Heliyon 8 (2022) e10857

Contents lists available at ScienceDirect

Heliyon

journal homepage: www.cell.com/heliyon

Research article

Mesenchymal stem cells-derived microvesicles versus platelet-rich plasma in the treatment of monoiodoacetate-induced temporomandibular joint osteoarthritis in Albino rats

Nermeen AbuBakr [a,b,*], Amira E. Fares [c], Abeer Mostafa [d], Dina B.E. Farag [a]

[a] Department of Oral Biology, Faculty of Dentistry, Cairo University, Cairo, Egypt
[b] Stem Cells and Tissue Engineering Unit, Faculty of Dentistry, Cairo University, Cairo, Egypt
[c] Department of Oral Biology, Faculty of Dentistry, Suez University, Suez, Egypt
[d] Medical Biochemistry and Molecular Biology Department, Faculty of Medicine, Cairo University, Cairo, Egypt

ARTICLE INFO

Keywords:
Temporomandibular joint
Osteoarthritis
Platelet-rich plasma
Microvesicles

ABSTRACT

Temporomandibular joint osteoarthritis (TMJ-OA) is a serious disease, designated by severe joint pain and dysfunction. Limitations of current therapeutics have led to an increased interest in regenerative strategies. Recently, the non-surgical treatment of OA has seen increased use of biologic injectable therapies like mesenchymal stem cells (MSCs) and platelet-rich plasma (PRP). Although these biotherapies represent an admirable effort, more studies are necessary to determine their efficacy. Thus, the aim of this study was to assess the curative potential of a single intra-articular injection of bone marrow MSCs-derived microvesicles (BM-MSCs-MVs) versus a single intra-articular injection of PRP in monoiodoacetate (MIA)-induced TMJ-OA model in Albino rats. Forty-eight male rats were used. A single intra-articular unilateral MIA injection was utilized to induce TMJ-OA. One week post induction, rats were sorted into 3 groups (16 rats each): group (I): received no treatment, groups (II) & (III): received BM-MSCs-MVs and PRP respectively. Scarification was done at 2 and 4 weeks from onset of treatment. Histological changes of the condylar TMJ were examined with H&E staining. Expression of *IL-1β*, *TNF-α*, *NF-κB*, *MMP-13*, *MMP-3*, and *collagen II* markers was detected using real-time PCR. Histologically, the osteoarthritic group exhibited degenerated condylar tissues which were aggravated at 4 weeks. Oppositely, a marked improvement in the condylar TMJ histology was noticed in both the BM-MSCs-MVs-and PRP-treated groups at both time intervals. Additionally, the treated groups showed a decrease in *IL-1β*, *TNF-α*, *NF-κB*, *MMP-13* and *MMP-3* and an increase in *collagen II* genes expression in contrast to the untreated group. Moreover, this difference was significant in the BM-MSCs-MVs group as compared to the PRP-treated group. Our results concluded that BM-MSCs-MVs as well as PRP treatments were able to target the key pathological features in OA, mainly inflammation and matrix degradation, and helped in restoring condylar structure in TMJ-OA rat model. However, BM-MSCs-MVs treatment exhibited more efficient therapeutic potential as compared to PRP treatment.

1. Introduction

Temporomandibular joint (TMJ) disorders are a collection of illnesses that result in discomfort and dysfunction in the jaw joint and the muscles controlling jaw movements. Such disorders have numerous and complex etiologies, including occlusal abnormalities, psychological, social, cognitive, and systemic factors [1, 2]. It was reported that patients with systemic sclerosis had trouble opening their mouth, besides difficulties in mandibular movements excursion, among other indicators of TMJ dysfunction [3]. Moreover, patients with juvenile idiopathic arthritis can suffer from unstable occlusion, changes in masticatory function, asymmetric loading of the joints and muscles, and TMJ pain [4].

TMJ osteoarthritis (OA) is a common form of TMJ disorders. It is a debilitating illness that is marked by progressive cartilage deterioration, synovitis, subchondral bone remodelling, and chronic pain [5]. The majority of TMJ-OA cases have a multifactorial or unclear etiology. One of the main causes of this disease is overloading of the TMJ, which involves significant malocclusion, muscle overuse, and skeletal jaw asymmetry [6]. The patients of TMJ-OA suffer from TMJ arthralgia, limited

* Corresponding author.
E-mail address: nermeen.abubakr@dentistry.cu.edu.eg (N. AbuBakr).

https://doi.org/10.1016/j.heliyon.2022.e10857
Received 12 February 2022; Received in revised form 14 March 2022; Accepted 26 September 2022

mandibular range of motion, impairment in chewing function, clicking sound, and stiffness [7].

Most of the traditional non-surgical clinical treatments for TMJ-OA including, occlusal splints, physical therapies, arthrocentesis, and nonsteroidal anti-inflammatory drugs, are directed towards alleviating pain and slowing the progression of the disease. However, these treatments can't fully heal deteriorated cartilage or subchondral bone lesions, as well as disc degeneration [8, 9].

Mesenchymal stem cells (MSCs) served as a precious cell reservoir for repair and regeneration of the TMJ in different animal models [10, 11]. The majority of their therapeutic effects are attributed to the secretion of a wide spectrum of mediators with potent regenerative, anti-inflammatory, and immunomodulatory properties [12, 13]. These mediators are transported within extracellular vesicles (EVs) released by the MSCs [14].

MSCs-EVs' regenerative activity has been reported in several animal models, including those for myocardial infarction, brain, lung, liver and acute renal injury [15], rheumatic diseases [16], fracture healing [17], and neurodegeneration [18]. When compared to direct cell transplantation, EVs are considerably simpler, safer, more practical, and easier to manipulate [19]. There are various forms of EVs, however the most typically reported vesicles are microvesicles, exosomes, and apoptotic bodies [20].

Microvesicles (MVs) are a diverse population of spherical structures with diameters ranging from 50–1000 nm [21]. Their biosynthesis necessitates vertical transportation of molecular content to the cell membrane, membrane lipid reallocation, and the utilization of contractile machinery at the surface to allow for vesicle pinching [22]. Researchers have realized the important role of MVs in modifying the extracellular environment and intercellular signaling via their capability to convey biologically active compounds [23].

Platelet-rich plasma (PRP) is blood plasma composed of concentrated platelets. It represents a promising regenerative therapy for injuries in the orthopaedic field, having anti-inflammatory, analgesic, and antibacterial properties [24, 25]. It has been used in intra-articular injections to treat temporomandibular disorders in a number of clinical trials with encouraging results where the included patients had to have imaging verified TMJ osteoarthritis or TMJ dysfunction. However, those with systemic illnesses were excluded [26, 27, 28]. PRP has been reported to restore intra-articular hyaluronic acid, enhance chondrocyte glycosaminoglycan synthesis, balance joint angiogenesis, and act as a scaffold for the migration of stem cells [26].

Due to the limitations of the current TMJ disorder therapies, there has been a surge in interest in regenerative approaches, which needs more supportive studies to draw a definite conclusion about their efficacy. So far, preclinical in-vivo studies comparing the effectiveness of different kinds of biotherapies in relation to each other in TMJ osteoarthritic models are very limited. Also, the ability of a single injection to induce positive therapeutic effect needs to be clarified. Thus, the aim of the present research was to assess and compare the curative potential of a single intra-articular injection of bone marrow (BM)-MSCs-derived MVs and PRP on TMJ-OA in Albino rats, through histopathological and quantitative real-time PCR (qRT-PCR) examination techniques.

2. Materials and methods

2.1. Preparation, isolation and identification of BM-MSCs

Extirpated bone marrow cells from 5 male Albino rats' (6 weeks old) tibias were washed in phosphate buffer saline (PBS) three times. 15 ml of extirpated bone marrow cells were layered over 15 mL Ficoll-Paque™ (Gibco/Invitrogen, USA) then centrifuged (400*g*, 35 min). After aspiration of the upper cell layer, the undisturbed mononuclear cell layer was aspirated, washed twice in PBS, and centrifuged (200*g*, 10 min). The isolated cells were then grown in RPMI-1640 media supplemented with 0.5% penicillin and streptomycin, 10% FBS (Gibco/Invitrogen, USA) and kept at 37 °C in a cell culture incubator (5% CO2) until reaching 80–90% confluence. BM-MSCs were then detached by trypsin/EDTA for 5–10 min at 37 °C and sub-cultured in new plates. BM-MSCs at third passage were used. MSCs were recognized by their shape. They were suspended (1×10^6 cells/ml) for further identification by fluorescence activated cell sorting (FACS) through assessment of $CD90^+$, $CD105^+$ positivity, and $CD14^-$ negativity [29, 30].

2.2. Preparation, isolation and characterization of BM-MSCs-MVs

MVs were acquired from the supernatant of BM-MSCs (5×10^6 cells/ml), cultured in RPMI without FBS and the cells were starved for 24 h, using a filtration/centrifugation-based protocol. Centrifugation at 2000*g* for 20 min at 4 °C was used to remove cellular debris. The supernatant was ultracentrifuged at 100000*g* for 1 h at 4 °C to pellet MVs, after which it was washed in serum-free media and subjected to another round of ultracentrifugation. The resulting pellets containing MVs were washed once with sterile PBS [31]. Protein concentration was quantified by Bradford protein assay kit (Thermo Scientific, USA). MVs were identified using transmission electron microscope (TEM) and by detecting CD63 and CD81 via enzyme-linked immunosorbent assay (ELISA).

