Title: Efficacy of Adipose 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 (IA) injections of adipose derived mesenchymal stem cells (ADSCs) and bone marrow derived mesenchymal stem cells (BMSCs) for regenerating articular cartilage in surgically induced knee osteoarthritis (OA) in Sprague-Dawely rats.

Design: A randomized-controlled study.

Animals: Thirty Sprague-Dawely rats were divided into 5 groups (6 rats in each group).

Procedures: OA was surgically induced by medial collateral ligament (MCL) transection and medial meniscal tear of the left knee joints to induce joint destabilization of the left knee. 4 weeks after the operation, single dose of ADSCs, BMSCs and phosphate buffer saline (PBS) was delivered to the operated knee by direct aseptic IA injection in each treated group (III), (IV) and (V) respectively. The healing was assessed clinically, grossly and microscopically at 10 weeks postoperatively.

Results: Rats that received ADSCs grossly showed better efficiency in regenerating of articular surface and closed to the normal knee morphology without any noticed defects and showed better cartilage quality and lower degree of cartilage degeneration histopathologically and there was a trend towards higher scores for all parameters in treated groups, and demonstrated that chondrocytes and chondroid matrix regained their histological architecture upon ADSCs more than BMSCs. ADSCs is mimicking the normal articular cartilage, the BMSCs alleviated inflammatory process and increased the lay down of chondroid matrix and the superficial cracks remains for some degree. ADSCs exhibited mild Immunohistochemical expression against Matrix Metalloproteinase 9 (MMP9) in comparison to other treated groups, at the time the control group showed no expression at all.

Conclusion and clinical relevance:

The results of the present study demonstrated that ADSCs and BMSCs can alleviate knee OA via their strong regenerative capacity on chondrocytes and increased chondroid matrix deposition. So, for such positive results, ADSCs are more effective and easily available compared to BMSCs.

Key words: OA, MCL, ADSCs, BMSCs, Rat.
1. INTRODUCTION

Osteoarthritis 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, and loss of joint space, marginal osteophytosis, and loss of joint function. Although the etiology of OA may differ across species or among individuals within a species, some components of the pathophysiology of the disease are consistent [2, 3]. OA is a condition not only associated with focal damage of the articular cartilage, but also with other intraarticular structures. It is characterized by inflammation and catabolic joint metabolism and biochemical changes which 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 effects, anti-inflammatory and immunomodulatory properties, beside their capability to differentiate in cells of chondrocyte lineage within the lesion site [5]. Different cell sources are exist but currently the most investigated cells for cartilage regeneration and OA treatment are BMSCs and ADSCs [6].

ADSCs have shown numerous advantages, including the high number, easier availability, and the evidence to be able to differentiate toward osteoblasts and chondrocytes [7]. Intra-articular (IA) injection of ADSCs reduced synovitis, osteophyte formation, cartilage degeneration [8], and improved the cartilage degradation and reduced knee synovitis in OA rabbit model [9].

BMSCs have multipotent differentiation potential (could differentiate into numerous tissues, such as bone, cartilage and fat), self-renewal capacity and immunomodulatory properties, has great potential for use in stem cell-based articular cartilage diseases [10]. BMSCs also have 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. Relationship between a specific molecular, cellular or pathological event and OA pain, requires therapeutic or prophylactic modification of that factor, severity of the pain defining the key changes that cause OA pain needs to be investigated in pre-clinical models where such factors can be therapeutically targeted [12].

This study aimed to evaluate the effectiveness of ADSCs and BMSCs derived MSCs in regeneration of surgically induced osteoarthritic knee in sprague-dawely (SD) rats.
2. MATERIALS AND METHODS

2.1. Animals, housing and feeding

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

2.2. Study design

All rats were divided into 5 groups: group (I) (negative control group, 6 rats). Surgical induction of OA was performed in 25 rats which were devided in to group (II) (positive control), group (III): osteoarthritic rats were treated with BMSCs, group (IV) osteoarthritic rats were treated with ADSCs, group (V) osteoarthritic rats were treated with PBS. Single dose of a million cells derived from bone marrow & adipose tissue and suspended in 100µl of PBS was delivered to the operated knee by direct aseptic IA injection 4 weeks postoperative [13].

