

# Mesenchymal Stem Cells Inhibit Proteoglycan Degeneration in a Rat Model of Osteoarthritis

Masoumeh Zirak Javanmard,<sup>1</sup> Davood Asgari,<sup>1</sup> Mojtaba Karimipour,<sup>1\*</sup> Fathemeh Atabaki,<sup>1</sup> Gholamhosain Farjah,<sup>1</sup> and Afsaneh Niakani

<sup>1</sup>Department of Anatomy, Urmia University of Medical Sciences, Urmia, IR Iran

\*Corresponding author: Mojtaba Karimipour, Department of Anatomy, Urmia University of Medical Sciences, Urmia, IR Iran. Tel: +98-9143486495, Fax: +98-4432780801, E-mail: mojtaba\_karimipour@yahoo.com

Received 2015 June 27; Revised 2015 October 7; Accepted 2015 October 8.

## Abstract

**Background:** Osteoarthritis (OA) is a degenerative disease, which is characterized pathologically by degeneration of articular cartilage. One of the mechanisms of cartilage lesion in OA is decreased synthesis of cartilage matrix and amongst the variety of treatment methods for OA, cell base therapies has a crucial role.

**Objectives:** The purpose of this study was to understand the regeneration effect of mesenchymal stem cells as well as secretions of these cells after induction of early-stage Osteoarthritis (OA).

**Materials and Methods:** To achieve this aim articular cartilages of rat knees were cured by monosodium iodoacetate (1 mg/50  $\mu$ L). Mesenchymal Stem Cells (MSCs) were cultured and injected into the knee joint of rats after two weeks. The left knee was kept as the control group and injected with either sterill saline. The animals were sacrificed at two weeks after transplantation. The knee joints were harvested and safranin-o and toluidine-blue microscopic analysis were performed. In order to assess changes, the amount of control and experimental cartilage extracellular matrix structure (proteoglycan) and cartilage fibrillation were determined by using histological approaches.

**Results:** The results revealed a decrease of proteoglycan content in the superficial and intermediate zones and presence of surface fibrillation. Therefore, OA induced matrix degeneration, and the reparative effects of MSCs were higher than cell secretions.

**Conclusions:** It can be concluded that due to some bioactive and nutrient factors, MSCs secretions could be used for recovery of OA as well as prevention of articular cartilage matrix degeneration, using a high dose of cell secretions.

**Keywords:** Osteoarthritis, Monosodium Iodoacetate, MSCs

## 1. Background

Osteoarthritis (OA) of the knee joint is characterized pathologically by loss of articular cartilage and clinically by pain and disability. Its prevalence will increase rapidly over the next 20 years (1). The occurrence of OA is related to age, obesity, trauma, genetics and various pathophysiological events within the joints (2). It is also associated with major cellular and molecular alterations in articular tissue. Inflammatory factors such as cytokines, nitric oxide, prostaglandin E2 (inflammatory pain mediators), neuropeptides and proteolytic enzymes are produced by the inflamed synovium (3). Chondrial changes include cartilage fragmentation (fibrillation), presence of tear, loss of type II collagen, chondrocyte apoptosis and loss of proteoglycans. As one grows older, the ability of chondrocytes to maintain and restore of articular cartilage decreases (4). Articular cartilage is composed of chondrocytes and extracellular matrix (ECM) and the ECM is composed mainly of collagen fibers type II (5). The balance of collagen and Proteoglycan (PG) is reject disrupted in OA, and proteoglycans are dis-

rupted in OA and trauma. Early stages of OA, are characterized by a decrease in proteoglycans and collagen, change in cartilage properties, activation of some degenerative enzymes and reduction of aggrecan size (6). The breakdown of type II collagen appears in later stages of OA and follows the degradation of PG (7). It is clear that both mechanical and biological factors are involved in degeneration of cartilage, decreased synthesis of ECM and formation of chondrocyte clusters (8). In other words, the number of cells remains unchanged yet distribution of their arrangement occurs, and highly cellular clone formation related to the disability of proliferated cells to migrate in damaged areas. Chondrocytes attach to ECM proteins, via integrin receptors and this adhesion is implicated in OA and leads to cartilage fibrillation (2). Growth factors such as IGF-1 or TGF- $\beta$  contribute to chondrocyte adhesion to fibronectin (9). It has been established by animal models of OA, that the intra-articular injection of Monosodium Iodoacetate (MIA) acts as an inhibitor of glycolysis and promotes loss

