Journal of Orthopaedic Translation 32 (2022) 59–68

Contents lists available at ScienceDirect

Journal of Orthopaedic Translation

journal homepage: www.journals.elsevier.com/journal-of-orthopaedic-translation

Original article

Anti-hypertrophic effect of synovium-derived stromal cells on costal chondrocytes promotes cartilage repairs

Yiyang Ma, Kaiwen Zheng, Yidan Pang, Fuzhou Xiang, Junjie Gao **, Changqing Zhang ***, Dajiang Du *

Department of Orthopedic Surgery, Shanghai Jiao Tong University Affiliated Sixth People's Hospital, Shanghai, 200233, China

ARTICLE INFO

Keywords:
Costal chondrocyte
Synovium-derived mesenchymal stromal cell
Hypertrophic differentiation

ABSTRACT

Background: Costal chondrocytes (CCs), as a promising donor cell source for cell-based therapy for cartilage repair, have strong tendency of hypertrophy and calcification, which limited CCs from further application in cartilage regenerative medicine. Synovium-derived stromal cells (SDSCs), have shown their beneficial effect for chondrocytes to maintain phenotype. This study aims to investigate whether SDSCs could help CCs to maintain chondrogenic phenotype and suppress hypertrophic differentiation in cartilage repairs.

Methods: CCs were directly cocultured with SDSCs in pellet or indirectly cocultured using a conditioned medium in vitro for 3 weeks. Cartilage matrix formation and hypertrophic differentiation of CCs were analyzed by RT-PCR, biochemical assays, and histological staining. Cocultured pellets were implanted into the osteochondral defects made on the femoral groove of the rats. Then, macroscopic and histological evaluations were performed.

Results: Pellets formed by CCs alone and CCs cocultured with SDSCs reveal equal cartilage matrix deposition. However, the gene expression of type X collagen was significantly downregulated in cocultured pellets. Immunohistochemistry analysis revealed suppressed expression of type X collagen in cocultured pellets, indicating SDSCs may suppress hypertrophic differentiation of chondrocytes. Further in indirect coculture experiment, SDSCs suppressed type X collagen expression as well and promoted the proliferation of CCs, indicating SDSCs may influence CCs by paracrine mechanism. The pellets implanted in the osteochondral defects showed good restoration effects, whereas the grafts constructed with CCs and SDSCs showed lower type X expression levels.

Conclusion: These results suggest that SDSCs may maintain the phenotype of CCs and prevent the hypertrophic differentiation of CCs in cartilage repair.

The Translational Potential of this Article: CCs is a promising donor cell source for cell-based therapy for cartilage repair. Based on our study, cocultured with SDSCs weakened the tendency of hypertrophy and calcification of CCs, which provide a potential usage of SDSCs in CCs-based cartilage repair therapy to suppress newly formed cartilage calcification and improve clinical outcomes.

1. Introduction

Cartilage defect is one of the most important causes of osteoarthritis [1]. It is devastating due to the limited capacity of cartilage for intrinsic healing. Autologous chondrocyte implantation (ACI) serves as a promising treatment for cartilage defect. Current clinical chondrocytes source usually involves harvesting tissue from the articular cartilage of damaged joints, which could only obtain a small quantity of chondrocytes yet create donor site morbidity in joints, hence exacerbate joint degeneration. To overcome these limitations, costal chondrocytes (CCs), an alternative cell source, have attracted great attention to be used in treatment of cartilage defect [2,3]. Costal cartilage is the largest permanent hyaline cartilage in the mammalian body that shows similar phenotype to articular cartilage [4]. CCs obtained from costal cartilage

Abbreviations: CCs, costal chondrocytes; ACI, Autologous chondrocyte implantation; SDSCs, synovium-derived mesenchymal stromal cells; CM, conditioned medium; RT-PCR, reverse transcription-polymerase chain reaction; GAG, glycosaminoglycan.

* Corresponding author.
** Corresponding author.
*** Corresponding author.
E-mail addresses: colingjj@163.com (J. Gao), zhangcq@sjtu.edu.cn (C. Zhang), dudajiang@sjtu.edu.cn (D. Du).

https://doi.org/10.1016/j.jot.2021.05.002
Received 17 March 2021; Received in revised form 3 May 2021; Accepted 7 May 2021
Available online 2 June 2021

hold the advantage of large cell quantity and high cell yield [5,6]. Besides, easy surgical accessibility and minimal donor side morbidity make CCs a promising donor cell source for ACI.

However, in comparison with other source of chondrocytes, CCs have stronger tendency of hypertrophy and calcification [7–10]. Hypertrophy is the terminal differentiation stage of growth plate chondrocyte, which is a necessary developmental stage in endochondral ossification during normal bone formation and growth [11]. During hypertrophic differentiation, chondrocytes experience drastic phenotypic changes, including increased cell volume, decreased cell proliferation, altered matrix production, enhanced remodeling, and calcification [12]. CCs hypertrophy and calcification may result in the inferior mechanical properties of tissue reparative effect as well as ACI treatment effects.

In synovial joints such as knee and hip, articular cartilage has gradual morphologic change from superficial zone to deep zone. Chondrocytes in the surface of articular cartilage facing the synovial cavity maintained chondrocyte phenotype with high cell stemness, while chondrocytes near the growth plate show hypertrophic phenotype. Among these different chondrocyte phenotypes, synoviocytes and their secreted factors may play a role in it. The effect of synoviocytes toward chondrocytes remains controversial. Recent studies have reported that when articular cartilage is injured, synoviocytes are recruited to the defect site and play important roles in repairing cartilage [13]. Coculture synoviocytes with articular chondrocytes are reported to enhance the chondrogenic potential of articular chondrocyte [14–16] and rescue the impact from hazard factors such as injury, infection, and inflammation factors [17–20]. Matrix formed by synoviocytes provides a more chondrogenic-preserving environment for chondrocyte expansion and delays chondrocyte senescence [21,22]. Ito et al. repaired rabbit full-thickness cartilage defect using layered chondrocyte sheets together with cultured synoviocytes and achieved better results than using layered chondrocyte alone [23]. Moreover, synovium-derived mesenchymal stromal cells (SDSCs), a cell subpopulation in synoviocytes, having a superior proliferation and chondrogenic potential compared with mesenchymal stromal cells (MSCs) derived from other tissue sources [24–26]. Studies using SDSCs to repair cartilage defect in vivo have also been conducted where cartilage engineered by SDSCs showed promising reparative potential for cartilage defect [27–31]. Conversely, several studies also reported adverse effect of synoviocytes on chondrocytes. Steinhagen et al. and Bonitz et al. reported that synovial supernatants and synovial fibroblasts reduce the biosynthetic activity and the matrix deposition of chondrocytes [32,33]. In a clinical trial, chondrocyte ACI grafts that contained higher abundance of synoviocytes had inferior structural repair quality and graft survival rate [34]. Perhaps the conflicting results are because synoviocytes are a mixture of several kinds of cells, each of which may have different effects on chondrocytes. As several studies have reported that MSCs may facilitate chondrogenesis and proliferation of chondrocytes [35,36], SDSCs as a subpopulation of synoviocytes and their effects on chondrocytes need to be further clarified.

In this study, we hypothesized that SDSCs and their secreted factors may play a role in maintaining CCs phenotype and suppress chondrocyte hypertrophic differentiation, which improves the repair effect of CCs on articular cartilage defect.

2. Materials and methods

2.1. Isolation of CCs and SDSCs

SD rats aged 3–4 months and weighing about 300 g were used as the source of CCs and SDSCs. Costal cartilages were isolated from the cartilaginous portion of the 4th to 8th rib specimens with carefully removing of the perichondrium. Synovial tissue was harvested from inside the knee joint. Cartilaginous and synovial tissue were then finely minced into 1 mm^3 pieces and rinsed with phosphate buffered saline (PBS) for three times. To obtain CCs, cartilages were first digested in 0.25% trypsin-ethylenediaminetetraacetic acid (EDTA; Hyclone) for 1 h, followed by digestion in 0.1% Type II collagenase (Gibco) and 0.1% Dispase (Sigma) in Dulbecco's modified Eagle's medium high-glucose (DMEM-hg; Hyclone) with 1% penicillin/streptomycin (PS; Hyclone) overnight. Primarly synoviocytes were isolated from synoial tissue as previously reported [37,38]. Briefly, synovial tissue was digested in 0.1% Type II collagenase and 0.1% Dispase in DMEM-hg with 1% PS overnight. After digestion, cells were filtered through 70 μm cell strainers, centrifuged, and resuspended in DMEM-hg supplemented with 10% fetal bovine serum(FBS, Gibco) and 1% PS. Isolated cells were cultured at 37 °C, 5% CO_2. CCs cultured for no more than passage 3 were used in further experiments. Synoviocytes cultured beyond passage 3 were considered as synovium-derived mesenchymal cells and used in further experiments. The medium was changed every 3 days.