2.3. Characterization of MVs using TEM

MVs were washed, then fixed for 2 h with 2.5% glutaraldehyde. 1% osmium tetroxide in 0.1 M PB was employed for 1 h for post-fixation. After rinsing with sodium maleate buffer, the fixed MVs were subjected to dehydration in graded ethanol followed by infiltration and embedding in beam capsules. Sections were mounted onto a carbon-formvar grid, negatively stained for 1 min with 1% uranyl acetate, and observed by TEM (JEOL, JEM -2100, Japan) [32].

2.4. Characterization of MVs using ELISA

Binding buffer (200 μl) (System Biosciences Inc.) was applied to the pellets. Pellets were then incubated for 10 min at room temperature. 50 μl of each marker's preparation was then applied to microtiter plate's wells. The plate was incubated overnight at 37 °C. Following washing with a working buffer (System Biosciences Inc.), 50 μl of anti-CD63 and anti-CD81 (ELISA kits) (System Biosciences Inc.) were added to the wells, and then incubated at 37 °C for 60 min. After another wash, the plate was incubated for an hour at room temperature with 50 μl of horseradish peroxidase (HRP) enzyme-linked secondary antibody (goat anti-rabbit). To stop the reaction, a colorimetric substrate and stop solution from the kit were applied. The amount of protein was calculated by reading the optical density at 450 nm on a microplate reader (Molecular Devices; Sunnyvale, CA). Protein amount was compared to the standard curve generated by the kit. For each concentration (pg/ml) of the two investigated proteins, the results were expressed as mean ± SD [32].

2.5. Preparation of PRP

A total of 3 ml of blood was taken from the retro-orbital vein of previously anesthetized animals. The tubes containing the blood samples were centrifuged at 500*g* for 10 min resulting in three layers; inferior red cells, intermediate white cells, and superficial plasma. The upper portion of the supernatant containing platelets and plasma was collected into fresh tubes and centrifuged for 10 min at 2200*g*. The resulting upper poor-platelet plasma was separated and stored in different tubes, while the residual material bearing the platelet pellet was gently aspirated and placed in a sterile tube originating the platelet-rich plasma portion [33].

2.6. Experimental study design

2.6.1. Animals

All animal protocols were carried out according to the Institutional Animal Care and Use Committee (IACUC) of Cairo University (approval number; CU/III/F/31/19). In this investigation, forty-eight adult male

Albino rats (3–4 months), weighing 150–170 gm, were employed. The animals were housed in a climate-controlled setting with a 12-hour light/dark cycle, as well as easy access to water and food.

2.6.2. Induction of TMJ-OA model

An intraperitoneal injection of a ketamine and xylazine mixture was used to anaesthetize all of the experimental rats. OA was induced with a single intra-articular injection of 2 mg/joint of monosodium iodoacetate (MIA) (Sigma-Aldrich Chemical Co., St. Louis, USA) in 50 μL sterile 0.9% saline solution into each rat's right TMJ [34].

2.6.3. Animal grouping

One week after OA induction, the animals were randomly separated into 3 groups, each with $n = 16$. Group I (OA) received 50 μL PBS. Group II (OA + MVs) received 100 μg MVs/50 μL PBS [35]. Group III (OA + PRP) received 50 μL PRP [36]. The injections were administrated as a single intra-articular injection in the right TMJ.

2.6.4. Animals' scarification and tissue preparation

At 2 and 4 weeks post-treatment, animals from each experimental group (n = 8) were euthanized, using anaesthetic overdose. The heads were then sagittally dissected. The rats' TMJ tissue specimens were then collected.

2.6.4.1. Histopathological examination. The obtained samples were fixed in 4% paraformaldehyde, and demineralized in 15% ethylene diamine tetra-acetic acid (EDTA). The samples were then dehydrated in increasing grades of alcohol, cleared in xylol, and embedded in paraffin blocks. Haematoxylin and Eosin (H&E) stained sections (4–5 mm thick) were put on standard glass slides for histological assessment.

2.6.4.2. Quantitative real-time PCR. The mRNA gene expression of interleukin-1-beta (*IL-1β*), tumour necrosis factor-alpha (*TNF-α*), nuclear factor kappa B (*NF-κB*), matrix metalloproteinase-13 (*MMP-13*), matrix metalloproteinase-3 (*MMP-3*), and *collagen II* was investigated. *GADPH* was used as the internal control. The condylar tissue samples were homogenized and the total RNA was extracted in accordance with the manufacturer's instructions utilizing a Qiagen extraction kit (Qiagen, USA). Total RNA was converted to complementary DNA (cDNA) using cDNA reverse transcription kit (Fermentas, USA).

Subsequent real-time qPCR amplification and analysis were performed. SYBR Green Master Mix (Applied Biosystems), and gene-specific primer pairs (Table 1) were used in the reaction. Amplification protocol involved: enzyme activation (95 °C, 15 min) then followed by 40 cycles of denaturation (95 °C, 30 s) and annealing/extension (60 °C, 60 s). The comparative threshold cycle approach was used to calculate the relative expression of the studied genes using Applied Bio system software [37].

2.7. Statistical analysis

Values were analyzed using SPSS (Statistical Package for Scientific Studies, SPSS, Inc., Chicago, IL, USA) computer software version 22. Data were expressed as mean ± SD. Data normality was explored using the Kolmogorov-Smirnov test. Results revealed normally distributed data. Thus, analysis of variance (ANOVA) test followed by Post-hoc Tukey test were employed to compare between groups. Comparisons between two durations within the same group were analyzed using a paired t-test. Correlation between various studied variables was performed using Pearson's correlation. P value < 0.05 was considered as statistically significant.

3. Results

3.1. Characterization of BM-MSCs

BM-MSCs were recognized by their spindle-fusiform appearance (Figure 1a). Furthermore, FACS analysis indicated the purity of BM-MSCs where they showed positivity for $CD90^+$ (99.3%), $CD105^+$ (97.5%), and negativity for $CD14^-$ (3.6%) (Figure 1b).

Table 1. Primers' sequence of examined genes.

Gene	Primer sequence from 5′- 3′	Gene bank accession number
IL-1β	Forward: TGGACCTTCCAGGATGAGGACA Reverse: GTTCATCTCGGAGCCTGTAGTG	NM_008361
TNF-α	Forward: CTCTTCTGCCTGCTGCACTTTG Reverse: ATGGGCTACAGGCTTGTCACTC	NM_000594
NF-κB	Forward: AACGGCCTTCTGCACAGCGG Reverse: CCAGGTAACAGGGCGTGGCC	NM_001024872.1
MMP-13	Forward: TGCGGTTCACTTTGAGGACA Reverse: TCTTCTATGAGGCGGGGATA	M60616.1
MMP-3	Forward: CACTCACAGACCTGACTCGGTT Reverse: AAGCAGGATCACAGTTGGCTGG	NM_002422
Collagen II	Forward: CCTGGCAAAGATGGTGAGACAG Reverse: CCTGGTTTTCCACCTTCACCTG	NM_001844
GAPDH	Forward: ACAGTCCATGCCATCACTGCC Reverse: GCCTGCTTCACCACCTTCTTG	NG_009348.3

3.2. Characterization of BM-MSCs-MVs

BM-MSCs-MVs were characterized by their size (average diameter 79 nm) and their spherical shape as identified under TEM (Figure 2a). In addition, MVs were positive for CD63 (179.4 ± 5.662 Pg/ml), and CD81 (241.6 ± 8.817 Pg/ml) markers as shown by ELISA (Figure 2b).

3.3. Histopathological results

3.3.1. At 2 weeks

The articular surface of the rat condylar TMJ of group I (OA) revealed the condyle with abnormal configuration. The fibrocartilaginous layer demonstrated disrupted cellular and fibrillar arrangement. The cartilaginous layer displayed focal regional loss of chondrocytes as well as areas of vacuolization and atrophy. Cartilage chondrocytes showed abnormal arrangement, clustering, as well as signs of degeneration with empty lacunae. The subchondral bone presented distorted bone architecture with absence of regular bone trabecular alignment. Most of the osteocytes were degenerated and showed disorganized alignment. Widened bone marrow cavities with apparent loss of osteoblastic lining as well as abnormal marrow fibrosis was noticed (Figure 3a).

Regarding group (II) (OA + MVs), well organized fibrocartilaginous layer overlying relatively thin but properly aligned hyaline cartilage layer was observed. The chondrocytes displayed well stained basophilic nuclei. Some mitotic figures were also observed in the cartilaginous layer. Subchondral bone demonstrated closely packed lamella enclosing organized osteocytes in their lacunae. Multiple resting lines were also detected. Osteoblasts were seen partially lining the intervening marrow cavities. Most of the marrow cavities enclosed obvious cellular infiltrate (Figure 3b).

In group III (OA + PRP), the fibrocartilaginous tissue demonstrated dense acellular fibrous layer that seemed to be hyalinized. The cartilaginous layer displayed appropriate chondrocyte arrangement. Empty chondrocytic lacunae were observed in some areas. The underlying subchondral bone assumed relatively regular appearance enclosing few osteocytes. Widened bone marrow cavities enclosing high cellular infiltrate, extravasated red blood cells, and vascular congestion were also detected (Figure 3c).

