The Design and Characterization of a Strong Bio-Ink for Meniscus Regeneration

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Abstract: The meniscus is vital to the mechanical function of the knee, while it is frequently harmed because it bears a heavy load. A strong bio-ink for meniscus regeneration was prepared for the future meniscal tissue engineering. The prepared bio-ink consists of poly (vinyl alcohol) and decellularized extracellular matrix (PVA/dECM). The mechanical properties and the rheological features were explored to evaluate the effects of freezing/thawing cycles and alkaline treatment process. The printability was verified using a three-dimensional printer. The endothelial cells were employed to assess the biocompatibility. Finally, a 12-week rabbit meniscus defect model was established to evaluate the meniscus regeneration capability. We found that the bio-ink by soaking in alkaline for 40 min and 20 freezing/thawing cycles demonstrated excellent mechanical properties. The Young’s modulus reached 0.49 MPa and the stress limitation was 2.9 MPa. The results also showed good printability and biocompatibility of the proposed bio-ink in vitro. The PVA/dECM hydrogel healed the meniscus defect after 12 weeks of implantation. The articular cartilage and subchondral bone exhibited normal microstructure and composition. These results suggested that the PVA/dECM hydrogel could be a promising solution to repair meniscal lesions with preventive effects against degenerative meniscal tears and post-traumatic arthritis.

Keywords: Meniscus; 3D printing; Strong bio-ink; Tissue regeneration; Decellularized extracellular matrix

1. Introduction

The menisci in the knee are two crescent-shaped fibrocartilage discs positioned between the femur and tibia surfaces in the medial and lateral compartments of the joint. Load transfer to a vast region of articular cartilage, joint stability, and shock absorption during dynamic motions are the fundamental roles of the menisci[1]. The meniscus is important for maintaining the knee joint’s...
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...homeostasis, biomechanics, and structural stability. Meniscus injury is very common in middle-aged and older adults. Knee discomfort and even reduced mobility are the most common symptoms of meniscal damage. The outcomes of case studies from the 1940s on partial regeneration of peripheral meniscal tissue following complete meniscectomy had bolstered our belief that radical excision would result in a better outcome than repair. However, a number of studies with a 10–20-year follow-up period following complete meniscectomy have been conducted. Following the operation, these trials all showed an unacceptably high prevalence of radiographic knee osteoarthritis (OA), knee pain, and impaired knee function.[2-8] Roos et al. reported that the risk of developing radiographic tibiofemoral OA was elevated six-fold at 21 years after total meniscectomy (relative risk: 6.4; 95% confidence interval: 2.7 – 15.2).[9] The meniscus is widely known for having a restricted ability for tissue regeneration. Only the peripheral vascularized section of damaged adult menisci heals on its own, but the interior avascular area hardly heals on its own.[10] Thus, promoting the meniscal repair has noticeably attracted surgeons’ attention.

Decellularized extracellular matrix (dECM) scaffolds have been studied as a natural substitute for the torn meniscus, which is claimed to have the potential to stimulate regeneration. dECM scaffolds may also be constructed by extracting cells and components from allogeneic or xenogeneic donor tissues. Physical (e.g., shocks and freeze-thaw cycles), chemical (e.g., detergents like Triton X-100 and sodium dodecyl sulfate [SDS]), and enzymatic (e.g., DNase and trypsin) therapies damage and solubilize the cell’s cytoplasmic and nuclear membranes.[11-15] The obtained dECM scaffolds showed excellent biomechanical properties and a minimum immunogenicity. Then, by recreating a similar tissue milieu to encourage cell infiltration and ECM formation, the dECM scaffolds were employed to repair injured menisci in the knee joint. The fibrous structure of dECM meniscus tissue, on the other hand, was thick and dense, making cell infiltration into the inner area of the implanted scaffolds challenging. Wu et al. converted acellular scaffolds into hydrogels, resulting in greater porosity and fast cell infiltration in the implanted material.[16] When compared to intact dECM meniscal scaffolds, processed dECM meniscal scaffolds, such as meniscal slices, powders, and hydrogels, may attain greater success in cell regeneration. The loss of the original structure of the processed dECM meniscal scaffolds could result in biomechanical disadvantages that may impact cellular behavior and metabolic activity in vivo. Therefore, the application of processed dECM meniscus was limited to the partial meniscus regeneration. Further studies should be therefore conducted to assess the regenerative capacity of processed dECM scaffolds in biomechanics and to repair meniscus defect in vivo.