2.2. Cell proliferation assay

For cell proliferation assay, CCs were seeded into bottom well of a 24-well transwell system (Corning Life Science) at density of 5000 cells/well and SDSCs were seeded on the transwell insert with 0.4 μm pore at density of 2500 cells/cm^2. Chondrocyte cell growth was determined on the basis of Cell Counting Kit-8 assay (Dojindo Molecular Technologies, Inc.). Cells were cultured for 1, 3, 5, and 7 days. To each well, we added 40 μL of CCK8 solution and incubated in the dark for 1.5 h. The absorbance of each well was recorded at 450 nm using Thermo Varioscan LUX.

2.3. Chondrogenic differentiation of CCs coculture with SDSCs

For mixed pellet culture, SDSCs and CCs were cultured in pellets. Pellets consisting of CCs alone and of 75% CCs+25% SDSCs were suspended in chondrogenic differentiation medium (CHGM) containing 10 ng/ml TGFβ3 (Peprotech), 100 nM dexamethasone, 50ug/ml ascorbic acid 2-phosphate, 1 mM sodium pyruvate, 40ug/ml proline, 1% ITS liquid media supplement (Sigma), 1% PS in DMEM-hg and centrifuged at 400 g for 4 min with a total number of 2.5×10^5 cells per pellet. For conditioned medium culture, CCs pellets were produced as described above and cultured in SDSCs-conditioned medium (CM). To obtain SDSCs-CM, SDSCs were cultured in CHGM for 3 days. After 3 days of culture, CM was collected and enriched with 1:1 fresh CHGM and immediately added to the pellet culture system. Medium was changed two times a week. After 3 weeks, pellets were harvested for histological evaluation and gene expression analysis. For chondrogenic differentiation of SDSCs, SDSCs were suspended in CHGM and produced as described above.

2.4. RT-PCR

Total RNA from pellets were extracted using Tissue RNA Purification Kit PLUS. Complementary DNAs were obtained by RT of 500 ng total RNA using 4 × EZscript Reverse Transcription Mix II. PCR was performed in a volume of 10 μl. CDNAs of volume 0.2 μl were added to the following 2 × Color SYBR Green qPCR Master Mix. All reagents used in RT-PCR were purchased from EZBioscience. PCR reactions were conducted in QuantStudio™ 7 Flex real-time PCR System.

2.5. Glycosaminoglycan (GAG) quantification

GAG content was quantified by dimethyl methylene blue (DMMB) assay, as previously described [39]. Briefly, pellets were washed twice with PBS and digested in 0.01% papain (Yeasen Biotech Co., Ltd.) at 65 °C overnight following addition of DMMB dye reagent (Sigma). The metachromatic reaction of GAG with DMMB was monitored spectrophotometrically at 525 nm using Thermo Varioscan LUX. The total amount of GAG was normalized to the total amount of DNA in the same sample.

2.6. Western blotting

For western blotting, CCs were seeded in 6-well plates. When cells reached 80% confluency, the culture medium was replaced with SDSCs-CM and replenished every 2 days. CCs were lysed by CelLytic™ M (Sigma) with 1% protease inhibitor cocktail and 1% phosphatase inhibitor cocktail (Epizyme Biotech) at day 7. Equal proteins were electrophoresed on 10% SDS-PAGE, transferred to 0.45 μm polyvinylidene fluoride (PVDF) membranes (Millipore), blocked with blocking buffer (New cell & Molecular Biotech Co., Ltd), and incubated with the primary antibodies against type X collagen and GAPDH (Affinity Biosciences) in tris-buffered saline tween-20 (TBST). The membranes were then washed and incubated with HRP-conjugated secondary antibody (Affinity Biosciences). Finally, the membranes were washed, reacted with the ECL kit (Epizyme Biotech), and scanned with Bio-Rad ChemiDoc imaging system to quantify the signals.

2.7. Implantation of pellets into an osteochondral defect model

Twelve-week-old male SD rats (n = 3, 3 groups) were used in this study. Briefly, under general anesthesia, a medial parapatellar incision was made on one knee. The patellae were dislocated laterally; subsequently, a 1-mm-diameter, 1-mm-deep osteochondral defect was created on the patellar groove of the femur. The defect was rinsed with normal saline, and allogenic pellets of CCs or CCs+SDSCs cultured for 3 weeks were then press fitted into the defect. The joint capsule and skin were then closed. The rats were allowed free cage activity after the operation.

2.8. Macroscopic evaluation of the defect repair

The rats were sacrificed with an overdose of intraperitoneal injection of pentobarbital sodium at 4 weeks after surgery. The defect sites were macroscopically assessed using the International Cartilage Repair Society (ICRS) macroscopic score [40].

2.9. Histological evaluation

After in vitro culturing for 3 weeks, the pellets were harvested, dehydrated in 25% sucrose solution, fixed in 4% paraformaldehyde, embedded in optimum cutting temperature (SAKURA Tissue-Tek), and frozen. Frozen embedded pellets were cut into 10-μm slices, mounted onto adhesive slides, and stored in −20 °C before further evaluation.

The distal portions of the femur were fixed in 4% paraformaldehyde, decalcified for 2 weeks in 10% EDTA, embedded in paraffin wax, cut into 7-μm slices, and mounted onto adhesive slides.

To evaluate tissue morphology, sections were stained with Hematoxylin & Eosin (H&E) staining. To evaluate proteoglycans, present in the pellets, sections were stained with Safranin-O and Alcian blue.

2.10. Immunohistochemistry

Immunohistochemical staining was performed for detecting collagen type II and collagen type X. Briefly, sections were probed with rabbit antibodies against collagen type II (Abcam) or collagen type X (Invitrogen) at a 1:100 dilution, followed by probing with a goat anti-rabbit secondary antibody conjugated with HRP (Yeasen Biotech Co., Ltd.) at 1:200 dilution. The area of the immunocomplex was visualized by chromogen 3,3′-diaminobenzidine (DAB) for 2 min. ImageJ software was used to analyze the integrated optical density (IOD) and area to calculate average of intensity (AOI) of images.

2.11. Histological grading score

The histology of the repaired tissue at 4 weeks was evaluated blindly using the ICRS histological grading system [41].

2.12. Statistics

All data are representative of at least three experiments of similar results performed in triplicates. Statistical analysis was performed using Prism 8 software (GraphPad). The results are presented as mean ± standard error of mean (SEM). $P < 0.05$ was considered to be statistically significant.

3. Results

3.1. SDSCs attenuate hypertrophic differentiation of CCs

To evaluate the effect of SDSCs on CCs during chondrogenic culture, mixed pellet cultures of CCs and SDSCs for 3 weeks were harvested for further analysis (Fig. 1A). Pellets of CCs or CCs+SDSCs were spherical in shape with ivory white and opaque appearance (Fig. 1B). RT-PCR was performed to investigate the chondrogenesis-related gene and hypertrophic-related gene expression (Fig. 1C). Interestingly, *Col2a1* was expressed significantly higher in CCs pellets than in CCs+SDSCs pellets ($P = 0.026$). *Acan* expression showed a statistically nonsignificant decrease in CCs+SDSCs pellets compared to CCs pellets. Next, *Col10a1*, and *Alpl* were analyzed as hypertrophic differentiation gene markers. *Col10a1* expression was comparatively lower in CCs+SDSCs pellets than in CCs pellets ($P = 0.048$). *Alpl* also exhibited trends of downregulation, although not statistically significant.

To validate the effect of SDSCs on the structure of pellets, we employed H&E staining (Fig. 1D) and Alcian blue staining (Fig. 1E) on CCs pellets. Results indicated that CCs pellets were rich in cartilage-like form with cartilage lacuna structure, and the CCs+SDSCs pellets were rich in cartilage matrix, but form less lacuna structure with looser structure, indicating SDSCs may impede cartilage lacuna formation, but not affect matrix formation. Glycosaminoglycan (GAG) is a crucial constituent of cartilage. The maintenance of chondrocyte phenotype and cartilage tissue requires synthesis of sufficient amounts of GAG. To determine GAG deposition and distribution, we performed Alcian blue staining and Safranin-O staining. Semi-quantitative analysis indicated that both Alcian blue and Safranin-O staining were similar in CCs pellets and CCs+SDSCs pellets ($P = 0.107$) (Fig. 1E, F and G). To further quantify GAG deposition, total GAG contents were measured using DMMB method. In line with the histology results, CCs pellets and CCs+SDSCs pellets contained similar GAG contents, when normalized to DNA contents ($P = 0.500$) (Fig. 1H), indicating that SDSCs have minimum effect on GAG deposition in direct coculture.