of articular cartilage similar to OA and produces necrosis with degeneration of chondrocyte in tibial plateaus and femoral condyles (10). Despite the high prevalence of OA, an effective treatment is currently lacking. Difficulties of studying OA in humans have led to highly dynamic animal models (11). There are different pharmacologic and non-pharmacologic methods for treatment of OA; however the application of cell-based therapies has been used for a number of decades (12). Bone marrow is the main source for isolation of Mesenchymal Stem Cells (MSCs), and these cells have the potential to differentiate to a variety of cells of different tissues (13). The application of MSCs for OA repair requires different techniques, although the local injection of MSCs may be the simplest procedure (14).

There are two administration methods of stem cells: direct or local and intravascular administration. Numerous researchers have discussed the use of MSCs to repair a variety of tissues (15). The structural damages of articular cartilage have been demonstrated in a variety of ways such as clefts. Proteoglycan loss and collagen breakdown have been observed by microscopic slides (16). It is clear that MSCs are suited for cartilage repair. These cells facilitate regeneration, by secreting nutrient and bioactive factors (17).

## 2. Objectives

In the current study, MSCs and their secretions were used to repair articular cartilage defects by assessment of ECM (PG) depletion in a rat model.

## 3. Materials and Methods

### 3.1. Animals

Eighteen healthy, 18-week-old, male Sprague-Dawley rats were used as sample rejections in this study. Animal experiments were reviewed and approved by the institutional animal care and use committee (IACUC) of our institution.

### 3.2. Experimental Protocol

The rats were selected randomly and divided to four groups, each consisting of six animals except the control group. Induction was done through intra-articular injection of MIA in right knee joints, while the other six left knee joints were considered as the control group. The MSCs were harvested in a separate procedure and a single dose of  $2.5 \times 10^5$  cells was injected for the samples of the MSCs group, while the same amount of medium plus cell secretions was injected for the M + CS group. All animals were sacrificed two weeks after therapy.

### 3.3. Intra-Articular Injection of Monosodium Iodoacetate (MIA)

Osteoarthritis was induced in rats briefly anaesthetized by an intraperitoneal injection of xylazine 10 mg/kg and ketamine 20 mg/kg, by intra-articular injection of MIA (Sigma, UK) into the right knee joint. The knee joint was

shaved and flexed at a 90 degree angle. Five microliters of 1 mg/50  $\mu$ L (MIA) in sterile saline (0.9%) was injected through the infrapatellar ligament into the joint space of the right knee with an insulin syringe. This concentration of MIA has been previously demonstrated to induce OA in mice. Control rats received an intra-articular injection of 50  $\mu$ L of sterile saline in the left knee joint [R5].

### 3.4. Cell Isolation and Culture

The bone marrow aspirate was diluted with Dulbecco's Modified Eagle's Medium (DMEM) (1:3) and 10% fetal serum bovine (FBS), and was then centrifuged at 1200 g for five minutes. The supernatant was discarded and the cells pellet was washed two times with PBS. The cells pellet was suspended with DMEM (1 mm) and was plated as primary culture in a culture flask at 0.5 mm. The flask was then incubated at 37°C in a humidified atmosphere containing 5% CO<sub>2</sub>. On day four of culture, the non-adherent cells were removed along with the change of medium every three days. Undifferentiated MSCs were transplanted upon reaching 70% - 80% confluency (12).

### 3.5. Histological Analysis

At two weeks post treatment, the animals were sacrificed. The knee joints of the three experimental (right knees) and six contra-lateral joints were dissected and fixed in 10% formalin at 4°C and decalcified in formic acid (5%) for three days. The specimens then underwent automated tissue processing for 24 hours. Sections were imbedded in paraffin and cut into 5  $\mu$ m slices. Each section was stained with toluidine-blue and safranin-O.

