European Review for Medical and Pharmacological Sciences 2021; 25: 3546-3556

Intra-articular human deciduous dental pulp stem cell administration *vs*. pharmacological therapy in experimental osteoarthritis rat model

D. CORREA MALDONADO[1], T. NICOLICHE[1], J. FABER[2], I. KERKIS[3], D. MARTINEZ SAEZ[1,4], R. TETSUO SASAKI[1,5], M. CAVENAGHI PEREIRA DA SILVA[1]

[1]Department of Morphology and Genetics, Universidade Federal de São Paulo, São Paulo, Brazil
[2]Department of Neurology and Neurosurgery, Universidade Federal de São Paulo, São Paulo, Brazil
[3]Genetics Laboratory, Instituto Butantan, São Paulo, Brazil
[4]Department of Anatomy, Universidade Federal de Alfenas, Alfenas, Minas Gerais, Brazil
[5]Department of Basic Sciences in Health, Universidade Federal de Mato Grosso, Cuiabá, Mato Grosso, Brazil

Abstract. – OBJECTIVE: The aim of the present study was to compare the molecular and morphological effects of diacerein and glucosamine-chondroitin drug treatment and intra-articular injection therapy of human deciduous dental pulp stem cells (hDPSCs) in a rat knee model of induced osteoarthritis (OA).

MATERIALS AND METHODS: Thirty-six adult male rats were randomly separated into six groups: Control group (without induction of OA), osteoarthritis group 60 (induction of OA, saline gavage started on day 14 and performed for 60 days, followed by euthanasia), osteoarthritis group (induction of OA and euthanasia after 14 days), diacerein group, glucosamine-chondroitin group, and mesenchymal stem cell group. The drug-treated groups were gavaged with 50 mg/kg of diacerein and 400/500 mg/kg of glucosamine-chondroitin starting on dat 14 for 60 days. The cell therapy-treated group received an intra-articular single dose of 8 × 10^5 hDPSCs on day 14, and euthanasia was performed after 60 days. Lateral femoral condyles were collected and prepared for immunohistochemistry and light microscopy procedures.

RESULTS: The morphological features and immunoexpression of SOX-5, IHH, MMP-8, MMP-13, and Type II collagen were statistically analysed. Our data suggest that hDPSC therapy contributes more actively and effectively in the structural reorganization of lateral femoral condyles. In contrast, the glucosamine-chondroitin sulphate treatment was more effective in inflammatory control, while diacerein showed better results associated with the maintenance of the primordial cartilage.

CONCLUSIONS: The positive therapeutic effect of daily administered conventional drugs can be confirmed in a rat model of OA. However, one single dose of locally administered hDPSCs provides significant improvement in tissue regeneration in an OA model.

Key Words:
Osteoarthritis, Glucosamine, Chondroitin, Dental pulp stem cells.

Introduction

Osteoarthritis (OA) is the most common articular disease in the world, and it causes several disabilities[1]. OA is characterized by a metabolic imbalance in which the overexpression of matrix metalloproteinases (MMPs)[2] results in extracellular matrix (ECM) changes, such as proteoglycan and collagen 2 (Col2) loss, matrix fibrillation, and initial erosion followed by complete cartilage loss; moreover, in a later stage, bone to bone contact can be observed[3].

Several transcription factors are involved in the formation and maintenance of a healthy articular cartilage, such as the SOX gene family (SOX-5, 6, and 9)[4], and they have been reported to be crucial in primordial cartilage development because the absence of these proteins leads to severe chondral impairment[5]. These transcription factors also play a role in Col2

Corresponding Author: Diogo Corrêa Maldonado, MD; e-mail: maldonado@unifesp.br

activation[6] and the maintenance of chondrogenic phenotypes because SOX-5 and SOX-6 promote cartilage matrix formation[7] and SOX-9 overexpression shows an improvement in chondrogenic performance[8]. In association with the SOX gene family, Indian hedgehog (IHH) is an important factor for cartilage analysis because it is involved in chondrocyte hypertrophic regulation and endochondral ossification[9].

Among the most commonly used drugs for OA therapy are diacerein[10] and a combination of glucosamine and chondroitin[11]. Diacerein shows moderate anti-inflammatory action and analgesic activity[12] and is mainly associated with interleukin 1(IL-1), inhibition, nitric oxide and MMPs[13]. Diacerein has recently been awarded the seal of excellence by the European Association for the Clinical and Economic Aspects of Osteoporosis and Osteoarthritis (ESCEO) because of its benefits in the treatment of OA[14].

Glucosamine acts on the synthesis of glycosaminoglycans and proteoglycans in cartilage[15] and induces chondrocytes to inhibit MMPs synthesis[16]. Chondroitin shows anti-inflammatory activity by stimulating the synthesis of hyaluronic acid and proteoglycans and inhibiting the synthesis of proteolytic enzymes and nitric oxide[17]. After a systematic review and meta-analysis of randomized clinical trials[18], the efficacy of diacerein and glucosamine sulphate/chondroitin in OA treatment has been verified. Because a cure for OA has not yet been discovered, treatments for OA involve inflammatory control and pain relief[19]. Mesenchymal stem cells (MSCs) are an important candidate for current research into OA treatment[20] because they can differentiate into chondrogenic line cells, directly promote new cartilage formation, and provide inflammatory control[21].

In the present paper, we investigate the hypothesis that human deciduous dental pulp-derived stem cells (hDPSCs) may represent a more effective treatment for OA compared with conventional drugs, such as diacerein and a combination of glucosamine and chondroitin, based on the morphology of three articular layers as indicated by morphometry (measurement) and stereology (chondrocytes quantification) analyses. The effects of hDPSCs, diacerein and glucosamine-chondroitin sulphates on the expression of transcription factors important for the genesis and maintenance of healthy cartilage (SOX-5 and IHH), the main protein that constitutes ECM (Col2) and ECM-degrading enzymes (MMP-8 and MMP-13) were also observed.

Materials and Methods

Study Subjects

The animal procedures were performed in accordance with the Ethical guidelines of the Animal Care Ethical Committee of the Universidade Federal de São Paulo (nº 04.023-061).

