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Autologous serum supplementation promotes the phenotype maintenance of human chondrocytes with increased cellular autophagy

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Articular cartilage lacks the capacity for self-repair after injury, rendering autologous chondrocyte transplantation a crucial treatment option for severe cartilage damage. However, the in vitro expansion of chondrocytes in elderly patients poses challenges, particularly due to inadequate cell numbers and rapid phenotypic loss, often exacerbated by the use of animal-derived serum. In this study, we propose the utilization of human autologous serum (HAS) as a supplement in the in vitro cell culture to preserve the phenotype of chondrocytes isolated from the same patient. Chondrocytes cultured in medium supplemented with 5% HAS demonstrated improved cell proliferation and adherence, whereas a 10% concentration promoted a more differentiated phenotype. While 10% fetal bovine serum stimulates chondrocyte proliferation in the short term, 10% HAS is more advantageous for the long-term viability of cells. Notably, chondrocytes treated with the optimal 10% HAS supplementation exhibited a higher level of autophagy compared to those treated with 5% HAS alone. Our findings suggest that 10% human autologous serum holds promise for the efficient expansion of patient-derived autologous chondrocytes and facilitates the maintenance of chondrocyte phenotype, in part through enhanced cellular autophagy.

Keywords Human autologous serum, Human chondrocytes, In vitro culture

Osteoarthritis (OA) is one of the most common orthopedic diseases in the world. Accompanied by pain, joint swelling, osteophyte formation, subchondral bone changes and joint deformity, OA is characterized by cartilage loss, progressive narrowing of joint space, spur formation as well as synovitis[1]. OA progression is common in the middle-aged and elderly people and is associated with high morbidity and a long course. According to statistics from western countries, approximately 12% of middle-aged and elderly people suffer from OA with varying degrees[2], which places a very heavy economic burden on families and society.

The current treatments for osteoarthritis include anti-inflammatory drugs and surgical interventions[3]; however, their therapeutic effects are still limited. Once the articular cartilage is severely damaged, it can cause long-term pain and mobility problems in patients. Autologous chondrocyte transplantation (ACI) has been widely used for the treatment of cartilage degeneration in the late stage[4], however, the dedifferentiation of chondrocytes during the large-scale preparation in vitro is still a bottleneck limiting its clinical application[5]. The most basic manifestations of chondrocyte dedifferentiation of in vitro culture are morphological fibrosis of chondrocytes, disturbance of chondrocyte matrix metabolism, and a reduction in the cellular secretion of proteoglycan and type II collagen. In the middle-aged and elderly patients, autologous chondrocytes are relatively more dedifferentiated, and the commonly used feral bovine serum (FBS) in culture medium is very likely to result in reduced cell proliferation capacity and loss of the chondrocyte phenotype during in vitro expansion. Thus, the serum supplementation greatly hinders the development of high-quality cell culture methods for autologous chondrocyte transplantation.

As an essential substance in cell culture, serum plays an important role in promoting the proliferation of chondrocytes and maintaining the chondrocytic phenotype. Numerous studies have demonstrated that human-derived platelet-rich plasma (PRP) is superior to fetal bovine serum in sustaining the growth and viability of

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human chondrocytes in cell culture[6]. It is suggested that human-derived serum can be used as an important source of serum for in vitro chondrocyte culture. Furthermore, for in vitro culture of human chondrocytes isolated from patients, previous studies have shown a protective role of autophagy in chondrocytes when mitochondrial function is dysregulated[7]. In addition, Yi Wang et al. have demonstrated that different serum concentrations had different regulation on autophagy in short-term cultured of skeletal muscle satellite cells[8].

In this study, chondrocytes harvested from OA patients were cultivated in the presence of autologous serum supplementation. We systematically explored the impact of autologous serum on cell survival, the preservation of chondrocyte phenotype, and the associated regulatory mechanisms. As a result, this study proposes a tailored, personalized strategy for the expansion of autologous chondrocytes in vitro, paving the way for potential future applications in cell transplantation to treat focal articular cartilage defects.

Methods

Statement of experiments

The studies involving human-derived blood samples and knee joint cartilage from medical wastes were reviewed and approved by Zhejiang Hospital Ethics Committee. All experiments in this study were performed in accordance with relevant guidelines and regulations.

Reagents and antibodies

Chondrocyte culture medium (DMEM/F-12), double antibiotics (P/S), Collagenase, dry trypsin powder (Shanghai Shenggong Biological, China), Fetal bovine serum (Hyclone, USA), EDTA, bovine serum albumin (Shanghai Shenggong Biological, China), Petri dishes (Thermo Fisher Scientific Ltd., USA), Cell Culture Plate (Corning Corporation, USA), Blood Count Plate (Yancheng Hengtai glass instrument factory, China). Western lysate (Biyuntian Biotechnology Corporation, China), BCA protein concentration assay kit (Biyuntian Biotechnology Corporation, China), protein molecular weight Marker (Invitrogen, USA), ECL Chemiluminescence Kit (Thermo Fisher Scientific Ltd., USA), rabbit-derived polyclonal LC3B (Cell Signaling, USA), mouse-derived polyclonal β-actin (Santa Cruz, USA), HRP-labeled goat anti-rabbit, goat anti-mouse secondary antibody (Santa Cruz, USA).

