Rat Bone Marrow Derived Mesenchymal Stem Cells (rBMMSCs) Encapsulated in Collagen Type I Containing Platelet-Rich Plasma for Osteoarthritis Treatment in Rat model

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Abstract

Osteoarthritis (OA) is the most common form of degenerative joint disease, affecting more than 25% of the adult though prevalent in the elderly population. Most of the current therapeutic modalities aim at symptomatic treatment and lingering the disease progression. In recent years, regenerative medicine such as stem cell transplantation and tissue engineering has been suggested as a potential curative intervention for OA. The objective of current study was to assess the safety and efficacy of an injectable tissue-engineered construct composed of BMMSCs, PRP, and Collagen type I in rat model of OA. To produce collagen type I, PRP and BMMSCs, male Wistar rats were ethically euthanized. After expansion and characterization of rat BMMSCs (rBMMSCs), tissue-engineered construct was formed by combination of appropriate amount of collagen type I, PRP and rBMMSCs. In vitro studies were conducted to evaluate the effect of PRP on chondrogenic differentiation capacity of encapsulated cells. Then tissue-engineered construct was injected in knee joint of rat model of OA (24 rat in 4 groups: OA, OA+MSC, OA+Collagen+MSC+PRP, OA+MSC+Collagen). After 6 weeks, the animals were euthanized and knee joint histopathology examinations were performed to evaluate the effect of each treatment on OA. Tissue-engineered construct was successfully manufactured and in vitro assays demonstrated that relevant chondrogenic genes and proteins expression were higher in PRP group than the others. Histopathological findings of the knee joint samples showed favorable regenerative effect of rBMMSCs+PRP+Collagen group comparing to others. In this study, we introduced an injectable tissue-engineered product composed of rBMMSCs+PRP+Collagen with potential regenerative effect on cartilage damage caused by OA.

Introduction

In the last decades, advancement in health care system improved the prevention, diagnosis and treatment of great number of disorders which restrict human life expectancy [1, 2]. These advancements led to population aging and high prevalence of degenerative disorders same as osteoarthritis (OA). OA considered as the fourth leading cause of physical disability in both the young and the old people in the world. Conventional medical treatment approaches for OA are focused mainly on pain relief and symptom management including nonsteroidal anti-inflammatory drugs (NSAIDs), physical exercises and braces. Besides, some invasive curative strategies consist of steroids, hyaluronic acids (HA), platelet-rich plasma (PRP) injection. When Conventional treatments strategies fail, final curative option may be the surgical approaches [3]. Unfortunately, there are no effective therapeutics, hence, scientists attempt to find novel treatment to restore the function and structure of damaged articular cartilage. Tissue engineering is an attractive field to regenerate damaged tissues and restore their functions with three components including cells, 3D scaffold and growth factors. Recently, the autologous chondrocyte implantation (ACI) has appeared as a promising cell-based therapy aim to provide functional repair of injured cartilage to manage the disease symptoms [4]. In fact, ACI approach is restricted by some hindrances including insufficient donor tissue supply, donor site injury and the rapid dedifferentiation of transplanted chondrocytes during two-dimensional expansion [5]. To overcome such obstacles, tissue
engineering recommends scaffolds in combination with stem cells as poly-therapy to provide three dimensional environment [6]. Multipotent mesenchymal stem cells (MSCs) with chondrocyte differentiation capacity proposed as an attractive source to OA injuries regeneration (direct intra-articular injection) [7–9]. Also, the homing ability of MSCs to injured sites has been shown [10] and after transplantation exerted its effectiveness to cartilage repair [11, 12]. One limitation of utilizing MSCs alone is insufficient ratio of implanted MSCs and chondrogenic differentiation difficulty. Leijs et al demonstrated the low homing of injected MSCs into cartilage while the majority of MSCs detected in joint space or adhered to synovial membrane [13]. Unlike MSCs transplantation, tissue engineering strategies using stem cells seeded or encapsulated in appropriate scaffold such as hydrogels which is impregnated with growth factors revealed more acceptable results for cartilage repair[10, 14, 15]. In cartilage tissue, chondrocytes are encapsulated in a thin rim named pericellular matrix (PCM) that play a critical role in chondrocyte anchorage to the extracellular matrix and preserving chondrogenic differentiation capability [16, 17]. The PCM and the entrapped chondrocyte make functional cartilage unit termed ‘chondron’. However, the injected stem cells are encapsulated with a biomimetic scaffold can facilitate cell migration to a targeted niche and promote chondrogenic potential capability [17]. For clinical application, the cells should be encapsulated in an injectable biocompatible scaffold with low immunogenic reaction which could be easily prepared in large scale. Because of the great importance of this factor, collagen type I appears to be a hopeful candidate with PCM mimicry to form an injectable chondron. MSCs encapsulated in collagen hydrogel reside in the specific niche and differentiate to chondrocyte properly [18, 19]. Incorporation of collagen hydrogel in various tissue-engineered-based scaffolds has gained priority in recent studies in order to increase the biocompatibility, biodegradability, and regenerative potential [6]. Collagen type I has a high connection affinity to fibronectin (FN) that is expressed at the damaged sites so collagen hydrogel provide a biocompatible injectable scaffold to find cartilage damaged and aim to chondrocyte differentiation promotion of encapsulated MSCs [20, 21]. Besides, the injected cells need adequate growth factors to accelerate their differentiation and conduct them to their determined fate. Recent studies detected the regenerative therapeutic effects of PRP because of the great number of growth factors presence that increase healing rate of damaged tissues by growth promotion in the site of injection [22–24]. In this study, we prepared an injectable collagen hydrogel that contains MSCs and PRP to evaluate the effect of this tissue-engineered construct transplantation in rat model of osteoarthritis.

