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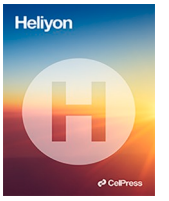

Research article

Human umbilical cord mesenchymal stem cells on treating osteoarthritis in a rabbit model: Injection strategies

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ABSTRACT

Human umbilical cord mesenchymal stem cells (UCMSCs) are a novel stem-cell source to treat osteoarthritis (OA). Here we investigated the therapeutic effects of UCMSCs injection strategies on knee OA in a rabbit model. Thirty OA rabbits randomly received normal saline, a single dose of 1×10^6 UCMSCs, or three injections of 1×10^6 UCMSCs at 2, 4, 6 weeks. Articular cartilages were collected after 8 weeks. Macroscopic and histological assessments indicated that intra-articular injection of UCMSCs, both single and multiple injection, significantly reduced the formation of periarticular osteophytes and articular cartilage degeneration when compared with the control. Furthermore, both UCMSCs injections increased the expression of chondrogenic markers in the articular cartilage, and reduced the levels of TNF-α and IL-6 in synovium. Micro-CT showed significant reduction of sub-chondral bone degeneration and osteophytes in the multiple-injection group compared to the control and single-injection group. Taken together, intra-articular injection of UCMSCs for OA treatment is safe and effective. Single and multiple injection of UCMSCs had comparable reparative effect on cartilage lesions, while multiple injection of UCMSCs further exerted effect on enhancing subchondral bone volume.

1. Introduction

Osteoarthritis (OA) is the most common joint disease which increases with ageing in both incidence and prevalence [1]. OA is represented by cartilage deterioration, osteophytosis, and chronic synovitis [2]. Owing to increased aging population all around world, the incidence of OA is increasing, which imposes a substantial economic burden on both individuals and society [3]. However, all the current clinical treatments (including physical therapy, drug therapy, and surgery) are symptom relieving (such as pain, stiffness and swelling), no curative therapy is available for OA [4].

Several cell-based therapies have been investigated with the purpose to regenerating articular cartilage (AC) [5]. Cartilage related cell implantation, including autologous chondrocyte transplantation, osteochondral transplantation, autologous perichondral and

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periosteal tissues were firstly conducted [6,7]. Although some improvements and pain relief were noticed, the effects were temporally. Also, autologous implantation has high demand on surgical techniques and requires to harvest healthy cartilages, which significantly limited the application of these therapies [8,9].

Mesenchymal stem cells (MSCs) can be harvested from various mesenchymal origins [10] with slight differences in multipotency and protein expression profile [11,12]. MSCs have received increasing attention in the cell treatments for OA [13] and other diseases [14–17]. Animal studies have demonstrated the benefits of MSCs therapy in cartilage repairment, including chondrocyte repopulation and extracellular matrix synthesis, immunomodulatory and anti-inflammatory effect [18,19]. Moreover, some phase I/II clinical trials of MSCs therapy on OA revealed significant pain relief and function improvement of joint without severe adverse events [20–22].

So far, no clear and standard operational guidelines is available for MSC therapies in OA treatment. Among various source of MSCs, umbilical cord MSCs (UCMSCs) are an increasingly popular cell source owing to its high proliferation capacity, low immunogenicity, noninvasively harvesting method and relatively minor ethic issue [23–25]. In addition, UCMSCs have similar levels of cell growth factors, chemokines, and cytokines with cartilage tissues, suggesting it as an appropriate candidate for OA therapy [26]. Although clinical study has shown the effectiveness and safety of UCMSCs therapy, studies have indicated that a single dose of injection seems insufficient to prevent OA progression in the long term [27]. Therefore, we aimed to optimize the protocol for the administration of UCMSCs by comparing the effect of a single dose and multiple doses of UCMSCs to treat OA, using a rabbit knee OA model by anterior cruciate ligament transection (ACLT), and further explore the underlying mechanisms.

2. Materials and methods

2.1. Isolation and expansion of human UCMSCs

All procedures involving humans and animals were performed following the ethical principles of the Helsinki declaration and the Ethics Committee of Guangdong Provincial People's Hospital (KY-Q-2022-094-03) guidelines. Human umbilical cords were got from healthy donors who underwent full-term cesarean section and had given prior informed consent (cell batches: RU201812001/P3, RU201812001/P10, RU201901002/P3, RU201901002/P10). The human UCMSCs were isolated from the Wharton's jelly of human umbilical cord by explant culture in complete medium (UltraCULTURE Serum Free Medium, 12–725F, LONZA, USA; 2%Ultroser G Serum Substitute, 15950-017, Pall, USA) at 37 °C under 5 % CO2. Cells at passage 4–8 were used for the following experiments. Cell viability was testified using Cellometer Auto 2000 Cell Profiler (Nexcelom Bioscience LLC).

2.2. Flow cytometry

The characterization of human UCMSCs was conducted by the expression of surface markers of MSCs. The fluorescently labeled antibodies, including CD11b, CD19, CD45, CD34, CD73, CD90, CD105 and human leukocyte antigen (HLA)-DR (Biolegend, USA), were incubated with the cells and analyzed on a flow cytometer (FACSCanto™, BD).

2.3. Biological safety evaluation

A cell tumorigenicity evaluation was conducted to examine the biological safety of the UCMSCs. Female Balb/C rats at seven weeks of age and weight 15–18g were purchased from the Southern Medical University (Guangzhou, China) and categorized into three groups: the experimental group, the negative control group, and the positive control group. Subcutaneous injection of 1×10^6 UCMSCs in 200 μl saline was conducted in the experimental group rats. The negative control group received a dose of 1×10^6 MRC-5 cells in 200 μl saline, and the positive control group received the same dose of HeLa cells. The rats were euthanized after 16 weeks of observation. Tissues with the injection sites at right axilla were harvested and fixed in 4 % paraformaldehyde. After the preparation of 4 μm thick tissue sections, hematoxylin and eosin (HE) staining were conducted for histological evaluation.