Extraction, isolation and culture of chondrocytes from patients

During the knee replacement operation for the patients (54–67 years old, 60 years old on average, 6 cases in total (the individual ages of the donors are 54, 58, 60, 61, 64, 67 years old), including 4 females and 2 males), the discarded knee cartilage was taken aseptically, avoiding osteophytes, the cartilage tissue with relatively intact structure in the center was taken, the tissue was cut into 0.5 mm × 0.5 mm sized fragments, PBS (containing 100 U/mL of penicillin and streptomycin respectively) was washed three times, 0.25% type II collagenase with 3 times the volume was added, and the cartilage was digested in a 37 ℃ constant temperature shaking table for 8–12 h (most of the cartilage was digested), Centrifuge (500*g*, 5 min) to precipitate undigested cartilage. Centrifuge the supernatant (1200 r/min, 10 min), wash the precipitated cells with PBS solution for three times, wash away the digestive enzymes on the cell surface, and dilute them with DMEM/F-12 containing 20% FBS (fetal bovine serum), 50 g/ml vitamin C, 0.4 mmol/L proline, 5ug/ml insulin and 1 mmol/L (NEAA) × Inoculate 10^5/ml into a 50 ml culture bottle at 37 ℃ with 5% CO_2 for overnight culture until it sticks to the wall. The next day, the culture medium containing different serum or serum with different concentration was changed for subsequent experiments. Each chondrocyte preparation was cultured with its own autologous serum, and all experiments were performed in all samples from the 6 donors.

Autologous serum extraction and Preparation from patients

After obtaining the consent of patients, each patient collected 2 ml (6 patients in total, and the mean age of the donors is 60) during blood routine test, and obtained a total of 20 ml of peripheral blood. After being injected into a sterile glass bottle, it was placed in a 37 ℃ incubator and kept still for 2 h. After the blood clots were completely separated out, the serum was drawn out in an ultra-clean workbench under sterile conditions and transferred into a centrifuge tube. Horizontal centrifugation (1000*g*, 20 min) was conducted at 4 ℃, the sediment was discarded, and stored at − 20 ℃. Before use, it was inactivated in a 56 ℃ water bath for 30 min, and stored at 4 ℃ for preparation.

Cell treatments and biological observation

For experimental use, the primary chondrocytes were cultured for 2 weeks until cells reach confluent, and passage 1 and passage 3 were seeded in the experimental wells. In this experiment, serum-free (control group), fetal bovine serum (FBS) and human autologous serum (HAS) were used to culture articular chondrocytes in vitro, and the growth and survival of the cells were observed. MTT assay was performed on the first day, the third day, the fifth day and the seventh day. The cell morphology and proliferation were observed under the inverted phase contrast microscope every day. The primary cells could stick to the wall after 24 h, and most of them were round. After 48 h, the cells began to proliferate, but the morphological changes were not obvious. After 72 h, the polygonal cells increased. By blood cell count boards, cells were counted. The cells were stained with safranine O, type II collagen immunofluorescent staining to observe the dedifferentiation of cells. In order to test the effect of different serum on proliferation activity and phenotype maintenance of chondrocytes, serum-free culture, 10% fetal bovine serum, and 10% human autologous serum were used.

Detection of chondrocyte viability

The cells of the second passage were seeded into 96-well plates at 2.0×10^3 cells/well and cultured at 37 ℃ with 5% CO_2. After 24 h, the cells were changed to medium containing different concentrations and types of serum

according to the groups and continued to be cultured. At the time of detection, the culture medium to be tested was sucked out, MTT 20 μl/well was added, incubated in 5% CO_2 incubator at 37 ℃ for 6 h, MTT was sucked away, 200 μl/well dimethyl sulfoxide was added, and the cell viability was calculated by shock for 10 min. The light absorption value (OD value) was measured on automatic microplate analyzer.

Cell imaging by laser confocal microscopy (LCSM)

The articular chondrocytes were seeded into 33 mm live cell confocal special dishes, and cultured with media containing different concentrations and types of serum in 3 groups, and detected by laser confocal. The small dishes were placed in the dedicated culture chamber of the live cell workstation during the test. Turn on the heating system and carbon dioxide culture system to maintain a normal cell growth environment. The cell proliferation, growth status and spindle shape of different experimental groups were observed under 60× objective lens (numerical aperture NA = 1.4). The excitation wavelength was selected as 405 nm and 488 nm, and the output power, pinhole, scanning speed, HV of photomultiplier tube and other parameters were adjusted. The effects of different concentrations of human serum on the proliferation activity and phenotype maintenance of articular chondrocytes isolated from the patients were observed under confocal.

Real Time-qPCR of chondrogenic genes

Chondrogenic gene expressions of chondrocytes were measured by a Real-time quantitative reverse transcription-polymerase chain reaction (RT-qPCR) system. RNA was extracted from cells and reverse transcribed into complementary DNA (cDNA) using Trizol reagent and PrimeScript RT reagent kit (Takara, Tokyo, Japan) according to manufacturer's instructions. Then diluted cDNA was mixed with SYBR Premix Ex TaqTM (Takara, Tokyo, Japan), forward and reverse primers and RNase free water to perform RT-qPCR. Chondrogenic differentiation markers including Col2, Sox9 and Acan of chondrocytes among 0%, 5%, 10% human autologous serum-supplemented medium, or among serum-free, 10% FBS, and 10% HAS-supplemented medium were evaluated, with GAPDH used as housekeeping gene. Relative expression level for each gene (fold change) to that of serum-free group by the delta delta Ct method was calculated according to a previously published study[9].