Traditional techniques, such as freeze-thawing and chemical crosslinking, have been utilized to create hydrogels from poly (vinyl alcohol) (PVA).[17] PVA scaffolds showed high mechanical properties and cytocompatibility, promoting regeneration of different tissues.[18-21] Under freeze-thaw cycles, Parameswaran-Thankam et al. created bionanocomposite hydrogels by combining HPG, PVA, and nano-hydroxyapatite, and osteoblastic activity was demonstrated in vitro.[21]. Thankam et al. used PVA to shape the rotator cuff ligament and shown its potential for use in the treatment of rotator cuff tendon injuries.[19] Therefore, a PVA hydrogel can mimic the mechanical properties of meniscus at some aspects, and it can be regarded as a candidate for the meniscus tissue engineering.

In addition, the photocrosslinked system may be advantageous to achieve a better gelling capability in the bio-ink. One of the most common procedures for fabricating biomaterials is free-radical photopolymerization, which has various benefits, including relatively high reaction rates at room temperature, spatial and temporal control of the initiation process, minimal energy input, and chemical diversity. The photocrosslinked system has been applied in a variety of polymers, including poly (ethylene glycol) (PEG) gelatin methacryloyl, and methacryloyl hyaluronic acid.[22-25] PEG hydrogels have been used in drug delivery, wound healing, and a variety of biomedical applications due to its beneficial qualities such as non-toxicity, strong water solubility, biocompatibility, and highly adjustable capabilities. Changing the monomer (e.g., PEG methacrylate, PEG acrylic amide, vinyl alcohol, methyl methacrylate, and methacrylic acid) can positively tailor the crosslinking density of PEG hydrogels to satisfy varied demands.[22,23]

In the present study, we designed a PVA/dECM bio-ink for three-dimensional (3D) printing of meniscal scaffolds. The proposed bio-ink formed hydrogels under photocrosslinking to mimic the mechanical properties of meniscus. The PVA could form a strong and stretchable network through freezing/thawing and alkaline treating process. The alginate chains formed the ionic networks, and the PEG provided the necessary bioactive factors for meniscus tissue regeneration. This strategy could realize the fabrication of PVA/dECM hydrogel scaffolds with high mechanical properties, being resistant to injury, and with properties of shape memory polymers. Concurrently, the PVA/dECM bio-ink simulated a natural tissue microenvironment to promote meniscal repair. The PVA/dECM bio-ink can be an ideal candidate for 3D printing and meniscus tissue regeneration.
2. Materials and methods

2.1. Preparation of PVA/dECM hydrogel

The meniscal dECM was prepared as previously reported\(^{[10]}\). Rabbit menisci were sliced into thin slices (1 mm thick), frozen at −80°C, and then pulverized into coarse powders. For 72 h, the powders were mixed in a solution of 1% SDS/phosphate-buffered saline (SDS/PBS) (w/v) (Sigma-Aldrich, St. Louis, MO, USA). Every 24 h, the solution was updated. The sample was then submerged overnight in a considerable volume of deionized water to remove the leftover compounds after being treated with 0.1% w/v ethylenediaminetetraacetic acid (EDTA)/PBS solution (Sigma-Aldrich) for 24 h. Finally, the resulting dECM was frozen at −80°C for 3 days before being lyophilized. The dECM was finely powdered before being added to a pepsin/0.01 M hydrochloride (HCl) solution (Sigma-Aldrich) and stirred at 15 mg/mL for 48 h at room temperature. 0.1 M sodium hydroxide (NaOH; Sigma-Aldrich) and PBS were used to neutralize the viscous solution (Sigma-Aldrich).

PVA (Sigma-Aldrich) was dissolved in deionized water with a 10% mass-to-volume ratio. The solution was stirred in 55°C water bath for 4 h until PVA crystals were completely dissolved. Then, the 10% w/v PVA and 10% w/v dECM were added to deionized water and stirred evenly. The 10% w/v PEGDA (Sigma-Aldrich) and 0.05% w/v 2-hydroxy-4′-(2-hydroxyethoxy)-2-methylpropophenone (Sigma-Aldrich) were added to the solution as crosslinking agent and photoinitiator, respectively. After that, 6% w/v alginic acid sodium salt (Sigma-Aldrich, St. Louis, MO, USA) was added and kept in a 4°C refrigerator overnight.

The prepared solution was placed into a 2 mL centrifuge tube and exposed to blue light for 20 min to complete the photocrosslink reaction. Hydrogel samples were stored at room temperature.

