Intra-articular Injection of Cell-laden 3D Microcryogels Empower Low-dose Cell Therapy for Osteoarthritis in a Rat Model

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Abstract

Intra-articular injection of mesenchymal stem cells (MSCs) in an osteoarthritic joint can help slow down cartilage destruction. However, cell survival and the efficiency of repair are generally low due to mechanical damage during injection and a high rate of cell loss. We, thus, investigated an improved strategy for cell delivery to an osteoarthritic joint through the use of three-dimensional (3D) microcryogels. MSCs were seeded into 3D microcryogels. The viability and proliferation of MSCs in microcryogels were determined over 5 d, and the phenotype of MSCs was confirmed through trilineage differentiation tests and flow cytometry. In Sprague Dawley rats with induced osteoarthritis (OA) of the knee joint, a single injection was made with the following groups: saline control, low-dose free MSCs (1×10^5 cells), high-dose free MSCs (1×10^6 cells), and microcryogels + MSCs (1×10^5 cells). Cartilage degeneration was evaluated by macroscopic examination, micro-computed tomographic analysis, and histology. MSCs grown in microcryogels exhibited optimal viability and proliferation at 3 d with stable maintenance of phenotype *in vitro*. Microcryogels seeded with MSCs were, therefore, primed for 3 d before being used for *in vivo* experiments. At 4 and 8 wk, the microcryogels + MSCs and high-dose free MSC groups had significantly higher International Cartilage Repair Society macroscopic scores, histological evidence of more proteoglycan deposition and less cartilage loss accompanied by a lower Mankin score, and minimal radiographic evidence of osteoarthritic changes in the joint compared to the other two groups. In conclusion, intra-articular injection of cell-laden 3D microcryogels containing a low dose of MSCs can achieve similar effects as a high dose of free MSCs for OA in a rat model. Primed MSCs in 3D microcryogels can be considered as an improved delivery strategy for cell therapy in treating OA that minimizes cell dose while retaining therapeutic efficacy.

Keywords

osteoarthritis, mesenchymal stem cells, microcryogels, hydrogels, cartilage repair

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Introduction

Osteoarthritis (OA) is the most prevalent chronic degenerative joint disease and is associated with a serious health burden both for individuals and society[1]. The incidence and prevalence of OA are constantly increasing due to the world's aging population[2]. OA is characterized by cartilage damage, subchondral bone sclerosis, and osteophyte formation, resulting in chronic pain and reduced mobility that severely impact an individual's quality of life[3]. Current treatments for OA include nonpharmacological, pharmacological, and surgical approaches, all of which can only provide a transient relief of symptoms[4,5]. No effective disease-modifying therapies targeting complicated chronic joint pathology have been developed for OA[6].

Mesenchymal stem cells (MSCs) have been tested over the last decade as a potential regenerative therapy for treating OA[7]. Preclinical and clinical studies have recently demonstrated some favorable effects on cartilage repair when MSCs were injected into OA joints in animal models[8] and in humans[9]. The standard method of MSC administration for treating OA is by direct intra-articular injection of cells into the affected joint, and any resulting effects are thought to be due to the anti-inflammatory and pro-regenerative paracrine functions of the MSCs within the lesion site[10,11]. However, the long-term therapeutic benefits of MSC injections remain limited, and the treatment effects are sometimes irreproducible due to extensive cell death or cell loss from the lesion site following injection[12]. We previously investigated the effects of injecting human umbilical cord-derived MSCs (hUC-MSCs) in a rat model of knee OA, and reported that a single injection could have temporary effects on slowing cartilage degeneration in the short term[13].

Several factors may contribute to the poor effects of cell therapy administered through direct injection, including mechanical damage to cells during injection, low rate of cell retention at the injection site due to leakage to surrounding tissues, and extensive cell death following injection due to lack of protection from the extracellular matrix (ECM)[14]. A simple and commonly used solution to enhance the efficacy and reproducibility of MSC therapy for treating OA is to inject a large dose of cells, which theoretically accounts for significant cell loss and ensures that a sufficient number of functional MSCs will remain in the intra-articular space following injection[15]. However, using high doses of MSCs not only reduces the practicality of cell therapy due to substantially increasing the costs for cell processing, but also brings additional risks associated with overexpansion of cells[16]. These risks may include uncontrollable cell growth or aberrant mutations, particularly when genetically modified MSCs are used[17]. There is hence a significant need for improved delivery strategies to enable low-dose cell therapy, which can simultaneously minimize the side effects and enhance the efficacy and reproducibility of treatment.

A number of biomaterial-assisted cell delivery approaches have been developed to improve MSC injections for OA[18,19]. For example, MSCs can be encapsulated in an aqueous suspension that forms a hydrogel following injection into an OA joint[19]. However, while delivery in a hydrogel can improve cell retention and survival, the hydrogel does not promote ECM accumulation and cell–cell interactions for the encapsulated cells. An optimal delivery method should provide protection to cells during injection, while providing a suitable environment for the cells to construct a native cellular niche following injection. We previously developed injectable three-dimensional (3D) microcryogels as improved cell delivery vehicles, which were microscopic porous gelatin hydrogels[14,20,21]. These microcryogels were biocompatible and biodegradable, and shown to promote cell retention and survival following injection in a range of animal models.

In this study, we hypothesized that using our 3D microcryogels as a delivery vehicle would enhance the survival, retention, and function of MSCs in treating OA, such that therapeutic effects could be achieved using a reduced cell dose. hUC-MSCs were "primed" by preculturing in 3D microcryogels for 3 d before the cell-laden microcryogels were injected into the knee joint of rats with induced OA. Although the microcryogels contained a low dose of MSCs, they achieved similar therapeutic effects as a high dose of free MSCs for up to 8 wk post-injection by macroscopic, histological, and radiographic analyses. This was the first evidence where microscopic porous hydrogels were able to deliver highly efficient injectable MSC therapy using a small dose of cells in an OA animal model. A similar method may be useful for improving the treatment efficacy of intra-articular MSC injection for OA while minimizing the cell dosage.