Thirty-six male Wistar rats (*Rattus norvegicus*) that were bred and housed until 12 weeks of age at the CEDEME (Central de Desenvolvimento de Modelos Experimentais para Biologia e Medicina) of the Federal University of São Paulo under a natural dark and light (12:12 h) cycle with *ad libitum* water and standard food access were used. The animals were randomly distributed into six groups (n=6 animals each). The control group (CG): OA was not induced, and gavage was started on day 14 and performed for 60 days, followed by euthanasia; the osteoarthritis group (OAG60): OA was induced and saline gavage was started on day 14 and performed for 60 days, followed by euthanasia the osteoarthritis group (OAG): OA was induced, and euthanasia was performed at day 14; the diacerein group (DG)/glucosamine-chondroitin group (GSCG): OA was induced and drug gavage was started on day 14 and performed for 60 days, followed by euthanasia; the mesenchymal stem cell group (MSCG): OA was induced, a single intra-articular application of 8×10^5 hDPSC in 4 mL of saline solution was administered on 14, and euthanasia was performed on day 60.

OA Induction and Treatment

OA was induced under anaesthesia (ketamine 100 mg/kg – Ceva, Paulinia, São Paulo, Brazil and xylazine 10 mg/kg - Ceva, Paulinia, São Paulo, Brazil) via an intra-articular injection in the left knee of a single dose of 1 mg zymosan (CAS number 58856-93-2, Merck KGaA, Darmstadt, Hesse, Germany) dissolved in up to 50 µL of sterile saline. After trichotomy and antisepsis with povidone (Vic Pharma, Taquaritinga, São Paulo, Brazil), a needle was carefully inserted into the left knee via a medial approach just below the femoro-patellar ligament. Thereafter, the animals rested for 14 days in order to develop the inflammatory process and chondral degeneration via the Rocha et al method[22]. The same procedures were performed in the CG using sterile saline.

Fourteen days after induction, 50 mg/kg of diacerein (Artrodar®, TRB Pharma Indústria Química e Farmacêutica Ltda, São Paulo, São Paulo, Brazil) and 400/500 mg/kg of glucosamine chondroitin (Artrolive®, Aché Laboratórios Far-

macêuticos S.A., São Paulo, São Paulo, Brazil) were administered daily for 60 days by gavage to the DG and GSCG. In the CG and OAG60, sterile saline was administered by gavage for 60 days. The MSCG received a single intra-articular application of 8×10^5 hDPSC in 4 mL of saline solution.

The tissue explant of dental pulp was used to isolate stem cells from exfoliated deciduous teeth. Isolation, cultivation and characterization of this population of MSC was obtained in accordance with the Kerkis et al method[23]. Early passages with normal karyotypes were grown in DMEM supplemented with 10% FBS, 100 U/ml penicillin, 100 g/ml streptomycin, and 2 mM L-glutamine (Thermo Fisher Scientific Cat. Number 11965092, Waltham, MA, USA). All cultures were incubated at 37°C in 5% CO_2 and high humidity. At the moment of the treatment, the cells were counted, washed in saline and injected in the knee.

The animals in the OAG were euthanized at 14 days post OA induction, while the other groups were treated for more than 60 days and euthanized intraperitoneally using a lethal anaesthetic dose (5 times the amount used to anaesthetize the animals). The knee capsule was sectioned and the meniscus was isolated. The distal epiphysis of the femur containing the lateral condyle cartilage was isolated and fixed with 4% paraformaldehyde in 0.1 M phosphate-buffered saline (PBS) at 7.4. The fixed specimens were decalcified in formic acid (20%, CAS Number 64-18-6, F0507, Merck KGaA, Darmstadt, Hesse, Germany) dissolved in distilled water for approximately 2 days. The specimens were then routinely paraffin-embedded (Paraplast, CAS Number 145686-99-3, Merck KGaA, Darmstadt, Hesse, Germany), and 3 µm sagittal cuts perpendicular to the articular surface of the femoral cartilage were obtained.

Quantitative Analysis

For the morphometrical studies, 50 cuts of each sample were performed to minimize variability. Every fifteenth cut the samples from each animal was stained with haematoxylin and eosin (HE). Two trained examiners determined the cartilage thickness and chondrocyte count in a blind manner. Histological sections were analysed using a digital image analysis system for quantitative histomorphometry (KS-300; Carl Zeiss Inc., Oberkochen, Baden-Wurttemberg, Germany). Each microscopic image was projected to a monitor, and the sectional cartilage area was divided into five equidistant points from the front to the back. Subsequently, the thickness of the entire cartilage was measured perpendicularly from the surface layer to the deep layer at each determined point. For each area, the surface, middle or deep layers and the number of chondrocytes were determined.

Immunohistochemistry

For immunohistochemistry, sections (3 µm thick) from the specimens were deparaffinized and rehydrated. The slides were heated to 95°C for 35 min in citrate buffer (pH 6.0) for antigen retrieval and incubated in a methanol and 6% hydrogen peroxide solution (1:1) for 30 min to quench endogenous peroxidase activity. The sections were incubated with 1% BSA (bovine serum albumin -CAS Number 9048-46-8, 5470 - Merck KGaA, Darmstadt, Hesse, Germany) for 45 min to block nonspecific reactions. The slides were incubated with primary antibodies: Col2 1:250 (M2139, Santa Cruz Biotechnology, Dallas, TX, USA), MMP-8 1:200 (SAB4501895, Merck KGaA, Darmstadt, Hesse, Germany), MMP-13 1:200 (SAB4501900, Merck KGaA, Darmstadt, Hesse, Germany), SOX-5 1:50 (H-90, Santa Cruz Biotechnology, Dallas, TX, USA) and IHH 1:100 (AV45230, Merck KGaA, Darmstadt, Hesse, Germany) overnight at 4°C. The Advanced HRP system (Dako, Carpinteria, CA, USA) was used to detect antibodies and specimens were counterstained with Mayer's haematoxylin, dehydrated and mounted for observation and quantification[24].

The quantification analyses were performed using WCIF ImageJ software. Three sections for each sample were selected, the surface, middle, and deep sections were obtained and calibrated, and colour deconvolution was performed. In the vectors, H DAB was chosen, and the brown image was selected. After many tests, the *Threshold* interval was selected (0 to 180), and the coloured *Area Fraction* was obtained and compared to the total area for each studied section.

Statistical Analysis

Statistical analyses were performed carried out using MATLAB® (version 9.0 R2016a, Mathworks Inc., Natick, MA, USA) software to compare the morphometrical data and the immunohistochemical staining percentage area in the surface, middle, and deep layers of the studied groups. The Kolmogorov-Smirnov test was ap-

plied to evaluate the normality index of the sample origin, and all indexes were rejected. Therefore, the Kruskal–Wallis nonparametric test was used to compare the variables of interest. Thereafter, a *post hoc* Dunn-Sidak test was used to identify the inter-group pair significance. A significance level of α =0.05 was considered in all tests.