All rats were anaesthetized by intra-peritoneal (IP) injection of a mixture XylazineHcl in dose 10 mg/kg (Xylajet (20mg/ml), ADWIA, Egypt) and ketamine Hcl in dose 75mg/kg (Ketamax(50mg/ml), Troikaa Pharmaceuticals Ltd, Gujarat, India) according to [14]. The skin over the medial aspect of the left knee joint was aseptically prepared. The rats were fixed in dorsal recumbency in plate of wax. A 2-3 cm medial parapatellar incision was performed. Blunt dissection was performed to expose the medial collateral ligament (MCL), which was next transected to expose the medial meniscus. The medial meniscus was cut across its full thickness to induce joint destabilization of the left knee. Following transection of the meniscus, the joint capsule and s/c tissues were sutured with simple continuous pattern using 4/0 Vicryl. The skin was opposed by simple interrupted stitches using 4/0 monofilament Nylon according to [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 given amoxicillin (E-MOX, EIPICO, Egypt) in dose (20 mg/kg IM), and analgesic...
Meloxicam (MOBITIL, MUP, Egypt) in dose (5 mg/kg SC) once a day for the first 3 days, and once animals regained complete consciousness, permitted free cage activity until the end of the study.

2.3. BMSCs 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 BMSCs culture medium, consisting of low-glucose Dulbecco’s modified Eagle’s medium (L-DMEM) containing 10% fetal bovine serum (FBS, 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). The confluent cells were dissociated with 0.25% trypsin and 0.01% EDTA, and subcultured in new six-well culture dishes at a plating 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 method of[16].

2.4. ADSCs isolation and culture

The steps of ADSCs preparation were performed under complete aseptic condition 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 tissue is no longer fibrous. Place tissues 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 4 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 para-film, and place in a 37°C shaking water bath at 75 rpm for 1.5 min or until the tissue becomes homogenous. Vortex for 15 s to thoroughly mix cells and then centrifuge at 1,200 rpm (300 × g) for 5 min. Vortex solution for 10 s to get as many cells out into the media as possible. Then centrifuge again at 1,200 rpm (300 × g) for 5 min., carefully, remove the supernatant consisting of lipids, primary adipocytes, and collagenase solution, leaving the pelleted stromal vascular fraction. Resuspend the pellet in 1% BSA solution. Transfer solution to a new 15-mL centrifuge tube, centrifuge cells at 1,200 rpm (300 × g) for 5 min and remove the supernatant. When aspirating, keep the tip of the pipette very superficial so the majority of liquid is removed. Resuspend the pellet in 1 mL of stromal
media. Centrifuge a 20-μL aliquot in a microcentrifuge tube at 1,200 rpm (300 × g) for 5 min. Remove the supernatant and resuspend the pellet in 20 μL of red cell lysis buffer. Incubate 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% CO2. Change the media after 24 h to remove nonadherent cells. Media should be changed about every 3 days according to method of [17].

2.5. Evaluation of OA

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–Sellito analgesiometer (Ugo Basil, Varese, Italy). Briefly, the 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 linear increasing force to test paw. The withdrawal threshold was taken as the point at which the rat vocalized or struggled vigorously, expressed as the force in grams (g). The withdrawal threshold (gram) was recorded at the first, second, third and fourth week respectively after treatment and compared to the control group [19].

All rats in each group were euthanized 10 weeks after surgery with IP injection of thiopental sodium in a dose of 120 mg/kg [20]. Assessment of joint stiffness by measurement of maximum extension angle of the knee. The left knee of each animal was dissected and the articular cartilage was left intact. After the dissection, the maximum extension angle of each knee was measured with zero degree represent as 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 treatments [22].
2.5.3. Microscopical evaluation

The preparation of knee specimen for the microscopic examination at 10 weeks postoperative. The fixation of the specimens was done in 10% buffered formalin, and then decalcified with EDTA for 8-10 weeks till they became soft. Fixed samples were processed by the paraffin embedding technique, sectioned, and stained by hematoxylin and eosin (H&E), Masson’s trichrome and Safranin O stains. Cartilage changes were graded according to histopathological Mankin score in (Table 1)[23].