Materials and Methods

Experimental Design

Three-dimensional gelatin-based microcryogels were seeded with hUC-MSCs and cultured for 3 d *in vitro*, before intra-articular injection into the knee joint in a rat model of OA. Cartilage degeneration was evaluated at 4 and 8 wk post-injection (Fig. 1).

Seeding of hUC-MSCs in Microcryogels

hUC-MSCs (EUBIO Technologies) at passage 4 were cultured in MSC growth medium (BioWit Technologies, Shenzhen, China), as previously described[21]. The microcryogels were packed and supplied as 5-mm-diameter discs (TableTrix[TM], CytoNiche Biotech, China)[13]. The hUC-MSCs were seeded in a 200-µl cell suspension (2.5×10^6 cells/ml) onto 20 mg microcryogel discs in a 40-mm-diameter cell culture dish. Upon contact with aqueous solution, the microcryogel disc immediately hydrated and dispersed into 1×10^5 microcryogels. The seeded microcryogels were incubated at 37°C in a humidified chamber
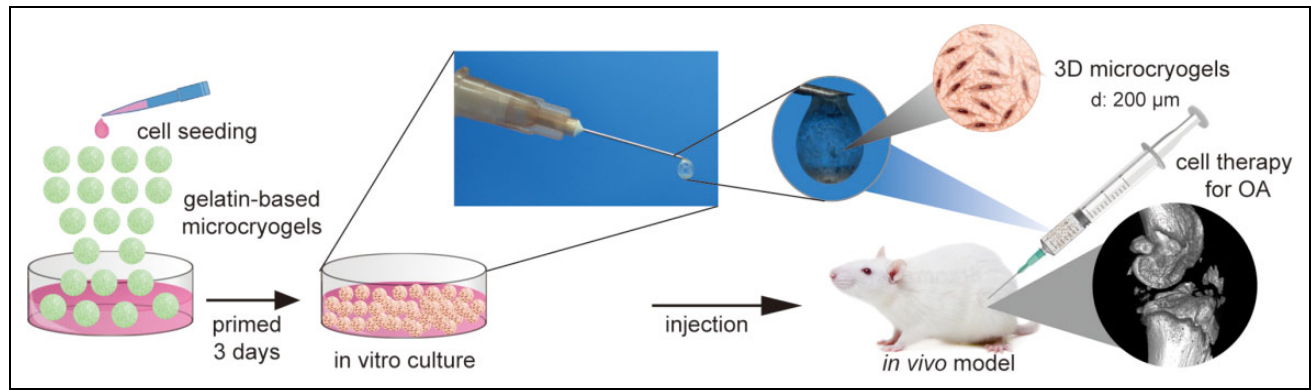
Figure 1. Schematic illustration of the study design. hUC-MSCs were seeded in 3D microcryogels and cultured for 3 d before intra-articular injection into the knee joint in a rat model of OA.
3D: three-dimensional; hUC-MSC: human umbilical cord-derived mesenchymal stem cell; OA: osteoarthritis.

for 2 h to allow cell adhesion[22], following which MSC growth medium was added.

Morphological Observation of Microcryogels

The morphology of microcryogels dispersed using phosphate-buffered saline (PBS) was observed using scanning electron microscopy (SEM; FEI Quanta 200). Samples were fixed using 10% neutral buffered formalin, dehydrated using graded ethanol, subjected to critical point drying, and sputter coated with gold for 90 s prior to SEM observation.

Flow Cytometry

Following 0, 3, and 5 d of culture in microcryogels, the hUC-MSCs were digested from the microcryogels and suspended in PBS. The anti-human antibodies CD34 (catalog number: 343513), CD44 (catalog number: 338809), CD73 (catalog number: 344003), CD90 (catalog number: 328113), and CD105 (catalog number: 323203) (BioLegend, San Diego, CA, USA) were used for flow cytometry according to the manufacturer's instructions. Antibody incubations were conducted on ice for 30 min. The hUC-MSCs were then washed and resuspended in PBS for analysis by flow cytometry (BD LSRFortessa SORP, USA).

Trilineage Differentiation Assay

For all differentiation assays, hUC-MSCs were first cultured in microcryogels for 0, 3, and 5 d before being digested, and were subsequently seeded in six-well plates. Cells were cultured in growth medium for 12 h followed by changing to differentiation medium. The trilineage differentiation ability of hUC-MSCs was assessed by culturing the cells in StemPro® differentiation media (Gibco, Life Technologies, USA) to induce osteogenesis, chondrogenesis, and adipogenesis. Osteogenesis was assessed at 14 d by staining the cells for alkaline phosphatase with the BCIP/NBT Kit (Sigma-Aldrich, USA) according to the manufacturer's

instructions. Chondrogenesis was assessed at 14 d by staining for sulfated proteoglycan deposits with Alcian-Blue Staining Solution (Sigma-Aldrich). Adipogenesis was assessed at 21 d by staining for lipid accumulation with an Oil Red O Kit (Sigma-Aldrich).

Cell Proliferation

Cell proliferation was assessed at 0, 3, and 5 d of culturing hUC-MSCs in microcryogels, by calculating the number of cells per 20 mg of microcryogels in microscope. The viability of hUC-MSCs within the microcryogels was visualized using live/dead staining (Life Technologies).