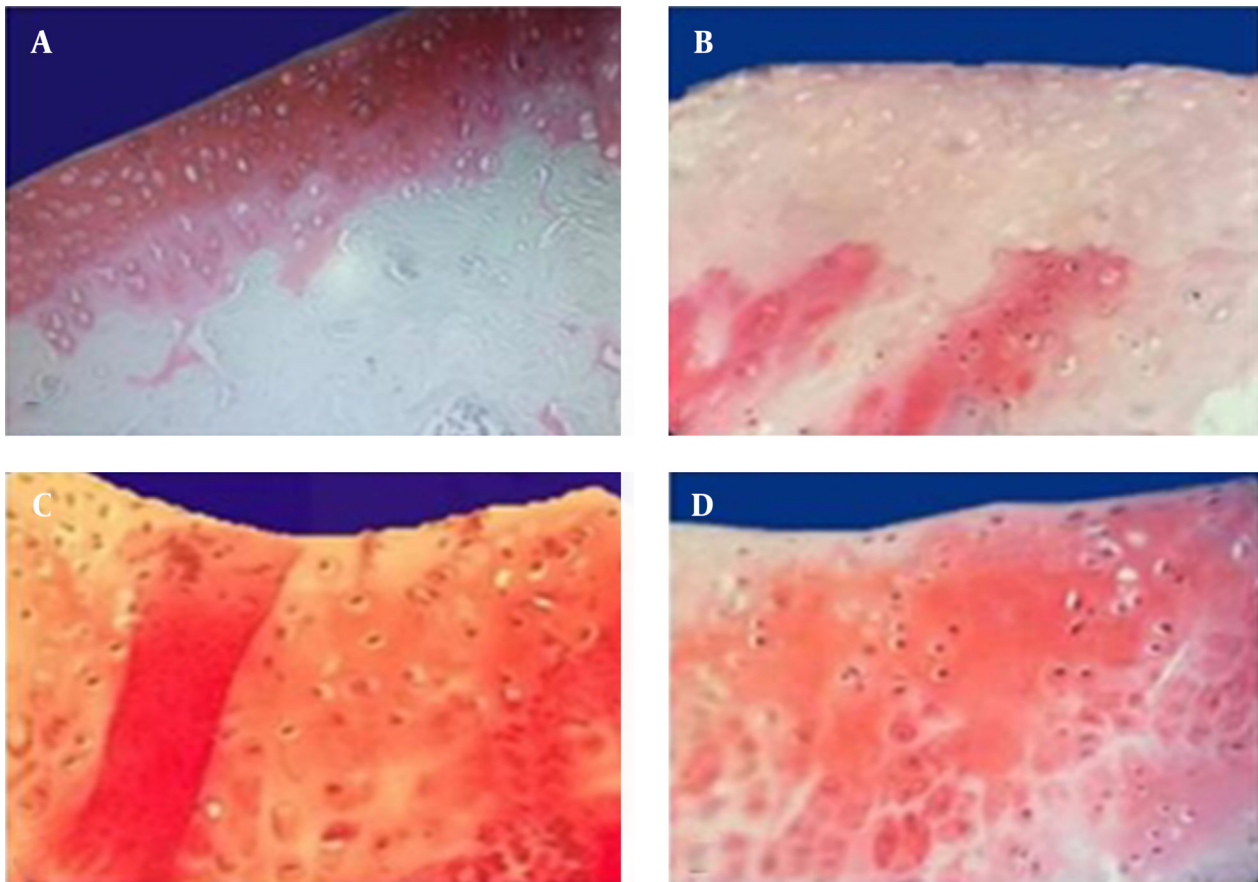
### 3.6. Safranin-O

The distal femurs were removed and fixed in 10% formaldehyde, decalcified in 10% formic acid, and embedded in paraffin. Serial sections of 6  $\mu$ m were cut sagittally. Histological staining was performed with safranin-O histo-chemical stain to analyze proteoglycan distribution. Slides were first placed in Mayer's hematoxylin for two minutes and rinsed in water. These slides were then placed in fast green fetal calf fluid (FCF) Yellowish for 10 minutes and rinsed in acetic acid (1%) until the glass was free of stain. Next, the slides were placed in safranin-O for 30 minutes, and rinsed in water and 95% EtOH until the glass was free of stain. After air drying, the slides were cover-slipped. The histological preparations were examined by a light microscope and PG depletion of cartilage matrix was graded as high, moderate and low.

The Mann-Whitney test for nonparametric analysis was used to compare the number of high, moderate and low density PGs. Values of  $P < 0.05$  were considered statistically significant.