2.4. Evaluation of the three-line differentiation potential of UCMSCs

Osteogenesis, chondrogenesis and adipogenesis were conducted to examine the three-lineage differentiation potential of the UCMSCs. For osteogenesis, UCMSCs were inoculated into six-well plates at cell density of 2×10^4 cells/cm^2 and treated in an osteogenic medium (OriCell, China) for 14–28 days and assessed by alizarin red staining. For chondrogenesis, UCMSCs were inoculated into six-well plates at cell density of 2×10^4 cells/cm^2 and treated in Chondrogenesis Differentiation Kit (Procell, China) for 20–30 days and assessed by Alican Blue staining. For adipogenesis, UCMSCs were inoculated into six-well plates at cell density of 2×10^4 cells/cm^2 and treated in an adipogenic medium (OriCell, China) for 16–28 days and assessed by oil red O staining.

2.5. OA rabbit model and UCMSCs transplantation

All the animal experiments were approved by the Animal Ethics Committee of South Medical University. A total of 30 healthy female New Zealand white rabbits at 37 weeks or older with the weight between 3 kg and 3.5 kg were randomly divided into four groups: blank group (healthy, n = 6), control group (OA, n = 8), single-injection group (OA + single MSCs, n = 8), and multiple-injection group (OA + multiple MSCs, n = 8). OA model was established by bilateral anterior cruciate ligament transection (ACLT). Briefly, after anesthesia with 3 % pentobarbital sodium (30 mg/kg), bilateral arthrotomy through medial parapatellar

approach and lateral dislocation of the patella was performed to fully expose the anterior cruciate ligament (ACL). After transection of the ACL, the knee capsules were closed and the rabbits were then kept in separate cages. The control group received one injection of 400 μl normal saline at 2 weeks postoperatively after the surgery of OA modeling. The single-injection group had one injection of 1×10^6 UCMSCs resuspended in 400 μl normal saline at 2 weeks after the surgery of OA modeling, while the multiple-injection group received three injections of 1×10^6 UCMSCs suspended in 400 μl normal saline at 2, 4, 6 weeks postoperatively. The blank group did not receive any surgery or extra administration. The sample size calculation and concentration and intervals of MSCs were referenced in previous studies [19,28].

2.6. Macroscopic assessment

The rabbits were euthanized with an overdose of 3 % pentobarbital sodium eight weeks after the first injection. The femoral condyles, tibial plateaus, meniscus were collected. Semi-quantitative scoring was applied to evaluate the degeneration of femoral condyles and tibial plateaus (Table S1) [29]. The formation of osteophytes was assessed on a scale of 0–3, where 0 represents nil formation, 1 represents mild formation, 2 represents moderate formation, and 3 represents severe formation) [19].

2.7. Micro-computed tomography (micro-CT) analysis

After macroscopic grading, the intact femoral condyles and tibial plateaus were scanned using a micro-CT system (SCANCO MEDICAL, μCT100, Switzerland) for three-dimensional (3D) reconstruction. A scanning time of 0.24 s with settings of 70 KVp, 200 μA and 30 calibrations was applied. The CT value was calibrated at 1200mgHA/cm. A 50.4-mm axial and trans-axial fields of view (FOV) were acquired. A 3D model was reconstructed and bone volume was analyzed to evaluate the knee OA progression.

2.8. Histological and immunohistochemical assessment

The collected femoral condyles, tibial plateaus, meniscus were fixed in 4 % paraformaldehyde at 4 °C for 2 days. The femoral condyles, tibial plateaus were decalcified with 10 % ethylenediaminetetraacetic acid solution (EDTA) for eight to ten weeks. After graded alcohol dehydration, the samples were embedded in paraffin and cut into 5 μm sections. Histological assessment was performed by Safranin-O/Fast green staining. Osteoarthritis Research Society International (OARSI) scoring systems was used to evaluate the