Immunofluorescent staining

Cells cultured on cover slips were fixed with 4% paraformaldehyde (PFA) for 30 min and then permeabilized with 0.01% Triton X-100 for 10 min at room temperature. After washed with PBS for 3 times, cells were blocked with 1% bovine serum albumin for 1 h at room temperature. The primary antibody (Anti-Collagen II antibody, diluted 200-fold, ab34712, Abcam) was incubated at 4 °C overnight. Cells were next incubated with secondary antibody (diluted 200-fold, G-Rabbit Alexa Fluor® 488, A11008; Invitrogen) for 1 h at room temperature. After incubation, the nuclei were lastly stained with 1 X DAPI. After staining, the cells were observed using a confocal microscope (OLYMPUS IX81-FV1000), and positive cell numbers were calculated with Image J software.

Western blot analysis

Cellular protein was extracted with RIPA Lysis Buffer (RIPA buffer, R0020, Solaribio.) containing protease inhibitor (P8340, Sigma, USA), phosphatase inhibitor (P5726, Sigma, USA) and PMSF (Phenylmethylsulfonyl fluoride, P0100, Solaribio), and the protein concentration was determined with a bicinchoninic acid assay kit (PierceTM BCA Protein Assay Kit, Pierce #23227). The total protein of the cells was extracted and subjected to polyacrylamide gel electrophoresis, then transferred to nitrate-cellulose membrane and blocked with 1%BSA at room temperature for 2 h. To detect the marker of autophagy in chondrocytes, LC3-II antibody (1:1000) was added and incubated overnight at 4 ℃. After washing the film with PBST, the corresponding horseradish peroxidase (HRP) labeled secondary antibody (1:5000) was added, and Excessive secondary antibody was rinsed off from the membrane with PBST, and a chemiluminiscent signal was generated by using the detecting reagents (ECL Western Blotting Substrate, 32106, Thermo Scientific, USA) according to the manufacturer's protocol, and quantitative data were analyzed with Image J software.

Statistical analysis

Values are expressed as mean ± SD or mean ± SEM. The significance between two groups was analyzed using two-tailed Student's t-tests. For multiple comparisons, one-way analysis of variance (ANOVA) with Tukey's post hoc test was used. Statistical analysis was performed using the Graphpad software. $P < 0.05$ was considered $^{**}P < 0.01$, $^{***}P < 0.001$, $^{****}P < 0.0001$.

Results

Human autologous serum is beneficial to maintain the survival and phenotype of chondrocytes isolated from the patients

In order to investigate the role of human autologous serum (HAS) in the culture of primary chondrocytes of, as well as to figure out the most suitable concentration for its use in chondrocyte culture, we took 0%, 5%, and 10% concentration of human autologous serum for the in vitro culture of primary chondrocytes isolated from the knee joint cartilage in the same corresponding patients. The cell proliferation and phenotype of the articular chondrocytes were then analyzed using laser confocal microscopy. The results showed that chondrocytes cultured with 0% serum (serum-free) were dendritic and polygonal in shape, whereas those with 5% and 10% concentration of human autologous serum were more favorable for the cell attachment and proliferation, and the chondrocytes were polygonal in cellular shapes (Fig. 1A). Further observation revealed that 5% concentration of human autologous serum was more favorable for cell proliferation, however, RT-qPCT results showed that only 5% HAS significantly promoted the mRNA expression of type II collagen (Fig. 1B). Besides, much extensive type II collagen and its fluorescent intensity, as well as the higher number of type II collagen positive cells were in the

