrow and adipose tissues [46]. It has been shown that accessibility, processing procedures, and cell yield of MSCs

obtained from adipose tissues are comparable to those obtained from bone marrow [47].

The cartilage covering the joint surface showed marked variability; therefore, safranin-O staining was used to demonstrate the degree of cartilaginous matrix restoration and/or loss in different groups. The stem cell-treated groups displayed favorable deposition of cartilaginous matrix, in contrast to the OA-treated group, which displayed marked loss of cartilaginous matrix. ADSCs were better than BMSCs, indicating the regenerative properties of stem cells [48–50]. However, in contrast to this study, Hennig *et al.* [51] found that BMSCs have enhanced potential for chondrogenesis compared with ADSCs.

The decreased safranin-O stain and changes between the different groups, along with the appearance of surface fibrillation on the surface of articular cartilage in the OA group, and was minimized in stem cell-treated groups, indicate that there is a direct correlation between stem cells and proteoglycan deposition [33], the stability of the matrix of articular cartilage is maintained by proteoglycans [52,53]. ADSCs have a stronger effect on proteoglycan precipitation.

The tidemark is the destination that separates the articular surface and underlying tissue, mostly affected by injury due to penetrating blood vessels to the avascular surface cartilage, in attempts to increase blood delivery to the surface and provide elements for the inflammatory and reparative process. The tidemarks were nearly normal without penetrating blood vessels and avascular upper cartilage in the ADSC and BMSC groups, while the OA group had proliferating angiogenesis, destroying the tidemark, which implies the ability of stem cells to manage and reduce the levels of inflammatory cytokines released in OA. This matches previous results reported by Jacer *et al.* [22]. The regenerative effects of MSCs are attributed to their paracrine and anti-inflammatory effects [54].

The synovial membrane affected by the inflammatory cytokines liberated in OA appeared to proliferate and hyperplastic in the OA group but decreased in thickness in the ADSC-treated and BMSC-treated groups, indicating that the anti-inflammatory action of stem cells extended from the articular surface to the neighboring structures. In addition, they possess powerful limiting effects on liberated cytokines, counteracting the adverse effects of harmful cytokines, which was approved by Ter Huurne *et al.* [8] and Desando *et al.* [9] who showed that ADSCs, when administered at an early phase of experimental OA, inhibit synovial lining thickening and formation of enthesophytes associated with ligaments, cartilage destruction, and protect against joint destruction by both anabolic and catabolic mediators.

Masson's trichrome is a specific stain for the collagen fiber in the chondroid matrix, which is lost in the diseased group. On the other hand, the ADSC-treated and BMSC-treated groups displayed marked restoration, which matched with previous results. Peng *et al.* [40] compared BMSC-treated group with the other groups using Masson's trichrome staining, it was found that the BMSC-injected group was closer to normal, protected cartilage. It reduces abnormal differentiation, proliferation, and fibrosis in the knee articular cartilage, thus slowing the process of cartilage degradation.

Histological staining was performed to evaluate the degree of fibrosis. The results indicated that ADSCs were the best in all the treated groups with minimal fibrosis, which means that the healing process passes in normal consequence without any accumulation of fibrotic bundles. This is reflected in cartilaginous regeneration in the normal consequences and has an impact on normal movement. This was in agreement with previous studies [52,53], which assessed the effect of ADSC-differentiated chondrocytes versus ADSC transplantation in a rat model of OA.

Immunohistochemical staining for MMP9 was done, in order to assess the degree of chondroid matrix destruction and the ability of stem cell therapy to reverse it. The results were compatible with gross and histological examination, where ADSCs showed minimal expression and BMSCs were the succeeding ones. The highest expression was observed in the OA group; therefore, the MMP9 results confirmed the degree of chondroid matrix destruction and loss of proteoglycans. Shirai *et al.* [55] and Dai *et al.* [56] determined the effectiveness of ADSCs, the expression of MMP, and the ability of ADSCs to lower their expression in OA models.

5. Conclusion

The results of this study demonstrated that ADSCs and BMSCs are capable of downregulating inflammatory factors and alleviating knee OA by their strong regenerative capacity on chondrocytes and increased chondroid matrix deposition. Considering that reconstructive stem cell therapy is becoming increasingly recognized in clinics, particularly in orthopedics, ADSCs are more effective and easily available compared with BMSCs.

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Data availability

Research data supporting this publication are available within this study.

Authors' contribution

A.A., M.S., A.R. and E.M. conceived the idea, contributed to its design and coordination, helped in the acquisition of the data, performed the statistical analysis of the data, and drafted the paper; M.F.H. performed and interpreted the histopathological section; M.A.M. contributed to the analysis and interpretation of the data, and revised the paper critically for important intellectual content. All authors have read and approved the final paper.

Conflict of interest

There are no conflicts of interest.

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