Materials And Methods

Experimental animals

Male Wistar rats (2 months old, 200–220g) were purchased from the animal house of Pasteur Institute (Tehran, Iran). The study was performed according to instructions approved by the Animal Care and Use Committees of Tehran University of Medical Sciences (ethical code: IR.TUMS. VCR.1398.034). The animals were kept in a room with controlled temperature (21–25°C) on a 12 hours light/12 hours dark cycle.
Rat bone marrow mesenchymal stem cells (rBMMSCs) isolation and culture

Adult rats were euthanized under proper anesthesia with ethically approved protocol. Femora and tibias were harvested aseptically. Connective tissues were removed and both ends of each bone were cut. Bone shafts were thoroughly washed with sterile PBS and the bone marrow was flushed-out with Dulbecco’s Modified Eagle’s Medium (DMEM). The cell suspension was collected in a 90 mm culture dish, then filtered by strainer (70µm) and centrifuged at 1200 rpm for 5 min and re-suspended in growth medium containing low glucose DMEM supplemented with 10% FBS, penicillin (100U/mL), L-glutamine (2mM), streptomycin (100µg/mL), and amphotericin-B (0.25µg/mL) (all from Thermo Fisher Scientific, USA). Finally, cells were plated at a density of 10^5 cells/cm^2 in a T25 tissue culture flask (Nunc, USA) and maintained at 37°C in a humidified atmosphere of 5% CO₂. The medium was refreshed after 24 hours and then every 3 days. Cells at 70–80% confluency were serially passaged. We used cells at passage 3 for subsequent experiments.

rBMMSCs characterization

To characterize the cell population as multipotent MSCs, Cells at 3rd passage were characterized to meet the minimal criteria such as morphology, plastic-adherent property, expression of MSC-specific surface markers by flow cytometry and the ability for multi-lineage differentiation in vitro.

Surface Marker Expression

Flow cytometry (FACS Calibur, BD Bioscience, USA) was performed to determine the phenotypic expression of rBMMSCs. At 3rd passages, 1×10^6 cells were stained with anti-CD-90, anti-CD-105, anti-CD-45, and anti-CD-31 (All from Abcam, USA) We used the following antibodies: primary antibodies anti-CD-105 mouse monoclonal (ab2529), anti-CD-90 mouse monoclonal (ab222781), anti-CD-34 mouse monoclonal (ab81289), and FITC-conjugated anti-CD-31 mouse monoclonal (ab33858). Unstained cells were used for iso type control. Data were analyzed by recording 10,000 events using FlowJo Version 7 software.