Table 1. Histopathological Mankin scores

<table>
<thead>
<tr>
<th>Cartilage structuring</th>
<th>Chondrocytes</th>
<th>Safranin-O Staining</th>
<th>Tidemark</th>
</tr>
</thead>
<tbody>
<tr>
<td>Score</td>
<td>Definition</td>
<td>Score</td>
<td>Definition</td>
</tr>
<tr>
<td>0</td>
<td>Normal</td>
<td>0</td>
<td>Normal</td>
</tr>
<tr>
<td>1</td>
<td>Superficial fibrillation</td>
<td>1</td>
<td>Diffuse hypercellularity</td>
</tr>
<tr>
<td>2</td>
<td>Pannus and superficial fibrillation</td>
<td>2</td>
<td>Cell clusters</td>
</tr>
<tr>
<td>3</td>
<td>Fissures to mid zone</td>
<td>3</td>
<td>Hypocellularity</td>
</tr>
<tr>
<td>4</td>
<td>Fissures to deep zone</td>
<td>4</td>
<td>No staining</td>
</tr>
<tr>
<td>5</td>
<td>Fissures to calcified zone</td>
<td>5</td>
<td>Total disorganization</td>
</tr>
</tbody>
</table>

2.5.4. Immunohistochemical Analysis

Paraffin embedded sections were rehydrated in graded alcohols, steamed in citrate buffer at pH 6 and probed at room temperature for 2 hours using the MMP-9 (rabbit polyclonal; 1:250; Abcam [AB38898]) and processed with a polymer-HRP kit (BioGenex) with diaminobenzidine development and Mayer hematoxylin counterstaining according to[24].

The Immunohistochemical expression against mmp9 for the enzyme matrix metalloproteinase to display marked expression in the articular cartilage in the diseased group in comparison to other group and calculate number of immunopositive cells per 1000 cells.
2.5.5. Statistical analysis

All data obtained from the experiment were expressed as means ± SEM. Statistical analysis of data was carried out by software SPSS program [25] using the one-way analysis of variance ANOVA followed by post hoc tukey for testing the significant differences between variables. Results were considered significant only at the level of (p ≤ 0.05).

3. RESULTS

3.1. Clinical findings

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

In mechanical hyperalgesia, the improvement of the decreased withdrawal threshold in ADSCs treated group was significantly higher compared to all other treated groups but this improvement still significantly lower than the control normal group (Table 2).

Assessment of joint stiffness by measurement of maximum extension angle of the knee, ADSCs treated group showed a significant enhancement of the increased maximum angle of the knee extension found in the diseased group, but still significantly higher than the control normal group, less than PBS treated group and BMSCs treated group (Table 2).

3.2. Gross finding

The isolated articular cartilage surfaces from 5 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 various treatment groups were compared with control normal group. Osteoarthritic knee joint showed formation of hard, thick, yellowish fibrotic tissue and exhibited severe erosion and fibrillation (granular appearance)(Fig. 1A) over the whole articular surface compared with the gross appearance of normal group with smooth, glistening surface with no fibrillation or erosions(Fig. 1B). Also, macroscopic observations from femoral condyle in BMSCs group represented intense reddish patches distributed in the almost of articular surface resulted from diminishing in cartilage thickness reflecting the colour of red marrow of 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 ADSCs group, the gross observation of articular surface showing...
homogeneity of grayish coloration near normal appearance without any noticed defects indicating better efficiency in regenerating of articular surface and is closer to the normal knee morphology (Fig. 1D,E). The gross appearance of the PBS group was like OA group, articular surface showing marked erosion of trochlear articular surface exposing underlying vascularized tissue (sponge bone of distal end of femur) (Fig. 1F), and partial erosion of condyloid articular surface with appearance of reddish coloration of sponge bone (Fig. 1G). In the ADSCs group, cartilage regeneration was significantly higher versus all treated groups whereas no significant regeneration was found in PBS group in comparison to other different treatment groups.