Type II collagen is the major structural component of the hyaline cartilage of the articular surfaces, which has a relatively restricted localization. We observed downregulated expression of *Col2a1* in CCs+SDSCs pellets and then employed immunohistochemistry analysis of type II collagen. Both CCs pellets and CCs+SDSCs pellets revealed positive staining of type II collagen from day 7 while most dense and homogenous staining was observed at day 21, yet no significant difference was found between CCs pellets and CCs+SDSCs pellets over time (Fig. 1I and J), indicating similar pellet quality among these two groups. As *Col10a1* expression was also downregulated in the RT-PCR results, we then performed immunohistochemistry analysis of type X collagen, which is a network-forming collagen mainly expressed in hypertrophic chondrocytes in the cartilage. Staining of type X collagen was similar

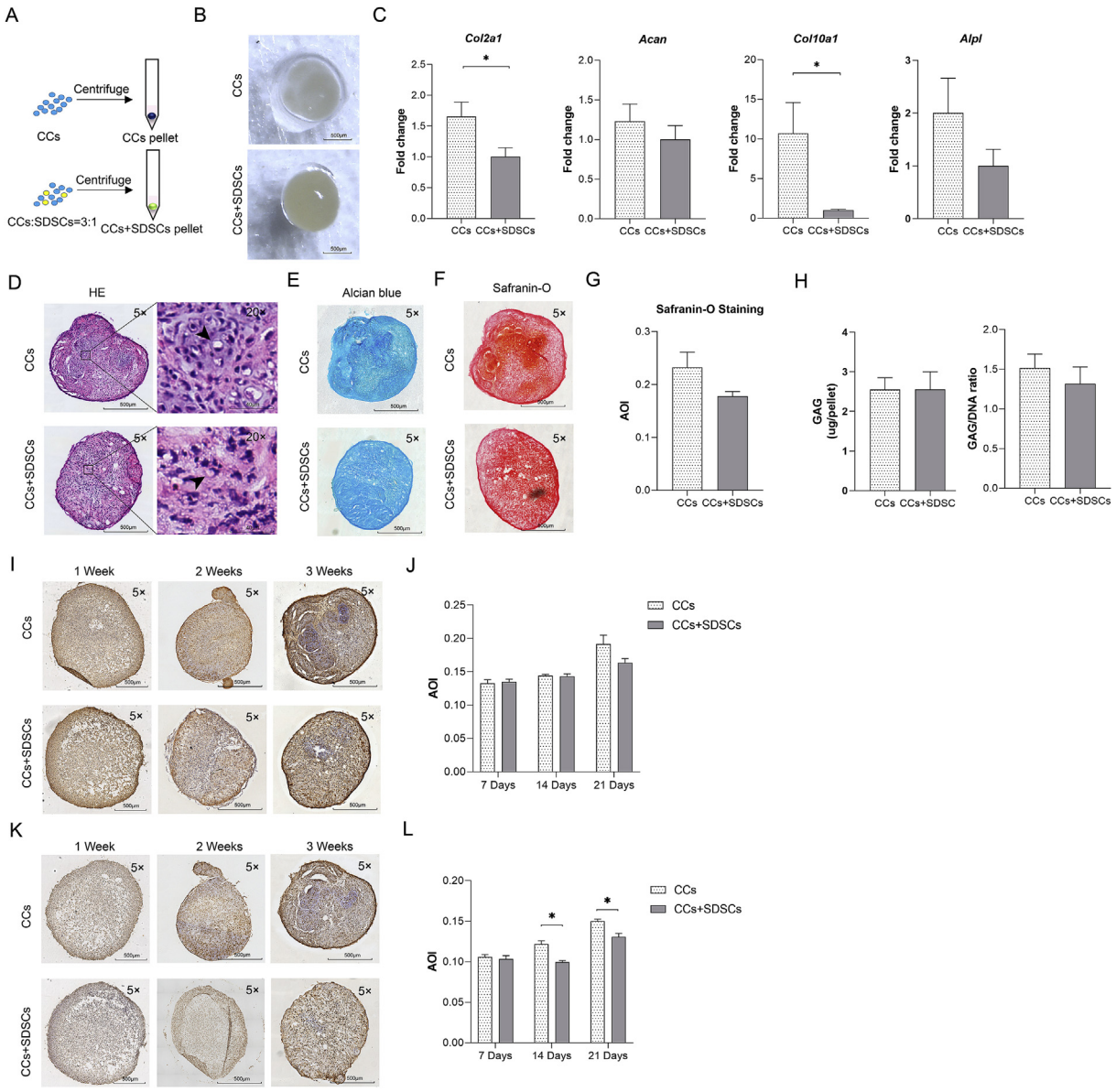

Figure 1. Effect of SDSCs on CCs in direct coculture. A) Schematic of mixed pellet coculture experimental outline. In light blue: CCs; In yellow: SDSCs; In dark blue: CCs pellet; In green: CCs+SDSCs pellet B) Macroscopic appearance of pellets. C) RT-PCR analysis for chondrogenesis and hypertrophic-related gene expression after 3 weeks of culture. D) H&E staining of pellets. Black arrows: lacuna-like structures. E) Alcian blue staining. F, G) Safranin-O staining. H) Biochemical evaluation of GAG content and GAG/DNA ratio of pellets. I, J) Immunohistochemistry analysis of type II collagen. K, L) Immunohistochemistry analysis of type X collagen. (For interpretation of the references to color/colour in this figure legend, the reader is referred to the Web version of this article.)

among two groups at day 7. However, CCs pellets showed stronger and more homogenous staining of type X collagen than CCs+SDSCs pellets at day 14 and day 21, with the periphery of CCs pellets stained the strongest while the inside of the pellets stained lighter and homogeneous (Fig. 1K and L), indicating less hypertrophic differentiation tendency in coculture pellets. These results demonstrated that SDSCs may impede the process of hypertrophic differentiation of CCs in pellet culture.

3.2. SDSCs suppressed chondrocyte hypertrophy by paracrine mechanism

To figure out the mechanism underlying the effect of SDSCs, we employed CM coculture system (Fig. 2A). Pellets cultured in SDSCs-CM show similar appearance to the control group (Fig. 2B). Similarly, RT-PCR results showed the culture medium from SDSCs significantly impeded the *Col10a1* expression ($P = 0.046$), but had minimum effects on *Col2a1*, *Acan*, and *Alpl* expression (Fig. 2C).

H&E staining and Alcian blue staining showed that pellets formed cartilage-like form appeared rich in GAG. The monoculture group appeared more compact and homogeneous than SDSCs-CM group (Fig. 2D and E).

The Alcian blue and Safranin-O staining showed no significant between both groups (Fig. 2E and F). The semi-quantitative results of Safranin-O staining remained no significant change ($P = 0.140$) (Fig. 2G). GAG quantification results using DMMB method matched RT-PCR results and histology results that total GAG contents of pellets have no significant difference in relation to culture medium ($P = 0.940$). When referenced to DNA contents, the GAG/DNA ratio remain no difference between the two groups ($P = 0.720$) (Fig. 2H), indicating no significant change in GAG deposition between the two groups, which was similar to mixed pellet culture. In line with RT-PCR results, immunohistochemistry results revealed a slightly denser and more homogeneous staining of type II collagen in monoculture group (Fig. 2I). The semi-quantitative results