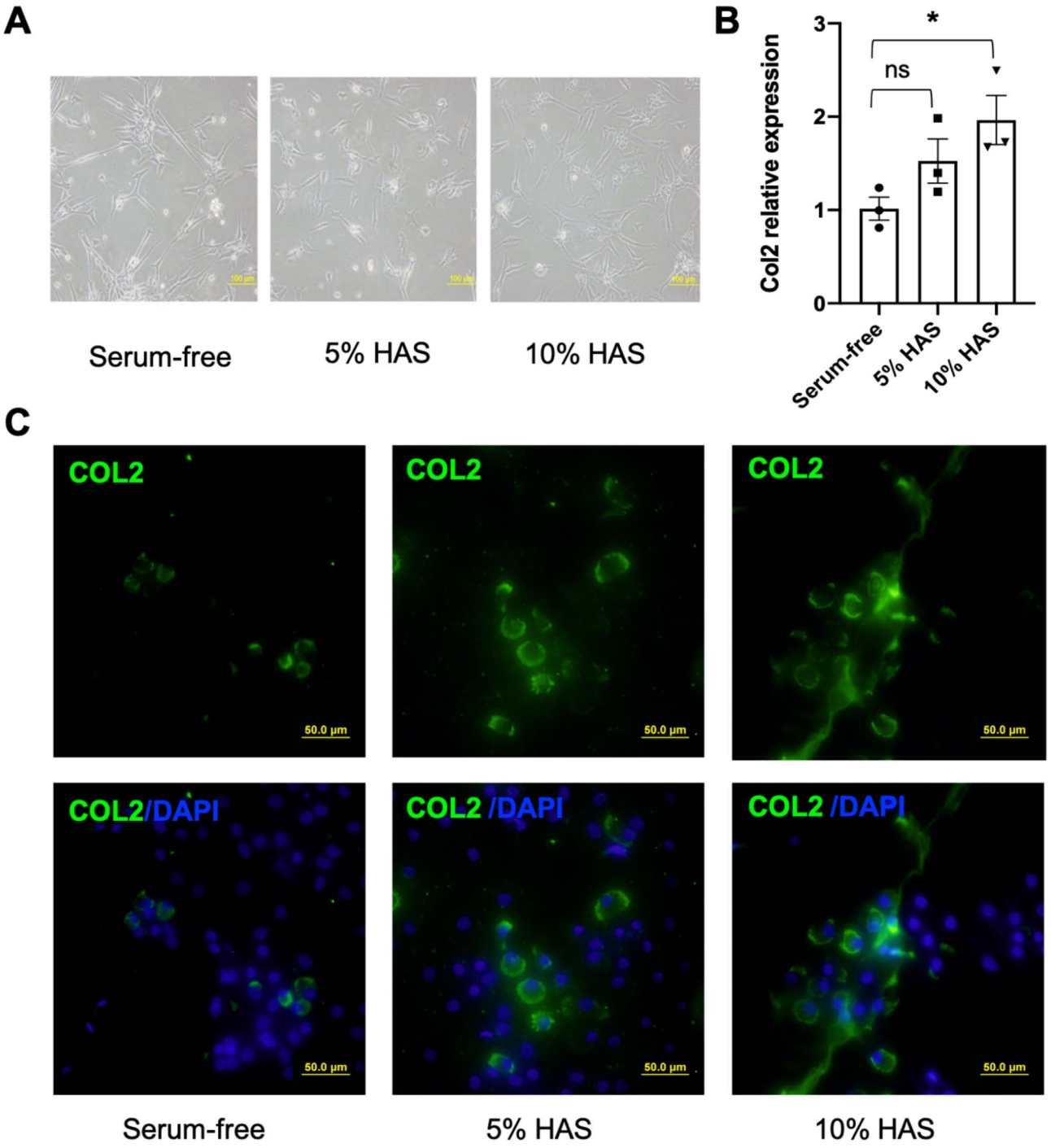

Fig. 1. Effects of different concentrations of human autologous serum on the proliferation and phenotype maintenance of articular chondrocytes isolated from the patients. **A** Cell morphology of Passage 1 chondrocytes after 7 days of culture with 0%, 5%, 10% human autologous serum-supplemented medium, respectively. **B**, **C** The Col2 mRNA level (Relative expression, replicates, $n = 3$) and protein level expression (Immunofluorescent staining, Bar = 50 μm) of type II collagen in human chondrocytes cultured in 0%, 5% and 10% human serum culture medium, respectively.

10% concentration group (Fig. 1C). Thus, for the in vitro culture of articular chondrocytes from the patients, and the optimal concentration of human autologous serum is suggested at 10%, which shows strong advantage in chondrocyte phenotype maintenance.

Human autologous serum increased the cell viability of chondrocytes isolated from the patients

To explore whether the human-derived autologous serum is superior to traditional fetal bovine serum (FBS) on promoting the cell proliferation and phenotype maintenance of in vitro cultured articular chondrocytes from the patients. Primary chondrocytes isolated from the patients were cultured in the medium supplemented with fetal bovine serum (FBS) and human-derived autologous serum, respectively. The medium containing serum-free (control group), 10% fetal bovine serum (FBS), or 10% human autologous serum (HAS) was used to culture the articular chondrocytes (at Passage 1) from the corresponding patient each. Firstly, the growth of chondrocytes could be observed from the overall cell proliferation curve at multiple time points. Both 10% FBS and 10% HAS significantly promoted the proliferation of chondrocytes (nearly a 2-fold increase in OD values) when compared with that in the serum-free group at day 5 (Fig. 2). Secondly, according to the data on the fifth day of cell viability experiment (Fig. 2, day 5 data), we found that the supplementation of 10% FBS seems to be beneficial for the cellular proliferation of chondrocytes in the short-term, while 10% HAS supplementation showed advantages on the long-term culture and the survival of chondrocytes (Fig. 2, day7 data).

Human autologous serum (HAS) simultaneously maintains cell proliferation and phenotype maintenance in chondrocytes isolated from patients

Having demonstrated that human-derived autologous serum (HAS) supplementation can promote the proliferation of in vitro cultured chondrocytes from the patients, the key question is whether HAS is also advantageous in maintaining chondrocyte phenotype. Cells in the 10% HAS culture group grew more faster and were more oval than those in the other groups (Fig. 3A a–c), indicating the lowest degree of chondrocyte dedifferentiation. The corresponding safranin-O staining showed a higher positive cells in the 10% HAS culture group (Fig. 3A d–f), indicating the highest level of matrix protein secretion and anabolism. Further validation experiment by immunostaining also showed increased intensity of ACAN expression and cytoplasmic retention (Fig. 3B), as well as the relative proportion of ACAN-positive cells (Fig. 3C) in 10% HAS culture. After cartilage explant culture for 7 days in vitro, the 10% HAS-supplemented medium was obviously advantageous for preventing the proteoglycan (as indicated by safranin-O staining) loss and the matrix degradation among groups