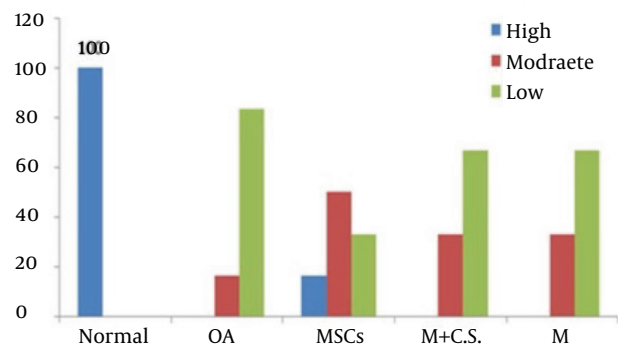
## 4. Results

Outcomes were evaluated microscopically with histological staining. Uniform safranin-O staining was seen

**Figure 1.** Histological Staining of Proteoglycan (Safranin-O) in Articular Cartilage

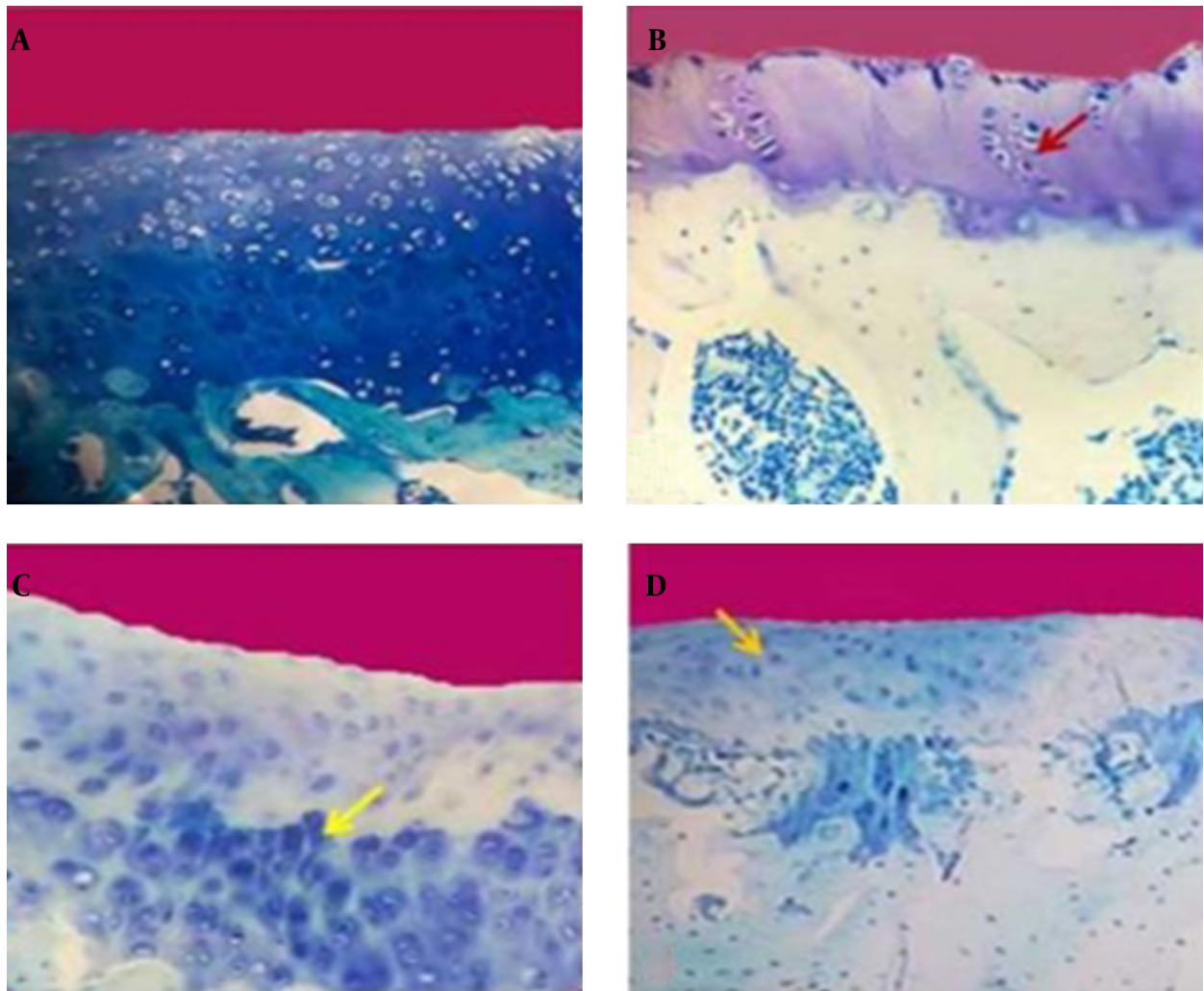
Intact articular cartilage with intense red (S-O) staining indicates the concentration of PG. A, A uniform red staining was seen in all regions of normal articular cartilage; B, Increases loss of red staining was seen in OA group; C, Significant increase of PG in MSCs and D, slight increase of PG in (M + C.S.).