In vitro differentiation of rBMMSCs

After three passages, cells were re-plated in growth medium at 2x10^5 cells/well in 24-well tissue culture plates. After 24 h incubation, the growth medium was replaced with osteogenic differentiation medium containing dexamethasone (10nmol), β-glycerophosphate (10mmol), L-ascorbic acid (0.3mM) (all from Sigma-Aldrich, USA). For adipogenic differentiation, 2x10^5 rBMMSCs/well were cultured in 24-well tissue culture plates and after 24h incubation, adipogenic differentiation media containing insulin (10µL/mL), dexamethasone (1µM), indomethacin (0.5mM), and 3-isobutyl-1-methylxanxine (60µM) (all from Sigma-Aldrich, USA) was added. The humidified atmosphere with 5% CO₂ was maintained all through the incubation. The differentiation media was replaced every three days. After 21 days of differentiation period, the media was removed and cells were fixed and stained by alizarin red for osteoblast cells and
Oil Red O staining for adipocyte cells. Calcium deposition and lipid droplets in the cells were observed using microscopy.

**PRP preparation**

Approximately 8ml blood was collected from each euthanized rat by cardiac puncture and transferred to a centrifuge tube pre-loaded with 2ml acid citrate dextrose solution. The tubes were then centrifuged at 2000 rpm for 10 min at 20°C and plasma collected carefully then further centrifuged at 4000 rpm for 10 min at same temperature. The supernatant alone is platelet-poor-plasma (PPP) and the precipitate at the bottom of the centrifuge tube with supernatant is the PRP. We activated PRP immediate prior to application with CaCl$_2$ 10% (0.2 ml CaCl$_2$ to 0.8 ml PRP).

**Collagen type I gel preparation and cell encapsulation**

Collagen type I was extracted following the protocol described by Navneeta Rajan and colleagues (25) from rat tails and processed using acetone, 70% (vol/vol) isopropanol, and 0.02N cold acetic acid. The pure acetone, isopropyl alcohol, acetic acid were purchased from Merck (Germany). The collagen solution was sterilized using 1% chloroform (Merck (Germany)) and the resultant solution was mixed with 10X DMEM-F12 (Gibco, USA) and triple-buffer system-HSS at a volume ratio of 8:1:1 vol/vol respectively. The medium and HSS buffer were filtered through 0.22 µm strainer prior to mixing. The HSS buffer composed of HEPES 4.77g and 2.2g sodium bicarbonate in 100 mL of 0.5M NaOH solution. All the components of HSS were purchased from Sigma Aldrich (USA). The final concentration of collagen type I hydrogel was 2mg/ml.

rBMMSCs encapsulation procedure is shown in Fig. 1. Briefly, the activated PRP and collagen type I solution were mixed properly (1:1 vol/vol) in a falcon tube and kept half an hour in room temperature. MSCs were harvested by using trypsin/EDTA and 2x10$^6$ cells suspended in 100µL of complete culture media. The cell suspension was added to PRP-Collagen solution and mixed by gentle pipetting. The composition transferred and cultured in 24-well plate.

**MTT assay**

MTT assay was performed to measure the viability of encapsulated cells alone and with PRP at different incubation period (day 1, 3, and 5) in a 96-well plate. This method is based on the reduction of the yellow MTT-salt [3-(4,5-dimethylthiazol-2-yl)-2, 5-diphenyltetrazolium-bromide] to blue-purple formazan crystals by mitochondrial enzyme succinate dehydrogenase. For this purpose, 1x10$^4$ cells (encapsulated) were plated on each well with PRP (controls without PRP). Then 200µL MTT solution (5 mg/mL) was added in each well and the plate was incubated at 37°C for 3–4 hours. Then the supernatant was replaced with 100µL DMSO in order to dissolve formazan crystal producing purple-blue colour. Optical density measured by using microplate reader at 570nm.

**In vitro chondrogenic differentiation of encapsulated rBMMSCs**
After passage 3, the cells, at a concentration of 2x10^6 cells/ml, encapsulated in collagen solution then cultured in the 24-well tissue culture plate. After 4h incubation, differentiation groups were divided to three groups. 1. TGF-β1 group: treated with chondrogenic differentiation media that contains DMEM/F12 supplemented with 10%ITS + 10^{-7} M dexamethasone (Sigma), 1µM ascorbate-2-phosphate, and 10ng/ml transforming growth factor-beta 1 (TGF-β1, SIGMA,USA) 2. PRP- TGF-β1 group: treated with chondrogenic differentiation media + PRP with a ratio of 1:1, and 3. Control group without differentiation media and PRP. The cell culture maintained for 21 days with media change in every 3 days. Finally, the cells were collected for the real time PCR and were fixed for immunocytochemistry for expression of related genes to chondrocyte.