Intra-articular Injection in an OA Animal Model

The animal experiment was performed with approval from the Peking University Biomedical Ethics Committee. Thirty Sprague Dawley rats (10 wk old; weighing 180 ± 47 g) were randomly divided into four groups: control ($n = 6$), low-dose free MSCs ($n = 8$), high-dose free MSCs ($n = 8$), and microcryogels + MSCs ($n = 8$). Bilateral knee OA was induced by anterior cruciate ligament (ACL) transection. Briefly, the rats were anesthetized and surgery was performed to transect the ACL to induce destabilization of the knee joint. After surgery, each rat was administered penicillin once a day for the first 3 d. Rats were treated with intra-articular injection in both knees at 4 wk after ACL surgery. The control group was injected with 100 μl saline. For the low-dose and high-dose free MSC groups, hUC-MSCs were injected at 1×10^5 and 1×10^6 cells/knee, respectively, in 100 μl saline. For the microcryogels + MSCs group, 1×10^5 hUC-MSCs in about 0.5 to 0.7 mg microcryogels in saline with a total volume of 100 μl were injected. Cartilage degeneration was evaluated at 4 and 8 wk after treatment by macroscopic examination, micro-computed tomography (μ-CT), and histology.
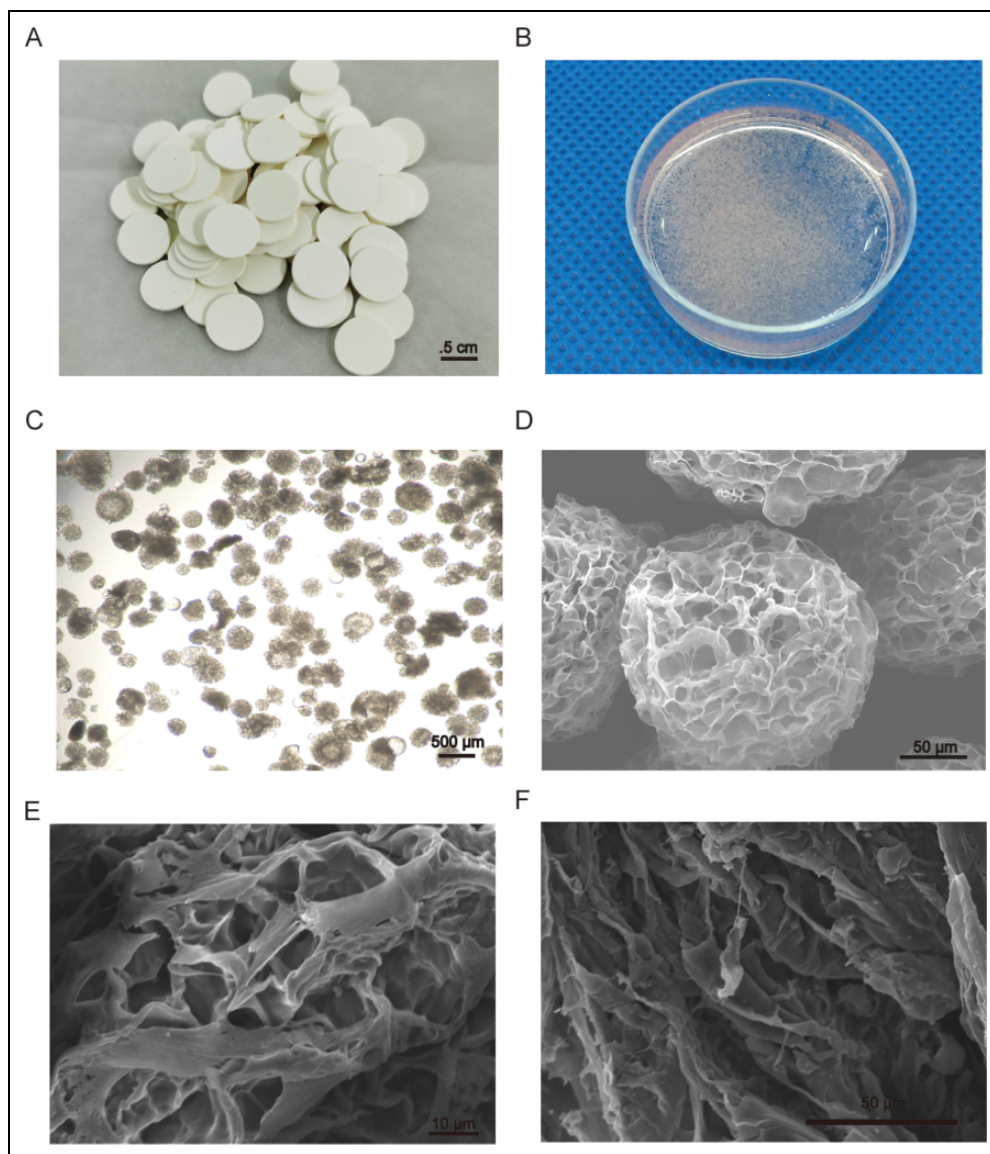
Figure 2. Characteristics of microcryogels. (A) Packed microcryogels in a tablet form. (B) Morphology of microcryogels dispersed in phosphate-buffered saline. (C) Microscopic observation of microcryogels. (D, E) Images of microcryogels. (F) SEM images of microcryogels seeded with mesenchymal stem cells.
SEM: Scanning electron microscopy.

μ-CT Analysis. After sacrificing the rats at 4 and 8 wk after treatment, intact knee samples were imaged using a μ-CT system (Inveon MM CT, Siemens AG, Munich, Germany). A scanning time of 0.21 s with settings of 80 kVp, 500 μA, and 30 calibrations was used. Axial and transaxial fields of view of 30 mm were acquired. 3D reconstructions were generated from 2D images using multimodal 3D visualization software (Inveon Research Workplace, Siemens). For the medial femoral condyle, the region of interest was acquired from subchondral bone. An appropriate threshold was adjusted to define the mineralized bone phase. Bone mineral density (bone volume/total volume) was calculated three times for each sample. A semi-quantitative method based on the degree of osteophyte formation and joint destruction was used to grade the degree of OA changes in the joint[23,24].