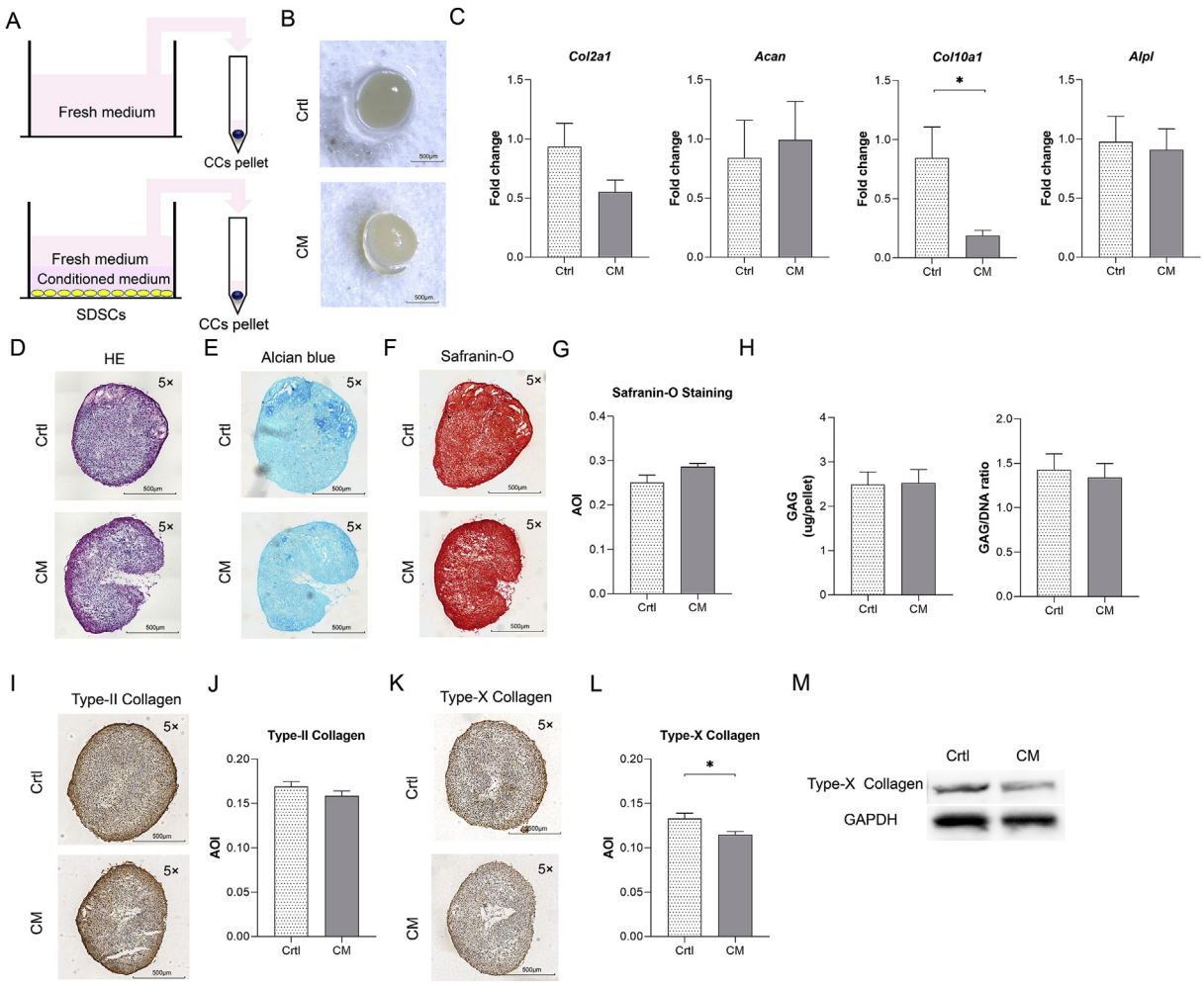

Figure 2. Paracrine effect of SDSCs toward CCs in indirect coculture. A) Schematic of conditioned medium coculture experimental outline. In light blue: CCs; In yellow: SDSCs; In dark blue: CCs pellet. B) Macroscopic appearance of pellets. C) RT-PCR analysis for chondrogenesis and hypertrophic-related gene expression after 3 weeks of culture. D) H&E staining. E) Alcian blue staining. F, G) Safranin-O staining. H) Biochemical evaluation of GAG content and GAG/DNA ratio of pellets. I, J) Immunohistochemistry analysis of type II collagen. K, L) Immunohistochemistry analysis of type X collagen. M) Western blot analysis of type X collagen. Crtl: Control group. CM: SDSCs-conditioned medium group. (For interpretation of the references to color/colour in this figure legend, the reader is referred to the Web version of this article.)

revealed slightly decrease of type II collagen in SDSCs-CM groups with no significant difference ($P = 0.215$) (Fig. 2J). These results indicate that SDSCs have little effect on type II collagen generation of CCs. Similar with results in mixed pellet coculture, type X collagen staining was stronger in monoculture pellets, especially in the periphery of the pellet (Fig. 2K). The semi-quantitative analysis revealed that AOI was significantly lower in the CM group ($P = 0.022$) (Fig. 2L), which was in line with RT-PCR results. We then performed western blotting and the results revealed that type X collagen expression in CCs cultured in SDSCs-CM was downregulated (Fig. 2M), which was in line with RT-PCR and immunohistochemistry, indicating that type X collagen expression was downregulated in SDSCs-CM.

3.3. SDSCs differentiate to chondrocyte and promote the proliferation of CCs in vitro

To test whether SDSCs could differentiate to chondrocyte in vitro, we also performed 3D pellet culture (Fig. 3A). Compared to CCs pellets, the pellets constructed by SDSCs were smaller in size ($P = 0.027$) and more translucent (Fig. 3B and C). H&E staining of SDSCs pellets displayed loose content with fibrous structure with far less matrix inside (Fig. 3D). This result indicates that SDSCs may generate cartilage with poor quality. Further, Alcian blue staining and Safranin-O staining revealed GAG formation in SDSCs pellets (Fig. 3E and F). However, the AOI of Safranin-O staining was 3.6 times lower than CCs pellets ($P < 0.001$) (Fig. 3G). GAG quantification results showed that total GAG content of SDSCs pellets were 1.7 times lower than CCs pellets ($P = 0.023$), and the GAG/DNA ratio was also higher in CCs pellets than SDSCs pellets ($P = 0.012$) (Fig. 3H), indicating SDSCs pellets formed less GAG than CCs pellets. Moreover, immunohistochemistry of SDSCs pellets showed positive results of both type II collagen and type X collagen, and the staining was significantly lower when compare to CCs (Fig. 3I–L), indicating worse matrix generation ability of SDSCs. To investigate the effect of SDSCs on chondrocyte proliferation, we employed transwell coculture system, with CCs seeded into bottom well of a 24-well transwell system and SDSCs seeded on the transwell insert with 0.4 μm pore (Fig. 3M). The CCs quantity increased faster in the transwell coculture system (Fig. 3N), indicating that SDSCs promote proliferation of CCs. These results indicate that SDSCs promote proliferation of CCs when cocultured indirectly.

3.4. In vivo cartilage repair of SDSC cocultured pellets

To validate the effect of SDSCs on cartilage repair, we implanted cocultured pellets into the osteochondral defect on the patellar groove of the femur and evaluated the tissue repair effect using the ICRS macroscopic scoring and histological grading systems 4 weeks after surgery.