**Real time PCR**

Quantitative reverse transcriptase-polymerase chain reaction (qRT-PCR) was used for the mRNA expression patterns of chondrogenic specific genes in control, TGF-β1 and PRP-TGF-β1 groups. Total RNA was prepared by using RNeasy Plus Mini kit (Qiagen, USA, 74134) as described by the manufacturer, and complementary DNA (cDNA) synthesis from 1µg of extracted RNA was performed by Revert Aid First Strand cDNA Synthesis kit (Takara, USA, K1632). qRT-PCR reactions were carried out in the 48-well optical reaction plates on StepOneTM Real-Time PCR machine. For each PCR reaction, 30ng synthesized cDNA was used for PCR by mixing with 10µl of Power SYBER Green master mix (2×, Applied Biosystems) primed with 0.5 µM of each primer (Table 1) in a total volume of 20µl at the annealing temperature. The comparative Ct method (ΔΔCT Method) was used for relative gene expression analysis. All Ct values calculated from the normalization of target genes to GAPDH as an internal control and calibrated using calculation from the undifferentiated BM-MSCs. The relative gene expression values presented as mean of three independent experiments.

| primer      | forward   | reverse             | Accessation number |
|-------------|-----------|---------------------|--------------------|
| Aggrecan    | TCCACATCAGAAGAGCCATAc | AGTCAAGGTCGCCAGAGG | NM_022190.1        |
| Collagen I  | GCTGTGGAAGTGGATGAAGA | TGAGGAACGTGGAGAGACG | NM_053304          |
| Collagen II | ACCTGGTACCCCTGGAAATC | CACCAGGATTGCCTTGAAT | NM_012929.1        |
| GAPDH       | TGAGGACCAGGGTTGTCTCCT | ATGTAGGCCATGAGGTCAC | NM_017008.4        |

**Immunocytochemistry**

After 21 days' post-induction to chondrogenic differentiation, encapsulated cells were fixed with 4% paraformaldehyde (PFA; Sigma-Aldrich) for 20 minutes. Subsequently permeabilized with 0.1 % Triton X-100 in PBS. The cells were blocked for 30 min at room temperature with 5 % bovine serum albumin (BSA), then incubated with primary antibodies (diluted in 5 % BSA in PBS) against aggrecan (mouse monoclonal antibody; Abcam, USA, 1:200), collagen type I (mouse monoclonal antibody; Abcam, 1:200), and collagen type II (mouse monoclonal antibody; Abcam,1:200) overnight at 4°C. Secondary antibodies included
Alexa fluor 488 donkey anti-mouse (1:500; Gibco) and the nuclei were counter-stained with DAPI (Sigma-Aldrich, D8417). For negative controls, only the secondary antibody was used.

**In vivo experiment**

The 8 weeks male wistar rats (200-220g) were randomized into four groups each of 6 rats: (i) OA group (OA induction without treatment), (ii) OA + MSC group, (iii) OA + Collagen + MSC + PRP group, and (iv) OA + MSC + Collagen group. OA of the knee joints was induced as previously described by Tang et al (2017). In brief, under sterile conditions, the animals were anesthetized and a medial parapatellar approach was used to release the joint capsule and to perform a medial collateral ligament transection (MCLT) of the right knee joints. The wounds were closed and covered with a local antibiotic ointment. All rats were returned to their cages after the operation and were allowed to move freely, and 0.2 mg/kg/day of intramuscular Meloxicam was administered for 5 days for pain relief and administrated by penicillin once a day for the first 3 days. On fourth weeks post-induction, OA was confirmed by radiography. Then the knee joints were injected by MSC, Collagen + MSC, and Collagen + MSC + PRP. The recipient rats were injected with 4X10^6 collagen-encapsulated MSCs (passage 3). All rats were euthanized 6 weeks post-treatment, and knee joint samples were collected for histopathological evaluations.

**Statistical analysis**

Tukey's HSD test and repeated measures analysis of variance test was used for multigroup comparisons according to the GraphPad Prism software, Version6.00 (GraphPad Prism, Inc., San Diego, CA). $P < 0.05$ was considered significant.

**Results**

**Isolation and characterization of rBMMSCs**

Bone marrow samples harvested from rat tibia and femur bones. Primary rBMMSCs were cultured and exhibited typical fibroblastic morphology (Fig. 2A). At passages 3, flow-cytometry analysis demonstrated that the cells were positive for CD90 and CD105, and negative for CD31 and CD34 (Fig. 2B). The osteogenic and adipogenic differentiation were approved by Alizarin red and oil red O staining, respectively (Fig. 2Ab, 2Ac).