Macroscopic Evaluation. Following μ-CT analysis, the surface of the distal femur of each knee joint was exposed. Macroscopic evaluation was conducted according to the cartilage repair assessment instrument of the International Cartilage Repair Society (ICRS)[25]. The assessment was performed based on macroscopic examination of the cartilage surface, which was scored from 4 to 0 (4: Intact smooth surface, 3: Fibrillated surface, 2: Small, scattered fissures or cracks, 1:
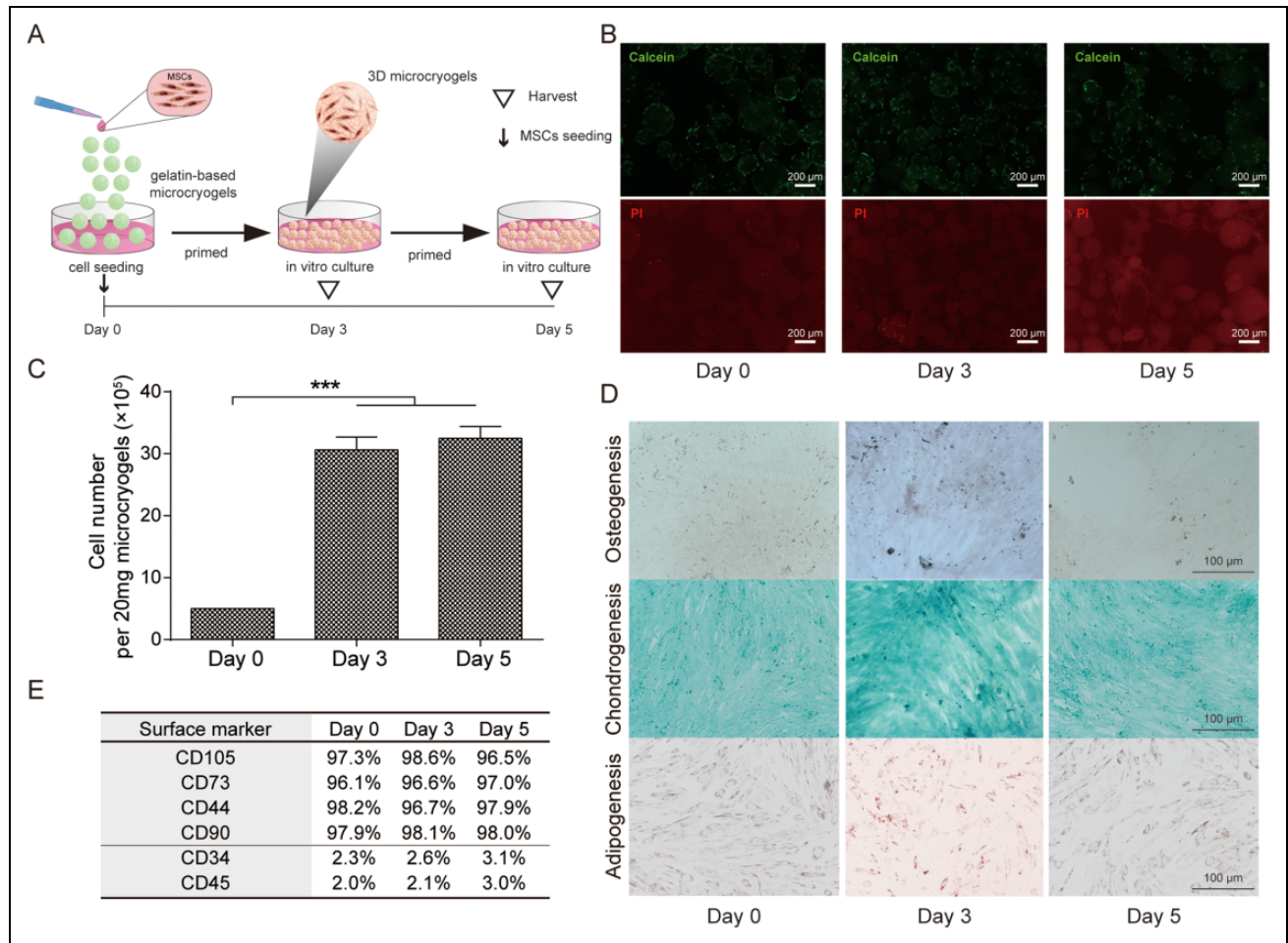
Figure 3. Characterization of hUC-MSCs cultured in microcryogels. (A) Schematic illustration of the timeline for *in vitro* culture of hUC-MSCs in microcryogels. (B) Fluorescence microscopy images of MSCs with live/dead staining and (C) number of MSCs in microcryogels over 5 d. (D) Trilineage differentiation ability and (E) surface marker expression by flow cytometry of MSCs following 0, 3, and 5 d of culture in microcryogels.
3D: three-dimensional; hUC-MSC: human umbilical cord-derived mesenchymal stem cell.

Several, small or few but large fissures, 0: Total degeneration of surface area).

Histology and Immunohistochemistry. Following macroscopic examination, the knee joints were fixed in 4% paraformaldehyde overnight. Decalcification was conducted in 4% ethylenediamine tetraacetic acid for 1 mo, with the decalcifying solution changed every 3 d. Decalcified joints were embedded in paraffin and 4 μm sections of the medial femoral condyle were cut. The sections were stained using hematoxylin and eosin (HE), safranin-O, and toluidine blue.

The severity of cartilage degeneration was assessed using the Mankin score[26] based on histological findings, namely (1) structure (score 0 to 6), (2) cells (score 0 to 3), (3) safranin-O staining (score 0 to 4), and (4) tidemark integrity (score 0 to 1). A higher score indicated a greater level of cartilage degeneration.