3.3. Microscopical findings

The histological score for the cartilage structure estimated that OA group showing significant cartilage destruction towards 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, also OA joint displayed articular capsule with exuberant fibrous proliferation protruding from capsule (Fig. 2C; arrow) and hyperplasia in synovial membrane appeared as multilayers cuboidal epithelium (Fig. 2D; arrow) and congested blood vessels. Meanwhile, the ADSCs group showed marked improvement of cartilage structure with only minimal superficial fibrillation and normal synovial membrane appeared as monolayer of flattened cuboidal epithelium (Fig. 3B; arrow) with normal underling stromanearly similar to control group. while in BMSCs group, the histological view displayed hyperplastic synovial membrane appears as multilayer of cuboidal to columnar epithelium (Fig. 3D; arrow) which showing significant improvement in histological score as compared to the OA group and PBS group but still less than control normal (Fig. 2E,F) and ADSCs treated group (Table 3).

The histological examination for the chondrocytes within the various groups demonstrated that OA group showing 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 the contrary, the ADSCs group showed marked significantly improvement of chondrocytes histological architecture with normal chondrocytes inside its lacunae and normal basophilic chondroid matrix (Fig. 3A; arrow) which only displayed mild hypercellularity. while The histological examination for the chondrocytes in BMSCs group...
displayed focal loss of chondrocytes appears and chondroid matrix as fade basophilic devoid from lacunae (Fig.3C;arrow) showing significant improvement in histological score as compared to PBS group which displayed fused lacunae (Fig.3E;arrow) and loss of chondroid matrix in articular surface which appeared pale esinophilic(Table 3).

The histological examination for cartilage stained with safranin O stain explore that OA group showing Severe reduction in the staining of chondroid matrix which appears faint from red safranin stain at which indicates marked loss of chondrocytes and loss of proteoglycan at chondroid matrix (Fig.4A;arrow) in comparison to other groups, in the contrary, the ADSCs group showed slight loss of proteoglycan at chondroid matrix(Fig.4B;arrow) while the remaining articular surface appears normal red stained against safranin stain in comparison to other treated groups. while The histological examination for cartilage in BMSCs treated group showing articular degeneration represented by fissured surface and uneven distribution of safranin O stain with loss of chondrocytes(Fig. 4C;arrow),The histological score for cartilage stained with safranin O stain in BMSCs treated OA group and PBS treated group (Fig.4D;arrow) but still significantly higher than normal group (Fig.4E;arrow) and no significant improvement in histological score as compared to ADSCs treated group(Table 3).

The histological findings in OA group showing decrease length of articular surface with tidemark near to articular surface (Fig.2B;arrow head) it was significantly destructed and penetrated blood vessels,more significantly in OA group in the comparison to all other groups(Table 3).

Using masson trichrome stain which is specific stain for the collagen fiber in the chondroid matrix revealed loss in OA group(Fig.5A) and PBS group (Fig.5D), meanwhile in the ADSCs (Fig.5B) and BMSCs(Fig.5C)displayed marked restoration.The normal group was normal histological appearance (Fig.5E).

The Immunohistochemical expression against MMP9 for the enzyme matrix metalloproteinase displayed strong marked expression in the articular cartilage in the OA group (Fig.6A) in comparison to other groups. ADSCs treated group exhibited mild expression of it (Fig.6B) in comparison to other treated groups at the time the control normal group showed no expression at all (Fig.6E) while in the BMSCs group displayed moderate expression in the articular cartilage (Fig.6C) that showed significantly increase in expression in the articular cartilage as compared to the control group and ADSCs treated group .But in the PBS treated group showed significantly
increase in expression in the articular cartilage (Fig. 6D) as compared to all treated groups but showed no significant improvement in histological score in expression in the articular cartilage as compared to OA group (Table 4).

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 normal group; congestion and intense red spots distributed in the almost of articular cartilage (black arrow; C) in BMSCs group; better efficiency in regenerating of articular surface (black arrow; D, E) in ADSCs group; and marked erosion of cartilage exposing underling vascularized tissue (black arrow; F, G) in PBS group.
Fig. 2. Histological view of femoral condyles at week 10 after surgery. H&E staining. The OA group at week 10 A, B, C (HE, 100x), D (HE, 400x); The control group E (HE, 100x), F (HE, 400x).

Fig. 3. Histological view of femoral condyles at week 10 after surgery. H&E staining. The ADSCs group at week 10 A (HE, 100x), B (HE, 400x); The BMSCs group at week 10 C (HE, 100x), D (HE, 400x); The PBS group E (HE, 100x).
Fig. 4. Histological view of femoral condyles at week 10 after surgery. The OA group at week 10 A (safranin O stain, 100x); the ADSCs group at week 10 B (safranin O stain, 100x); The BMSCs group at week 10 C (safranin O stain, 100x); The PBS group D (safranin O stain, 100x); The control group E (safranin O stain, 100x).