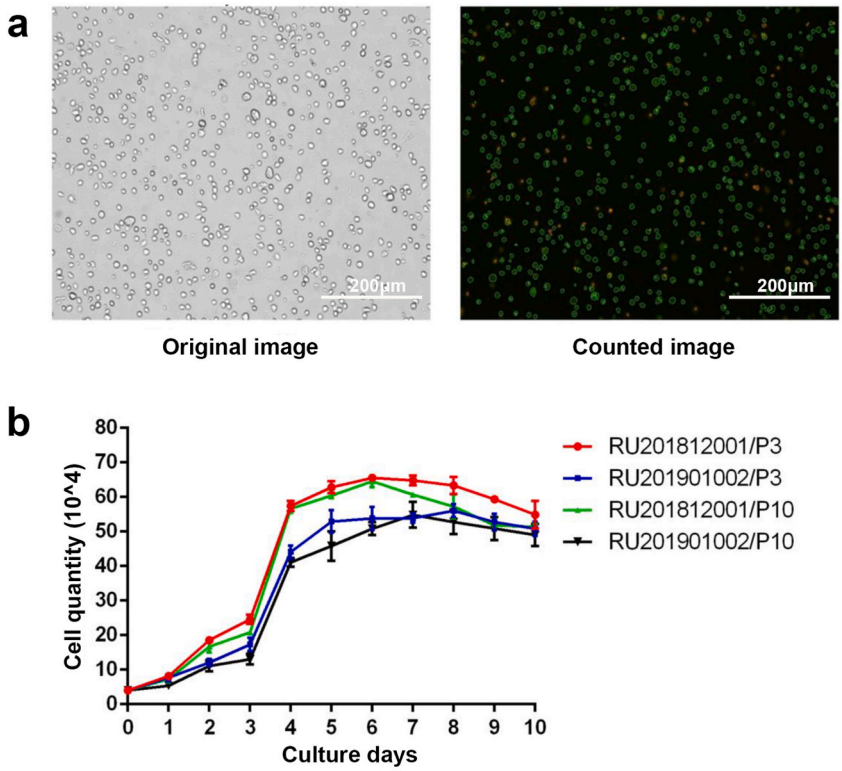

Fig. 1. The viability of four batches of UCMSCs. (a) The original images of UCMSCs detected in light microscope and counted images of UCMSCs detected in Cellometer Auto 2000 Cell Profiler. Scale bar = 200 μm; (b)The growth curve of four batches of UCMSCs. UCMSCs: human umbilical cord mesenchymal stem cells.

changes of AC(29). Histological meniscal scoring was used to evaluate the changes of meniscus, which gives histological scores between zero (best) to nine (worst) [19].

2.9. Inflammatory cytokines detection

At eight weeks after the first injection, the synovial tissues of the experimental animals were collected after euthanasia, and prepared into homogenate using a mortar on ice. Then they were centrifuged at 4000 rpm for 10 min and the supernatants were collected. Enzyme-linked immunosorbent assay (ELISA) was conducted following a standard protocol for assessing the level of inflammatory factors including tumor necrosis factor-α (TNF-α), transforming growth factor-β (TGF-β), interleukin-6 (IL-6) and interleukin-10 (IL-10) in the homogenate of synovial tissues.

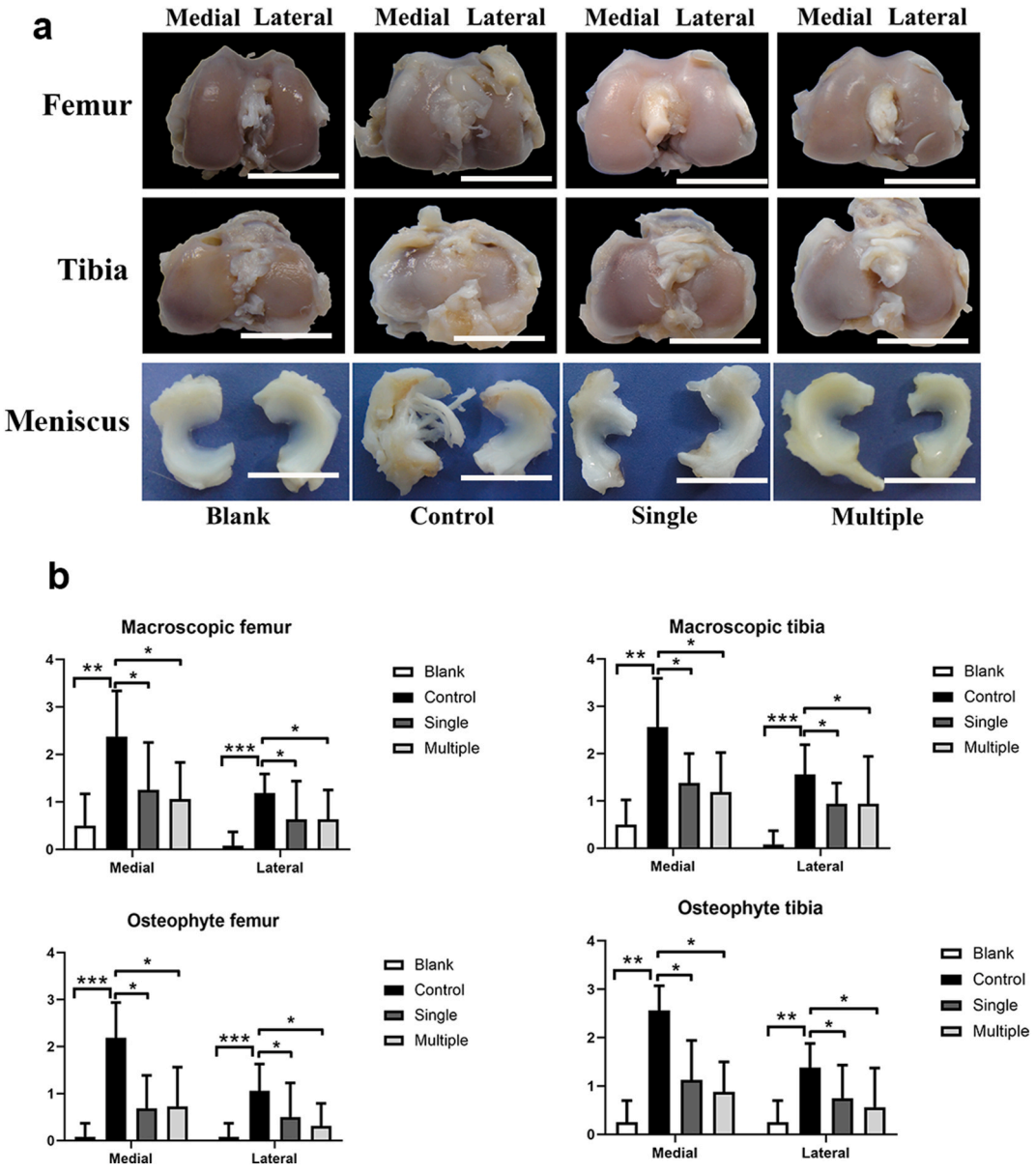

Fig. 2. Macroscopic features and scores of knee joint. (a) Representative macroscopic features of femoral condyles, tibial plateaus and meniscus of blank, control, single-injection and multiple-injections groups. Scale bar = 1 cm. (b) Macroscopic and osteophyte grading in the blank(n = 6), control(n = 8), single-injection(n = 8), and multiple-injections groups(n = 8). *, P < 0.05; **, P < 0.01; ***, P < 0.001.