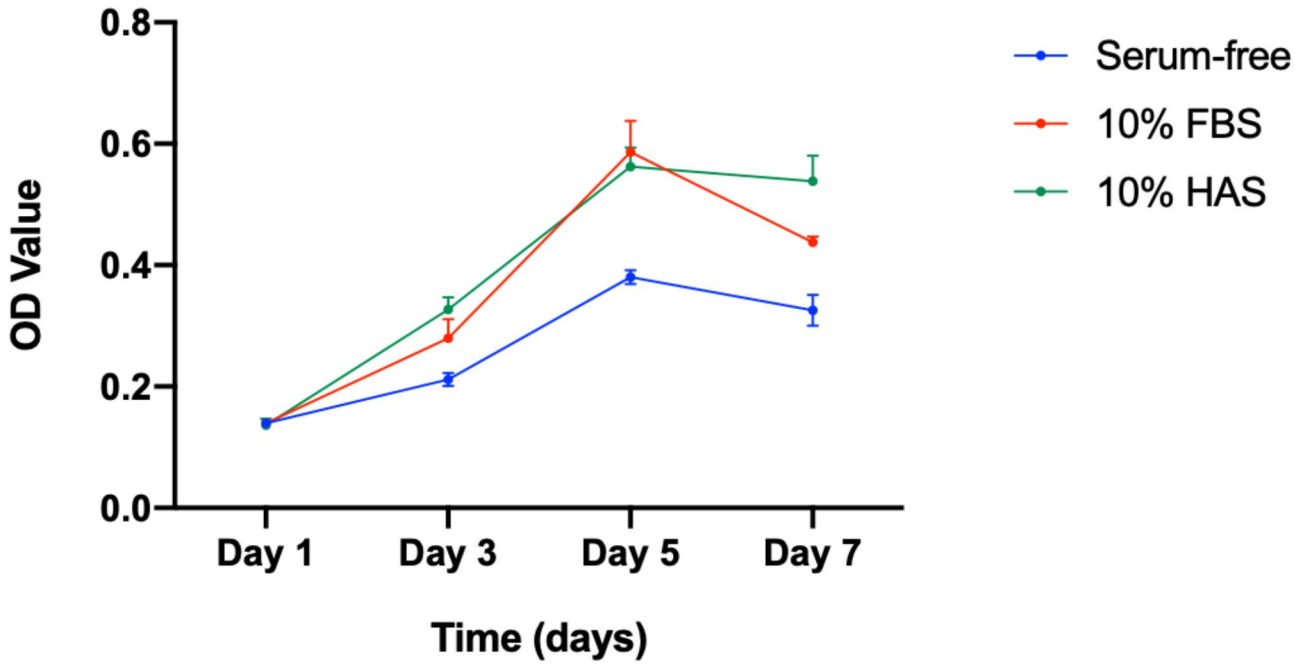

Fig. 2. Chondrocyte proliferation test (MTT) in multi-time points, cell culture time: 1, 3, 5, 7 days. The proliferation curves of the Passage 1 chondrocytes isolated from the patients in the condition of serum-free (Control), 10% FBS, 10% human serum (HS). Statistically significant differences in quantitative data were analyzed. Values are means ± SD ($n = 3$). The exact p-value calculated with one-way ANOVA Tukey's multiple comparisons test in 10% HAS VS Serum-free : day3 ***$p < 0.0001$, day5 ***$p < 0.0001$, day7 ***$p < 0.0001$; the exact p-value calculated with one-way ANOVA Tukey's multiple comparisons test in 10% FBS VS Serum-free: day3 ***$p < 0.0001$, day5 ***$p < 0.0001$, day7 ***$p < 0.0001$; and the exact p-value calculated with one-way ANOVA Tukey's multiple comparisons test in 10% HAS VS 10% FBS : day3 **$p = 0.006$, day5 not significant, day7 ***$p < 0.0001$.