Fig. 5. Histological view of femoral condyles at week 10 after surgery. The OA group at week 10 A (Masson trichrome, 400x); the ADSCs group at week 10 B (Masson trichrome, 400x); The BMSCs group at week 10 C (Masson trichrome, 400x); The PBS group D (Masson trichrome, 400x); the control group E (Masson trichrome, 400x).
**Fig. 6.** Histological view of femoral condyles at week 10 after surgery. The OA group at week 10 A (IHC, DAB immunostain, hematoxylline as counter stain, 100x); the ADSCs group at week 10 B (IHC, DAB immunostain, hematoxylline as counter stain, 100x); The BMSCs group at week 10 C (IHC, DAB immunostain, hematoxylline as counter stain, 100x); The PBS group D (IHC, DAB immunostain, hematoxylline as counter stain, 100x); The control group E (IHC, DAB immunostain, hematoxylline as counter stain, 100x).

**Table 2.** 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.

<table>
<thead>
<tr>
<th>Groups</th>
<th>Withdrawal threshold (g)</th>
<th>Maximum extension angle of the knee</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control group</td>
<td>23.94±0.45&lt;sup&gt;a&lt;/sup&gt;</td>
<td>22.50±2.26&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td>Diseased group</td>
<td>12.07±3.57&lt;sup&gt;c&lt;/sup&gt;</td>
<td>53.83±3.06&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>ADSCs treated group</td>
<td>16.59±1.12&lt;sup&gt;b&lt;/sup&gt;</td>
<td>27.26±2.14&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td>BMSCs treated group</td>
<td>13.37±0.97&lt;sup&gt;bc&lt;/sup&gt;</td>
<td>39.15±2.26&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>PBS group</td>
<td>14.30±1.42&lt;sup&gt;bc&lt;/sup&gt;</td>
<td>51.50±2.26&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
</tbody>
</table>

Means with different superscript litters for each parameter are significantly different at P≤0.05.
Table 3. Median and range of histopathological evaluation of articular cartilage in the knees of the rat using Histopathological Mankin score.

<table>
<thead>
<tr>
<th>Groups</th>
<th>Cartilage structure</th>
<th>Chondrocytes</th>
<th>Safranin-O Staining</th>
<th>Tidemark</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control group</td>
<td>0.17&lt;sup&gt;d&lt;/sup&gt;(0.00-1.00)</td>
<td>0.00&lt;sup&gt;c&lt;/sup&gt;(0.00-0.00)</td>
<td>0.00&lt;sup&gt;c&lt;/sup&gt;(0.00-0.00)</td>
<td>0.11&lt;sup&gt;b&lt;/sup&gt;(0.00-0.00)</td>
</tr>
<tr>
<td>Diseased group</td>
<td>3.50&lt;sup&gt;a&lt;/sup&gt;(3.00-4.00)</td>
<td>2.50&lt;sup&gt;a&lt;/sup&gt;(2.00-3.00)</td>
<td>3.33&lt;sup&gt;a&lt;/sup&gt;(3.00-4.00)</td>
<td>1.00&lt;sup&gt;a&lt;/sup&gt;(1.00-1.00)</td>
</tr>
<tr>
<td>ADSCs treated group</td>
<td>1.33&lt;sup&gt;c&lt;/sup&gt;(1.00-2.00)</td>
<td>1.00&lt;sup&gt;b&lt;/sup&gt;(1.00-1.00)</td>
<td>1.00&lt;sup&gt;b&lt;/sup&gt;(1.00-1.00)</td>
<td>0.00&lt;sup&gt;b&lt;/sup&gt;(0.00-0.00)</td>
</tr>
<tr>
<td>BMSCs treated group</td>
<td>2.33&lt;sup&gt;b&lt;/sup&gt;(2.00-3.00)</td>
<td>1.67&lt;sup&gt;ab&lt;/sup&gt;(1.00-3.00)</td>
<td>1.33&lt;sup&gt;b&lt;/sup&gt;(1.00-2.00)</td>
<td>0.00&lt;sup&gt;b&lt;/sup&gt;(0.00-0.00)</td>
</tr>
<tr>
<td>PBS treated group</td>
<td>3.33&lt;sup&gt;a&lt;/sup&gt;(3.00-4.00)</td>
<td>2.33&lt;sup&gt;b&lt;/sup&gt;(2.00-3.00)</td>
<td>3.17&lt;sup&gt;a&lt;/sup&gt;(3.00-4.00)</td>
<td>0.67&lt;sup&gt;a&lt;/sup&gt;(0.00-1.00)</td>
</tr>
</tbody>
</table>