Results

Morphological Data and Immunohistochemical Quantification

The morphological changes observed in the articular cartilage of OA in the treatments' groups based on HE staining (Figure 1) showed an increased thickness of the joint cartilage in OAG60 compared to the other groups but did not show significant differences in the middle and deep layers between the MSCG and CG (Figure 2A). Moreover, a decreased number of chondrocytes was observed in the OAG and DG compared with the CG in the middle layer while no significant differences in chondrocyte number was observed for the GSCG and MSCG compared with OAG or CG (Figure 2B).

Col2 immunostaining was present in all cartilage layers of all groups, with the MSCG showing more expression in certain layers than the DG and OAG60 (Figure 2C). IHH immunostaining was observed in the chondrocyte cytoplasm and nucleus, and its expression was decreased in the OAG60 superficial layer compared with that of the CG and in the MSCG middle layer compared with that of the OAG and OAG60 (Figure 2D).

MMP-8 and MMP-13 immunostaining was observed in the ECM and chondrocyte cytoplasm (Figure 3). MMP-8 expression was mainly decreased in the GSCG compared with the CG in all layers. Moreover, the staining was weaker in the GSCG than the DG and OAG in the middle and deep layers (Figure 4A). MMP-13 immunostaining was weaker in the GSCG compared with the MSCG, but no difference was observed between the MSCG and the CG or DG (Figure 4B).

SOX-5 immunostaining was observed in the chondrocyte cytoplasm and nucleus, and the expression was increased in the DG superficial and middle layers compared with that of the OAG and increased in the CG superficial layer compared with that of the OAG. The MSCG middle layer also showed an increase in comparison to that of the OAG (Figure 4C).

Discussion

Treatments with fewer side effects, such as cellular therapies, are needed. MSCs differentiate into cartilage and can provide a therapeutic benefit in injured tissue due to their paracrine affect. hDPSC are of particular interest because they are not subject to the ethical and morbidity problems observed with other MSC sources.

It is important to notice that several sources of hDPSCs were described in the literature, using deciduous teeth, third molars, clinical indicated extraction or endodontic treatment. These sources have shown that DPSC being mesenchymal origin has great potency for various therapeutic and regenerative purposes in different fields of the Medicine like cartilage tissue engineering[25,26] and when compared DPSC lines derived from children, adolescents, adults and elderly donors, DPSCs derived from aged teeth displayed a decrease on their differentiation capacity[27].

Concerning the use of induced pluripotent stem cells (iPS) and embriological stem cells (ESCs), iPS cells have been considered as an alternative to human ES cells in order to avoid the ethical problems of embryo destruction. To produce patient-matched pluripotent cells but the efficiency of reprogramming varies significantly between different starting cell types mainly when the procedure to obtain iPS cells are compared to the DPSC process[28].

OA treated with common drugs, such as diacerein and glucosamine-chondroitin sulphate were previously reported in rats[29] and humans[30]. However, hDPSC intra-articular injection has not been described. Several preclinical and clinical studies with scaffolds and MSCs reported beneficial results in the treatment of chondral lesions but also demonstrated some disadvantages, such as the need for two surgical procedures and the expansion of chondrocytes in culture, which may lead to the production of fibrocartilage instead of hyaline cartilage[31,32]. To minimize or solve these problems, the effects of MSC intra-cellular injections in the treatment of OA were researched using animal models[33] and humans[34].

The first clinical trial phase I/II using 18 patients with discontinued pharmacological therapy used abdominal liposuction of MSCs that were grown and administered in three different doses in saline solution: low dose = 1×10^7, moderate dose = 5×10^7, and high dose = 1×10^8. After radiological, arthroscopic, histologic and immunohistochemical analyses for type I and II col-