Immunohistochemical staining was conducted using the histological sections. Briefly, after deparaffinization, sections were incubated with 0.3% hydrogen peroxide for 30 min and treated with hyaluronidase for 60 min. The sections were then incubated with COL-I or COL-II monoclonal antibodies (Santa Cruz Biotechnology, USA). All antibody dilutions were made in PBS. After an overnight reaction with the primary antibody at 4°C, sections were incubated with labeled polymer-horseradish peroxidase anti-mouse immunoglobulin G at room temperature for 30 min.

Statistical Analysis

All data were expressed as mean $\pm$ standard deviation. After testing for homogeneity of variances, one-way analysis of variance followed by Tukey's post hoc multiple comparisons test were used to determine significant differences between
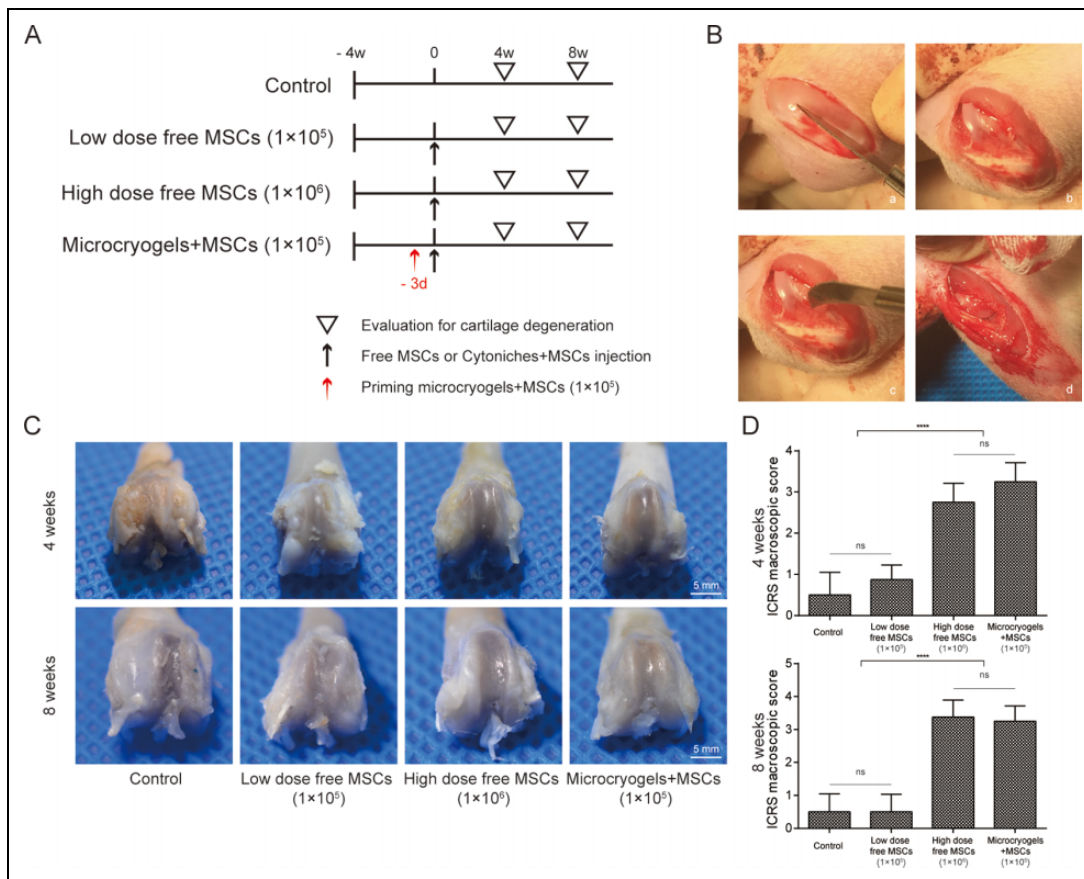
Figure 4. Macroscopic evaluation. (A) Schematic of the *in vivo* study timeline. (B) Surgical procedure for anterior cruciate ligament transection to establish the rat osteoarthritis model. The skin and fascia on the knee cap region of the hind limb were vertically incised in the midline for a distance of approximately 4 cm. The patella was retracted laterally to expose the articular cavity. The anterior cruciate ligaments were transected. The patella was relocated back to its original position, and the fascia and skin were closed with sutures. (C) Representative macroscopic images of the femoral condyle in different groups at 4 and 8 wk after injection. (D) ICRS macroscopic scores of the femoral condyle at 4 and 8 wk after injection. ****$P < 0.0001$.
ICRS: International Cartilage Repair Society; MSC: mesenchymal stem cell.

groups. SPSS 11.0 was used to perform statistical analyses, and values of $P <0.05$ were considered statistically significant.

Results

Characteristics of Microcryogels

The gelain-based 3D microcryogels were packed into a tablet form for storage (Fig. 2A). Upon contact with PBS (or any other liquid), the tablets dispersed into loose microcryogels (Fig. 2B). Observations using a light microscope showed that the microcryogels had a round, translucent, and uniform appearance (Fig. 2C). SEM images of microcryogels showed their interconnected and macroporous structure (Fig. 2D, E), which would greatly facilitate cell loading.

Characterization of hUC-MSCs Cultured in Microcryogels

hUC-MSCs were seeded in microcryogels and cultured over 5 d for *in vitro* analyses (Fig. 3A). Live/dead staining showed that the vast majority of cells were alive at 0, 3, and 5 d of culture in microcryogels (Fig. 3B). The number of cells in one tablet worth of microcryogels increased significantly from 0 to 3 d, but remained relatively stable between 3 and 5 d (Fig. 3C).

hUC-MSCs cultured for 0, 3, and 5 d in microcryogels and subsequently digested for trilineage differentiation testing showed the ability to undergo osteogenesis, chondrogenesis, and adipogenesis through differentiation-specific staining assays (Fig. 3D). At 0, 3, and 5 d, the cells also showed positive staining for the MSC-specific surface markers CD44, CD73, CD90, and CD105, as well as negative staining for CD45 and CD34 (Fig. 3E).