through all zones in the controls, with dense underlying bony plate, yet it was lighter near the articular surface where the PG content was lower. In the OA group, a progressive loss of PG staining was found at the cartilage surface and progressed to deeper layers. Direct injection of MSCs to articular space caused a subsequent marked synthesis of PG in the MSCs group, and after cell transfer these samples showed uniformly stronger staining in the proliferative zone than in the calcified region (Figure 1). Similar results with low intensity were obtained when the cells were removed and medium plus secretions of cells was used as a second treatment method. Analysis with Mann-Whitney test using the SPSS software (Figure 2) indicated considerable increase of PG synthesis in the MSCs group compared with the control cases ( $P < 0.05$ ). Presence of fibrillation was identified in all groups at the femoral condyles of the cartilage surface with toluidine blue staining. In fibrillated cartilage (OA), the cluster shape of chondrocytes was formed and some areas of the cartilage presented a cellular formation, which was filled with ECM. Cartilage fibrillation was observed in OA groups, and it decreased significantly in the MSCs group ( $P < 0.05$ ) after injection of a single dose of  $2.5 \times 10^6$  cells (Figures 3 and 4).

**Figure 2.** The Grading of Cartilage Matrix Staining With Safranin-O

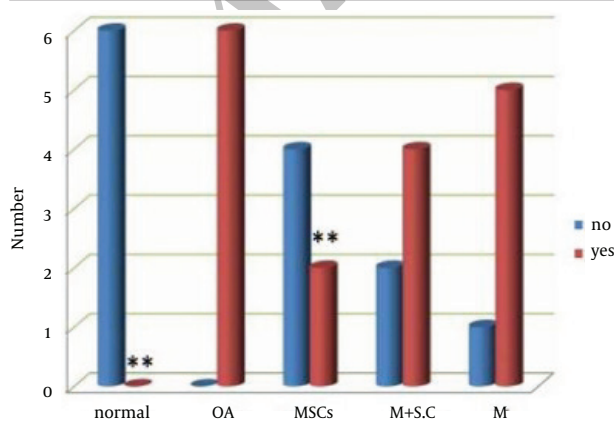
The control group (normal) showed high intensity of PG. Analysis with Mann-Whitney test indicates an increase of PG synthesis in the MSCs group compared with the control. Significance is indicated by  $P < 0.05$ : in OA (osteoarthritis) and MSCs groups compared with the control.

**Figure 3.** Histological Staining of Proteoglycan (Safranin-O) in Articular Cartilage



A, Intact articular cartilage with chondrocytes in all zones of the cartilage; B, Cluster shape of chondrocytes in fibrillated cartilage on OA; C, marked increase of chondrocytes and did not show fibrillation after two weeks from treatment by MSCs; D, moderate increase of chondrocytes in the M + C.S. group.

**Figure 4.** Presence of Cartilage Fibrillation



In the control group (normal) all samples were normal. In OA presence of fibrillation were markedly visible, and the MSCs group showed a significant decrease of cartilage fibrillation compared to the controls.