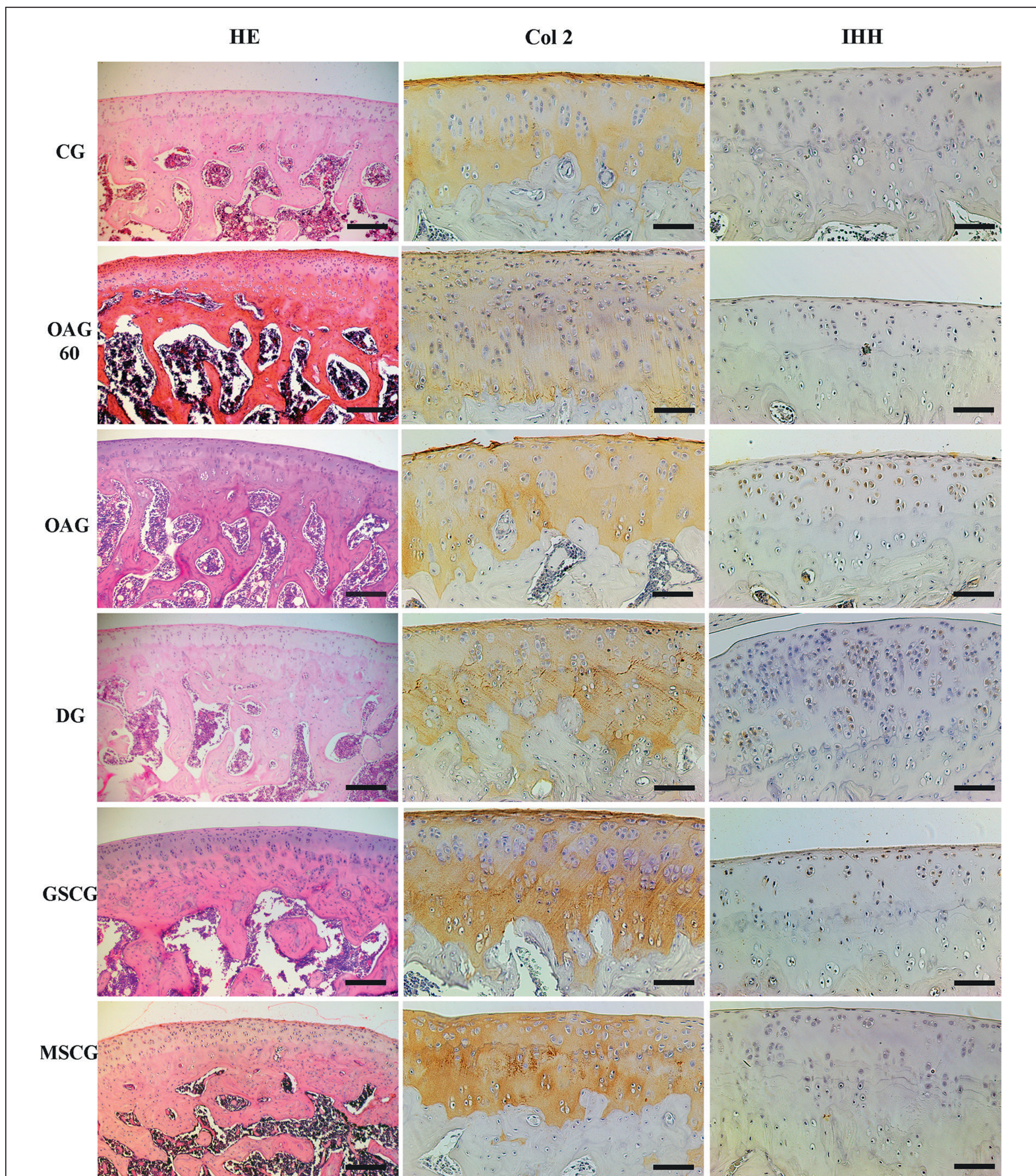

Figure 1. Histological sagittal sections of the rat femoral articular cartilage of the CG, OAG60, OAG, DG, GSCG and MSCG stained with HE, and the surface, middle, and deep layers were observed. Immunohistochemical expression of Col2 and IHH observed in articular layers (surface, middle, and deep) of the studied groups. HE scale bar: 200 µm, immunohistochemistry scale bar: 50 µm.

lagen, a reduction in pain and articular cartilage regeneration was observed with the high dose (1×10^8)[35]. These results are similar to the ones obtained in the present study both achieved the reestablishment of Col2 expression in the MSCG and a trend towards normalize of the number of chondrocytes and joint thickness was observed compare to the CG.

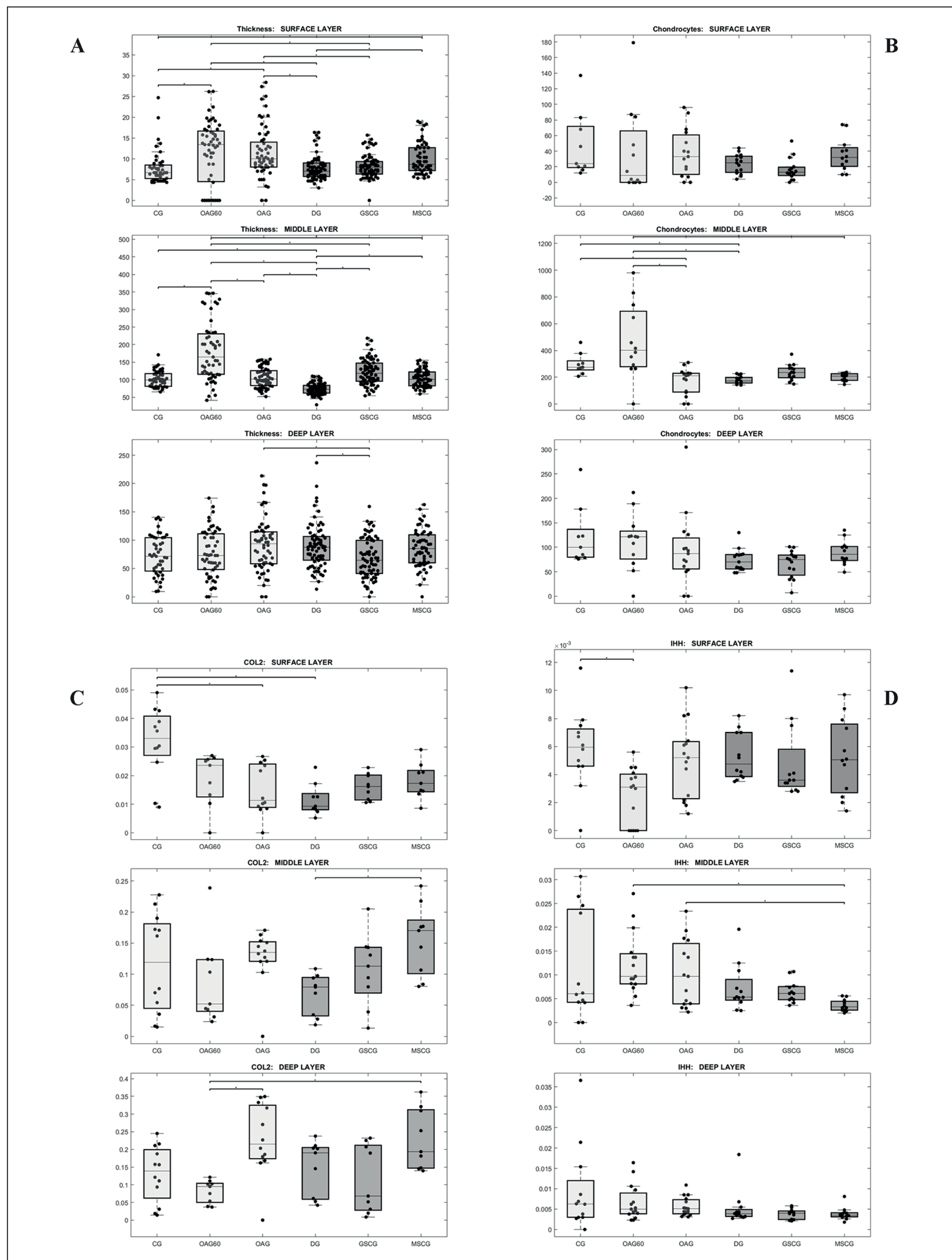

Figure 2. **A**, Measurement of articular cartilage thickness in all layers. In the surface layer CG *vs.* OAG ($p<0.0001$), OAG60 ($p<0.0003$) and MSCG ($p<0.0175$), OAG *vs.* DG ($p<0.0003$) and GSCG ($p<0.0063$), and DG *vs.*GSCG ($p<0.0184$). In the middle layer CG *vs.* OAG60 ($p<0.0004$), OAG *vs.* DG ($p<0.0002$), OAG60 *vs.* OAG ($p<0.0005$), GD ($p<0.0009$) and MSGC ($p<0.0001$); DG *vs.* GSCG ($p<0.0003$) and MSCG ($p<0.0001$). In the deep layer OAG *vs.* GSCG ($p<0.0315$) and GD *vs.* GSCG ($p<0.0328$). **B**, Quantification of chondrocytes in all articular cartilage. In the middle layer CG *vs.* OAG ($p<0.0493$) and DG ($p<0.0021$), OAG *vs.* OAG60 ($p<0.0022$), OAG60 *vs.* DG ($p<0.0001$) and MSCG ($p<0.0071$). **C**, Quantification of Col2 in the ECM in all articular layers. Immunostaining was observed as follows: surface layer CG *vs.* OAG ($p<0.0143$) and DG ($p<0.0006$); middle layer DG *vs.* MSCG ($p<0.0338$); deep layer OAG *vs.* OAG60 ($p<0.0137$) and MSCG ($p<0.0330$). **D**, IHH quantification in the chondrocyte nuclei and cytoplasm. Immunostaining was observed as follows: middle layer OAG *vs.* MSCG ($p<0.0389$) and OAG60 *vs.* MSCG ($p<0.0002$).