2.10. Statistical analysis

All data were assessed normality distribution by a frequency distribution chart and described as mean ± standard deviation. The statistical analysis was carried out by SPSS 22.0 statistical software (Chicago, IL, USA). Comparisons of macroscopic scoring, OARSI scores, meniscal histological scores and levels of inflammatory factors were performed by one-way analysis of variance (ANOVA) test. A $p < 0.05$ was considered to be a statistically significance.

3. Results

3.1. Characterization of UCMSCs

The isolated UCMSCs showed a satisfactory cell viability of 98.1 % (Fig. 1a) with a relatively slow cellular growth in the first three days and an exponential growth at 4–6 days in culture (Fig. 1b). They were positive (>95 %) for CD73、CD90、CD105 expression, and negative (<2 %) for CD11b、CD19、CD34、CD45 and HIA-DR expression (Fig. S1), satisfying the criteria of MSCs. Cell tumorigenicity evaluation showed no positive findings around the injection sites and other organs in the experimental group from both macroscopic and histological findings, which was close to the negative control group (Figs. S2 and S3). On the contrary, progressive growth of neoplasms was observed in all the rats injected with HeLa cells, which verified the biological safety of UCMSCs. Three-line differentiation potential assessment confirmed the capacities of osteogenesis, chondrogenesis and adipogenesis of the UCMSCs (Fig. S4).

3.2. Macroscopic findings

Compared with the blank group (healthy group), significant osteoarthritic changes were observed in the control group (OA model group), including moderate to severe degeneration of the AC of the femoral condyle and tibial plateau, osteophyte formation, synovial

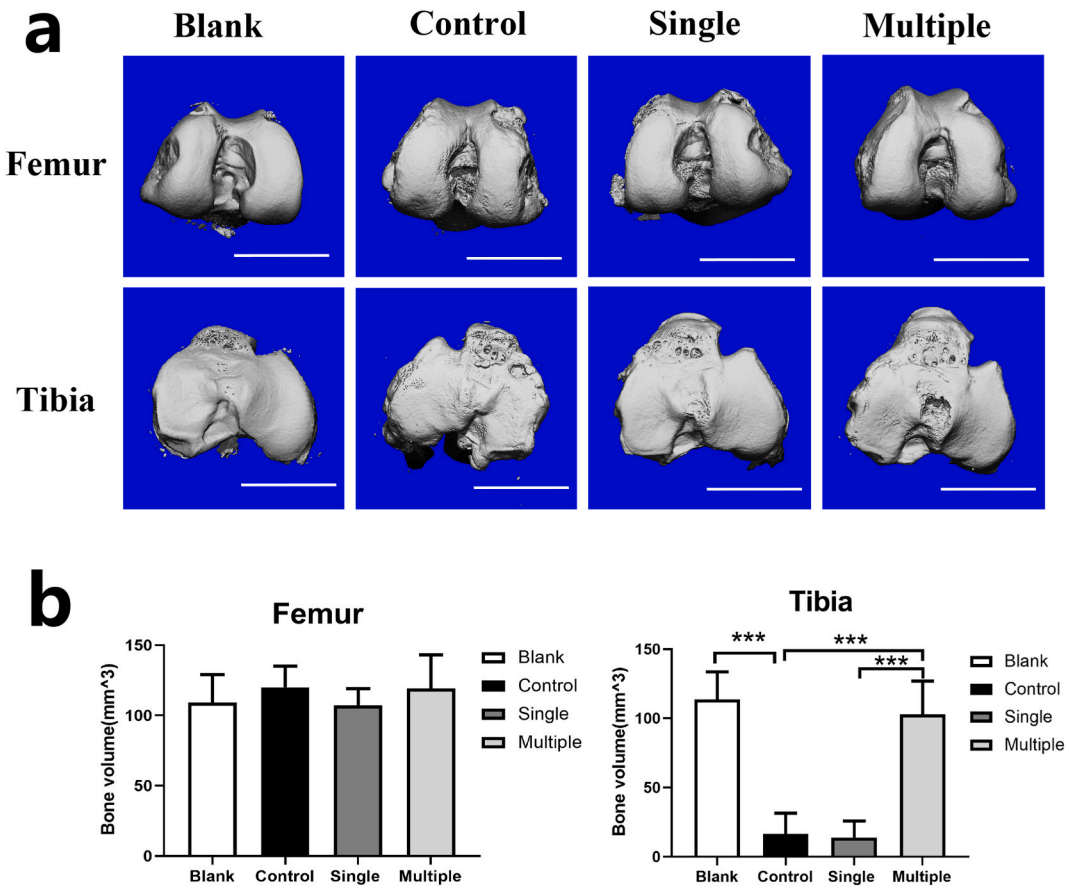

Fig. 3. Micro-CT scanning of knee joint. (a) Representative micro-CT 3D images of femoral condyles and tibial plateaus of blank, control, single-injection and multiple-injections groups. Scale bar = 1 cm. (b) Quantitative analysis of bone volume in the blank(n = 6), control(n = 8), single-injection(n = 8), and multiple-injections groups(n = 8). ***, P < 0.001.

hyperplasia and meniscal tears (especially medially). In single-injection group, improvement of the AC, less osteophyte presence, milder synovial hyperplasia and meniscal tears were noticed compared to control group. In multiple-injection group, slight degenerative changes of the AC and few osteophytes were found in femoral condyles and tibial plateaus, while no synovial thickening and meniscal tears were observed (Fig. 2a). Concerning macroscopic grading, both single and multiple-injection groups showed significantly higher improvement in relieving the degeneration of the AC and osteophyte formation, compared to the control group. The multiple-injection group had slightly higher effect compared with the single-injection group, but the difference was not significant (Fig. 2b).