## 5. Discussion

Mesenchymal stem cells with inherent chondrogenic potential and anti-inflammatory function are the best candidates for repairing cartilage damage in OA. These cells facilitate regeneration by secreting various factors (12). Osteoarthritis makes changes in ECM and these changes are transmitted to the environment of chondrocytes. This condition, thus promotes the progression of disease. In this disease, many inflammatory factors, such as cytokines and proteases change the quality of ECM (18). The changes in ECM component can prevent the differentiation of MSCs to chondrocytes (19). It has been revealed that PG content is decreased in OA, which is in concordance with our results. Because of the incidence of proliferation of chondrocytes and the formation of cluster shaped cells, this cellular arrangement can change the quantity and composition of ECM (20). Our findings revealed a significant loss of proteoglycan synthesis. Both

treated groups (MSCs, M + C.S.) demonstrated potential regeneration of cartilage compared to the control group with a higher degree in the MSCs group. Previous in vitro studies have shown that the induction of bioactive factors, such as TGF- $\beta$  and IGF-1, promote the synthesis of PG. The present study also reported that fibrillated cartilage was seen significantly in OA cartilage samples from femoral ends and injection of MSCs could significantly decrease the percentage of fibrillated cartilage. The association between both highly cellular clusters and acellular areas is known as a common finding in OA (21). It was observed that some chondrocytes do not have the ability to migrate to damaged areas. Decrease in cell adhesion to fibronectin could be related to presence of apoptotic or necrotic chondrocytes in affected cartilage (22). Mesenchymal stem cells have the potential of differentiating into chondrocytes and can migrate to injured areas (23). In conclusion, MSCs can be used for healing and regeneration of cartilage. One of the most interesting findings of this study was the progression in repair of OA caused by factors that were secreted from MSCs. It has been emphasized that, possibly because of growth factors needed for cartilage healing, the absence of cells cannot stop the regeneration process.

## Acknowledgments

Department of Anatomy, Urmia university of medical sciences, Urmia, IR Iran.

## Footnotes

**Authors' Contribution:** Fathemeh Atabaki; Gholamhosain Farjah, Masoumeh Zirak Javanmard, Mojtaba Karimi-pour: Leaders. Davood Asgari performed the laboratory experiments. Afsaneh Nikani: statically analyzer.

**Funding/Support:** Department of anatomy, Urmia university of medical sciences, Urmia, IR Iran

## References

- Nanri A, Mizoue T, Poudel-Tandukar K, Noda M, Kato M, Kurotani K, et al. Dietary patterns and suicide in Japanese adults: the Japan Public Health Center-based Prospective Study. *Br J Psychiatry*. 2013;**203**(6):422-7. doi: 10.1192/bjp.bp.112.114793. [PubMed: 24115342]
- Mobasheri A, Kalamegam G, Musumeci G, Batt ME. Chondrocyte and mesenchymal stem cell-based therapies for cartilage repair in osteoarthritis and related orthopaedic conditions. *Maturitas*. 2014;**78**(3):188-98. doi: 10.1016/j.maturitas.2014.04.017. [PubMed: 24855933]
- Mobasheri A, Richardson S, Mobasheri R, Shakibaei M, Hoyland JA. Hypoxia inducible factor-1 and facilitative glucose transporters GLUT1 and GLUT3: putative molecular components of the oxygen and glucose sensing apparatus in articular chondrocytes. *Histol Histopathol*. 2005;**20**(4):1327-38. [PubMed: 16136514]
- Hudelmaier M, Glaser C, Hohe J, Englmeier KH, Reiser M, Putz R, et al. Age-related changes in the morphology and deformational behavior of knee joint cartilage. *Arthritis Rheum*. 2001;**44**(11):2556-61. doi: 10.1002/1529-0131(200111)44:11<2556::aid-art436>3.0.co;2-u. [PubMed: 11710712]
- Hollander AP, Dickinson SC, Kafienah W. Stem cells and cartilage development: complexities of a simple tissue. *Stem Cells*.