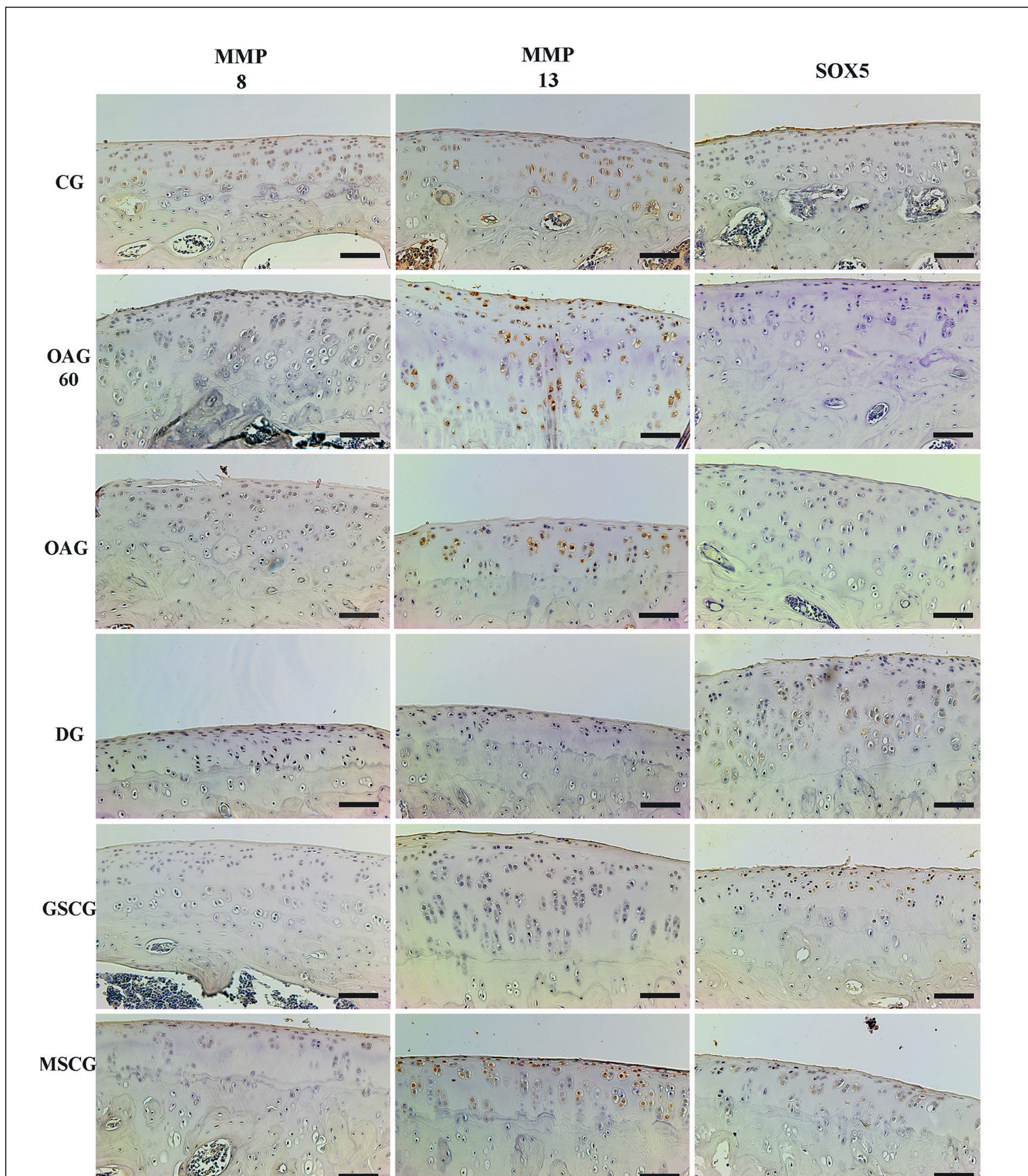

Figure 3. Immunohistochemical expression of MMP-8, MMP-13 and SOX-5 in articular layers (surface, middle, and deep) of the CG, OAG60, OAG, DG, GSCG and MSCG. Scale bar: 50 µm.

OA is a mesenchymal disease in which no chondral repair is observed because of MSC depletion and the reduction in proliferative capacity and cell differentiation[20]. In our MSCG, a reduction in MMP-8 expression and consistent MMP-13 expression were observed compared with that of CG. These results indicate the occurrence of direct chondrogenic differentiation and MSC regulation of the OA inflammatory process by inflammatory cytokine production[36].