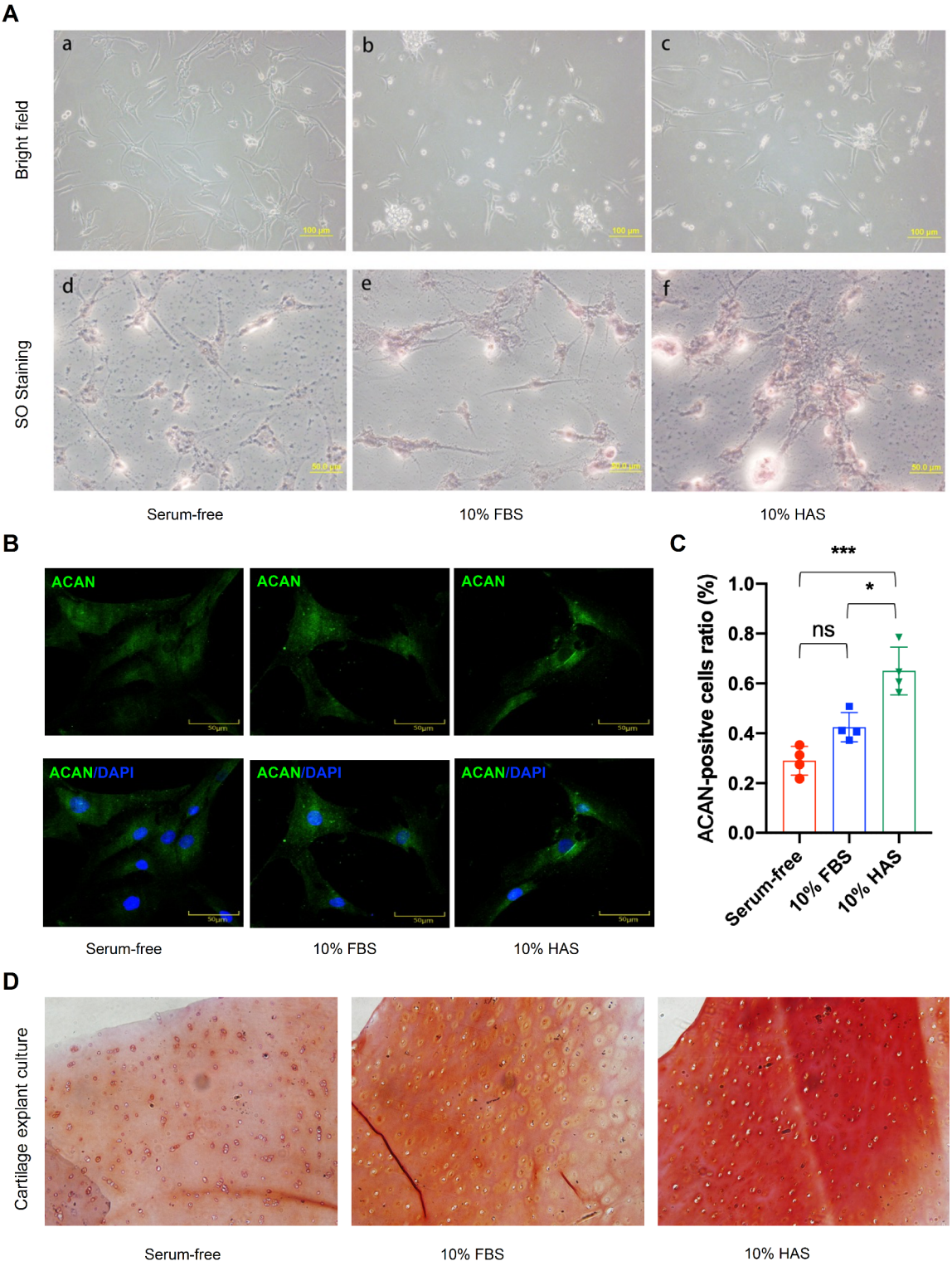

Fig. 3. Effects of HAS on chondrocyte morphology and proteoglycan maintenance. (A) Cell morphology of chondrocytes in serum-free, 10% FBS, and 10% HAS-supplemented media, respectively. Bar = 100 µm. (B) Immunostaining of Aggrecan expression (ACAN) in Human chondrocytes at Passage 3 among serum-free, 10% FBS, and 10% HAS-supplemented media, respectively. Bar = 50 µm. (C) Relative ACAN-positive cell ratio among serum-free medium, 10% FBS, 10% human serum culture medium, respectively. (replicates, $n = 3$; samples, $n = 4$). (D) Safranin-O staining of OA cartilage after explant culture for 7 days, and culture with serum-free, 10% FBS, and 10% HAS-supplemented media, respectively. Bar = 50 µm.

(Fig. 3D). These results demonstrate that the 10% HAS supplementation is available for the in vitro culture and shows advantages in cell expansion and maintenance of chondrogenic phenotype.

Interestingly, after 7 days of in vitro culture, the mRNA expression of Col2, Sox9, Acan of chondrocytes were significantly higher among different groups (Fig. 4A–C). Compared with the serum-free group, both 10%FBS and 10%HAS group significantly increased the expression and secretion of type II collagen in cultured chondrocytes (Fig. 4D). Compared with the 10%FBS group, the 10%HAS showed much higher Col2-positive cell ratio, which indicates a more stable capability to maintain the expression of type II collagen (Fig. 4E). These results indicate that human serum is more suitable for chondrocyte expansion in vitro than serum-free and fetal bovine serum (FBS), and can better maintain the chondrocyte phenotype in short-term culture.

10% HAS maintains the chondrocyte function with increased autophagy level

Some studies have shown that serum affects cell mitochondrial activity and autophagy level of chondrocytes. The difference of cell phenotype was observed and the mechanism of autophagy was elucidated by observing the level of cellular autophagy. In this study, in order to further explore whether chondrocytes cultured with human autologous serum initiate the autophagy process, chondrocyte proteins cultured with 0%, 5% and 10%