3.3. Micro-CT evaluation

Fig. 3 showed the micro-CT reconstructed images of the rabbit knee joints. In the OA control group, the femoral condyle and tibial plateau presented with rough and irregular articular surface and significant subchondral bone loss in tibia with a large number of osteophytes around the knee margin, compared to the blank group (Fig. 3a). Single-injection of UCMSCs resulted in reduced osteophytes and more regular surface of the joint, but did not significantly improved bone volume compared to the OA control group. In multiple-injection group, a significant improvement of bone volume was found in the tibial plateau when compared to the control group and the single-injection group (Fig. 3b).

3.4. Histological evaluation

Fig. 4 shows the histological results of femoral condyles and tibial plateaus. Safranin-O/Fast green staining of cartilage identified typical degenerative changes of OA in the control group, including significantly decreased cartilage staining, erosion of hyaline cartilage, multifocal confluent decrease in chondrocyte cells and increased cluster formation (Fig. 4a). In the single-injection group, the loss of cartilage staining and increased cluster formation were improved compared to the control group. Mild fissures and cracks were observed in the superficial cartilage surface with focal decrease in chondrocyte cells. In multiple-injection group, the cartilage matrix was in normal architecture with moderate staining, the cartilage volume was increased, the chondrocytes were distributed regularly in columnar form. Only mild cracks were seen in the superficial cartilage surface. OARSI scores were significantly improved in the UCMSCs injection groups compared to the control group, but there was no significant difference between single-injection group and multiple injection group(Fig. 4b).

The result of Safranin-O/Fast green stain of the meniscus was shown in Fig. 5. In the control group, both the medial and lateral

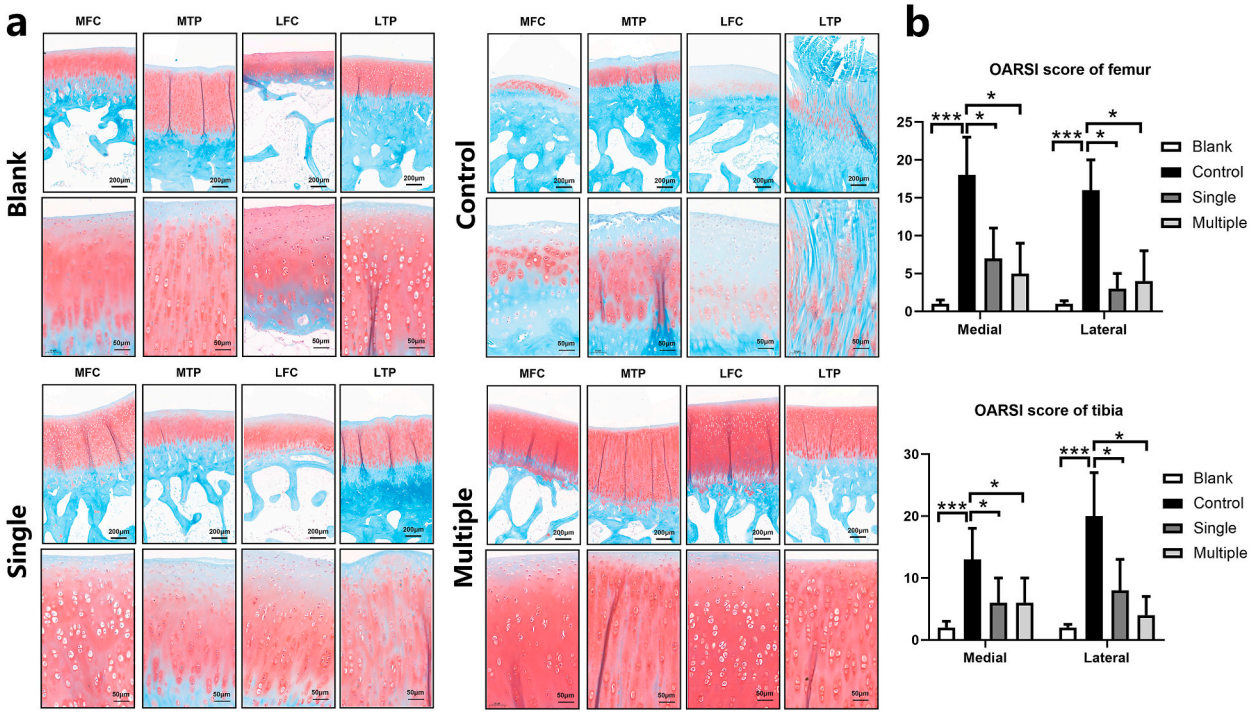

Fig. 4. Histological features and scores of articular cartilages. (a) Representative histological features of articular cartilage under Safranin-O/Fast green staining of blank, control, single-injection and multiple-injections groups. (b) OARSI score of femur and tibia in blank(n = 6), control(n = 8), single-injection(n = 8), and multiple-injections groups(n = 8). Scale bar = 200 μm; *, P < 0.05; ***, P < 0.001. MFC, medial femoral condyle; MTP, medial tibial plateau; LFC, lateral femoral condyle; LTP, lateral tibial plateau.