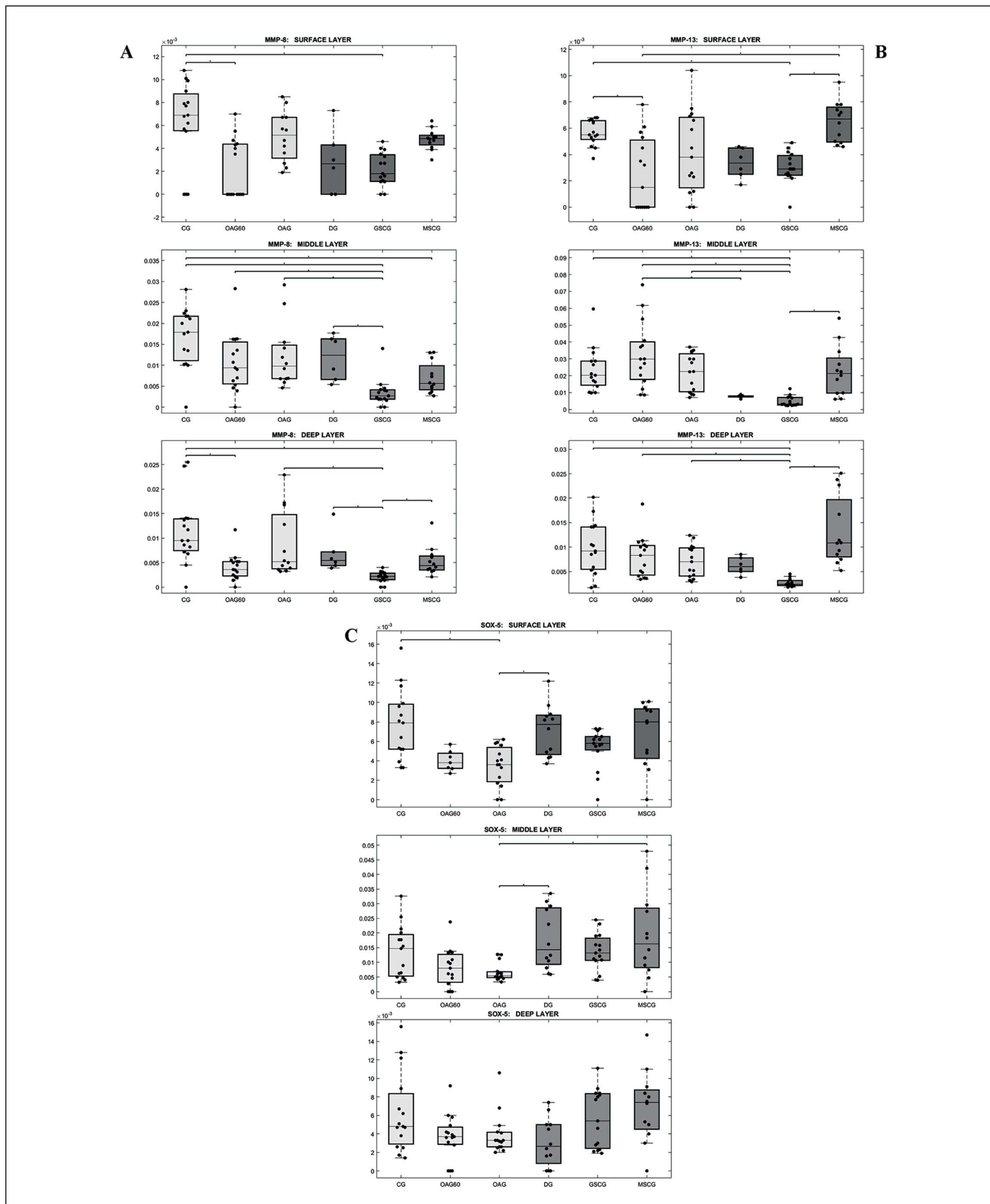

Figure 4. **A**, Quantification of MMP-8 expression in the chondrocyte nuclei and cytoplasm. Immunostaining was observed as follows: surface layer CG *vs.* OAG60 ($p<0.0033$) and GSCG ($p<0.0015$); middle layer CG *vs.* GSCG ($p<0.0001$); OAG *vs.* GSCG ($p<0.0037$) and OAG60 *vs.* GSCG ($p<0.0135$); CG *vs.* MSCG ($p<0.0211$) and GD x GSCG ($p<0.0321$); and deep layer CG *vs.* OAG60 ($p<0.0083$) and CG *vs.* GSCG ($p<0.0002$); OAG *vs.* GSCG ($p<0.0009$); DG *vs.* GSCG ($p<0.0197$) and GSCG *vs.* MSCG ($p<0.0442$). **B**, Quantification of MMP-13 expression in chondrocyte nuclei and cytoplasm. Immunostaining was significant as follows: surface layer CG *vs.* OAG60 ($p<0.0093$) and GSCG ($p<0.0103$), OAG60 *vs.* MSCG ($p<0.0008$) and GSCG *vs.* MSCG ($p<0.0009$); middle layer CG *vs.* GSCG ($p<0.0001$), OAG *vs.* GSCG ($p<0.0002$), OAG60 *vs.* GSCG ($p<0.0003$), and GSCG *vs.* MSCG ($p<0.0019$); and deep layer CG *vs.* GSCG ($p<0.0006$), OAG *vs.* GSCG ($p<0.0078$), OAG60 *vs.* GSCG (0.0014), and GSCG *vs.* MSCG ($p<0.0003$). **C**, Quantification of SOX-5 expression in chondrocyte cytoplasm and nucleus. Immunostaining was observed as follows: surface layer CG *vs.* OAG ($p<0.0068$) and OAG60 ($p<0.0062$); OAG *vs.* DG ($p<0.0190$) and OAG60 *vs.* DG ($p<0.0207$) in the middle layer OAG *vs.* MSCG (0.0437).

Analysis of IHH and SOX-5 expression in the CG, OAG and MSCG groups showed that OA causes a reduction in these transcription factors while MSCs enhance the expression of these markers, indicating that cell therapy led to chondrocyte hypertrophy and cartilage maintenance.

MSCs are attracted by chemokines present in diseased tissue[37]; therefore, intra-articular injection of MSCs facilitates adhesion to the chondral defect and leads to cell proliferation and tissue recovery. Our findings corroborate previous studies[38] in that the number of chondrocytes is similar between the CG and MSCG.

Our drug-treated groups showed effectiveness of the treatment of OA. An analysis of the diacerein effects in ECM degradation by MMPs showed that MMP-13 expression was reduced in the middle articular layer, which indicates that diacerein reduces chondral lesions caused by OA as previously described[39,40], and in chondrocyte culture[41].

The stimulating effect of diacerein in the production of Col2 was shown in humans and animals with OA[42]. These results differ from those of our study because we observed a reduction in Col2 expression in the superficial layer in comparison to the CG.

Our Col2 expression results indicated that the GSCG showed no difference compared to the other groups. A randomized double-blind placebo-controlled study involving 32 patients with OA to evaluate the effects of glucosamine-chondroitin conjugate showed low expression of Col2 degrading biomarkers, suggesting that the group treated with a combination of drugs showed protection of the main protein of articular cartilage[30].

Diacerein presents a more significant pain control effect than glucosamine/chondroitin sulphate; however, both treatments demonstrated efficacy in articular function[18]. The effects of glucosamine/chondroitin sulphate in human joints with OA[43] and the results presented in this study revealed that a decrease in MMPs had a chondroprotective effect. A comparison of the chondroprotective effects of glucosamine and diacerein in animals indicated that diacerein was more effective due to the articular movement amplitude[44].

Cartilage thickness is altered by different conditions, such as the transection of the anterior cruciate ligament in rats, which showed a decrease in the thickness of the superficial layer and increase in the intermediate and deep layers[45]. After OA, hypertrophy of the joint thickness is reported and represents an attempt to repair the damage caused by the chondral lesion[46]. In our findings, the superficial articular layer thickness was increased in the two groups, with OA being more evident in OAG60, whereas in the middle layer, there was increase of the thickness in the OAG60 and no significant differences among the CG, OAG, GSCG compared to the MSCG. The middle layer is an important measurement because it accounts for the majority of the total cartilage thickness and chronicity of the chondral lesion directly interferes with an increase in cartilage thickness.