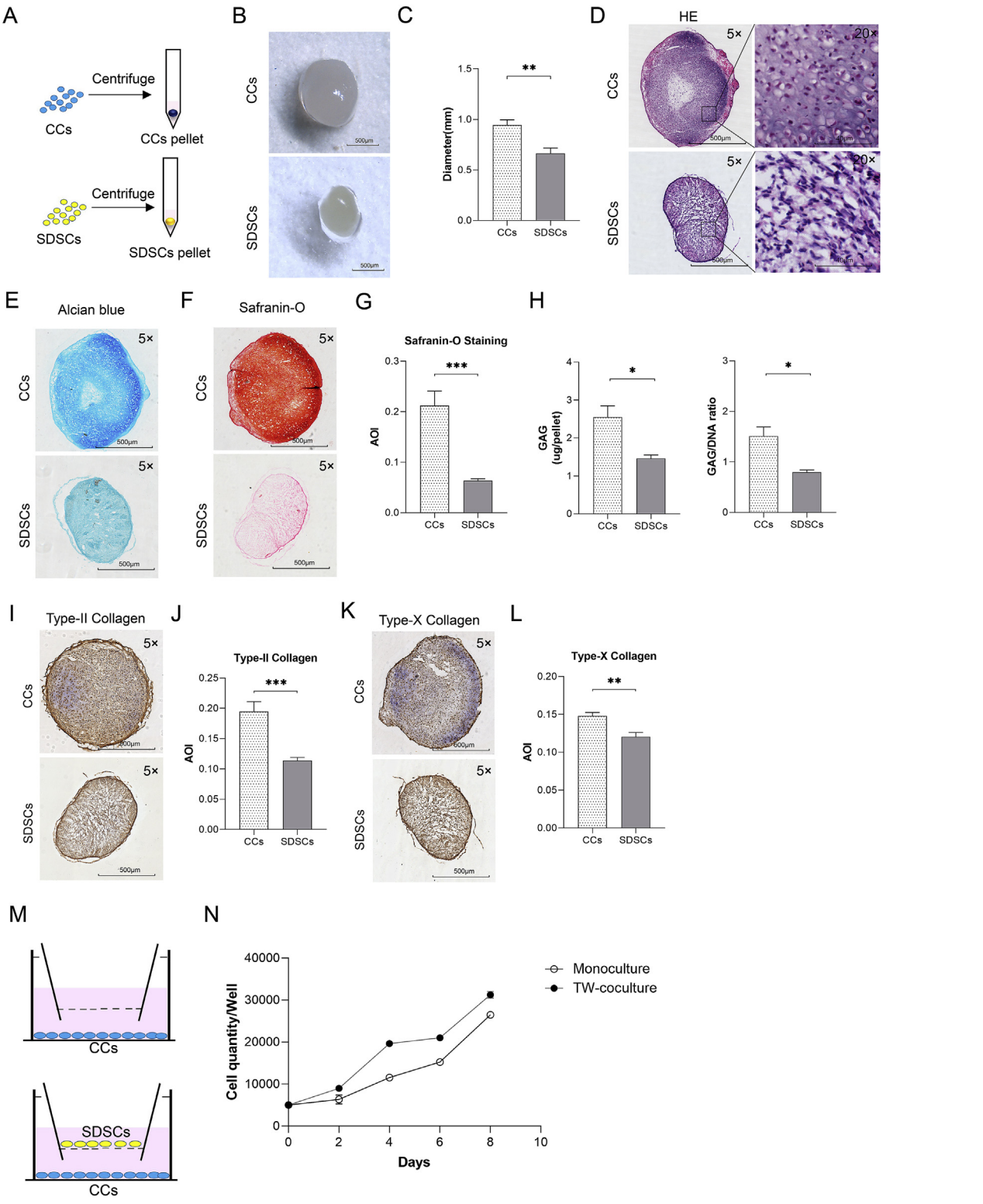

Figure 3. Chondrogenic induction of SDSCs and its promotion of CCs proliferation. A) Schematic of chondrogenic induction of SDSCs experimental outline. In light blue: CCs; In yellow: SDSCs; In dark blue: CCs pellet; In orange: SDSCs pellet. B) Macroscopic appearance of pellets. C) Quantitative analysis of diameters of pellets constructed by CCs and SDSCs. D) H&E staining. E) Alcian blue staining. F, G) Safranin-O staining. H) Biochemical evaluation of GAG content and GAG/DNA ratio of pellets. I, J) Immunohistochemistry analysis of type II collagen. K, L) Immunohistochemistry analysis of type X collagen. M) Schematic of cell proliferation experimental outline. N) Cell quantities of CCs transwell cocultured with SDSCs. (For interpretation of the references to color/colour in this figure legend, the reader is referred to the Web version of this article.)

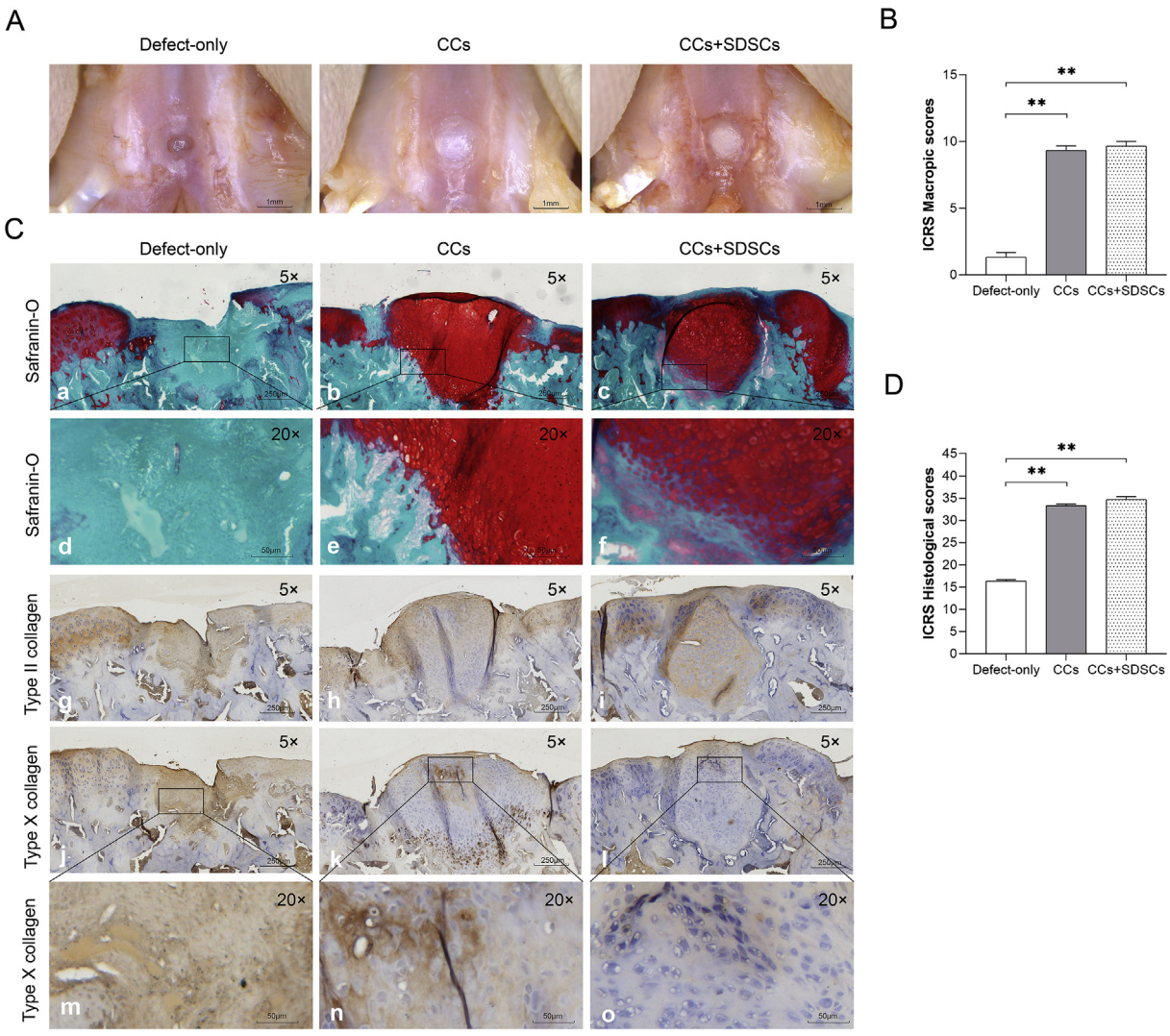

Figure 4. In vivo cartilage repair of SDSC cocultured pellets. A) Macroscopic appearance of operated knees at 4 weeks. B) ICRS macroscopic scores for the defect-only and pellet-implanted groups. C) Histological and immunohistochemical findings. a-f are Safranin-O staining image; g-i are immunohistochemistry analysis of type II collagen; j-o are immunohistochemistry analysis of type X collagen; D) ICRS histological scores for the defect-only and pellet-implanted groups.

The defect-only sites showed an irregularly concaved surface, while both the CCs and CCs+SDSCs groups showed good restoration in the defect sites. The implanted pellets had integrated well with the surroundings, and none was lost. The restored surfaces were white and smooth, resembling the native articular cartilage, with no signs of degradation (Fig. 4A). The ICRS macroscopic scores were significantly higher in both pellet-implanted groups than in the defect-only group ($P < 0.001$), albeit no significant difference was observed between the CCs and CCs+SDSCs groups ($P = 0.519$) (Fig. 4B). In the defect-only group, the histological evaluation revealed that the surface was depressed with fibrous-like tissue partially filling the defect. By contrast, both implanted groups showed restoration of the articular surface without sign of immune rejection. The cartilage matrix of the grafts was stained equally strong by Safrain-O, indicating that the grafts were GAG-rich, with no signs of degradation. There was a continuous integration between the pellet and the surrounding host bone without any gap at 4 weeks after implantation, with Safranin-O showed a light red stain with green interspersed (Fig. 4C a-f). The ICRS histological scores were significantly higher in both pellet-implanted groups than in the defect-only group ($P < 0.001$), with no significant difference between the CCs and CCs+SDSCs groups ($P = 0.148$) (Fig. 4D), suggesting equally effective repair abilities of the CCs and CCs+SDSCs pellets. Immunohistochemistry staining revealed that type II collagen was uniformly distributed in the CCs+SDSCs grafts, while the CCs groups were stained slightly lighter (Fig. 4C g-i). With regard to type X collagen, the CCs+SDSCs grafts showed only slight staining in the grafts' superficial layer, but the staining was stronger in the CCs grafts, predominantly in the central portion of the grafts (Fig. 4C j-o). These results were in line with an in vitro study that reported that SDSCs may impede the hypertrophic differentiation of CCs pellets.