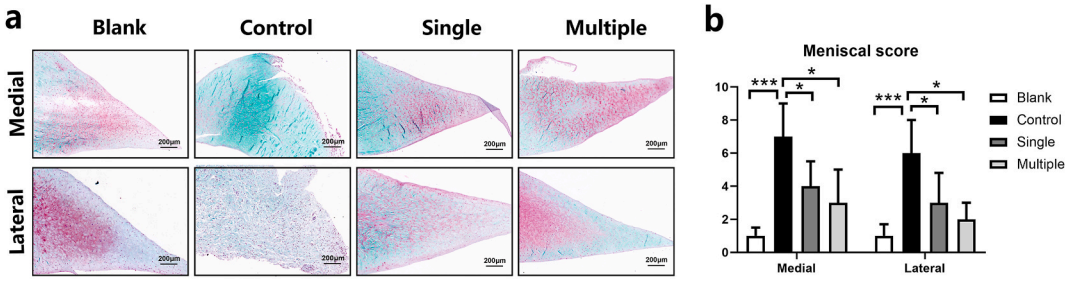

Fig. 5. Histological features and scores of a meniscus. (a) Representative Safranin-O/Fast green staining result of the meniscus of blank, control, single-injection and multiple-injections groups. (b) Meniscal score in blank (n = 6), control (n = 8), single-injection (n = 8), and multiple-injections groups (n = 8). Scale bar = 200 μm; *, P < 0.05; ***, P < 0.001.

meniscus exhibited significantly reduced matrix staining with hypocellularity and fibrillation of surface(Fig. 5a). In the single-injection group, better distribution of meniscal cells at the lateral meniscal border and improved matrix staining were noted, but loss of meniscal cells still existed in the medial meniscus. In the multiple-injection group, staining of the meniscus matrix was moderate and the shape of the meniscus was close to the blank group, with a smooth surface and regular meniscal cells arrangement. Concerning the histological scoring of meniscus, both single and multiple UCMSCs injection group had significantly reduced scores compared with the control group, but there was no significant difference between the single and multiple injection group(Fig. 5b).

3.5. *Inflammatory cytokines detection*

Inflammatory cytokines play critical roles in the pathogenesis of OA. Here we evaluated the levels of TNF-α, TGF-β1, IL-6, and IL-10 in homogenate of synovial tissues by ELISA (Fig. 6). The control group (OA model group) had significantly upregulated pro-inflammatory cytokines (TNF-α and IL-6) (Fig. 6a and b), and decreased expression of anti-inflammatory cytokines (TGF-β1 and IL-10) (Fig. 6c and d) (P < 0.05) compared to the blank group (healthy group). After treatment of UCMSCs, both the injection groups

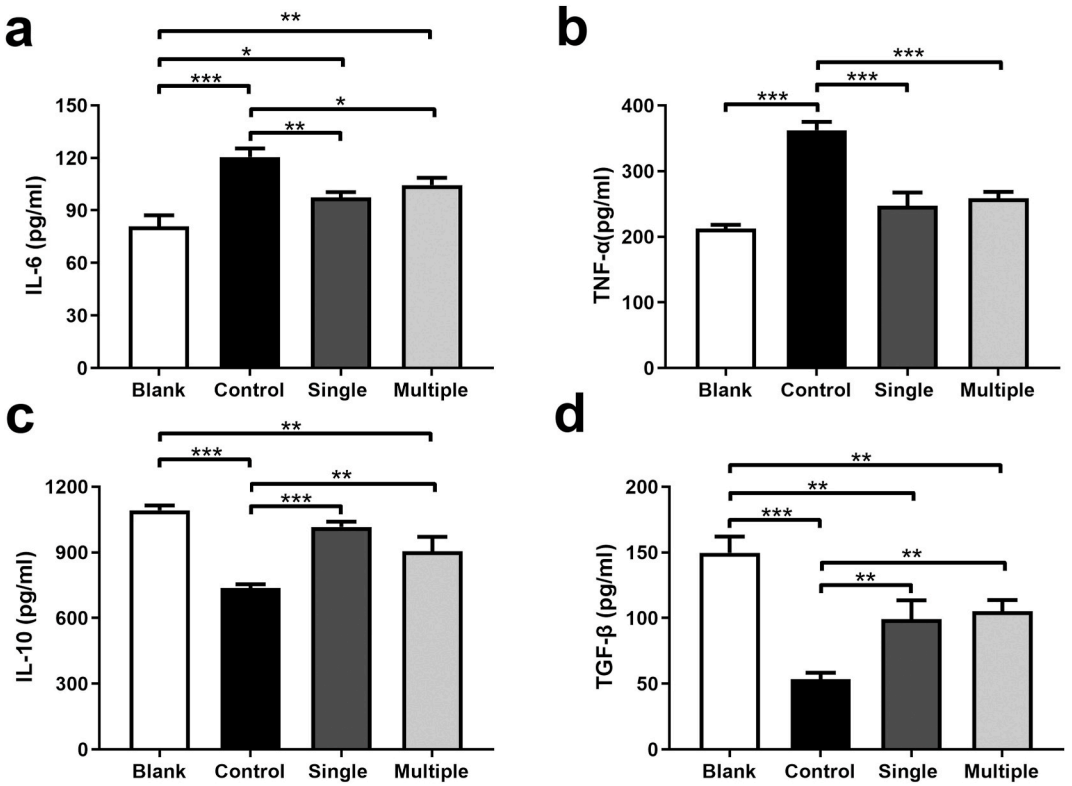

Fig. 6. Quantitative analysis of inflammatory factors in the synovial tissues in the blank (n = 6), control (n = 8), single-injection (n = 8), and multiple-injections groups (n = 8). a,b Injections of UCMSCs significantly down-regulated the level of IL-6 and TNF-α. c,d The level of IL-10 and TGF-β was significantly improved after treatments of UCMSCs by contrast with the control group. *, P < 0.05, **, P < 0.01 and ***, P < 0.001.

expressed significantly reduced TNF-α and IL-6, and enhanced TGF-β1 and IL-10, compared to the control group ($P < 0.05$), which indicating the effect of UCMSCs on alleviating articular inflammation. No significant difference was found between the single and multiple injection group.