Since hUC-MSCs grown in microcryogels for 3 d gave rise to peak cell numbers and viability, as well as maintenance of their MSC phenotype, microcryogels containing hUC-MSCs precultured for 3 d were used as the microcryogels + MSCs group for subsequent *in vivo* testing.

In Vivo Effects of Microcryogels Containing hUC-MSCs in an OA Model

Macroscopic Evaluation. The effects of treatments by intra-articular injection in a rat OA model were evaluated at 4 and 8 wk after injection (Fig. 4A). OA was induced in the rat model by ACL transection (Fig. 4B). Macroscopic examination of the joint surface of the distal femur at 4 wk showed that the control and low-dose free MSC groups had marked signs of OA progression, including cartilage surface roughness and osteophyte formation (Fig. 4C). In contrast, the high-dose free MSCs and microcryogels + MSC groups showed a well-preserved cartilage surface. At 8 wk, more severe OA progression had occurred in the control and low-dose free MSC groups, which showed a highly disrupted cartilage surface. In the high-dose free MSCs and microcryogels + MSC groups, the cartilage surface was smooth and had a relatively normal appearance. Accordingly, the ICRS macroscopic scores for the high-dose free MSCs and microcryogels + MSC groups were significantly higher than the other two groups at both 4 and 8 wk (Fig. 4D). There were no significant differences between ICRS scores of the high-dose free MSCs and microcryogels + MSC groups at either time point.

μ-CT Analysis. Representative μ-CT images of knee samples at 4 and 8 wk post-treatment showed radiological osteophyte formation around the knee joint in all groups (Fig. 5A). However, a greater number of samples in the high-dose free MSCs and microcryogels + MSC groups showed lower grades of OA change than the control and low-dose free MSC groups by μ-CT analysis (Fig. 5B). The groups showed no significant differences in bone mineral density (BV/TV) at 4 and 8 wk (Fig. 5C).

Histological and Immunohistochemical Analyses. Representative histological images of the medial femoral condyle are shown for all groups stained using HE, safranin-O, and toluidine blue at 4 and 8 wk (Fig. 6A). At 4 wk, the articular cartilage in the control and low-dose free MSC groups showed surface irregularity, loss of cellularity, and reduced area of safranin-O staining, indicating significant OA changes. In contrast, the high-dose free MSCs and microcryogels + MSC groups showed abundant proteoglycan in the articular cartilage and reduced cartilage loss, without significant features of OA progression. The same joint structure was preserved at 8 wk. On the other hand, knee joints in the control and low-dose free MSC groups showed increased OA progression at 8 wk, with significant loss of joint integrity and proteoglycan
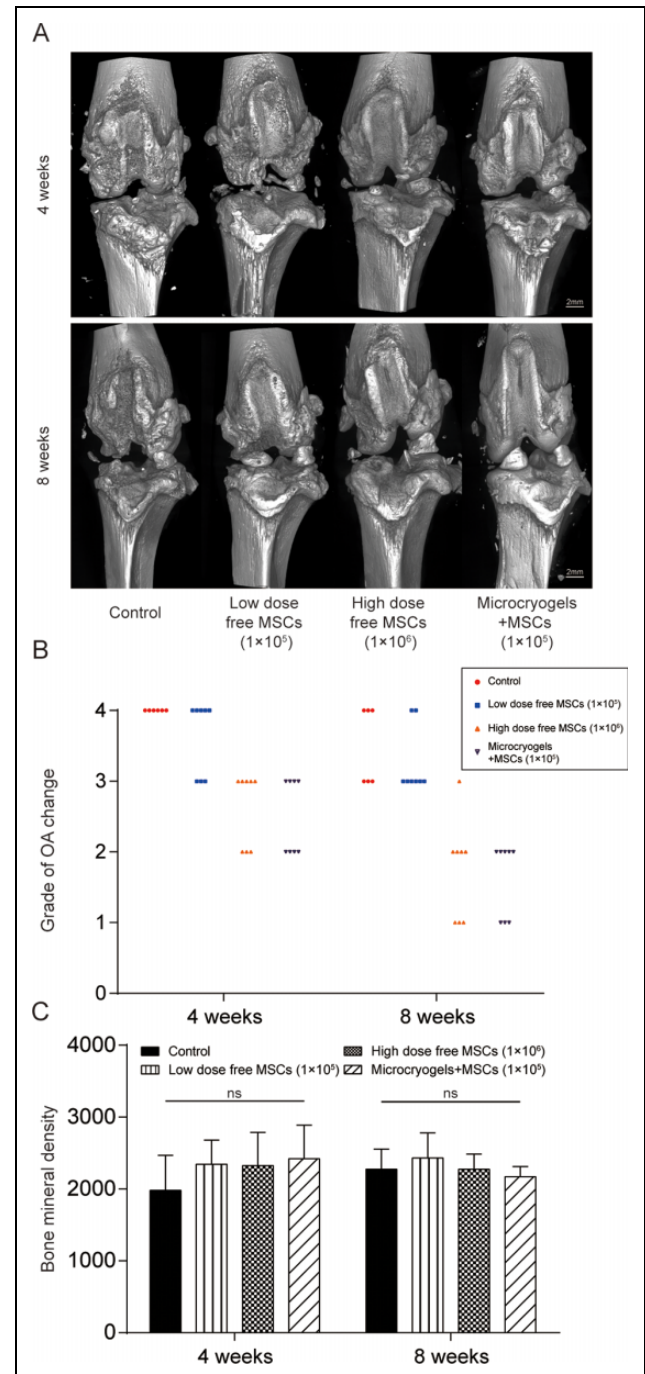