3.3.2. At 4 weeks

Marked histological distortion in the microstructure of the condylar TMJ of group I (OA) was observed. The fibrocartilaginous tissue showed abnormal fibers' orientation and disrupted cellular arrangement. Complete loss of the underlying hyaline cartilaginous layer was

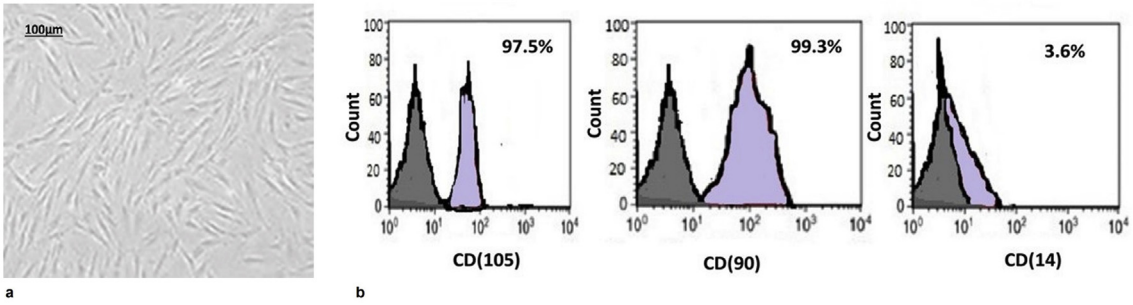

Figure 1. **(a)** BM-MSCs, having spindle fusiform shape under inverted microscope [scale bar = 100 µm]. **(b)** FACS analysis of BM-MSCs, positive for $CD105^{+}$, $CD90^{+}$ and negative for $CD14^{-}$.

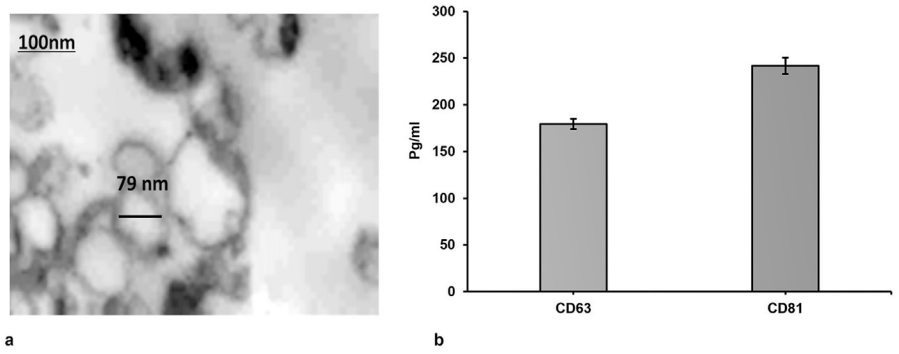

Figure 2. **(a)** TEM photomicrograph revealing the size and morphology of BM-MSCs-MVs [scale bar = 100 nm]. **(b)** A graph showing the expression of CD63 and CD81 protein markers as examined by ELISA. The values are presented as mean ± SD for each concentration (Pg/ml) of the 2 examined proteins.

demonstrated. The structure of the subchondral bone was obviously deteriorated. Fibrous tissue infiltration was seen advancing into some areas of subchondral bone replacing distorted bony trabeculae. Other bone areas enclosed irregular oriented few osteocytes where some lacunae were empty. Spaces resembling marrow cavities were also observed where most of them were obscured by fibrous tissue as well as extravasated red blood cells (Figure 3d).

In group II (OA + MVs), a fibrocartilaginous layer with evenly aligned fibers and cells was seen overlying a well-developed cartilage layer. The chondrocytes were intact and properly oriented. The subchondral bone displayed uniform configuration with regular trabeculation enclosing orderly arranged osteocytes. Most of the bone marrow cavities were outlined by osteoblastic cells and filled with fibrocellular tissue (Figure 3e).

A thick fibrocartilaginous layer with focal matrix discontinuity was observed in group III (OA + PRP). Its cellular and fiber orientation were relatively disoriented in many areas. The underlying cartilaginous layer was well identified with its characteristic chondrocytes. In some areas, the arrangement of the chondrocytes was disordered. The subchondral bone enclosed osteocytes in their lacunae. The bone trabecular boundaries enclosed multiple widened marrow cavities lined by osteoblasts and partially filled with fibrocellular tissue (Figure 3f).

3.4. Quantitative real-time PCR analysis

A difference was assessed in the expression of genes associated with pro-inflammation (*IL-1β*, *TNF-α* and *NF-κB*), matrix degradation (*MMP-13* and *MMP-3*) and matrix synthesis (*collagen II*) at 2 and 4 weeks, between all groups using ANOVA ($p < 0.001$). Multiple pairwise group comparisons were then performed within each time interval (Figure 4).

Within group I (OA), there was an increase in *IL-1β* ($p = 0.001$), *TNF-α* ($p = 0.017$), *NF-κB* ($p = 0.015$), *MMP-13* and *MMP-3* ($p = 0.017$) expression at 4 weeks compared to 2 weeks. On the other hand, the expression was decreased with time within group II (OA + MVs) and III (OA + PRP); *IL-1β* ($p = 0.017$, $p = 0.002$), *TNF-α* ($p < 0.001$, $p = 0.001$), *NF-κB* ($p < 0.001$, $p = 0.008$), *MMP-13* ($p = 0.038$, $p = 0.004$) and *MMP-3* ($p = 0.004$, $p < 0.001$) respectively (Figure 4a-e).

Relative to OA group, mRNA gene expression of *IL-1β*, *TNF-α*, *NF-κB*, *MMP-13* and *MMP-3* in the OA + MVs group was decreased at 2 and 4 weeks ($p < 0.001$). Similarly, OA + PRP group showed a decrease in *IL-1β* ($p = 0.002$ at 2 weeks and $p < 0.001$ at 4 weeks), *TNF-α*, *NF-κB*, *MMP-13* and *MMP-3* ($p < 0.001$ at 2 and 4 weeks) as compared to OA group (Figure 4a-e).

For *IL-1β* gene expression in MVs treated group, it was lower compared to that of PRP treated group, however this decrease was not statistically significant at 2 weeks ($p = 0.079$). By 4 weeks, this decrease was statistically significant ($p = 0.021$). Regarding other markers, a decrease in their expression was recorded in MVs group as compared to PRP group during 2 and 4 weeks time intervals (*TNF-α*, $p = 0.025$, $p = 0.007$; *NF-κB*, $p < 0.001$, $p = 0.038$; *MMP-13*, $p = 0.039$, $p = 0.03$ and *MMP-3*, $p = 0.01$, $p = 0.002$) respectively (Figure 4a-e).

For *collagen II* gene expression, within group I, there was a decrease in its expression at 4 weeks compared to 2 weeks ($p = 0.01$) while, within group II and III, the expression was increased with time ($p = 0.001$ and $p < 0.001$ respectively). Moreover, an increase in *collagen II* expression in OA + MVs group ($p < 0.001$ at 2 and 4 weeks) compared to OA group was evident. In parallel, the OA + PRP group demonstrated an increase in the gene expression ($p = 0.003$ at 2 weeks and $p < 0.001$ at 4 weeks) relative to OA group. The increase in *collagen II* expression in MVs group as compared to PRP group was non-significant at 2 weeks ($p = 0.1$), however, it was significant at 4 weeks ($p = 0.02$) (Figure 4f).

At 2 and 4 weeks time interval, Pearson's correlation among the studied genes showed a positive correlation present among *IL-1β*, *TNF-α*, *NF-κB*, *MMP-13* and *MMP-3*. While *collagen II* showed a negative correlation with *IL-1β*, *TNF-α*, *NF-κB*, *MMP-13* and *MMP-3* (Tables 2 and 3).