4. Discussion

In our study, we evaluated the potential benefits of single/multiple intra-articular injections of UCMSCs in treating OA by establishing an ACLT-induced rabbit OA model. All animals receiving the UCMSCs therapy were well tolerated. Macroscopic and immunohistochemical assessments indicated that intra-articular injection of UCMSCs significantly ameliorated OA lesions, represented by reduced formation of periarticular osteophytes and reduced AC damages. Investigation of inflammatory factors suggested a reduced inflammation level by the injection of UCMSCs. Taken together, both single and multiple injection of UCMSCs exerted reparative effect on cartilage lesions in the OA model. However, when it comes to the comparation of single injection and multiple injection of UCMSCs, multiple injection did not deliver significantly higher effect than the single injection in the majority of the parameters tested, except that multiple UCMSCs injection significantly improved bone volume of the subchondral bone over the single injection group.

The pathogenesis of OA is known as degenerative changes of AC accompanied by calcification of cartilage, subchondral and trabecular bone, which may relate with imbalanced autophagy and mitophagy [30]. However, treatment of OA is challenging due to the difficulty in spontaneous-healing of AC [2]. In this condition, MSCs therapy has received increasing attention due to the potential capacity to repair cartilage tissues [5]. In fact, MSCs is the most intensively investigated type of adult stem cells in regeneration studies because of their abundance, as they can be isolated from various sources such as bone morrow (BM), umbilical cord and synovial fluid. They also have low immunogenicity, minimal tumorigenicity with no ethical issues [31]. Past studies have demonstrated their safety and efficacy in treating cartilage diseases. Several studies on OA have shown improvement of joint function, reduction of pain, and enhancement of life quality after MSCs therapy [13]. Most of the published studies used MSCs isolated from bone marrow, which is most widely used due to its high differentiation potential. However, bone marrow MSCs have some limitations, including difficulty to harvest, cellular variation in differentiation and repair potential because of disease condition and age of donor and risk of infection [13]. Consequently, alternative sources of MSC are desired with less invasive harvest procedure. UCMSCs, which is easy to harvest causing no pain, as well as the rapid proliferation and multipotency, comes into sight as the possible upgradation of MSCs from bone marrow for OA treatment [32].

Although there is a great potential of UCMSCs in OA treatment, in vivo and clinical studies in this research field are limited. Zhang et al. investigated the potential benefits of UCMSCs on OA by using rat OA model induced by monosodium iodoacetate [18]. After intra-articular injection of UCMSCs (2.5×10^5 cells) for 2 weeks, researchers found significant increase of chondrocytes and cartilage volume and decrease of fissures. Tang et al. used 5×10^5 UCMSCs and their small extracellular vesicles for treating ACLT-induced rat OA model and found UCMSCs alleviated cartilage damage by inducing cartilage repair-related proteins via small extracellular vesicles [33]. In the present study, we used ACLT-induced rabbit OA model with intra-articular injection of 1×10^6 UCMSCs and found reduction of periarticular osteophytes formation and improvement of AC damages, which was similar with previous study using rat model [34]. Furthermore, we conducted cell tumorigenicity evaluation and found no positive findings around the injection sites and other organs in the experimental group from both macroscopic and histological findings, which verified the biological safety of UCMSCs.

Apart from the source of MSCs, the frequency of intra-articular injection also matters. It was reported that a single injection of MSCs may not be sufficient to deliver long-term cartilage protection as the amount of MSCs could decrease rapidly, leaving insufficient cells to protect the AC [35]. Mahmoud et al. compared the therapeutic effect of knee OA between single injection and multiple injections of BM-MSCs and found a single injection of MSCs could not effectively restore the joints while multiple injection of MSCs can replenish the cell population and to reduce inflammation [19]. Xing et al. investigated the effects of UCMSCs in treating OA and concluded that a single intra-articular injection of UCMSCs can only slow the progression of cartilage degeneration temporally without sustained long-term benefits [36]. Ju et al. compared the therapeutic effect of UCMSCs in a single or repeated twice injection in OA rat model and found no significant differences were observed between single injections and repeated twice injections [34]. In the present study, we also compared the effect of UCMSCs on restoration of AC damages between single and multiple injection. We found that multiple injection of UCMSCs showed comparative repair effect of cartilage lesions with a single injection. These results indicated that UCMSCs might superior to other source of MSCs in AC regeneration and single injection was able to inhibit OA progression. Limited clinical trial found that both single and repeated UCMSCs injections improved pain and function of OA patients while repeated injection group had advantage of steady improvement of WOMAC and VAS compared with single injection group [37]. Further studies were needed in investigating whether the total number of UCMSCs or the multiple injections play the vital role in steady improvement of OA progression.

The mechanisms of UCMSCs in regenerating damaged cartilage consist of cellular aspect and environmental aspect [38]. On the one side, UCMSCs had great differentiation capabilities, which might result in the induction of chondrocytes differentiation in OA condition. However, the association between the differentiation potential of MSCs and cartilage regeneration has never been identified directly [39]. Hsu et al. used rat OA model and found significant increase of SOX-9 and COL-2 in UCMSCs group, as well as upregulation of other cartilage development-specific markers (TGF-β and RUNX-2) in cartilage [40]. Further in vivo experiments tracking injected MSCs and their differentiation potentials were desiderated.