Conclusions

Our proposed treatments demonstrated positive effects in the symptoms and characteristics of OA by changing the articular morphology, transcription factor and degrading enzyme expression, and ECM components. An analysis of these effects showed that hDPSCs were more effective in restoring structural (Col2) and morphological features, such as cellular hypertrophy (IHH), articular thickness and chondrocyte count. However, glucosamine-chondroitin sulphate demonstrated better results in inflammatory control (MMP-8 and MMP-13) and diacerein showed better results in factors associated with the maintenance of primordial cartilage (SOX-5).

Conflict of Interest

The author Marcelo Cavenaghi Pereira da Silva has received a research Grant from FAPESP (grant number 2018/11235-2). The other authors declare that they have no conflict of interest.

Acknowledgements

We are thankful to Drª. Adriana da Costa Neves from Instituto Butantan for technical assistance in the immunohistochemical procedures.

Funding

This work was supported by the São Paulo Research Foundation (FAPESP) [grant number 2018/11235-2], Fundação Butantan and Instituto Butantan, São Paul, Brazil.

References

1) Cisternas MG, Murphy L, Sacks JJ, Solomon DH, Pasta DJ, Helmick CG. Alternative methods for defining osteoarthritis and the impact on es-

timating prevalence in a US population-based survey. Arthritis Care Res (Hoboken) 2016; 68: 574-580.

2) Goldring MB, Goldring SR. Osteoarthritis. J Cell Physiol 2007; 213: 626-634.

3) Shiomi T, Lemaitre V, D'Armiento J, Okada Y. Matrix metalloproteinases, a disintegrin and metalloproteinases, and a disintegrin and metalloproteinases with thrombospondin motifs in non-neoplastic diseases. Int J Surg Pathol 2010; 60: 477-496.

4) Akiyama H. Control of chondrogenesis by the transcription factor Sox9. Mod Rheumatol 2008; 18: 213-219.

5) Smits P, Li P, Mandel J, Zhang Z, Deng JM, Behringer RR, Crombrugghe B De, Lafebvre V. The transcription factors L-Sox5 and Sox6 are essential for cartilage formation. Dev Cell 2001; 1: 277-290.

6) Nishimura R, Hata K, Takahata Y, Murakami T, Nakamura E, Yagi H. Regulation of cartilage development and diseases by transcription factors. J Bone Metab 2017; 24: 147-153.

7) Akiyama H, Chaboissier M-C, Martin JF, Schedl A, Crombrugghe B De. The transcription factor Sox9 has essential roles in successive steps of the chondrocyte differentiation pathway and is required for expression of Sox5 and Sox6. Genes Dev 2002; 16: 2813-2828.

8) Takigawa Y, Hata K, Muramatsu S, Amano K, Ono K, Matsuda A, Takada K, Nishimura R, Yoneda T. The transcription factor Znf219 regulates chondrocyte differentiation by assembling a transcription factory with Sox9. J Cell Sci 2010; 123: 3780-3788.

9) Wang W, Lian N, Li L, Moss HE, Wang W, Perrien DS, Elefteriou F, Yang X. Atf4 regulates chondrocyte proliferation and differentiation during endochondral ossification by activating Ihh transcription. Development 2009; 136: 4143-4153.

10) Lohberger B, Kaltenegger H, Weigl L, Mann A, Kullich W, Stuendl N, Leithner A, Steinecker-frohnwieser B. Mechanical exposure and diacerein treatment modulates integrin-FAK- MAPKs mechanotransduction in human osteoarthritis chondrocytes. Cell Signal 2019; 56: 23-30.

11) Fransen M, Agaliotis M, Nairn L, Votrubec M, Bridgett L, Su S, Jan S, March L, Edmonds J, Norton R, Woodward M, Day R. Glucosamine and chondroitin for knee osteoarthritis : a double-blind randomised placebo-controlled clinical trial evaluating single and combination regimens. Ann Rheum Dis 2015; 74: 851-858.

12) Bruyère O, Cooper C, Pelletier JP, Branco J, Luisa Brandi M, Guillemin F, Hochberg MC, Kanis JA, Kvien TK, Martel-Pelletier J, Rizzoli R, Silverman S, Reginster JY. An algorithm recommendation for the management of knee osteoarthritis in Europe and internationally: a report from a task force of the European Society for Clinical and Economic Aspects of Osteoporosis and Osteoarthritis (ESCEO). Semin Arthritis Rheum 2014; 44: 253-263.

13) Mendes AF, Caramona MM, Carvalho AP De, Lopes MC. Diacerhein and rhein prevent interleukin-1 b -induced nuclear factor- k B Activation by inhibiting the degradation of inhibitor k B- a. Pharmacol Toxicol 2002; 91: 22-28.

14) Pavelka K, Bruye O, Cooper C, Kanis JA, Leeb BF, Maheu E, Martel-pelletier J, Monfort J, Pelletier J, Rizzoli R, Reginster J. Diacerein : benefits, risks and place in the management of osteoarthritis. An opinion-based report from the ESCEO. Drugs Aging 2016; 33: 75-85.

15) Jawed H, Anjum S, Awan SI, Simjee SU. Anti-arthritic effect of GN1 , a novel synthetic analog of glucosamine, in the collagen-induced arthritis model in rats. Inflammation 2011; 60: 1113-1120.

16) Dalirfardouei R, Karimi G, Jamialahmadi K. Molecular mechanisms and biomedical applications of glucosamine as a potential multifunctional therapeutic agent. Life Sci 2016; 152: 21-29.

17) Imagawa K, Andrés M de, Hashimoto K, Pitt D, Itoi E, Goldring MB, Roach HI, Oreffo RO. The epigenetic effect of glucosamine and a nuclear factor-kappa B (NF-kB) inhibitor on primary human chondrocytes – Implications for osteoarthritis. Biochem Biophys Res Commun 2011; 405: 362-367.

18) Fidelix T, Macedo C, Maxwell L, Trevisani VFM. Diacerein for osteoarthritis. Cochrane Collab 2014; 2: 1-68.

19) Jüni P, Nartey L, Reichenbach S, Sterchi R, Dieppe PA, Egger M. Risk of cardiovascular events and rofecoxib : cumulative meta-analysis. Lancet 2004; 364: 2021-2029.

20) Barry F, Murphy M. Mesenchymal stem cells in joint disease and repair. Nat Rev Rheumatol 2013; 10: 584-594.

21) Nöth U, Steinert AF, Tuan RS. Technology insight : adult mesenchymal stem cells for osteoarthritis therapy. Nat Cinical Pract Rheumatol 2008; 4: 371-380.

22) Rocha FAC, Aragão Jr AGM, Oliveira RC, Pompeu MML, Vale MR, Ribeiro RA. Periarthritis promotes gait disturbance in zymosan-induced arthritis in rats. Inflamm Res 1999; 48: 485-486.