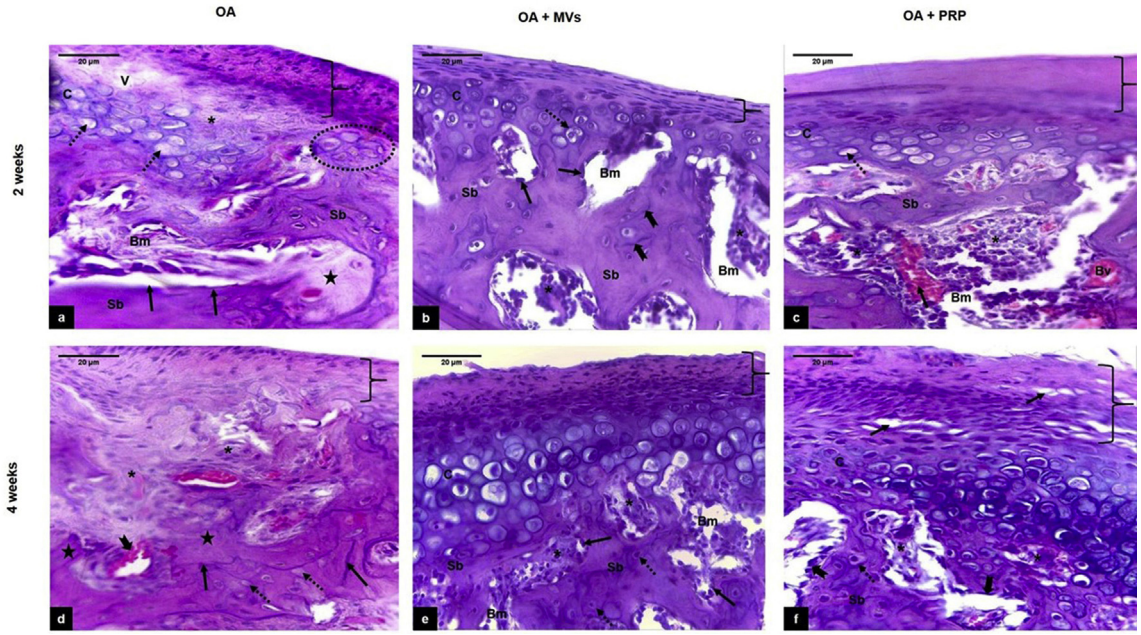

Figure 3. Photomicrographs of sagittal sections in the articular surface of rat condylar TMJ **(a)** Disrupted fibrocartilaginous layer (bracket), matrix vacuolization (V), misaligned cartilaginous layer (C), regional loss of chondrocytes (asterisk), chondrocytes clustering (dotted circle), degenerated chondrocytes (dotted arrow), distorted subchondral bone (Sb), widened bone marrow cavities (Bm), loss of osteoblastic lining (arrows), abnormal marrow fibrosis (star). **(b)** organized fibrocartilaginous layer (bracket), properly aligned cartilaginous layer (C), mitotic figure (dotted arrow), subchondral bone (Sb), resting lines (notched arrows), marrow cavities (Bm), partial osteoblastic lining (arrows), marrow cellular infiltrate (asterisks). **(c)** fibrocartilaginous layer with cellular atrophy (bracket), relatively aligned cartilaginous layer (C), empty chondrocytic lacunae (dotted arrow), subchondral bone (Sb), widened marrow cavities (Bm), marked marrow cellular infiltrate (asterisks), extravasated red blood cells (arrow), congested blood vessels (Bv). **(d)** disrupted fibrocartilaginous layer (bracket); Note: complete loss of underlying cartilaginous layer, distorted bone trabeculae (stars), fibrillar infiltration (asterisks), empty osteocytic lacunae (dotted arrows), resting lines (arrows), extravasated red blood cells (notched arrow). **(e)** well-arranged fibrocartilaginous layer (bracket), well developed cartilaginous layer (C), subchondral bone (Sb) with osteocytes in their lacunae (dotted arrows), marrow cavities (Bm), osteoblastic lining (arrows), fibrocellular tissue (asterisks). **(f)** fibrocartilaginous layer (bracket) with focal matrix discontinuity (arrows), relatively disordered cartilaginous layer (C), subchondral bone (Sb), osteocytes (dotted arrow), marrow cavities lined by osteoblasts (notched arrows), fibrocellular tissue (asterisks) **(a-f; H&E, Orig. Mag. × 400)**.

4. Discussion

TMJ disorders are a broad category of clinical conditions that affect the TMJ, and other structures connected to the masticatory system. They have a range of etiologies, including congenital, inflammatory, and traumatic [38]. Due to the shortcomings of existing treatments for TMJ problems, interest in regenerative approaches; combining cells, implantable scaffolds, and carefully targeted bioactive chemicals has increased [39]. TMJ-OA is a serious form of TMJ dysfunctions [40]. The use of biologic injectable treatments such as MSCs and PRP in the non-surgical therapy of OA have recently become popular [41]. Although these biotherapies are a commendable effort, further research is required to determine their effectiveness.

In this research, the therapeutic potential of a single intra-articular injection of BM-MSCs-MVs in MIA-induced TMJ-OA model was assessed and compared with a single intra-articular PRP injection. We demonstrated that injection of BM-MSCs-MVs could effectively restore the damaged condylar tissue better than PRP.

Male rats were employed in this study to prevent hormonal fluctuations from affecting the findings since the expression of estrogen receptors α and β in TMJ suggests that the TMJ is an estrogen target. Additionally, it was established that estrogen accelerated the progression of TMJ-OA as it caused cartilage deterioration, subchondral bone sclerosis, and erosion in a rat model of induced TMJ-OA [42].

In this investigation, the TMJ-OA model was set up by injection of MIA. The intra-articular injection paradigm of MIA is frequently utilized for pain research and therapeutic intervention efficacy evaluation, since this chemical model closely resembles the alterations seen in patients [43]. However, one of the MIA-induced TMJ-OA model disadvantages is the lack of serious histopathological alterations associated with TMJ-OA, such as cartilage vertical splitting and subchondral bone exposure [44].

Regarding the histological results in the current study, the condylar tissue including the fibrocartilage, cartilage as well as subchondral bone of the untreated OA group (I), showed time dependent degenerative changes in response to intra-articular MIA injection. Marked chondrocyte degeneration was observed at 2 weeks. These changes were aggravated after 4 weeks with complete loss of the cartilaginous layer. Our results were in line with previous studies which demonstrated successful OA-like lesions induced by MIA [35, 44]. It has been previously reported that MIA blocks glyceraldehyde-3-phosphate dehydrogenase, which causes chondrocyte death [45]. Moreover, MIA was found to induce an imbalance between anabolic and catabolic genes, concerned with cartilage and subchondral bone metabolism, which is considered a crucial aspect that contributes to condylar disintegration [46, 47]. Obvious distortion of osteochondral junction was also observed in the OA group of the present research. This might suggest a disturbance in the biochemical and biomechanical crosstalk between cartilage and bone that may contribute to joint deterioration [48].

In this study, rats with induced TMJ-OA were treated with either BM-MSCs-MVs (group II) or PRP (group III). An intra-articular injection was the administration method used for both types of treatment. This was based on previous reported protocols which proved the effectiveness of such a method of administration [49, 50].

The present results showed a marked histological improvement with almost complete structural restoration in condylar cartilage as well as subchondral bone after 4 weeks in the BM-MSCs-MVs-treated group (II). Chondrocyte proliferation was denoted by the presence of mitotic figures. Consistent with our findings, MSCs-EVs were reported to enhance migration and proliferation of chondrocytes as well as stimulate the

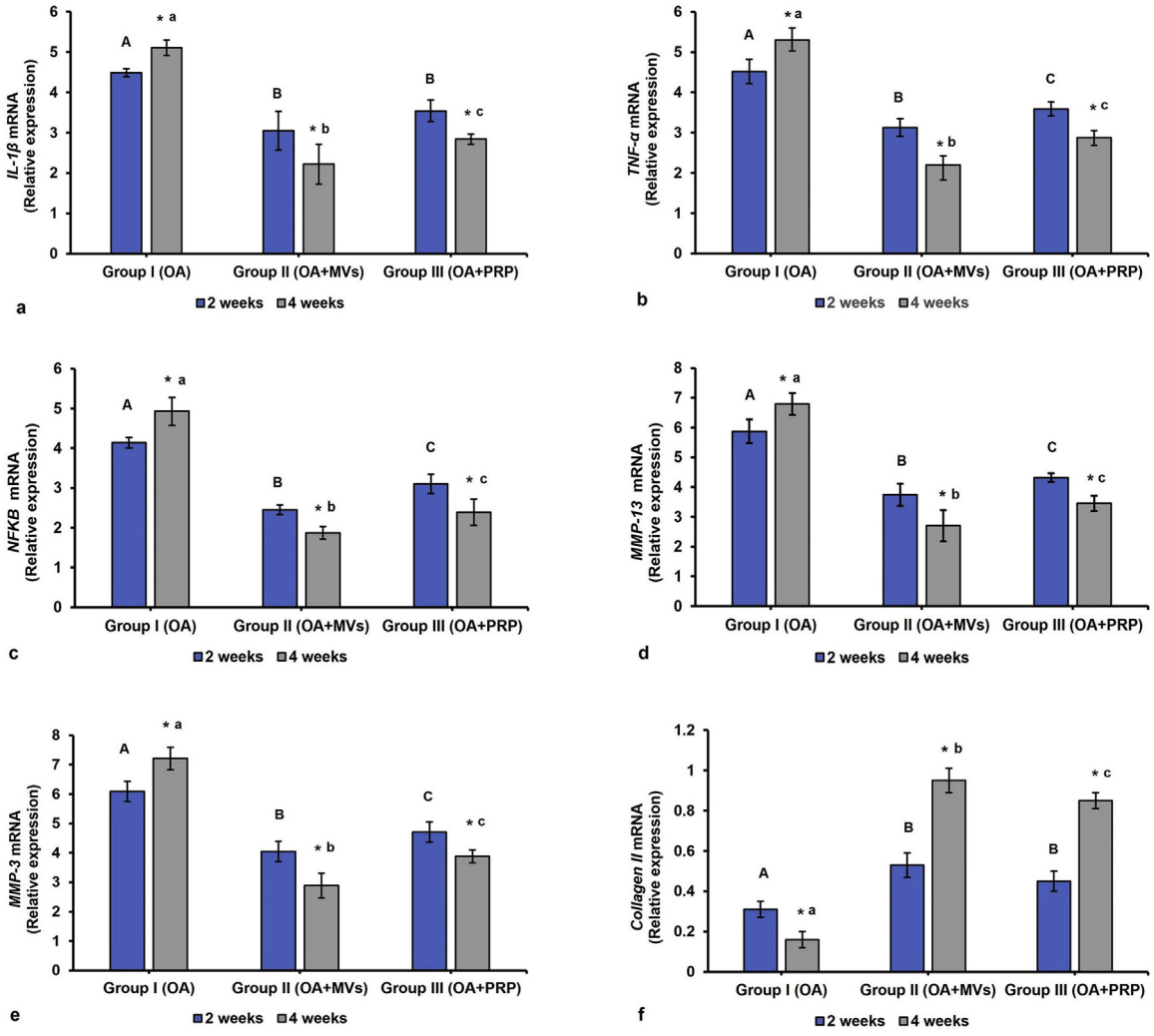

Figure 4. A graph showing the gene expression of **(a)** *IL-1β*, **(b)** *TNF-α*, **(c)** *NF-κB*, **(d)** *MMP-13*, **(e)** *MMP-3*, **(f)** *Collagen II*, in all study groups at 2 and 4 weeks. Mean values with different capital letters indicate statistically significant difference between groups utilizing Post-hoc Tukey test at 2 weeks. Mean values with different small letters indicate statistically significant difference between groups utilizing Post-hoc Tukey test at 4 weeks. * denotes significant difference within each group across various time intervals (2 and 4 weeks) using paired t-test. Significance level was adjusted at $P < 0.05$.