On the other side, UCMSCs promote repair via secretion of regulatory factors in synovium fluid, which also regarded as paracrine modulatory effect [41]. MSCs can exert immunomodulatory and immunosuppressive effects on various immune cells [42]. MSCs

inhibit the proliferation and differentiation of native T lymphocytes toward the Th1 or Th17 phenotype by programmed death-ligand 1 (PD-L1) and Fas ligand (FasL). Also, MSCs are able to suppress the inflammatory responses of natural killer (NK) cells by secreting TGF-β [43] and inhibit mononuclear cell proliferation and M2 macrophage polarization by JAK-STAT1 signaling pathway. MSCs improved cellular oxidative stress and mitochondrial dysfunction by reduced the production of TNFα by M1 macrophages while enhancing TGF-β1 and IL-10 release by M2 macrophages [44]. Synovitis plays a substantial role in the pathogenesis of OA through up-regulating the level of pro-inflammatory cytokines such as TNF-α, IL-6 and IL-1, which promote the progression of chondrocyte apoptosis and cartilage degradation [45,46]. Anabolic anti-inflammatory processes also participate in OA development by regulating anti-inflammatory factors such as TGF-β, IL-4, and IL-10, which regulate chondrogenesis and maintain the differentiated phenotype of articular chondrocytes [47,48]. In the present study, we found that intra-articular injection of UCMSCs inhibited the inflammation significantly in the rabbit OA model by down-regulating pro-inflammatory factors (IL-6, TNF-α) and up-regulating anti-inflammatory factors (IL-10, TGF-β).

Apart from immunomodulatory properties, MSCs can also secret trophic factors for cartilage repairment. The chondrogenic differentiation of MSCs is tightly regulated by growth factors and signaling pathways such as TGF-β, BMP and Wnt/β-catenin [49], where cytokines like TGF-β was increased significantly in the present study. Ozeki et al. detected increase of several trophic factors containing PRG-4, BMPs, and TSG-6 within the synovium of rat model after intra-articular injection of MSCs [50]. Recently, exosomes, which are crucial molecules for cellular communication, have been increasingly addressed in the therapeutic potential of OA [51]. Wu et al. showed that infrapatellar fat pad MSCs protected AC and alleviated gait abnormalities via miR-100-5p-abundant exosomes by inhibiting mTOR pathway in osteoarthritis [52]. Only one study on human articular chondrocytes revealed that UCMSCs-derived exosomes inhibited ROS generation and cell apoptosis through miR-100-5p/NOX4 regulation [53]. Further iv vivo studies concerning UCMSCs-derived exosomes in treating OA animals and the underlying mechanisms are warranted.

This study has some limitations. These include a lack of histological evaluation of the synovial tissue and collateral ligaments. We have found that UCMSCs could suppress inflammation in the OA model, which may be one of the underlining mechanisms of their reparative effect, however, the mechanisms of how the UCMSCs could regulate inflammation requires further investigation. The scalability of the cell production process for clinical use is still a long way to go, for example, the number of UCMSCs for injection needs to be optimized for clinical application. The UCMSC potency varies due to donor differences, which may result in batch differences and affect their therapeutic effect. A limited time frame is used in this study, therefore further test of the effect of UCMSCs on a late OA model is desired. Further clinical studies are wanted to identify therapeutic effect of UCMSCs on different stage of OA patients.

5. Conclusions

In conclusion, we reported the safety and effectiveness of intra-articular injection of UCMSCs for OA treatment. Our results exert a comparative therapeutic effect of both single and multiple injections of UCMSCs on OA damage by attenuating degeneration of AC, loss of cartilage matrix, osteophyte formation and meniscus wear. Apart from these, we revealed that UCMSCs prevented OA progression through regulating the production of inflammatory factors and the expression of chondrogenic genes. Our study demonstrates the benefits of UCMSCs therapy and provides a potential dosage protocol for future clinical application.

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Institutional review board statement

The animal study protocol was approved by the Animal Ethics Committee of South Medical University (SMUL2021001, 2021). The human umbilical cords were obtained from healthy donors following full-term cesarean section with prior informed consent (RU201812001, RU201901002). All procedures involving humans and animals were performed following the ethical principles of the Helsinki declaration and the local ethical committee guidelines for laboratory animal science.

Informed consent statement

The human umbilical cords were obtained from healthy donors following full-term cesarean section with prior informed consent (RU201812001, RU201901002).

Data availability statement

The original data is available on request from Zhantao Deng (dengzhantao@gdph.org.cn).

CRediT authorship contribution statement

Zhantao Deng: Writing – original draft, Investigation, Funding acquisition, Formal analysis. **Xiaoli Zeng:** Writing – review & editing, Resources, Conceptualization. **Bofu Lin:** Writing – original draft, Investigation, Formal analysis. **Lixuan Chen:** Writing – original draft, Investigation, Data curation. **Jiwei Wu:** Validation, Investigation. **Jie Zheng:** Resources, Methodology. **Yuanchen Ma:** Resources, Investigation. **Feng-Juan Lyu:** Writing – review & editing, Project administration, Funding acquisition. **Qiujian Zheng:** Writing – review & editing, Project administration, Funding acquisition, Conceptualization.

Declaration of competing interest

The authors declare the following financial interests/personal relationships which may be considered as potential competing interests:

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Appendix A. Supplementary data

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