**Mtt Assay**

As shown in Fig. 3, the proportion of live cells in the Collagen + Cell + PRP group was obviously higher than that in the Cell-group and Collagen + Cell group. This result confirmed that PRP enhances the survival rate of MSCs.

**Rbmmscs Encapsulation And Chondrogenic Differentiation**

The collagen scaffolds were successfully produced and micro-encapsulated with MSCs. All of them were bead-like and, during the 21 days of chondrogenic differentiation induction, remained in its original gel
state. All scaffolds were able to induce and maintain the most basic morphological feature of a chondrocyte, its round shape. The cell distribution was homogeneous, occupying all the scaffold area. Two hours after seeding, the cells were in close contact with the scaffold but after one week, lacunae were observed housing the cells, due to ECM production confirmed at histological sections. Some chondrocyte-like features were observed on the phase-contrast microscopy such as cells inside lacunae and cells grouped together resembling isogenous groups commonly found in hyaline cartilage.

**Cartilage Genes Expression**

At 21 day of differentiation, the expression of chondrogenic of genes aggrecan, collagen type I and collagen type II in different groups were analyzed with qRT-PCR. The chondrogenic gene expression were increased and these levels in group of TGF-β1 and PRP-TGF-β1 were obviously higher than in control group. Furthermore, aggrecan expression in PRP-TGF-β1 significantly higher than TGF-β group (Fig. 4).

**Chondrogenic Differentiation**

The effect of PRP on cartilage-specific proteins are shown in Fig. 5. Collagen type I, II and aggrecan are the major structural proteins of cartilage extracellular matrix (ECM). The amount of collagen type I, II and aggrecan protein in the PRP-TGF-β1 group was obviously more than TGF-β1 group. In comparison, when treated with PRP along with TGF-β1, the cells synthesized more collagen type I, II and aggrecan compared with the TGF-β1 group (Fig. 5).

**In vivo experiment**

Osteoarthritis was confirmed by severe loss of joint space in medial compartment using radiographical images (Fig. 6)

Histopathological findings of the knee joint in various groups are presented in the Figs. 7 and 8.

Severe abrasion or superficial fibrillation characterized by microscopic cracks into the superficial, mid and deep zones of articular cartilage was present in the control group (Fig. 7A). Cartilage matrix loss resulted in delamination of superficial, mid, and deep layers and cyst formation associated with chondrocyte death, regeneration (clusters), hypertrophy, replication and proliferation in the control group. The deformative change cause articular bone plate micro-fracture. The organization of the subchondral and trabecular bone was disrupted. Sever subchondral sclerosis and extensively increased in bone volume and bone marrow (BM) distance from cartilage was detected. Osteonecrosis associated with the area of bone resorption and infiltration of osteoclastic-like cells associated with severe infiltration of multinucleated giant cells indicating granulomatous inflammation was seen in the control group.

Moderate abrasion or fibrillation characterized by microscopic cracks into the mid and deep zones of articular cartilage was present in the OA + MSC group (Fig. 7B). Mild abrasion or fibrillation characterized by microscopic cracks into the deep zone of articular cartilage was present in the OA + MSC + Collagen group (Fig. 7C). A mild subchondral sclerosis as well as micro-fractures in the trabecular bone was seen.
All histopathological features including chondrocytes proliferation, clusters and death, articular bone plate micro-fracture, and osteonecrosis without infiltration of multinucleated giant was also detected in the OA + MSC group with the lesser severity for the OA + MSC + Collagen group. The disrupted organization of the subchondral bone was improved in the OA + MSC + Collagen compared to both OA + MSC and control groups. The OA + MSC group also showed the better organization of the subchondral bone than that of the control group. There were no microscopic cracks into all three zones of articular cartilage in the OA + MSC + Collagen + PRP group. The cartilage was intact in the most part of knee joint and cartilage matrix swelling leads to cartilage hypertrophy in this group. There was no evident subchondral sclerosis. The subchondral plate and trabecular bone was approximately intact. The induced osteoarthritis was improved in the MSC + PRP + Collagen group compared to other groups (Fig. 7D). High magnification of histopathological features is shown in Fig. 8.