Table 2. Correlation between all 6 examined genes at 2 weeks.

		IL-1β	TNF-α	NF-κB	MMP-13	MMP-3	Collagen II
IL-1β	r	1	.936**	.867**	.864**	.881**	-.767**
	p value		<0.001	<0.001	<0.001	<0.001	0.001
TNF-α	r	.936**	1	.897**	.927**	.896**	-.760**
	p value	<0.001		<0.001	<0.001	<0.001	0.001
NF-κB	r	.867**	.897**	1	.912**	.942**	-.899**
	p value	<0.001	<0.001		<0.001	<0.001	<0.001
MMP-13	r	.864**	.927**	.912**	1	.899**	-.885**
	p value	<0.001	<0.001	<0.001		<0.001	<0.001
MMP-3	r	.881**	.896**	.942**	.899**	1	-.902**
	p value	<0.001	<0.001	<0.001	<0.001		<0.001
Collagen II	r	-.767**	-.760**	-.899**	-.885**	-.902**	1
	p value	0.001	0.001	<0.001	<0.001	<0.001	

** Correlation is significant at the 0.01 level (2-tailed).

expression of chondrocyte markers *in-vitro* [14]. Moreover, improvements in cartilage histological parameters with subsequent cartilage regeneration and prevention of OA progression in osteoarthritic models treated with MSC-EVs were demonstrated *in-vivo* [14, 51, 52].

In the current study, some signs of condylar tissue degeneration as well as bone marrow lesions were still evident in the PRP-treated group. This suggests incomplete restoration of condylar tissue histology. However, as compared to the OA untreated group, PRP treatment had

Table 3. Correlation between all 6 examined genes at 4 weeks.

		IL-1β	TNF-α	NF-κB	MMP-13	MMP-3	Collagen II
IL-1β	r	1	.986**	.955**	.935**	.992**	-.954**
	p value		<0.001	<0.001	<0.001	<0.001	<0.001
TNF-α	r	.986**	1	.969**	.947**	.985**	-.959**
	p value	<0.001		<0.001	<0.001	<0.001	<0.001
NF-κB	r	.955**	.969**	1	.960**	.975**	-.983**
	p value	<0.001	<0.001		<0.001	<0.001	<0.001
MMP-13	r	.935**	.947**	.960**	1	.949**	-.976**
	p value	<0.001	<0.001	<0.001		<0.001	<0.001
MMP-3	r	.992**	.985**	.975**	.949**	1	-.970**
	p value	<0.001	<0.001	<0.001	<0.001		<0.001
Collagen II	r	-.954**	-.959**	-.983**	-.976**	-.970**	1
	p value	<0.001	<0.001	<0.001	<0.001	<0.001	

** Correlation is significant at the 0.01 level (2-tailed).

noticeably attenuated condylar tissue degeneration. This is consistent with previous studies, where they reported decreased cartilage degenerative changes and increased cartilage thickness in OA models treated with PRP [53, 54]. Oppositely, a former study reported no statistical difference in cartilage and subchondral bone healing between the OA and the PRP-treated group in the TMJ-OA model in rabbits [55]. This inconsistency among different reported research may be attributed to the different methods utilized to prepare PRP solutions in the different experimental models [56, 57], leading to a lack of standardized procedure and thus the evaluation of outcomes may have been influenced. Furthermore, the PRP content, such as the concentration of certain growth factors or cytokines, could affect the PRP properties and thus affect its effectiveness [58].

IL-1β and *TNF-α* are two important proinflammatory cytokines associated with TMJ-OA [59]. These mediators play an important role in the degeneration of cartilage and bone joint by releasing proteinases and other inflammatory molecules [60]. Both inflammatory cytokines were found to activate *NF-κB* signaling pathway [61]. *NF-κB* proteins are found inactively attached to inhibitory protein IκB in the cytoplasm. Cell stimulation through *IL-1β* or *TNF-α* induces nuclear translocation of *NF-κB* to trigger the expression of a variety of regulatory genes involved in inflammation, apoptosis, and other immune responses [62]. *NF-κB* is an important transcription factor in the pathology of OA [63]. Several studies have shown that *NF-κB* signaling is highly activated in OA [59, 64, 65]. Thus, mRNA gene expression of *IL-1β*, *TNF-α* and *NF-κB* was examined by qRT-PCR in the current work.

We demonstrated that treatment with either MVs or PRP was associated with a decrease in the expression of *IL-1β*, *TNF-α* and *NF-κB* genes in contrast to the OA control group. These findings indicate that both types of treatment were able to down modulate *NF-κB* pathway mainly through down regulation of the inflammatory cytokines *(IL-1β* and *TNF-α*), thereby triggering the resolution of inflammation in TMJ-OA.

In line with our results, a previous study showed that weekly intra-articular administration of human embryonic MSCs derived vesicles in immunocompetent rat model of TMJ-OA reduced the expression of genes associated with pro-inflammation such as *IL-1β* and *TNF-α* compared to OA untreated rats [35]. Another study reported that binding of *NF-κB* to DNA in the nucleus of OA chondrocytes was reduced by MVs-derived from human adipose tissue MSCs [66]. Moreover, it was demonstrated that the phosphorylation status of *NF-κB* signaling molecules was blocked by human BM-MSCs- EVs [67].

As for PRP, it was found that PRP inhibited the inflammatory process in OA chondrocytes through the reduction of *IL-1β*-induced *NF-κB* activation [68]. Additionally, PRP in a dose-dependent manner suppressed the *NF-κB* pathway, with a marked reduction in IκBα phosphorylation and *NF-κB* nuclear translocation [69]. On the contrary, another study demonstrated that pure platelet-rich plasma showed no effect on the translocation of *NF-κB* in human articular osteoarthritic chondrocytes [70].

In the pathophysiology of OA, matrix metalloproteinases (MMPs) are a class of enzymes that induce cartilage extracellular matrix turnover and disintegration [71]. *MMP-13* is linked to the aggressive breakdown of type II collagen in OA, which leads to articular cartilage deterioration [72]. Other matrix molecules targeted by *MMP-13* include types I, III, IV, IX, X collagen, perlecan, osteonectin, and proteoglycan [73]. A former study documented that increased *MMP-13* mirrored the cartilage disintegration in MIA-induced OA [74]. *MMP-3* is another MMP that is primarily expressed in OA cartilage. It is capable of cleaving denatured collagens, aggrecan, link proteins, laminin, and fibronectin [75]. *MMP-3* levels have been found to be higher in arthritic conditions [76, 77], pointing to the significance of this MMP in cartilage proteoglycan release [78].

In our work, *MMP-13* and *MMP*-3 mRNA expression was assessed in the condylar tissue. We observed that MVs treatment reduced the gene expression of both *MMPs* as contrasted to the OA control group at time intervals of 2 and 4 weeks. Our findings went in accordance with former investigations that demonstrated the ability of MSCs-EVs to reduce *MMP-13* and *MMP-3* expression in an OA model [35, 66].

In line with group II, treatment of osteoarthritic rats with PRP (group III) in this work, decreased the expression of *MMP-13* and *MMP-3* in contrast to the OA untreated group. Our results supported a previous publication which found that PRP treatment reduced catabolic markers including *MMP-3, MMP-13,* and *IL-6* in osteoarthritic chondrocytes *in-vitro* [79]. The statistical reduction in *MMP-13* and *MMP-3* expression in the current study, observed in both groups II and III, suggests attenuation in tissue matrix degradation, which could provide a way to restore matrix haemostasis.

The extracellular matrix (ECM) of articular cartilage is composed mainly of a network of type II collagen, other minor collagens including VI, IX, X, XI, proteoglycans as well as non-collagenous proteins [80]. The integrity of cartilage is maintained by the presence of type II collagen and proteoglycans which are degraded by the expression of MMPs [81]. Thus, in the present study, the restoration of cartilage ECM was analyzed through investigating gene expression of collagen type II. Our results demonstrated that the gene expression of collagen II was increased in both groups II and III in comparison to group I. This finding indicated that MVs as well as PRP treatment succeeded in stimulating the anabolic response of the OA damaged cartilage.

A series of previous studies agreed with our results. BM-MSC-EVs were shown to improve the content of proteoglycans and upregulates COL2A1 gene expression in OA chondrocyte cultures [82]. Additionally, Zhang et al. [51] demonstrated that MSC-EVs promoted cartilage regeneration and enhanced matrix synthesis of type II collagen in a model

of osteochondral defect in immunocompetent rats. Similarly, the beneficial effect of PRP in stimulating the deposition of collagen type II, and regeneration of articular cartilage in arthritis model was previously reported [83, 84, 85].

Statistical correlation between all the studied genes in the current investigation was also examined. A positive correlation was observed among *IL-1β, TNF-α, NF-κB, MMP-13 and MMP-3*. While there was a negative correlation present with *collagen II* and (*IL-1β, TNF-α, NF-κB, MMP-13, MMP-3)*. Our findings demonstrated that there is a collaborative activity between the inflammatory mediators and the induction of matrix degradation as well as inhibiting collagen synthesis. It was reported that in OA, the chondrocytes shift to a degenerative phenotype and produce various *NF-κB* mediated catabolic chemokines and cytokines, which enhance the production of numerous degenerating enzymes, including *MMP-13,* reduce proteoglycan and collagen formation, causing articular cartilage damage and act in a positive feedback loop to enhance *NF-κB* stimulation [86, 87].