Figure 5. μ-CT analysis. (A) Posterior-anterior views of the knee joint in all groups by μ-CT at 4 and 8 wk after injection. (B) The grade of OA change for individual samples in all groups at 4 and 8 wk. (C) Bone mineral density (BV/TV) of subchondral bone in the medial femoral condyle at 4 and 8 wk.
μ-CT: micro-computed tomography; MSC: mesenchymal stem cell; OA: osteoarthritis; BV: bone volume; TV: total volume.

content. Accordingly, the high-dose free MSCs and microcryogels + MSC groups showed similar Mankin scores at 4 and 8 wk, which were significantly higher than the other two
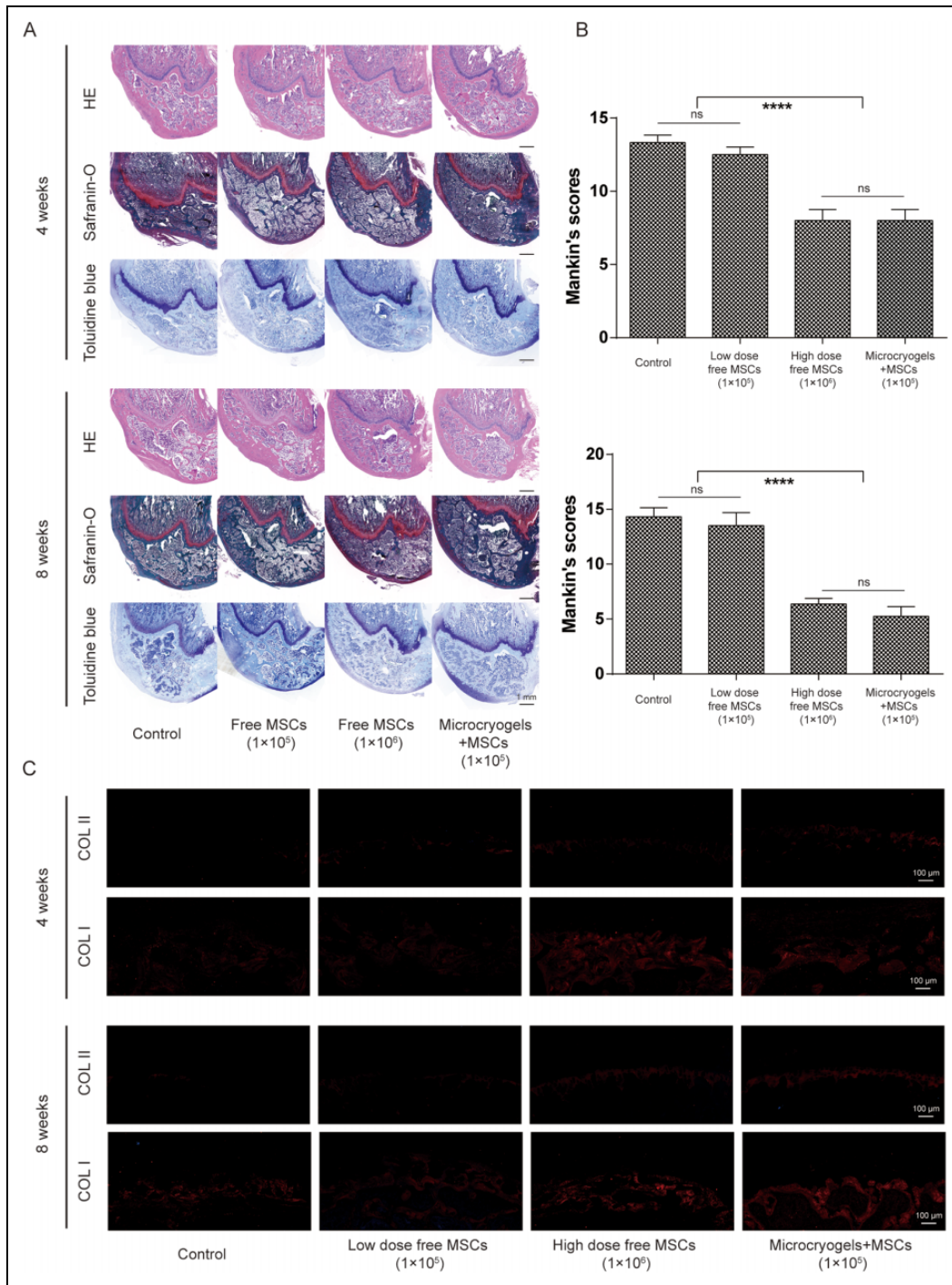
Figure 6. Histological and immunohistochemical analyses. (A) Histological images of articular cartilage in the medial femoral condyle of all groups, stained by HE, safranin-O, and toluidine blue, and (B) Mankin scores at 4 and 8 wk after injection. (C) Immunohistochemical staining for collagen types I and II at 4 and 8 wk. ****$P < 0.0001$.
HE: hematoxylin and eosin; MSC: mesenchymal stem cell.

groups (Fig. 6B). Immunohistochemical staining showed that collagen types I and II were both more abundant in the high-dose free MSCs and microcryogels + MSC groups compared to the other two groups at 4 and 8 wk (Fig. 6C).

Discussion

Over the last decade, cell therapy using MSCs has been increasingly explored as a potential new approach for treating OA, with some early-phase clinical studies

demonstrating promising outcomes[27–29]. However, our recent reviews on the current evidence of using intra-articular injections of MSCs to treat knee OA in animal studies and clinical trials showed inconclusive benefits, and indicated low confidence of recommending this as a reliable long-term therapy for OA[8,30]. Our recent study in a rat model of OA also revealed that a single injection of MSCs can only have temporary, early-stage effects on the progression of cartilage degeneration rather than prolonged effectiveness in disease modification[13].