4. Discussion

CCs as a promising cell source for ACI, have stronger tendency of hypertrophy and calcification. We hypothesized that SDSCs and their secreted factors may play a role in maintaining costal chondrocyte phenotype and suppress chondrocyte hypertrophic differentiation. In this study, pellets constructed by CCs and SDSCs showed similar appearance and cartilage matrix deposition with pellets constructed by CCs. Besides, this pellet contained less type X collagen, indicating that cocultured pellets may have less tendency toward calcification. Further in indirect coculture experiment, SDSCs suppressed type X collagen expression as well and promoted the proliferation of CCs, indicating that SDSCs may influence CCs by paracrine mechanism. We further conducted in vivo cartilage repair experiment and found that pellets implanted in the

defects showed good restoration effects, whereas the CCs+SDSCs pellets showed lower type X expression levels, indicating less calcification tendency.

Previous studies have reported that chondrocytes cocultured with MSCs at ratio of 3:1 was one of the favorable ratios for pellet formation [42]. Thus, we conducted our research by using this ratio. An interesting discovery in our study is that in contrast with other sources of MSCs such as bone marrow mesenchymal stromal cells (BMSCs) and adipose-derived mesenchymal stromal cells (ADSCs) [36,43], SDSCs stimulated the proliferation of chondrocytes, but did not stimulate GAG formation and extracellular deposition. Based on our study, the ability of chondrocyte to form cartilage matrix varies between individuals and with cell generation. Regardless of this diversity, the total GAG content per pellet and GAG/DNA ratio showed no significant difference whether coculture with SDSCs or not. However, CCs pellets yield slightly higher amount of type II collagen. These results were partially in line with those reported by Chen et al., wherein they cocultured SDSCs with nucleus pulposus cells in pellet and found GAG and type II collagen was most predominant in pellets formed by nucleus pulposus cells alone [44]. One plausible explanation is that SDSCs generate relatively lower cartilage matrix compared to CCs. When CCs directly cocultured with SDSCs, though the matrix formation remains unchanged, due to the increasing total cell number and decreasing CCs proportion, type II collagen deposition and *Col2a1* gene expression were decreased. To further prove this explanation, we applied indirect coculture system using CM. In this culture system, the type II collagen remained unchanged when cocultured with SDSCs while the GAG expression was slightly increased in Safranin-O staining and RT-PCR results. These results are similar to those of Levorson et al. [45], which stated chondrocytes indirectly cocultured with MSCs generated high amounts of glycosaminoglycan and collagen in a poly(∈ -caprolactone) scaffold. We further evaluated pellets formed by SDSCs alone and found relatively low expression of GAG and type II collagen. Although SDSCs generate comparatively lower cartilage matrix alone, we cannot conclude that SDSCs have no contribution to cartilage matrix formation in mixed pellets. Nevertheless, previous studies stated that cartilage-forming capacity was mainly ascribed to chondrocytes while MSCs appear to promote chondrocyte proliferation and stimulate chondrocyte to generate cartilage matrix rather than generating the matrix themselves [35,36,43,46]. Our results indicate that SDSCs do not hinder cartilage matrix formation of CCs. The slight decrease of type II collagen in mixed pellets may result from the dilution effect of SDSCs.

Compared to articular chondrocytes, CCs have a stronger tendency of switching to hypertrophy and calcification [7,8]. In our study, we found that expression of type X collagen, a marker of hypertrophic differentiation, was significantly decreased both in gene expression and protein deposition in pellets formed by CCs cocultured with SDSCs. We further examined type X collagen expression in SDSCs. The results showed that type X collagen expressed by SDSCs was relatively low compared to CCs. To figure out whether decrease of type X collagen was due to anti-hypertrophic effect of SDSCs or just dilution effect, we conducted indirect cocultured experiment using CM system. The results revealed that gene expression of type X collagen was significantly decreased when CCs pellets cultured in SDSCs-CM, and the immunohistochemistry analysis revealed consistent results. These results could demonstrate that the decrease of type X collagen in pellets was at least partially due to the anti-hypertrophic effect of SDSCs through paracrine effect. However, the underlying cellular mechanism responsible for this effect yet remains unclear. Numerous researches focused on cellular communication pathways involved in coculture of MSCs with chondrocytes [47]. Cultured MSCs from other sources produce a multitude of cytokines and growth factors, including several cartilage-protecting factors such as PGE2, TIMPs, OPG, DDK-1, and HFG [48–50]. As SDSCs is one of the subtypes of MSCs, the underlying mechanism may be similar with other type of MSCs; however, the exact mechanism needs to be further clarified.

Furthermore, in indirect coculture method using a transwell system, we found that SDSCs could promote proliferation of CCs in monolayer culture. Our results prove our hypothesis that SDSCs may hold trophic role in maintaining chondrocyte stemness with high proliferation ability. These results were similar to those of studies by Acharya et al. and Pleumeekers et al. [36,51], wherein they found that BMSCs and ADSCs derived CM could promote articular cartilage proliferation when chondrocytes were cultured in pellets or embedded in alginate beads. The mechanism of proliferative effect of MSCs has been reported before. Different modalities of communication might be responsible for this proliferative effect, including cell–cell contact, release of soluble factors, or their combination [36,52,53]. So, as SDSCs are a subtype of MSCs, they may have similar effect as other kinds of MSCs. Nevertheless, the underlying mechanism needs to be further clarified.

As we found that SDSCs have a potential anti-hypertrophic effect, we further conducted an in vivo experiment using cocultured pellets to restore osteochondral defects. CCs is a promising chondrocytes cell source to generate tissue-engineered cartilage. Both scaffold and scaffold-free tissue-engineered cartilage showed encouraging results in repairing articular cartilage [54–56]. In our study, we observed that in comparison with the defect-only group, pellets constructed by CCs restored osteochondral defect and facilitated articular resurfacing. Besides, Both CCs pellets and CCs+SDSCs pellets restored defects with satisfactory outcome according to the ICRS scores, consistent with the findings of Ito et al. [23]. Moreover, the continuous integration between the pellet and the surrounding host bone showed a light red stain with green interspersed staining in Safranin-O. Therefore, it may be an early stage of subchondral bone regeneration, which would be further remodeled later and finally reconstructed subchondral bone. According to studies conducted by Meng et al. [57,58], most studies focusing on subchondral bone regeneration in rat often involved animal studies with endpoint of 12 weeks or even longer. Therefore, animal studies with longer time may be needed to investigate the effect of this mixed culture system on the repair of subchondral bone. We also observed that while the GAG was equally stained, there was a decreased expression of type II collagen in the implanted pellets of CCs group, mainly occurred in the central portion of the pellets. Immunohistochemistry analysis revealed that the central portion were predominantly stained with type X collagen. Type X collagen is a network-forming collagen which is critical for calcification and bone remodeling. With the stimuli from host bone, CCs may experience hypertrophic differentiation and subsequently generate type X collagen which facilitate angiogenesis and osteogenesis that might be beneficial for pellets integration and subchondral bone formation. However, expression of type X collagen in the central portion of the graft may lead to calcified nodule formation as other studies postulated [7], hence affect the biomechanical property of newly-formed articular cartilage. In the CCs+SDSCs groups, type X collagen was only slightly stained with no sign of type X collagen expression in the central portion, indicating that grafts were less liable to calcified. The results of our study can be used as a basis for the clinical use of SDSCs during costal chondrocyte implantation.

A limitation of our study is that the time-course of animal study was only limited to 4 weeks. The implanted pellet may undergo further remodeling beyond 4 weeks, such as subsequence remodeling of subchondral bone [57,58]. In vivo studies with longer period would be performed in the subsequent experiments. Moreover, we have not assessed the exact secreted factors of SDSCs in this study. More work is needed to clarify the relative mechanisms in the future.

In conclusion, we have shown for the first time that SDSCs had anti-hypertrophic effect toward CCs. This study provides valuable findings for using CCs as a new cell source and preliminary data of using SDSCs to prevent CCs hypertrophy and calcification to promote articular cartilage repair.

Authors' contributions

Y. Ma, C. Zhang, and D. Du designed the research protocol. Y. Ma, F. Xiang, K. Zheng, and Y. Pang conducted the research. Y. Ma, D. Du, J. Gao

analyzed the data. Y. Ma and D. Du wrote the manuscript. J. Gao, C. Zhang, and D. Du critically revised the manuscript. All the authors approved the final manuscript.

Funding

This study was supported in part by the National Natural Science Foundation of China (81820108020) and Innovative Research Team of High-Level Local Universities in Shanghai (SSMU-ZDCX20180800).

Ethical statement

The animal studies were performed after receiving the approval of the Institutional Animal Care and Use Committee (IACUC) in Shanghai Jiaotong University affiliated Shanghai Sixth People's Hospital (IACUC approval No. DWLL2021-0058).

Declaration of competing interest

The authors have no financial conflicts of interest.