Going through analyzing our qRT-PCR data at 4 weeks time interval, there was a decrease in the expression of inflammatory and matrix degrading genes as well as an increase in matrix construction gene in the MVs-treated group in comparison to the PRP-treated group. These results supported our histological results in that BM-MSCs-MVs had better therapeutic potential as compared to PRP treatment.

MVs are one of many other trophic secretions including exosomes, growth factors, chemokines, cytokines as well as ECM released by MSCs. All these secretions are contained in the conditioned medium of MSCs [88]. The potentiality of BM-MSCs-MVs to exert a protective and pro-regenerative effect in this study, might be explained by its ability to communicate with recipient cells in a variety of ways. They can activate intracellular signaling pathways by interacting with cell surface receptors via their transmembrane proteins. Furthermore, they can deliver cargo to target cells either directly by fusing into the cell membrane or through endocytosis [21]. The combined functional complexity of MSCs-EVs cargo provides them with the ability to exert broad therapeutic efficacies. MSCs- MVs were reported to possess complex regulatory components including proteins, and different nucleic acids including messenger RNAs, non-coding RNAs, and microRNAs [89, 90]. Cargo proteins play a significant role in cellular communication, structure and dynamics, immunological regulation, and tissue regeneration [91]. Notably, microRNAs are considered the key component of EVs. Through their modulation of the inflammatory response, chondrocyte survival, and extracellular matrix deposition, microRNAs play crucial roles in treatment of OA [92].

The presence of various important biological active molecules in PRP is considered the biological rationale behind the positive therapeutic effect demonstrated by PRP-treated group in the current study. PRP was reported to contain a great amount of growth factors released from the highly concentrated platelets, such as platelet-derived, transforming, vascular endothelial, insulin-like, fibroblast, and epidermal growth factors [93]. It has been suggested that these growth factors work synergistically, resulting in stimulating the proliferation and differentiation of fibroblasts, osteoblasts, chondrocytes, and MSCs [94]. PRP alpha granules are also considered a source of chemokines, cytokines, and other proteins that aid in chemotaxis, cell proliferation and maturation, inflammatory molecule modulation, and leukocyte attraction [95]. Moreover, histamine, adenosine, dopamine, serotonin, and calcium ions stored in platelets' dense granules play a complex function in tissue regulation and regeneration [96].

Finally, according to our discussion, although several previous preclinical studies provided evidence on the therapeutic effect of both MVs and PRP, comparing the effectiveness of a single injection dose of each kind of therapy in relation to each other was not previously reported as far as our knowledge. Our results provided evidence that BM-MSCs-MVs treatment was more effective than PRP treatment regarding delaying the progression of OA and that a single injection of both kinds of treatment succeeded in providing positive therapeutic activity.

However, this study still has several limitations. First, we did not evaluate the effect of the used treatments in-vitro as our primary aim was to evaluate the structural changes involved in cartilage reconstruction in-vivo. Second, we did not analyze post-treatment OA progression using OA pathology grading system and we didn't detect cytokines protein expression using Western Blot, which are recommended to be performed as additional tools in studying OA models.

5. Conclusion

In conclusion, we showed in a rat model of TMJ-OA, that intra-articular injection of BM-MSCs-MVs or PRP helped in restoring damaged condylar structure via attenuating inflammation and matrix degradation genes and promoting matrix synthesis gene collagen II. As discussed, microRNAs contained in MSCs-MVs, or growth factors contained in PRP could be strong candidates involved in the regenerative activity of these biotherapies. Our findings suggested a superior therapeutic potential for BM-MSCs-MVs over PRP.

Recommendations

Additional research about the superiority of biological approaches, especially stem cell derived-EVs, with respect to traditional approaches in the treatment of osteoarthritic lesions, need to be fully proven in order to draw clear clinical application. Also, the signaling pathways involved in cartilage reconstruction are worth exploring in future research. Moreover, further studies are required to set up a standardized preparation method, and to determine the optimal dosage and best protocol for administration of various types of biological treatments.

Declarations

Author contribution statement

Nermeen AbuBakr, Amira E. Fares, Abeer Mostafa and Dina B.E. Farag: Conceived and designed the experiments; Performed the experiments; Analyzed and interpreted the data; Contributed reagents, materials, analysis tools or data; Wrote the paper.

Funding statement

This research did not receive any specific grant from funding agencies in the public, commercial, or not-for-profit sectors.

Data availability statement

Data included in article/supp. material/referenced in article.

Declaration of interests statement

The authors declare no conflict of interest.

Additional information

No additional information is available for this paper.

References

[1] S. Sharma, D.S. Gupta, U.S. Pal, S.K. Jurel, Etiological factors of temporomandibular joint disorders, Natl. J. Maxillofac. Surg. 2 (2011) 116–119.

[2] V. Crincoli, M.G. Piancino, F. Iannone, M. Errede, M. Di Comite, Temporomandibular disorders and oral features in systemic lupus erythematosus patients: an observational study of symptoms and signs, Int. J. Med. Sci. 17 (2020) 153–160.

[3] G. Matarese, G. Isola, A. Alibrandi, et al., Occlusal and MRI characterizations in systemic sclerosis patients: a prospective study from Southern Italian cohort, Joint Bone Spine 83 (2016) 57–62.

[4] G. Isola, L. Ramaglia, G. Cordasco, A. Lucchese, L. Fiorillo, G. Matarese, The effect of a functional appliance in the management of temporomandibular joint disorders in patients with juvenile idiopathic arthritis, Minerva Stomatol. 66 (2016) 1–8.

[5] G.A. Zarb, G.E. Carlsson, Temporomandibular disorders: osteoarthritis, J. Orofac. Pain 13 (1999) 295–306.

[6] E. Tanaka, M.S. Detamore, L.G. Mercuri, Degenerative disorders of the temporomandibular joint: etiology, diagnosis, and treatment, J. Dent. Res. 87 (2008) 296–307.

[7] E. Schiffman, R. Ohrbach, E. Truelove, et al., Diagnostic criteria for temporomandibular disorders (DC/TMD) for clinical and research applications: recommendations of the international RDC/TMD consortium network* and orofacial pain special interest group, J. Oral Facial Pain Headache 28 (2014) 6–27.

[8] V. Machon, D. Hirjak, J. Lukas, Therapy of the osteoarthritis of the temporomandibular joint, J. Cranio-Maxillo-Fac. Surg. 39 (2011) 127–130.

[9] R.F. de Souza, C.H. Lovato da Silva, M. Nasser, Z. Fedorowicz, M.A. Al-Muharraqi, Interventions for the management of temporomandibular joint osteoarthritis, Cochrane Database Syst. Rev. 2012 (2012), Cd007261.

[10] K. Ahtiainen, J. Mauno, V. Ellä, et al., Autologous adipose stem cells and polylactide discs in the replacement of the rabbit temporomandibular joint disc, J. R. Soc. Interface 10 (2013), 20130287.

[11] Y. Wu, Z. Gong, J. Li, Q. Meng, W. Fang, X. Long, The pilot study of fibrin with temporomandibular joint derived synovial stem cells in repairing TMJ disc perforation, BioMed Res. Int. 2014 (2014), 454021.

[12] S. Meirelles Lda, A.M. Fontes, D.T. Covas, A.I. Caplan, Mechanisms involved in the therapeutic properties of mesenchymal stem cells, Cytokine Growth Factor Rev. 20 (2009) 419–427.

[13] C. Manferdini, M. Maumus, E. Gabusi, et al., Adipose-derived mesenchymal stem cells exert antiinflammatory effects on chondrocytes and synoviocytes from osteoarthritis patients through prostaglandin E2, Arthritis Rheum. 65 (2013) 1271–1281.

[14] S. Cosenza, M. Ruiz, K. Toupet, C. Jorgensen, D. Noël, Mesenchymal stem cells derived exosomes and microparticles protect cartilage and bone from degradation in osteoarthritis, Sci. Rep. 7 (2017), 16214.

[15] C. Akyurekli, Y. Le, R.B. Richardson, D. Fergusson, J. Tay, D.S. Allan, A systematic review of preclinical studies on the therapeutic potential of mesenchymal stromal cell-derived microvesicles, Stem Cell Rev. Rep. 11 (2015) 150–160.

[16] M. Maumus, C. Jorgensen, D. Noël, Mesenchymal stem cells in regenerative medicine applied to rheumatic diseases: role of secretome and exosomes, Biochimie 95 (2013) 2229–2234.

[17] T. Furuta, S. Miyaki, H. Ishitobi, et al., Mesenchymal stem cell-derived exosomes promote fracture healing in a mouse model, Stem Cells Transl. Med. 5 (2016) 1620–1630.

[18] D.K. Sarko, C.E. McKinney, Exosomes: origins and therapeutic potential for neurodegenerative disease, Front. Neurosci. 11 (2017) 82.

[19] D.G. Phinney, M.F. Pittenger, Concise review: MSC-derived exosomes for cell-free therapy, Stem Cell. 35 (2017) 851–858.

[20] I.M. Bjørge, S.Y. Kim, J.F. Mano, B. Kalionis, W. Chrzanowski, Extracellular vesicles, exosomes and shedding vesicles in regenerative medicine - a new paradigm for tissue repair, Biomater. Sci. 6 (2017) 60–78.