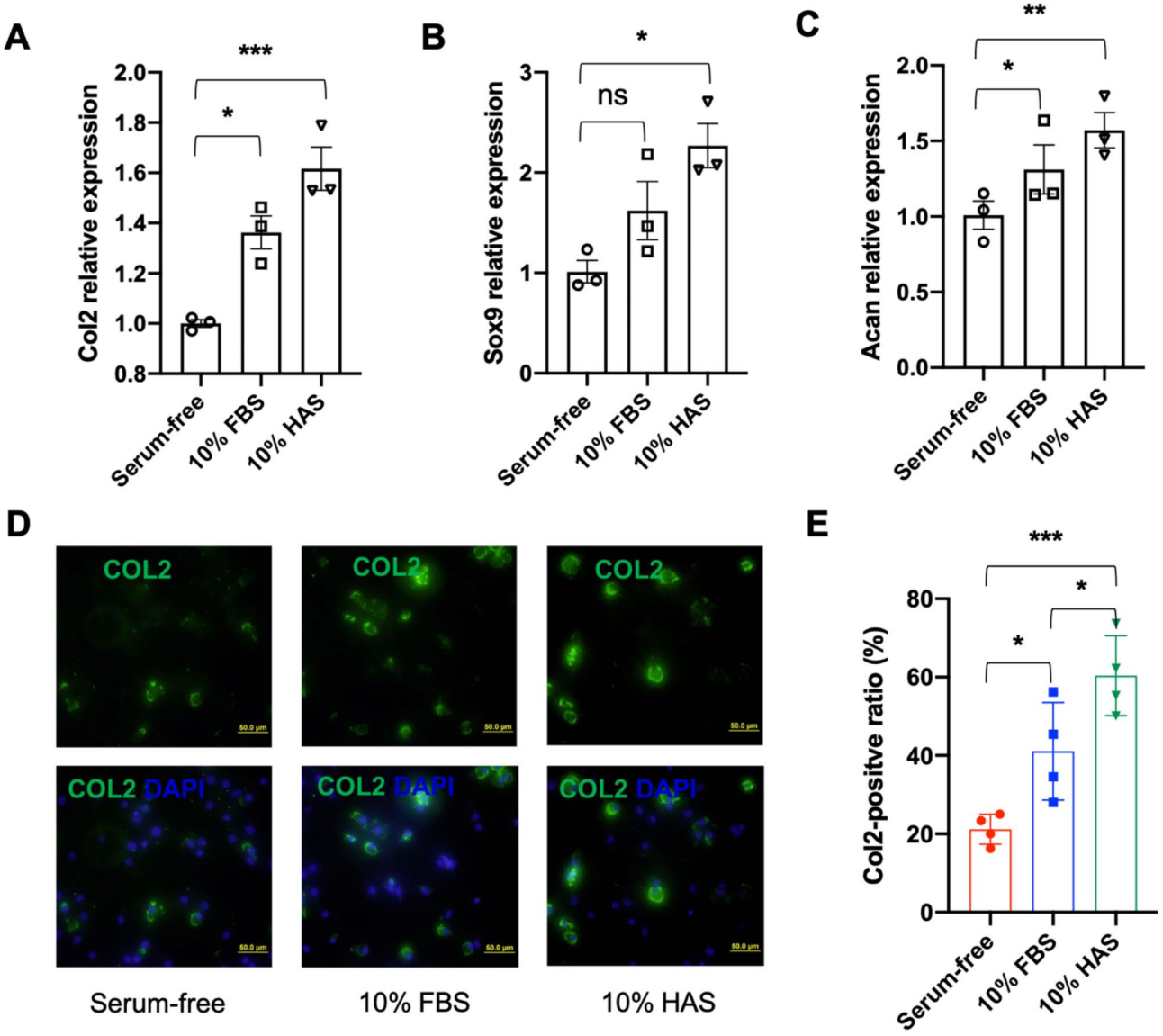

Fig. 4. Effects of HAS on chondrocyte phenotype maintenance at both mRNA and protein level. **A**–**C** The Col2 mRNA level (Relative expression) of Col2, Sox9, Acan in human chondrocytes (Passage 3) cultured in serum-free, 10% FBS, and 10% HAS-supplemented media, respectively. (replicates, $n = 3$). **D** The immunofluorescent staining of type II collagen (COL2, green) expression of Passage 3 human chondrocytes in serum-free medium, 10% FBS, 10% human serum culture medium, respectively. Bar 50 μm. E. Relative Col2-positive cell ratio among serum-free medium, 10% FBS, 10% human serum culture medium, respectively. (replicates, $n = 3$; samples, $n = 4$).

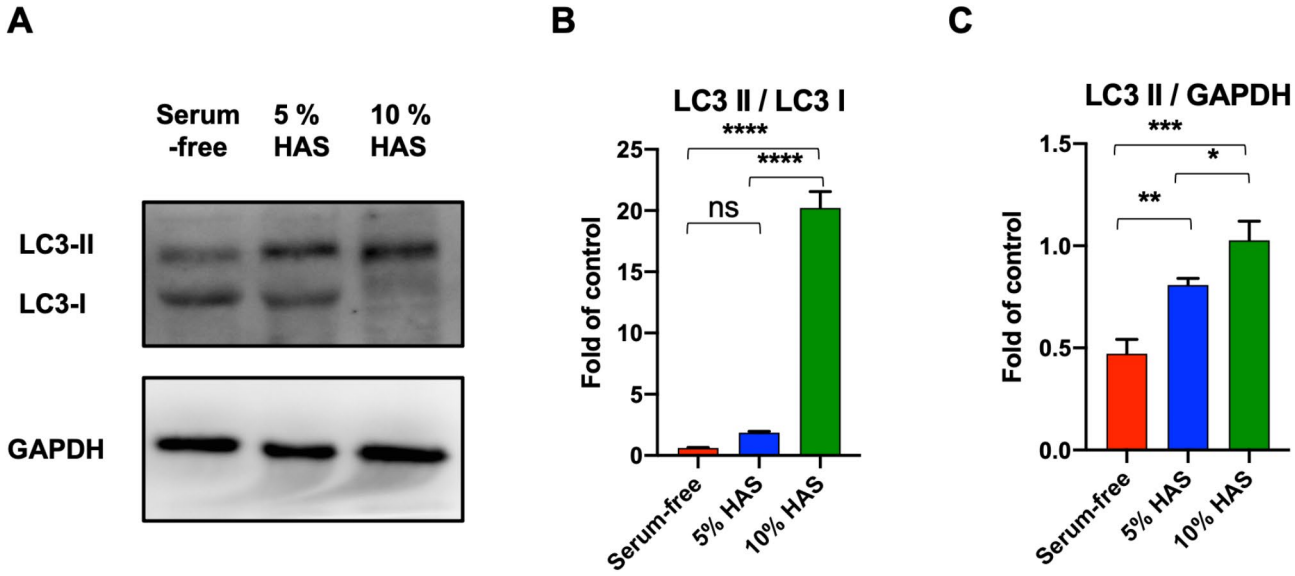

Fig. 5. Effects of different concentrations of human autologous serum on autophagy of chondrocytes. **A** The LC3-II expression level of Passage 3 human chondrocytes under the conditions of 0%, 5%, or 10% human autologous serum (HAS) -supplemented culture medium. **B** Relative expressions of LC3-II/I and C. LC3-II/GAPDH (and quantitative data were analyzed with Image J software.), in the chondrocytes (samples, $n = 3$) that were detected by Western blot, respectively.

human autologous autologous serum were extracted, respectively. The results of Western blot showed that 10% human autologous serum was more conducive to LC3-II expression in chondrocytes when compared with 0% and 5% human autologous serum groups (Fig. 5A). From the above results, it can be found that both 5% and 10% human autologous serum significantly promoted the expression of type II collagen in chondrocytes and maintained the phenotype. In contrast, 10% concentration of human autologous serum increased expression level of relative autophagy marker type II LC3 (LC3-II/GAPDH and LC3-II/LC3-I), demonstrating higher levels of cellular autophagy (Fig. 5B, C). In conclusion, the 10% concentration of human autologous serum may delay the dedifferentiation of chondrocytes partially by promoting the cellular autophagy.