References

[1] Gelber AC, Hochberg MC, Mead LA, Wang NY, Wigley FM, Klag MJ. Joint injury in young adults and risk for subsequent knee and hip osteoarthritis. Ann Intern Med 2000;133:321–8. https://doi.org/10.7326/0003-4819-133-5-200009050-00007.
[2] Yoon KH, Yoo JD, Choi CH, Lee J, Lee JY, Kim SG. Costal chondrocyte-derived pellet-type Autologous chondrocyte implantation versus microfracture for repair of articular cartilage defects: a prospective randomized trial. Cartilage 2020. https://doi.org/10.1177/1947603520921448. 1947603520921448.
[3] Yoon KH, Park JY, Lee JY, Lee E, Lee J, Kim SG. Costal chondrocyte-derived pellet-type Autologous chondrocyte implantation for treatment of articular cartilage defect. Am J Sports Med 2020;48:1236–45. https://doi.org/10.1177/0363546520905565.
[4] He A, Xia H, Xiao K, Wang T, Liu Y, Xue J. Cell yield, chondrogenic potential, and regenerated cartilage type of chondrocytes derived from ear, nasoseptal, and costal cartilage. J Tissue Eng Regen Med 2018;12:1123–32. https://doi.org/10.1002/term.2613.
[5] Lee J, Lee E, Kim HY, Son Y. Comparison of articular cartilage with costal cartilage in initial cell yield, degree of dedifferentiation during expansion and redifferentiation capacity. Biotechnol Appl Biochem 2007;48:149–58. https://doi.org/10.1042/BA20060233.
[6] Huwe LW, Brown WE, Hu JC, Athanasiou KA. Characterization of costal cartilage and its suitability as a cell source for articular cartilage tissue engineering. J Tissue Eng Regen Med 2018;12:1163–76. https://doi.org/10.1002/term.2630.
[7] Kusuhara H, Isogai N, Enjo M, Otani H, Ikada Y, Jacquet R. Tissue engineering a model for the human ear: assessment of size, shape, morphology, and gene expression following seeding of different chondrocytes. Wound Repair Regen 2009;17:136–46. https://doi.org/10.1111/j.1524-475X.2008.00451.x.
[8] Jurk V, Kampmann H, Iblher N, Bannasch H, Gubisch W. Long-term comparison of rib and ear cartilage grafts in autologous and allogenic fascia lata: an experimental study in a white rabbit model. Plast Reconstr Surg 2016;137:1465–74. https://doi.org/10.1097/PRS.0000000000002133.
[9] Mueller MB, Tuan RS. Functional characterization of hypertrophy in chondrogenesis of human mesenchymal stem cells. Arthritis Rheum 2008;58:1377–88. https://doi.org/10.1002/art.23370.
[10] Chen S, Fu P, Cong R, Wu H, Pei M. Strategies to minimize hypertrophy in cartilage engineering and regeneration. Genes Dis 2015;2:76–95. https://doi.org/10.1016/j.gendis.2014.12.003.
[11] Mackie EJ, Ahmed YA, Tatarczuch L, Chen KS, Mirams M. Endochondral ossification: how cartilage is converted into bone in the developing skeleton. Int J Biochem Cell Biol 2008;40:46–62. https://doi.org/10.1016/j.biocel.2007.06.009.
[12] Singh P, Marcu KB, Goldring MB, Otero M. Phenotypic instability of chondrocytes in osteoarthritis: on a path to hypertrophy. Ann N Y Acad Sci 2019;442:17–34. https://doi.org/10.1111/nyas.13930.
[13] Hunziker EB, Rosenberg LC. Repair of partial-thickness defects in articular cartilage: cell recruitment from the synovial membrane. J Bone Joint Surg Am 1996;78:721–33. https://doi.org/10.2106/00004623-199605000-00012.
[14] Kokubo M, Sato M, Yamato M, Mitani G, Kutsuna T, Ebihara G. Characterization of chondrocyte sheets prepared using a co-culture method with temperature-responsive culture inserts. J Tissue Eng Regen Med 2016;10:486–95. https://doi.org/10.1002/term.1764.
[15] Kim TW, Lee MC, Bae HC, Han HS. Direct coculture of human chondrocytes and synovium-derived stem cells enhances in vitro chondrogenesis. Cell J 2018;20:53–60. https://doi.org/10.22074/cellj.2018.5025.
[16] Kokubo M, Sato M, Yamato M, Mitani G, Uchiyama Y, Mochida J. Characterization of layered chondrocyte sheets created in a co-culture system with synoviocytes in a hypoxic environment. J Tissue Eng Regen Med 2017;11:2885–94. https://doi.org/10.1002/term.2192.
[17] Lee CM, Kisiday JD, McIlwraith CW, Grodzinsky AJ, Frisbie DD. Synoviocytes protect cartilage from the effects of injury in vitro. BMC Muscoskel Disord 2013;14:54. https://doi.org/10.1186/1471-2474-14-54.
[18] Kurz B, Steinhagen J, Schunke M. Articular chondrocytes and synoviocytes in a co-culture system: influence on reactive oxygen species-induced cytotoxicity and lipid peroxidation. Cell Tissue Res 1999;296:555–63. https://doi.org/10.1007/s004410051317.
[19] Ryu JS, Jung YH, Cho MY, Yeo JE, Choi YJ, Kim YI. Co-culture with human synovium-derived mesenchymal stem cells inhibits inflammatory activity and increases cell proliferation of sodium nitroprusside-stimulated chondrocytes. Biochem Biophys Res Commun 2014;447:715–20. https://doi.org/10.1016/j.bbrc.2014.04.077.
[20] Langenmair ER, Kubosch EJ, Salzmann GM, Beck S, Schmal H. Clinical trial and in vitro study for the role of cartilage and synovia in acute articular infection. Mediat Inflamm 2015;2015:430324. https://doi.org/10.1155/2015/430324.
[21] Kean TJ, Ge Z, Li Y, Chen R, Dennis JE. Transcriptome-wide analysis of human chondrocyte expansion on synoviocyte matrix. Cells 2019;8:85. https://doi.org/10.3390/cells8020085.
[22] Pei M, He F. Extracellular matrix deposited by synovium-derived stem cells delays replicative senescent chondrocyte dedifferentiation and enhances redifferentiation. J Cell Physiol 2012;227:2163–74. https://doi.org/10.1002/jcp.22950.
[23] Ito S, Sato M, Yamato M, Mitani G, Kutsuna T, Nagai T. Repair of articular cartilage defect with layered chondrocyte sheets and cultured synovial cells. Biomaterials 2012;33:5278–86. https://doi.org/10.1016/j.biomaterials.2012.03.073.
[24] Sakaguchi Y, Sekiya I, Yagishita K, Muneta T. Comparison of human stem cells derived from various mesenchymal tissues: superiority of synovium as a cell source. Arthritis Rheum 2005;52:2521–9. https://doi.org/10.1002/art.21212.
[25] Koga H, Muneta T, Nagase T, Nimura A, Ju YJ, Mochizuki T. Comparison of mesenchymal tissues-derived stem cells for in vivo chondrogenesis: suitable conditions for cell therapy of cartilage defects in rabbit. Cell Tissue Res 2008;333:207–15. https://doi.org/10.1007/s00441-008-0633-5.
[26] Kubosch EJ, Heidt E, Niemeyer P, Bernstein A, Sudkamp NP, Schmal H. In-vitro chondrogenic potential of synovial stem cells and chondrocytes allocated for autologous chondrocyte implantation - a comparison : synovial stem cells as an alternative cell source for autologous chondrocyte implantation. Int Orthop 2017;41:991–8. https://doi.org/10.1007/s00264-017-3400-y.
[27] Pei M, He F, Boyce BM, Kish VL. Repair of full-thickness femoral condyle cartilage defects using allogeneic synovial cell-engineered tissue constructs. Osteoarthritis Cartilage 2009;17:714–22. https://doi.org/10.1016/j.joca.2008.11.017.
[28] Shimomura K, Ando W, Tateishi K, Nansai R, Fujie H, Hart DA. The influence of skeletal maturity on allogenic synovial mesenchymal stem cell-based repair of cartilage in a large animal model. Biomaterials 2010;31:8004–11. https://doi.org/10.1016/j.biomaterials.2010.07.017.
[29] Ando W, Tateishi K, Hart DA, Katakai D, Tanaka Y, Nakata K. Cartilage repair using an in vitro generated scaffold-free tissue-engineered construct derived from porcine synovial mesenchymal stem cells. Biomaterials 2007;28:5462–70. https://doi.org/10.1016/j.biomaterials.2007.08.030.
[30] Nakamura T, Sekiya I, Muneta T, Hatsushika D, Horie M, Tsuji K. Arthroscopic, histological and MRI analyses of cartilage repair after a minimally invasive method of transplantation of allogeneic synovial mesenchymal stromal cells into cartilage defects in pigs. Cytotherapy 2012;14:327–38. https://doi.org/10.3109/14653249.2011.638912.
[31] Shimomura K, Ando W, Moriguchi Y, Sugita N, Yasui Y, Koizumi K. Next generation mesenchymal stem cell (MSC)-Based cartilage repair using scaffold-free tissue engineered constructs generated with synovial mesenchymal stem cells. Cartilage 2015;6:13s–29s. https://doi.org/10.1177/1947603515571002.
[32] Steinhagen J, Bruns J, Niggemeyer O, Fuerst M, Ruther W, Schunke M. Perfusion culture system: synovial fibroblasts modulate articular chondrocyte matrix synthesis in vitro. Tissue Cell 2010;42:151–7. https://doi.org/10.1016/j.tice.2010.03.003.
[33] Bonitz M, Schaffer C, Amling M, Poertner R, Schinke T, Jeschke A. Secreted factors from synovial fibroblasts immediately regulate gene expression in articular chondrocytes. Gene 2019;698:1–8. https://doi.org/10.1016/j.gene.2019.02.065.
[34] Ackermann J, Merkely G, Mestriner AB, Shah N, Gomoll AH. Increased chondrocytic gene expression is associated with improved repair tissue quality and graft survival in patients after autologous chondrocyte implantation. Am J Sports Med 2019;47:2919–26. https://doi.org/10.1177/0363546519868213.
[35] Wu L, Prins HJ, Helder MN, van Blitterswijk CA, Karperien M. Trophic effects of mesenchymal stem cells in chondrocyte co-cultures are independent of culture conditions and cell sources. Tissue Eng 2012;18:1542–51. https://doi.org/10.1089/ten.TEA.2011.0715.
[36] Pleumeekers MM, Nimeskern L, Koevoet JLM, Karperien M, Stok KS, van Osch G. Trophic effects of adipose-tissue-derived and bone-marrow-derived mesenchymal stem cells enhance cartilage generation by chondrocytes in co-culture. PloS One 2018;13:e0190744. https://doi.org/10.1371/journal.pone.0190744.
[37] De Bari C, Dell'Accio F, Tylzanowski P, Luyten FP. Multipotent mesenchymal stem cells from adult human synovial membrane. Arthritis Rheum 2001;44:1928–42. https://doi.org/10.1002/1529-0131(200108)44:8<1928::AID-ART331>3.0.CO;2-P.
[38] Djouad F, Bony C, Häupl T, Uzé G, Lahlou N, Louis-Plence P. Transcriptional profiles discriminate bone marrow-derived and synovium-derived mesenchymal stem cells. Arthritis Res Ther 2005;7:R1304–15. https://doi.org/10.1186/ar1827.
[39] Farndale RW, Buttle DJ, Barrett AJ. Improved quantitation and discrimination of sulphated glycosaminoglycans by use of dimethylmethylene blue. Biochim Biophys Acta 1986;883:173–7. https://doi.org/10.1016/0304-4165(86)90306-5.