Median and range with different superscript letters for each parameter are significantly different at P≤0.05.

Table 4. Median and range of the Immunohistochemical expression against MMP9 for the enzyme matrix metalloproteinase.

<table>
<thead>
<tr>
<th>Groups</th>
<th>The MMP9 relative expression</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control group</td>
<td>0.00&lt;sup&gt;d&lt;/sup&gt;(0.00-0.00)</td>
</tr>
<tr>
<td>Diseased group</td>
<td>111.20&lt;sup&gt;a&lt;/sup&gt;(105.00-116.00)</td>
</tr>
<tr>
<td>ADSCs treated group</td>
<td>23.00&lt;sup&gt;c&lt;/sup&gt;(18.00-29.00)</td>
</tr>
<tr>
<td>BMSCs treated group</td>
<td>45.00&lt;sup&gt;b&lt;/sup&gt;(43.00-47.00)</td>
</tr>
<tr>
<td>PBS treated group</td>
<td>105.60&lt;sup&gt;a&lt;/sup&gt;(102.00-112.00)</td>
</tr>
</tbody>
</table>

Median and range with different superscript letters for each parameter are significantly different at P≤0.05.
4. DISCUSSION

Osteoarthritis 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 is still a great challenge so in this study we have tried to find an effective using of ADSCs and BMSCs for treatment surgically induced OA that may be a promising in future clinical application.

It is known that OA treatment is usually symptomatic and non-curative, only aiming for pain reduction and symptoms control [27], like analgesics and non-steroid anti-inflammatory drugs [28]. Even surgical interventions, such as total knee or hip arthroplasty, could relieve pain and deformity, but also induce postoperative complications [29]. So the main target of this study, is therapeutic effect targeting reconstitution of articular surface and alleviating inflammation and pain to optimal state which mimic the normal condition. In the present study, the ADSCs and BMSCs treated rats showed a significant increase in clinical parameter, while OA-induced rat demonstrated marked decrease in mechanical hyper-analgesia and increased joint maximum extension. This indicates decreased pain sensation and improves the state of the joint to higher degree. It was noticed that ADSCs induced the best improvement and was time dependent, where rats after four weeks of treatment were greatly resembling the others in the normal group, this may be due to its paracrine actions by secreting various soluble and insoluble cytokines and chemokines that alleviates the pain sensation [30, 31].

Gross lesions show a remarkable normal cartilage covering in ADSCs and BMSCs and absence of fibrillation or minutes erosion, in spite of the lesion were somewhat more pronounced in BMSCs, which stated by [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 and were found to be located in new cartilage. Also [33,22] agreed with this study in observing femoral condyle in ADSCs treated group founding 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 a lot of differing opinions on the disease pathogenesis but the gross lesion is still 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 cracking, which is considered identical signs of OA, matching with the previous results [35, 36].

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

On the contrary, BMSCs treated group showed enhancement in the histological parameters and restoration of the chondroid matrix as previously mentioned [38-40].

While this study results estimated that ADSCs treated group was more efficient in the restoration of the chondroid matrix. The inflammatory process was pronounced attenuated in adipose tissue group with nearly normal tidemark and normal vascularization without invasion of articular surface comparable with other group which may be attributed to the paracrine anti-inflammatory response that was initiated by adipose tissue derived cells [41-43].

The chondrocytes were actively divided and surrounded by normal lacuna and well-organized pattern in ADSCs and BMSCs rather than necrosis and depletion in PBS treated group. This indicates the ability of differentiation and extremely regenerative role of MSCs which possess multilineage differentiation potential and have ability in cell therapy for OA [44, 45]. Similarly [22] agreed with this result and suggested that co cultures using ADSCs and chondrocyte possessing peri-cellular matrix (PCM) are able to produce cartilaginous tissue in-vivo.