- 2010;**28**(11):1992-6. doi: 10.1002/stem.534. [PubMed: 20882533]
- Lin PM, Chen CT, Torzilli PA. Increased stromelysin-1 (MMP-3), proteoglycan degradation (3B3- and 7D4) and collagen damage in cyclically load-injured articular cartilage. *Osteoarthritis Cartilage*. 2004;**12**(6):485-96. doi: 10.1016/j.joca.2004.02.012. [PubMed: 15135145]
- Hollander AP, Heathfield TF, Webber C, Iwata Y, Bourne R, Rorabeck C, et al. Increased damage to type II collagen in osteoarthritic articular cartilage detected by a new immunoassay. *J Clin Invest*. 1994;**93**(4):1722-32. doi: 10.1172/JCI117156. [PubMed: 7512992]
- Lee AS, Ellman MB, Yan D, Kroin JS, Cole BJ, van Wijnen AJ, et al. A current review of molecular mechanisms regarding osteoarthritis and pain. *Gene*. 2013;**527**(2):440-7. doi: 10.1016/j.gene.2013.05.069. [PubMed: 23830938]
- Guzman RE, Evans MG, Bove S, Morenko B, Kilgore K. Mono-iodoacetate-induced histologic changes in subchondral bone and articular cartilage of rat femorotibial joints: an animal model of osteoarthritis. *Toxicol Pathol*. 2003;**31**(6):619-24. [PubMed: 14585729]
- Mokbel AN, El Tookhy OS, Shamaa AA, Rashed LA, Sabry D, El Sayed AM. Homing and reparative effect of intra-articular injection of autologous mesenchymal stem cells in osteoarthritic animal model. *BMC Musculoskelet Disord*. 2011;**12**:259. doi: 10.1186/1471-2474-12-259. [PubMed: 22085445]
- Peterson L, Minas T, Brittberg M, Nilsson A, Sjogren-Jansson E, Lindahl A. Two- to 9-year outcome after autologous chondrocyte transplantation of the knee. *Clin Orthop Relat Res*. 2000;**374**:212-34. [PubMed: 10818982]
- Ohgushi H, Okumura M. Osteogenic capacity of rat and human marrow cells in porous ceramics. Experiments in athymic (nude) mice. *Acta Orthop Scand*. 1990;**61**(5):431-4. [PubMed: 2173333]
- Bendele AM. Animal models of osteoarthritis in an era of molecular biology. *J Musculoskelet Neural Interact*. 2002;**2**(6):501-3. [PubMed: 15758375]
- Fahmy LS, Hegazy AA, Abd El Hamid MA, Shamaa AA, Schimke E. Retrospective studies on equine arthritis with special reference to its treatment. *Vet Med J*. 1996.
- Thibault M, Robin Poole A, Buschmann MD. Cyclic compression of cartilage/bone explants in vitro leads to physical weakening, mechanical breakdown of collagen and release of matrix fragments. *J Orthopaed Res*. 2002;**20**(6):1265-73. doi: 10.1016/s0736-0266(02)00070-0.
- Gupta PK, Das AK, Chullikana A, Majumdar AS. Mesenchymal stem cells for cartilage repair in osteoarthritis. *Stem Cell Res Ther*. 2012;**3**(4):25. doi: 10.1186/srct116. [PubMed: 22776206]
- Maldonado M, Nam J. The role of changes in extracellular matrix of cartilage in the presence of inflammation on the pathology of osteoarthritis. *Biomed Res Int*. 2013;**2013**:284873. doi: 10.1155/2013/284873. [PubMed: 24069595]
- Vidal MA, Robinson SO, Lopez MJ, Paulsen DB, Borkhsenius O, Johnson JR, et al. Comparison of chondrogenic potential in equine mesenchymal stromal cells derived from adipose tissue and bone marrow. *Vet Surg*. 2008;**37**(8):713-24. doi: 10.1111/j.1532-950X.2008.00462.x. [PubMed: 19121166]
- Piperno M, Reboul P, Hellio Le Graverand MP, Peschard MJ, Anefeld M, Richard M, et al. Osteoarthritic cartilage fibrillation is associated with a decrease in chondrocyte adhesion to fibronectin. *Osteoarthritis Cartilage*. 1998;**6**(6):393-9. doi: 10.1053/joca.1998.0138. [PubMed: 10343772]
- Mathieu P. [A new mechanism of action of chondroitin sulfates ACS4-ACS6 in osteoarthritic cartilage]. *Presse Med*. 2002;**31**(29):1383-5. [PubMed: 12375394]
- Loeser RF. Integrins and chondrocyte-matrix interactions in articular cartilage. *Matrix Biol*. 2014;**39**:11-6. doi: 10.1016/j.matbio.2014.08.007. [PubMed: 25169886]
- Solchaga LA, Penick KJ, Welter JF. Chondrogenic differentiation of bone marrow-derived mesenchymal stem cells: tips and tricks. *Methods Mol Biol*. 2011;**698**:253-78. doi: 10.1007/978-1-60761-999-4\_20. [PubMed: 21431525]