[40] van den Borne MP, Raijmakers NJ, Vanlauwe J, Victor J, de Jong SN, Bellemans J, Saris DB. International cartilage repair society (ICRS) and oswestry macroscopic cartilage evaluation scores validated for use in autologous chondrocyte implantation (ACI) and microfracture. Osteoarthritis Cartilage 2007;15:1397–402. https://doi.org/10.1016/j.joca.2007.05.005.
[41] Mainil-Varlet P, Van Damme B, Nesic D, Knutsen G, Kandel R, Roberts S. A new histology scoring system for the assessment of the quality of human cartilage repair: ICRS II. Am J Sports Med 2010;38:880–90. https://doi.org/10.1177/0363546509359068.
[42] Amann E, Wolff P, Breel E, van Griensven M, Balmayor ER. Hyaluronic acid facilitates chondrogenesis and matrix deposition of human adipose derived mesenchymal stem cells and human chondrocytes co-cultures. Acta Biomater 2017;52:130–44. https://doi.org/10.1016/j.actbio.2017.01.064.
[43] Meretoja VV, Dahlin RL, Kasper FK, Mikos AG. Enhanced chondrogenesis in co-cultures with articular chondrocytes and mesenchymal stem cells. Biomaterials 2012;33:6362–9. https://doi.org/10.1016/j.biomaterials.2012.05.042.
[44] Chen S, Emery SE, Pei M. Coculture of synovium-derived stem cells and nucleus pulposus cells in serum-free defined medium with supplementation of transforming growth factor-beta1: a potential application of tissue-specific stem cells in disc regeneration. Spine 2009;34:1272–80. https://doi.org/10.1097/BRS.0b013e3181a2b347.
[45] Levorson EJ, Santoro M, Kasper FK, Mikos AG. Direct and indirect co-culture of chondrocytes and mesenchymal stem cells for the generation of polymer/extracellular matrix hybrid constructs. Acta Biomater 2014;10:1824–35. https://doi.org/10.1016/j.actbio.2013.12.026.
[46] Wang M, Rahnama R, Cheng T, Grotkopp E, Jacobs L, Limburg S. Trophic stimulation of articular chondrocytes by late-passage mesenchymal stem cells in coculture. J Orthop Res 2013;31:1936–42. https://doi.org/10.1002/jor.22466.
[47] Zou J, Bai B, Yao Y. Progress of co-culture systems in cartilage regeneration. Expet Opin Biol Ther 2018;18:1151–8. https://doi.org/10.1080/14712598.2018.1533116.
[48] Niada S, Giannasi C, Gomarasca M, Stanco D, Casati S, Brini AT. Adipose-derived stromal cell secretome reduces TNFα-induced hypertrophy and catabolic markers in primary human articular chondrocytes. Stem Cell Res 2019;38:101463. https://doi.org/10.1016/j.scr.2019.101463.
[49] Maumus M, Manferdini C, Toupet K, Peyrafitte J-A, Ferreira R, Facchini A. Adipose mesenchymal stem cells protect chondrocytes from degeneration associated with osteoarthritis. Stem Cell Res 2013;11:834–44. https://doi.org/10.1016/j.scr.2013.05.008.
[50] Niada S, Giannasi C, Gualerzi A, Banfi G, Brini AT. Differential proteomic analysis predicts appropriate applications for the secretome of adipose-derived mesenchymal stem/stromal cells and dermal fibroblasts. Stem Cell Int 2018;2018:7309031. https://doi.org/10.1155/2018/7309031.
[51] Acharya C, Adesida A, Zajac P, Mumme M, Riesle J, Martin I. Enhanced chondrocyte proliferation and mesenchymal stromal cells chondrogenesis in coculture pellets mediate improved cartilage formation. J Cell Physiol 2012;227:88–97. https://doi.org/10.1002/jcp.22706.
[52] Zhang Y, Cao L, Kiani C, Yang BL, Hu W, Yang BB. Promotion of chondrocyte proliferation by versican mediated by G1 domain and EGF-like motifs. J Cell Biochem 1999;73:445–57.
[53] Wu L, Leijten J, van Blitterswijk CA, Karperien M. Fibroblast growth factor-1 is a mesenchymal stromal cell-secreted factor stimulating proliferation of osteoarthritic chondrocytes in co-culture. Stem Cell Dev 2013;22:2356–67. https://doi.org/10.1089/scd.2013.0118.
[54] Cheuk YC, Wong MW, Lee KM, Fu SC. Use of allogeneic scaffold-free chondrocyte pellet in repair of osteochondral defect in a rabbit model. J Orthop Res 2011;29:1343–50. https://doi.org/10.1002/jor.21339.
[55] Zhao M, Chen Z, Liu K, Wan YQ, Li XD, Luo XW. Repair of articular cartilage defects in rabbits through tissue-engineered cartilage constructed with chitosan hydrogel and chondrocytes. J Zhejiang Univ - Sci B 2015;16:914–23. https://doi.org/10.1631/jzus.B1500036.
[56] Pomahac B, Zuhaili B, Kudsi Y, Aflaki P, Eriksson E. Use of a smooth, resorbable template for delivery of cultured pellets of autologous chondrocytes to articular cartilage defects–preliminary report. Eplasty 2009;9:e35.
[57] Meng X, Ziadlou R, Grad S, Alini M, Wen C, Lai Y. Animal models of osteochondral defect for testing biomaterials. Biochem Res Int 2020;2020:9659412. https://doi.org/10.1155/2020/9659412.
[58] Meng X, Grad S, Wen C, Lai Y, Alini M, Qin L. An impaired healing model of 865 osteochondral defect in papain-induced arthritis. J Orthop Translat 2021;26:101–10. https://doi.org/10.1016/j.jot.2020.07.005.