Collectively, these results showed that human autologous serum was superior to FBS for the cell proliferation of articular chondrocytes isolated from the patients, and the optimal concentration of human autologous serum is 10% for phenotype maintenance. Moreover, the chondrocyte phenotype was maintained partially through increased cellular autophagy levels under the regulation of human autologous serum. It also strongly indicates that a concentration of 10% human autologous serum is an optimal concentration for in vitro cell expansion, especially for autologous chondrocyte transplantation of the patients who suffered from severe cartilage damage.

Discussion

For patients with severe cartilage damage, an important problem facing autologous chondrocyte transplantation is that the chondrocytes are highly dedifferentiated in the regular culture conditions (e.g., the addition of fetal bovine serum). During the in vitro cell culture, fetal bovine serum are not effective in maintaining the phenotype of human chondrocytes. Moreover, the in vitro culture of chondrocytes could deepen the degree of dedifferentiation with time[10]. In the present study, in vitro culture of human chondrocytes supplemented with autologous serum demonstrated a significant enhancement in chondrocyte proliferation as well as the synthesis of proteoglycans and type II collagen, when compared to fetal bovine serum. This assertion has been supported by previous experiments in which chondrocytes were cultured in human autologous serum[11] and chondrogenic induction of stem cells[12,13]. In addition, other studies have found that for fibroblast culture, fetal bovine serum and human serum do not differ in their proliferative effects in short-term culture, but there is a significant difference in survival and proliferation in long-term culture[14]. The in vitro expansion of cells for human autologous chondrocyte transplantation (ACT), which is theoretically longer than one week, poses greater demands on the serum requirements for chondrocytes derived from patients.

In common orthopedic procedures, cartilage-related diseases face the dilemma of difficult chondrocyte regeneration. For patients with early osteoarthritis[15] or severe cartilage exfoliation[16], more cutting-edge studies have utilized autologous platelet-rich plasma injections as an adjunct to therapy. For patients, the extraction of a small amount of autologous blood not only makes the access easy and safe, but also the subsequent chondrocyte transplantation shows low immune rejection after expansion with autologous serum supplement[17–19]. Autologous plasma is rich in multiple growth factors, stable in source, accessible in a timely manner in the hospital itself, and can address immune rejection[20]. Therefore, the use of human autologous-derived serum for chondrocyte culture in vitro is all the more necessary, both from a safety and an economic point of view. Our study provides

a reference for mining the molecular mechanism of the influence of different types as well as different working concentrations of serum on the culture of chondrocytes.

In clinical studies, human autologous blood included platelet derivatives such as platelet lysate (PL), platelet releasing factor (PRF) and serum to replace fetal bovine serum (FBS). From the results of the study, it was observed that human serum was more favorable for cell proliferation and maintenance of type II collagen expression, and was smoother in cell proliferation, suggesting that human serum may trigger the autophagic process in chondrocytes. The development of osteoarthritis is often accompanied by apoptosis of chondrocytes, and autophagy is partially similar to its characteristics[21]. 10% human serum promotes the slow proliferation of chondrocytes most likely triggering autophagy. In addition, in (platelet rich plasma, PRP) studies, Borzi RM et al.[22]. found that PRP promotes chondrocytes to produce autophagic activity. All these studies explain the possible mechanisms by which human serum produces advantages on cell culture. Compared to fetal bovine serum, human-derived serum is more likely to cause cellular autophagy in chondrocytes, rescue cells when conditions of the in vivo microenvironment are lost, and maintain the balance of chondrocyte proliferation and survival in vitro.

Conclusion

The findings indicate that human autologous serum serves as a viable alternative for the in vitro culture of chondrocytes derived from relatively middle-aged and elderly osteoarthritis patients. Furthermore, a concentration of 10% human autologous serum has been identified as optimal for the in vitro expansion of chondrocytes harvested from these individuals prior to undergoing autologous chondrocyte transplantation surgery. Under the influence of human autologous serum, the maintenance of chondrocyte phenotype was companied by increased cellular autophagy level. Human autologous serum instead of fetal bovine serum can maintain chondrocyte function and improve chondrocyte autophagy at the same time. These findings cannot be ignored in the in vitro cell expansion before autologous chondrocyte transplantation, and also provide positive implications in the clinical translation of tissue engineering strategies.

Data availability

The raw data that supporting the conclusion of this article generated and/or analyzed during the current study are not publicly available, but are available from the corresponding author on reasonable request.

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Author contributions
QS contributed to the experimental concept and designed the research; WY and ZQ are responsible for the manuscript writing, experiment performance, data analysis; BS and CL are responsible for material preparations.

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Declarations

Competing interests
The authors declare no competing interests.

Ethics statement
The studies involving human-derived blood samples and knee joint cartilage from medical wastes were reviewed and approved by Zhejiang Hospital Ethics Committee. The patients were informed and consent to the medical waste samples that used in this study. The Ethical number : 2023 clinical examination No. 6k.

Additional information
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