The lack of long-term effects of MSC therapy in OA and other pathologies may be due to poor retention, survival, and function of the transplanted cells[31,32]. To circumvent these problems and increase the efficacy of cell therapy for OA, simply increasing the cell dose or increased number of injections may be an option. In this study, we confirmed that a high dose of 1×10^6 free MSCs produced significantly better therapeutic effects than a low dose of 1×10^5 cells in a rat OA model. Another study in a small cohort of patients also found that a single intra-articular injection of 100 compared to 10 million MSCs was more effective in attenuating joint damage in knee OA[15]. However, this approach may not be economically viable for large-scale clinical use due to the limited sources of MSCs and the costs of cell harvesting and expansion[33,34]. Although various peptides and antibodies can be used to direct cells to targeted repair sites[35,36], which can improve cell retention and potentially reduce the required cell dose, these approaches are associated with immune reactions and safety concerns. In this study, we used a new approach comprising injectable microcryogels as MSC carriers, which not only can enhance cell survival and retention following injection into the OA joint, but also improve cell function by allowing native cell–cell interactions and ECM deposition within the carrier[14,20,21]. We showed that the microcryogels loaded with a low dose of MSCs achieved similar therapeutic effects as a high dose of MSCs in a rat model of knee OA.

The microcryogels we previously developed could be aggregated into a tablet form for long-term preservation, enabling their use as an off-the-shelf product. These microcryogels were fabricated by cryogelation of gelatin, and once dispersed in aqueous solution can maintain their highly porous and interconnected structure, which facilitates efficient cell loading by automatic absorption[20]. In this study, the hUC-MSCs were easily loaded into the microcryogels by simply pipetting the cell suspension onto the surface of the microcryogel tablet. We considered the timing for preculture of MSCs in microcryogels as a critical parameter for optimal therapeutic efficacy, as long-term culture could lead to senescence, which ultimately reduces cell quality[37]. We chose 3 d as the duration for *in vitro* preculture of MSCs in microcryogels prior to *in vivo* injection, as this time period allowed peak MSC viability and proliferation, as well as stable maintenance of phenotypic characteristics, all of which were suggestive of an optimal balance between cell function and ECM accumulation (eventually leading to cell senescence).

The cell number for intra-articular injection in low-dose free MSCs and microcryogels + MSCs was 1×10^5. Although priming MSCs in microcryogels led to the proliferation of MSCs, we confirmed that the cell number for injection was 1×10^5 in total 10 ml saline. By MSCs' digestion from the microcryogels, we estimated about 1/30 20 mg microcryogels including 1×10^5 MSCs. Thus, 20 mg microcryogels with MSCs that were primed for 3 d was dispersed in 3 ml saline before injection. At 4 and 8 wk after *in vivo* injection, joints injected with high-dose free MSCs and microcryogels + MSCs showed similar histological features characterized by relatively normal articular cartilage structure and cell distribution, appropriate thickness, consistent safranin-O staining, and only mild irregularities in the surface layer. In contrast, joints injected with the control and low-dose free MSCs showed structural disorganization in the cartilage and significant OA progression. These results were consistent with the macroscopic and radiological findings, which collectively suggested that by using the microcryogels as cell carriers, a low dose of MSCs can exert similar treatment effects as a high dose of free MSCs in protecting joint cartilage from degradation during OA progression. Moreover, the protective effects were evident over a relatively long period of 8 wk, and no adverse effects were observed in the microcryogels + MSCs group throughout the duration of the study.

The therapeutic efficacy seen in the microcryogels + MSCs group is likely due to the microcryogels creating a microenvironment for the MSCs that maximizes their paracrine functions. The beneficial properties of MSCs in treating OA are thought to be linked to their release of immunomodulatory factors and chemoattractants, which exert an indirect effect by enriching the repair environment[38]. Subjecting the MSCs to a microenvironment that promotes natural ECM accumulation can contribute to modulating cell behavior and fate, as the biophysical and biochemical cues generated during ECM formation can activate distinct signaling pathways and enhance cell function[39,40]. The microcryogels can provide the MSCs with such a microenvironment and facilitate natural cell–cell and cell–ECM interactions, which can increase their paracrine secretions and result in a range of effects that are beneficial for cartilage repair, including cell homing, anti-inflammation, and immune regulation[41–43]. Moreover, the microcryogels containing accumulated ECM may better preserve the MSC-secreted exosomes to help suppress OA development[44,45]. The microcryogels may also offer physical protection to the MSCs by shielding them from mechanical insult and necrosis during and after cell injection[14,46,47].

Collectively, the results of this study suggested that MSC-laden microcryogels were a safe and effective approach for achieving therapeutic efficacy in a small animal model of OA while minimizing the required cell dose. The outcomes of using MSC-laden microcryogels with different sources of

MSCs and initial cell doses, and testing in larger animal models of OA will be explored in future studies.

Conclusion

Intra-articular injection of 3D microcryogels loaded with a low dose of hUC-MSCs (1×10^5 cells) had similar therapeutic effects as a high dose of free hUC-MSCs (1×10^6), which decelerated the progression of cartilage destruction in a rat OA model. This MSC delivery strategy using microcryogels may hold potential as a superior cell therapy approach for OA that reduces cost and limits the risk of side effects by minimizing the required cell dose to achieve therapeutic efficacy.

Ethical Approval

All animal procedures used in this study were approved by Peking University Biomedical Ethics Committee.

Statement of Human and Animal Rights

All of the experimental procedures involving animals were conducted in accordance with Institutional Animal Care guidelines of Peking University People's Hospital, China and approved by the Administration Committee of Experimental Animals of Peking University, Beijing, China.

Statement of Informed Consent

There are no human subjects in this article and informed consent is not applicable.

Declaration of Conflicting Interests

The author(s) declared no potential conflicts of interest with respect to the research, authorship, and/or publication of this article.

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