23) Kerkis I, Kerkis A, Dozortsev D, Stukart-Parsons GC, Massironi SMG, Pereira L V, Caplan AI, Cerruti HF. Isolation and characterization of a population of immature dental pulp stem cells expressing OCT-4 and other embryonic stem cell markers. Cells Tissues Organs 2006; 184: 105-116.

24) Da Cunha JM, Da Costa-Neves A, Kerkis I, Da Silva MCP. Pluripotent stem cell transcription factors during human odontogenesis. Cell Tissue Res 2013; 353: 435-441.

25) Martinez Saez D, Sasaki RT, Neves AD, da Silva MC. Stem cells from human exfoliated deciduous teeth: a growing literature. Cells Tissues Organs 2016; 202: 269-280.

26) Rosaian AS, Rao GN, Mohan SP, Vijayarajan M, Prabhakaran RC, Sherwood A. Regenerative ca-

pacity of dental pulp stem cells: a systematic review. J Pharm Bioallied Sci 2020; 12: S27-S36.

27) Wu W, Zhou J, Xu CT, Zhang J, Jin YJ, Sun GL. Derivation and growth characteristics of dental pulp stem cells from patients of different ages. Mol Med Rep 2015; 12: 5127-5134.

28. Kerkis I, Caplan AI. Stem cells in dental pulp of deciduous teeth. Tissue Eng Part B Rev 2012; 18: 129-138.

29) Li Z, Meng D, Li G, Xu J, Tian K, Li Y. Celecoxib combined with diacerein effectively alleviates osteoarthritis in rats via regulating JNK and p38MAPK signaling pathways. Inflammation 2015; 38: 1563-1572.

30) Nakasone Y, Watabe K, Watanabe K, Tomonaga A, Nagaoka I, Yamamoto T, Yamaguchi H. Effect of a glucosamine-based combination supplement containing chondroitin sulfate and antioxidant micronutrients in subjects with symptomatic knee osteoarthritis : a pilot study. Exp Adn Ther Med 2011; 2: 893-899.

31) Bornes TD, Jomha NM, Mulet-sierra A, Adesida AB. Hypoxic culture of bone ma rrow-derived mesenchymal stromal stem cells differentially enhances in vitro chondrogenesis within cell-seeded collagen and hyaluronic acid porous scaffolds. Stem Cell Res Ther 2015; 6: 1-17.

32) Knutsen G, Drogset J, Engebretsen L, Al. E. A randomized trial comparing autologous chondrocyte implantation with microfracture: finding at five years. J Bone Surg Am 2007; 89: 2105-2112.

33) Mokbel AN, Tookhy OS El, Shamaa AA, Rashed LA, Sabry D, Sayed AM El. Homing and reparative effect of intra-articular injection of autologus mesenchymal stem cells in osteoarthritic animal model. BMC Musculoskelet Disord 2011; 12: 259.

34) Emadedin M, Aghdami N, Taghiyar L, Fazeli R, Moghadasali R, Jahangir S, Farjad R, Eslaminejad MB. Intra-articular injection of autologous mesenchymal stem cells in six patients with knee osteoarthritis. Arch Iran Med 2012; 15: 422-428.

35) Jo C, Lee Y, Shin W, Kim H, Chai J, Jeong E, Kim J, Shim H, Shin J, Shin I, Ra J, Oh S, Yoon K. Intra-articular injection of mesenchymal stem cells for the treatment of osteoarthritis of the knee: a prof-of-concept clinical trial. Stem Cells 2014; 32: 1254-1266.

36) Acharya C, Adesida A, Zajac P, Mumme M, Riesle J, Martin I, Barbero A. Enhanced chondrocyte proliferation and mesenchymal stromal cells chondrogenesis in coculture pellets mediate improved cartilage formation. J Cell Physiol 2012; 227: 88-97.

37) Sordi V. Mesenchymal stem cell homing capacity. Transplantation 2009; 87: S42-45.

38) Sato M, Uchida K, Nakajima H, Miyazaki T, Guerrero AR, Watanabe S, Roberts S, Baba H. Direct transplantation of mesenchymal stem cells into the knee joints of Hartley strain guinea pigs with spontaneous osteoarthritis. Arthritis Res Ther 2012; 14: 1-9.

39) Martel-Pelletier J, Mineau F, Jolicoeur F, Cloutier J, Pelletier J. In vitro effects of diacerhein and rhein on interleukin 1 tumor necrosis factor-alpha systems in human osteoarthritic synovium and chondrocytes. J Rheumatol 1998; 25: 753-762.

40) Legendre F, Bogdanowicz P, Martin G, Domagala F, Leclercq S, Pujol J, Ficheux H. Rhein , a diacerhein-derived metabolite, modulates the expression of matrix degrading enzymes and the cell proliferation of articular chondrocytes by inhibiting ERK and JNK-AP-1 dependent pathways. Clin Exp Rheumatol 2007; 25: 546-555.

41) Álvarez-Soria M, Herrero-beaumont G, Sáchanz-Pernaute O, Bellido M, Largo R. Diacerein has a weak effect on the catabolic pathway of human osteoarthritis synovial fibroblast — comparison to its effects on osteoarthritic chondrocytes. Rheumatology 2008; 47: 627-633.

42) Wang P, Song J, Qian D. CTX - II and YKL - 40 in early diagnosis and treatment evaluation of osteoarthritis. Exp adn Ther Med 2019; 17: 423-431.

43) Calamia V, Mateos J, Fernández-Puente P, Lourido L, Rocha B, Fernández-Costa C, Montell E, Vergés J, Ruiz-Romero C, Blanco FJ. A pharmacoproteomic study confirms the synergistic effect of chondroitin sulfate and glucosamine. Scient 2014; 4: 1-10.

44) Toegel S, Huang W, Piana C, Unger FM, Wirth M, Goldring MB, Gabor F, Viernstein H. Selection of reliable reference genes for qPCR studies on chondroprotective action. BMC Mol Biol 2007; 8: 1-10.

45) Panula HE, Hyttinen MM, Arokoski JPA, Långsjö TK, Pelttari A, Kiviranta I, Helminen HJ. Articular cartilage superficial zone collagen birefringence reduced and cartilage thickness increased before surface fibrillation in experimental osteoarthritis. Ann Rheum Dis 1998; 57: 237-245.

46) Brandt KD, Myers SL, Burr D, Albrecht M. Osteoarthritic changes in canine articular cartilage, subchondral bone, and synovium fifty-four months after transection of the anterior cruciate ligament. Arthritis Rheum 1991; 34: 1560-1570.