[21] O.G. De Jong, B.W. Van Balkom, R.M. Schiffelers, C.V. Bouten, M.C. Verhaar, Extracellular vesicles: potential roles in regenerative medicine, Front. Immunol. 5 (2014) 608.

[22] C. D'Souza-Schorey, J.W. Clancy, Tumor-derived microvesicles: shedding light on novel microenvironment modulators and prospective cancer biomarkers, Genes Dev. 26 (2012) 1287–1299.

[23] D. Karpman, A.L. Ståhl, I. Arvidsson, Extracellular vesicles in renal disease, Nat. Rev. Nephrol. 13 (2017) 545–562.

[24] D. Wang, Y. Weng, S. Guo, et al., Platelet-rich plasma inhibits RANKL-induced osteoclast differentiation through activation of Wnt pathway during bone remodeling, Int. J. Mol. Med. 41 (2018) 729–738.

[25] R.T. Nguyen, J. Borg-Stein, K. McInnis, Applications of platelet-rich plasma in musculoskeletal and sports medicine: an evidence-based approach, Pm r 3 (2011) 226–250.

[26] A.F. Hegab, H.E. Ali, M. Elmasry, M.G. Khallaf, Platelet-rich plasma injection as an effective treatment for temporomandibular joint osteoarthritis, J. Oral Maxillofac. Surg. 73 (2015) 1706–1713.

[27] M. Pihut, M. Szuta, E. Ferendiuk, D. Zeńczak-Więckiewicz, Evaluation of pain regression in patients with temporomandibular dysfunction treated by intra-articular platelet-rich plasma injections: a preliminary report, BioMed Res. Int. 2014 (2014), 132369.

[28] S. Cömert Kiliç, M. Güngörmüş, M.A. Sümbüllü, Is arthrocentesis plus platelet-rich plasma superior to arthrocentesis alone in the treatment of temporomandibular joint osteoarthritis? A randomized clinical trial, J. Oral Maxillofac. Surg. 73 (2015) 1473–1483.

[29] N. AbuBakr, T. Haggag, D. Sabry, Z.A. Salem, Functional and histological evaluation of bone marrow stem cell-derived exosomes therapy on the submandibular salivary gland of diabetic Albino rats through TGFβ/Smad3 signaling pathway, Heliyon 6 (2020), e03789.

[30] T. Soliman, Z. Ali, M. Zayed, D. Sabry, N. AbuBakr, Assessing the bone-healing potential of bone marrow mesenchymal stem cells in jawbone osteoporosis in albino rats, Dent. Med. Probl. 59 (2022) 75–83.

[31] A. Shabbir, A. Cox, L. Rodriguez-Menocal, M. Salgado, E. Van Badiavas, Mesenchymal stem cell exosomes induce proliferation and migration of normal and chronic wound fibroblasts, and enhance angiogenesis in vitro, Stem Cell. Dev. 24 (2015) 1635–1647.

[32] Z.A. Salem, A.H.M. Kamel, N. AbuBakr, Salivary exosomes as a new therapy to ameliorate diabetes mellitus and combat xerostomia and submandibular salivary glands dysfunction in diabetic rats, J. Mol. Histol. 52 (2021) 467–477.

[33] J. Zhang, T. Yuan, N. Zheng, Y. Zhou, M.V. Hogan, J.H. Wang, The combined use of kartogenin and platelet-rich plasma promotes fibrocartilage formation in the wounded rat Achilles tendon entheses, Bone Joint Res. 6 (2017) 231–244.

[34] N. AbuBakr, Z. Salem, Z. Ali, M.E. Assaly, Comparative evaluation of the early effects of the low-level laser therapy versus intra-articular steroids on temporomandibular joint acute osteoarthritis in rats: a histochemical, molecular and imaging evaluation, Dent. Med. Probl. 55 (2018) 359–366.

[35] S. Zhang, K.Y.W. Teo, S.J. Chuah, R.C. Lai, S.K. Lim, W.S. Toh, MSC exosomes alleviate temporomandibular joint osteoarthritis by attenuating inflammation and restoring matrix homeostasis, Biomaterials 200 (2019) 35–47.

[36] Y. Akatsu, T. Enomoto, S. Yamaguchi, et al., Age-dependent differences in response to partial-thickness cartilage defects in a rat model as a measure to evaluate the efficacy of interventions for cartilage repair, Cell Tissue Res. 375 (2019) 425–435.

[37] D.B. Farag, C. Yousry, A.M. Al-Mahallawi, H.I. El-Askary, M.R. Meselhy, N. AbuBakr, The efficacy of Origanum majorana nanocubosomal systems in ameliorating submandibular salivary gland alterations in streptozotocin-induced diabetic rats, Drug Deliv. 29 (2022) 62–74.

[38] S.K. Gopal, R. Shankar, H.B. Vardhan, Prevalence of temporo-mandibular joint disorders in symptomatic and asymptomatic patients: a cross-sectional study, Int. J. Adv. Sci. 1 (2014) 14–20.

[39] X. Van Bellinghen, Y. Idoux-Gillet, M. Pugliano, et al., Temporomandibular joint regenerative medicine, Int. J. Mol. Sci. 19 (2018) 446.

[40] S.J. Scrivani, D.A. Keith, L.B. Kaban, Temporomandibular disorders, N. Engl. J. Med. 359 (2008) 2693–2705.

[41] J.C. Alcerro, C.J. Lavernia, Stem cells and platelet-rich plasma for knee osteoarthritis: prevalence and cost in south Florida, J. Am. Acad. Orthop. Surg. 27 (2019) 779–783.

[42] X.D. Wang, X.X. Kou, Z. Meng, et al., Estrogen aggravates iodoacetate-induced temporomandibular joint osteoarthritis, J. Dent. Res. 92 (2013) 918–924.

[43] K. Lampropoulou-Adamidou, P. Lelovas, E.V. Karadimas, et al., Useful animal models for the research of osteoarthritis, Eur. J. Orthop. Surg. Traumatol. 24 (2014) 263–271.

[44] X.D. Wang, X.X. Kou, D.Q. He, et al., Progression of cartilage degradation, bone resorption and pain in rat temporomandibular joint osteoarthritis induced by injection of iodoacetate, PLoS One 7 (2012), e45036.

[45] L. Grossin, C. Cournil-Henrionnet, A. Pinzano, et al., Gene transfer with HSP 70 in rat chondrocytes confers cytoprotection in vitro and during experimental osteoarthritis, Faseb. J. 20 (2006) 65–75.

[46] V.M. Baragi, G. Becher, A.M. Bendele, et al., A new class of potent matrix metalloproteinase 13 inhibitors for potential treatment of osteoarthritis: evidence of histologic and clinical efficacy without musculoskeletal toxicity in rat models, Arthritis Rheum. 60 (2009) 2008–2018.

[47] J.D. Sandy, C. Verscharen, Analysis of aggrecan in human knee cartilage and synovial fluid indicates that aggrecanase (ADAMTS) activity is responsible for the catabolic turnover and loss of whole aggrecan whereas other protease activity is required for C-terminal processing in vivo, Biochem. J. 358 (2001) 615–626.

[48] G. Li, J. Yin, J. Gao, et al., Subchondral bone in osteoarthritis: insight into risk factors and microstructural changes, Arthritis Res. Ther. 15 (2013) 223.

[49] S.E. Headland, H.R. Jones, L.V. Norling, et al., Neutrophil-derived microvesicles enter cartilage and protect the joint in inflammatory arthritis, Sci. Transl. Med. 7 (2015) 315ra190.

[50] O. Dolkart, O. Chechik, Y. Zarfati, T. Brosh, F. Alhajajra, E. Maman, A single dose of platelet-rich plasma improves the organization and strength of a surgically repaired rotator cuff tendon in rats, Arch. Orthop. Trauma. Surg. 134 (2014) 1271–1277.

[51] S. Zhang, W.C. Chu, R.C. Lai, S.K. Lim, J.H. Hui, W.S. Toh, Exosomes derived from human embryonic mesenchymal stem cells promote osteochondral regeneration, Osteoarthritis Cartilage 24 (2016) 2135–2140.

[52] S.C. Tao, T. Yuan, Y.L. Zhang, W.J. Yin, S.C. Guo, C.Q. Zhang, Exosomes derived from miR-140-5p-overexpressing human synovial mesenchymal stem cells enhance cartilage tissue regeneration and prevent osteoarthritis of the knee in a rat model, Theranostics 7 (2017) 180–195.

[53] N. Kütük, B. Baş, E. Soylu, et al., Effect of platelet-rich plasma on fibrocartilage, cartilage, and bone repair in temporomandibular joint, J. Oral Maxillofac. Surg. 72 (2014) 277–284.

[54] S.L. Lin, C.C. Tsai, S.L. Wu, S.Y. Ko, W.F. Chiang, J.W. Yang, Effect of arthrocentesis plus platelet-rich plasma and platelet-rich plasma alone in the treatment of temporomandibular joint osteoarthritis: a retrospective matched cohort study (A STROBE-compliant article), Medicine (Baltim.) 97 (2018), e0477.

[55] U. Coskun, C. Candirli, G. Kerimoglu, F. Taskesen, Effect of platelet-rich plasma on temporomandibular joint cartilage wound healing: experimental study in rabbits, J. Cranio-Maxillo-Fac. Surg. 47 (2019) 357–364.

[56] E. Anitua, Plasma rich in growth factors: preliminary results of use in the preparation of future sites for implants, Int. J. Oral Maxillofac. Implants 14 (1999) 529–535.