**Discussion**

Knee OA (KOA) is one of the most prevalent degenerative joint diseases with persistent pain and loss of function. It was published that among elders, the risk of lower limb movement disability caused by KOA is at least 40% (26). Natural polymers such as collagen has been studied the most owing to their biocompatibility, making them suitable for cell therapy (27). Tissue engineering approaches using MSCs and scaffolds is new strategy for cartilage regeneration (28). Therapies with encapsulated MSCs have been new approach for knee OA. Encapsulated MSCs show prolonged survival subsequent injection that could be beneficial for long-term efficacy (29). However, the absence of functional inducing chondrogenic differentiation is drawback. In this study, the effect of cartilage regenerative capacity of a tissue-engineered composite of PRP + MSCs + collagen hydrogel was evaluated in vitro and in vivo.

The growth factors, such as TGF-β, indicate that has important capacity as a tool for stimulating chondrocyte proliferation and repair of cartilage defects (30). PRP is obtained from peripheral blood, which has pain-relief and anti-inflammatory effects, exhibited good efficacy in knee-osteoarthritis and other musculoskeletal morbidity (31). In our study, we investigated that collagen hydrogel that contain MSCs and PRP might improve the efficiency of injured articular cartilage treatment in animal model. We theorized that PRP affects MSC proliferation and differentiation, especially chondrogenic differentiation.

Drengk et al. reported that PRP activated proliferation of mesenchymal stem cells in 3D culture (32). PRP as a source of important growth factors could efficiently facilitate differentiation and proliferation of MSCs (33). In this study, the viability and proliferation of MSCs was evaluated by MTT assay. The proliferation rate of MSCs-collagen-PRP group significantly higher that other groups.

PRP contains a natural cocktail of growth factors such as TGF-β demonstrated upregulated cartilage gene expression and differentiation (32). The capacity of chondrogenic effects of PRP are attributed to growth factors such a TGF-β, vascular endothelial growth factor, basic fibroblast growth factor produced in the alpha granules of platelets (34). In this study, Immune-staining of differentiated cells in TGF-β- PRP group shown that, aggrecan, collagen type I and collagen type II protein expression are higher than TGF-β.
-PRP group and confirmed that enhancive effect of TGF-β transcription factor in chondrogenic differentiation.

Next, we investigated the gene expression of chondrogenic pathway. Zhu et al. reported that TGF-β is reported to initiate the chondrogenic differentiation process by induction of collagen and aggrecan genes (35). Collagen type I, collagen type II and aggrecan are the main genes of cartilage extracellular matrix and play an essential role in maintenance of cartilage (27, 36). The gene expression of aggrecan in PRP-TGF-β1 group was higher than that of PRP and control groups. In this study, RNA levels of cartilage-specific markers, including collagen type I, collagen type II and aggrecan were similarly higher in the PRP-TGF-β1 and TGF-β1 group than control group. Liou et al. found that MSCs encapsulated in a PRP-containing hydrogel, resulting in optimal MSC chondrogenic differentiation (37). Intra-articular injection of MSCs has limitations being considered to cell death after injection and leakage from the injection site. Encapsulation of MSCs by biomaterial has therefore been developed to overcome these drawbacks and to deliver therapeutic factors in intra-articular knee (38). Rogan et al. reported that MSCs encapsulation obviously resulted in more cartilage formation in KOA models. Luca et al, founded that encapsulated cells show long-standing survival following injection that could be useful for long-term efficacy of MSCs in cell-based therapy of OA. In current study, histological assessment of articular cartilage was conducted 6-week post injection. Cartilage repair exhibited better healing result in MSC-PRP-Collagen group compared to other groups. Although the chondrogenic differentiation effect of PRP was poorer than TGF-β. The result is consistent with the research of Chellini et al. (39), and the anti-fibrotic potential of PRP underlies the formation of high-quality cartilage.

Conclusions

Our study revealed that MSCs encapsulated in a PRP-containing collagen hydrogel can be used as a potent chondrogenic inducer substitute on cartilage tissue engineering. *In vivo* experiment demonstrated that these composite have a beneficial effect on OA treatment via the stimulation of ECM synthesis and chondrocyte proliferation and provides a new insight into cartilage tissue engineering strategies.

Declarations

Ethics approval and consent to participate:

The study was performed according to instructions approved by the Animal Care and Use Committees of Tehran University of Medical Sciences (ethical code: IR.TUMS. VCR.1398.034).

Consent for publication:

All of the authors confirm publication of this paper

Availability of data and materials:
Competing interests:

The authors have no conflicts of interest.

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All of the authors contributed in different part of this paper.

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