Also, the chondrocytes in ADSCs was apparently more normal than BMSCs, which was approved by the most studied MSCs which were derived from bone marrows 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 provided by the bone marrow [47].

The cartilage covering the joint surface showed marked variability so safranin O stain was used to demonstrate the degree of cartilaginous matrix restoration and/or loss in different groups. The stem cells treated groups displayed favorable deposition of cartilaginous matrix, on the contrary of OA treated group, which displayed marked loss of cartilaginous matrix. ADSCs were more better than
BMSCs, implying the regenerative properties in cartilage by stem cells [48-50]. But in contrast to this study, [51] found that BMSCs have an enhanced potential for chondrogenesis compared to 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 OA group and was minimized in stem cells treated groups indicates that there is a direct correlation between stem cells and proteoglycans deposition [33]. The stability of matrix of articular cartilage is maintained by proteoglycans [52, 53]. ADSCs has stronger effect on the proteoglycans precipitation.

The tide mark, is the destination that separates between articular surface and underlying tissue, mostly affected by injury due to penetrating blood vessels to avascular surface cartilage, in attempts to increases blood delivery to the surface and provides the elements for the inflammatory and reparative process. The tide marks were nearly normal without penetrating blood vessels and avascular upper cartilage in ADSCs and BMSCs groups, meanwhile the OA group was with proliferating angiogenesis destructing the tide mark that implies the ability of stem cells to manage and reduce the levels of inflammatory cytokines liberated in OA. This matched the previous results that reported by [22]. The regenerative effects of MSCs are attributed to its paracrine mechanisms with anti-inflammatory effects [54].

The synovial membrane affected by the inflammatory cytokines liberated in OA appeared proliferated and hyperplastic in OA group but decreased in thickness in ADSCs and BMSCs treated groups. This indicates that the anti-inflammatory action of stem cells extended from the articular surface to the neighboring structures. Also, they possess powerful limiting effect on liberated cytokines, counteracting the adverse effect of harmful cytokines which was approved by [8, 9] who showed that ADSCs, when given at an early phase of experimental OA, inhibit synovial lining thickening and forming of enthesophytes associated with ligaments, and cartilage destruction and protect against joint destruction by both anabolic and catabolic mediators.

Masson trichrome is a specific stain for the collagen fiber in the chondroid matrix. It revealed loss in diseased group. on the other hand, in ADSCs and BMSCs treated groups displayed marked restoration and this matched with previous results [40]. Compared BMSCs treated group with the other groups using Masson's trichrome staining, it was found that BMSCs-injected group was closer to normal, protected cartilage. It reduced the abnormal differentiation and proliferation and fibrosis in the knee articular cartilage, thus slow down the process of cartilage degradation.
Histochemical staining was done to evaluate the degree of fibrosis. The results estimated that ADSCs were the best in all the treated groups with minimal fibrosis, this means that the healing process passes in normal consequence without any accumulation of fibrotic bundles which was reflected on cartilaginous regeneration in the normal consequences and have impact on the normal movement. This was agreed by [52, 53] who assessed the effect of ADSCs differentiated chondrocytes (DCs) versus ADSCs transplantation into the 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 was compatible with gross and histological examination where the ADSCs were the minimal expression and BMSCs was the succeeding one. The highest expression was in OA group so the MMP9 results were to confirm the degree of chondroid matrix destruction and loss of its proteoglycans. [55, 56] determined the effectiveness of ADSCs, the expression of matrix metalloproteinase and the ability of ADSCs in lowering expression in OA models.

5. Conclusion

The results of the present study demonstrated that ADSCs and BMSCs can alleviate knee OA via their strong regenerative capacity on chondrocytes and increased chondroid matrix deposition. So, for such positive results, ADSCs are more effective and easily available compared to BMSCs.

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Conflict of interest

The authors declare that there is no any conflict of interest in the current research work.

Animal ethics committee permission
This study was approved by the Local Ethical Committee, Faculty of Medicine, Mansoura University in accordance with the Ethics committee of national research Center-Egypt with registration number (09/189).

Authors’ contribution

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

6. REFERENCE


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