[57] L.F. Rodella, G. Favero, R. Boninsegna, et al., Growth factors, CD34 positive cells, and fibrin network analysis in concentrated growth factors fraction, Microsc. Res. Tech. 74 (2011) 772–777.

[58] W. Lim, S.H. Park, B. Kim, S.W. Kang, J.W. Lee, Y.L. Moon, Relationship of cytokine levels and clinical effect on platelet-rich plasma-treated lateral epicondylitis, J. Orthop. Res. 36 (2018) 913–920.

[59] J. Yuan, W. Ding, N. Wu, S. Jiang, W. Li, Protective effect of genistein on condylar cartilage through downregulating NF-κB expression in experimentally created osteoarthritis rats, BioMed Res. Int. 2019 (2019), 2629791.

[60] M.I. Campos, P.S. Campos, S.R. Line, Inflammatory cytokines activity in temporomandibular joint disorders: a review of literature, Braz. J. Oral Sci. 5 (2006) 1054–1062.
[61] T. Luedde, R.F. Schwabe, NF-kappaB in the liver—linking injury, fibrosis and hepatocellular carcinoma, Nat. Rev. Gastroenterol. Hepatol. 8 (2011) 108–118.
[62] H. Ishinaga, H. Jono, J.H. Lim, et al., Synergistic induction of nuclear factor-κB by transforming growth factor-β and tumour necrosis factor-α is mediated by protein kinase A-dependent RelA acetylation, Biochem. J. 417 (2009) 583–591.
[63] J. Tang, Q. Dong, Knockdown of TREM-1 suppresses IL-1β-induced chondrocyte injury via inhibiting the NF-κB pathway, Biochem. Biophys. Res. Commun. 482 (2017) 1240–1245.
[64] P. Wojdasiewicz, A. Poniatowski Ł, D. Szukiewicz, The role of inflammatory and anti-inflammatory cytokines in the pathogenesis of osteoarthritis, Mediat. Inflamm. 2014 (2014), 561459.
[65] L. Xu, C. Sun, S. Zhang, et al., Sam68 promotes NF-κB activation and apoptosis signaling in articular chondrocytes during osteoarthritis, Inflamm. Res. 64 (2015) 895–902.
[66] M. Tofiño-Vian, M.I. Guillén, M.D. Pérez Del Caz, A. Silvestre, M.J. Alcaraz, Microvesicles from human adipose tissue-derived mesenchymal stem cells as a new protective strategy in osteoarthritic chondrocytes, Cell. Physiol. Biochem. 47 (2018) 11–25.
[67] S. Li, S. Stöckl, C. Lukas, et al., hBMSC-derived extracellular vesicles attenuate IL-1β-induced catabolic effects on OA-chondrocytes by regulating pro-inflammatory signaling pathways, Front. Bioeng. Biotechnol. 8 (2020), 603598.
[68] G.M. Van Buul, W.L. Koevoet, N. Kops, et al., Platelet-rich plasma releasate inhibits inflammatory processes in osteoarthritic chondrocytes, Am. J. Sports Med. 39 (2011) 2362–2370.
[69] H. Zhao, W. Zhu, W. Mao, C. Shen, Platelet-rich plasma inhibits Adriamycin-induced inflammation via blocking the NF-κB pathway in articular chondrocytes, Mol. Med. 27 (2021) 66.
[70] W. Yin, H. Xu, J. Sheng, Z. Xu, X. Xie, C. Zhang, Comparative evaluation of the effects of platelet-rich plasma formulations on extracellular matrix formation and the NF-κB signaling pathway in human articular chondrocytes, Mol. Med. Rep. 15 (2017) 2940–2948.
[71] P.S. Burrage, K.S. Mix, C.E. Brinckerhoff, Matrix metalloproteinases: role in arthritis, Front. Biosci. 11 (2006) 529–543.
[72] P.G. Mitchell, H.A. Magna, L.M. Reeves, et al., Cloning, expression, and type II collagenolytic activity of matrix metalloproteinase-13 from human osteoarthritic cartilage, J. Clin. Invest. 97 (1996) 761–768.
[73] T. Shiomi, V. Lemaître, J. D'Armiento, Y. Okada, Matrix metalloproteinases, a disintegrin and metalloproteinases, and a disintegrin and metalloproteinases with thrombospondin motifs in non-neoplastic diseases, Pathol. Int. 60 (2010) 477–496.
[74] J. Lu, T. Zhang, H. Sun, S. Wang, M. Liu, Protective effects of dioscin against cartilage destruction in a monosodium iodoacetate (MIA)-indcued osteoarthritis rat model, Biomed. Pharmacother. 108 (2018) 1029–1038.
[75] S. Sun, A.C. Bay-Jensen, M.A. Karsdal, et al., The active form of MMP-3 is a marker of synovial inflammation and cartilage turnover in inflammatory joint diseases, BMC Muscoskel. Disord. 15 (2014) 93.
[76] X.Y. Wang, Y. Chen, X.J. Tang, L.H. Jiang, P. Ji, AMD3100 attenuates matrix metalloprotease-3 and-9 expressions and prevents cartilage degradation in a monosodium iodo-acetate–induced rat model of temporomandibular osteoarthritis, J. Oral Maxillofac. Surg. 74 (2016) 927.e1–927.e13.
[77] E.S. Mehana, A.F. Khafaga, S.S. El-Blehi, The role of matrix metalloproteinases in osteoarthritis pathogenesis: an updated review, Life Sci. 234 (2019), 116786.
[78] D.L. Xie, F. Hui, R. Meyers, G.A. Homandberg, Cartilage chondrolysis by fibronectin fragments is associated with release of several proteinases: stromelysin plays a major role in chondrolysis, Arch. Biochem. Biophys. 311 (1994) 205–212.
[79] M. Moussa, D. Lajeunesse, G. Hilal, et al., Platelet rich plasma (PRP) induces chondroprotection via increasing autophagy, anti-inflammatory markers, and decreasing apoptosis in human osteoarthritic cartilage, Exp. Cell Res. 352 (2017) 146–156.
[80] M. Maldonado, J. Nam, 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.
[81] G. Murphy, V. Knäuper, S. Atkinson, et al., Matrix metalloproteinases in arthritic disease, Arthritis Res. Ther. 4 (2002) S39–49.
[82] L.A. Vonk, S.F. van Dooremalen, N. Liv, et al., Mesenchymal stromal/stem cell-derived extracellular vesicles promote human cartilage regeneration in vitro, Theranostics 8 (2018) 906–920.
[83] C.M.R. Pacheco, Kerppers II, R.B. Parreira, et al., Use of platelet-rich plasma in an experimental rheumatoid arthritis model, Imper. J. Interdiscipl. Res. 2 (2016) 353–357.
[84] S. Lippross, B. Moeller, H. Haas, et al., Intraarticular injection of platelet-rich plasma reduces inflammation in a pig model of rheumatoid arthritis of the knee joint, Arthritis Rheum. 63 (2011) 3344–3353.
[85] A. Khoshbin, T. Leroux, D. Wasserstein, et al., The efficacy of platelet-rich plasma in the treatment of symptomatic knee osteoarthritis: a systematic review with quantitative synthesis, Arthroscopy 29 (2013) 2037–2048.
[86] M.B. Goldring, K.B. Marcu, Cartilage homeostasis in health and rheumatic diseases, Arthritis Res. Ther. 11 (2009) 224.
[87] M. Kapoor, J. Martel-Pelletier, D. Lajeunesse, J.P. Pelletier, H. Fahmi, Role of proinflammatory cytokines in the pathophysiology of osteoarthritis, Nat. Rev. Rheumatol. 7 (2011) 33–42.
[88] F. Soleimanifar, F.S. Hosseini, H. Atabati, et al., Adipose-derived stem cells-conditioned medium improved osteogenic differentiation of induced pluripotent stem cells when grown on polycaprolactone nanofibers, J. Cell. Physiol. 234 (2019) 10315–10323.
[89] R.C. Lai, S.S. Tan, R.W. Yeo, et al., MSC secretes at least 3 EV types each with a unique permutation of membrane lipid, protein and RNA, J. Extracell. Vesicles 5 (2016), 29828.
[90] T.S. Chen, R.C. Lai, M.M. Lee, A.B. Choo, C.N. Lee, S.K. Lim, Mesenchymal stem cell secretes microparticles enriched in pre-microRNAs, Nucleic Acids Res. 38 (2010) 215–224.
[91] R.C. Lai, S.S. Tan, B.J. Teh, et al., Proteolytic potential of the MSC exosome proteome: implications for an exosome-mediated delivery of therapeutic proteasome, Int. J. Proteomics 2012 (2012), 971907.
[92] A. Fathollahi, S. Aslani, A. Jamshidi, M. Mahmoudi, Epigenetics in osteoarthritis: novel spotlight, J. Cell. Physiol. 234 (2019) 12309–12324.
[93] R.E. Marx, Platelet-rich plasma: evidence to support its use, J. Oral Maxillofac. Surg. 62 (2004) 489–496.
[94] L.A. Fortier, J.U. Barker, E.J. Strauss, T.M. McCarrel, B.J. Cole, The role of growth factors in cartilage repair, Clin. Orthop. Relat. Res. 469 (2011) 2706–2715.
[95] S.G. Boswell, B.J. Cole, E.A. Sundman, V. Karas, L.A. Fortier, Platelet-rich plasma: a milieu of bioactive factors, Arthroscopy 28 (2012) 429–439.
[96] A. Mishra, J. Woodall Jr., A. Vieira, Treatment of tendon and muscle using platelet-rich plasma, Clin. Sports Med. 28 (2009) 113–125.