2.2. Surface characterization of the PVA/dECM hydrogel

A scanning electron microscope (SEM) was used to examine the surface topography (SU8020; Hitachi, Tokyo, Japan). All samples (n = 3 for each group) were dehydrated and dried at room temperature using a series of graded alcohols. The dried samples were sputter-coated with gold-palladium and viewed with a SEM at 1 kV. A diffractometer was used to measure the X-ray diffraction patterns of materials at 40 kV and 40 Ma using Cu radiation. Diffractograms were set to 6 – 70° (2θ), with a step size of 0.02° (2θ), at a rate of 1.20°/min (2θ).

2.3. Rheological test

A Haake Mars 40 Rotational Rheometer was used for the rheological test (Thermo Fisher Scientific, Waltham, MA, USA). Thin slices of the samples were cut (1 cm in diameter and 1 mm in thickness). The frequency range for the frequency sweep experiment was adjusted at 0.1 – 100 Hz with a strain of 0.1%. The strain range for the strain sweep experiment was adjusted at 0.1 – 100% with a frequency of 1 Hz. G’ and G” were measured at 0.1% strain and 6.28 rad/s rotational velocity for the destroy-recovery experiment. The strain was then increased to 300% for 60 s to destroy the hydrogels, and then reduced to 0.1% for 600 s to monitor mechanical property recovery while keeping the angular velocity at 6.28 rad/s. All of the trials were carried out 3 times.

2.4. Compression test

The samples with a height of 10 mm and a diameter of 8 mm were used for the compressive test. The compressive stress-strain test was carried out using an Instron-5944 universal instrument (Thermo Fisher Scientific) equipped with a 2 kN sensor in air at room temperature. In the compression-relaxation cycle test and the compression-crack test, the rate of compression was maintained at 2 mm/min. In the stress relaxation test, the strain in each compression process was set to 10% and repeated 5 times. Afterward, the hydrogel was quickly preloaded to different strains at a rate of 60 mm/min and kept for 5 min to achieve the stress-relaxation curves. All the experiments were repeated thrice.

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Ef = \int_{x_0}^{x_f} x(t) \, dt
\]

where \(x_0\) and \(x_f\) denote the starting distance and fracture distance of the compression, respectively, was calculated from the area below the distance and fracture distance of the compression, and the experiments were repeated thrice.
compression, respectively. The approximate linear fitting results at a strain deformation of 20 – 40% were used to calculate the Young’s modulus.

2.5. 3D printing test

A Bio-Architect® WS 3D printer was used for the 3D printing test (Regenovo, Hangzhou, China). The cylindrical scaffolds with angles of 0° and 90°, as well as the filaments, were extruded using a nozzle with a diameter of 310 m and a 0.25 MPa air pressure. The printing speed was set at 6 mm/s, and the filament separation distance was set to 600 m. The model’s thickness was set at 280 m. A BX-53 microscope (Olympus, Tokyo, Japan) was used to inspect the printed samples for printing correctness.

2.6. Cytotoxicity assay

The biocompatibility test was performed using human umbilical vein endothelial cells (HUVECs; Warner, Wuhan, China). The cells were kept in a 5% CO₂ environment at 37°C in Dulbecco’s modified Eagle’s medium (Gibco, New York, NY, USA) supplemented with 10% fetal bovine serum (Gibco) and 100 IU/mL penicillin/streptomycin. The materials were cut into cylinders of 8 mm diameter and 2 mm height and sterilized by UV irradiation overnight before in vitro testing. The samples were then transferred into 24-well plates, and the cells suspension (3000 cells/cm²) was dropped onto the surface of samples. The blank group received the same amount of HUVECs in the blank dishes. After culturing for 48 h, the cell counting kit-8 solution was added to each group (10 μL per each group). After incubation at 37°C for 2 h, the optical density (OD) was measured at 450 nm, and the cell viability was calculated. The cells were then dyed with live/dead assay. Briefly, the cells and samples were dyed with calcein AM and propidium iodide (PI) for 45 min in the dark and were then fixed by UV irradiation overnight before a laser scanning confocal microscope (141 FV3000; Olympus).

Cell proliferation was calculated using the following equation: Cell proliferation (%) = (ODₜₐₜₜₜₜ - ODₜₜₜₜₜₜ / ODₜₜₜₜₜₜ × 100%. The absorbance of cells cultured on the surface of hydrogels and dishes for 4 h was taken as ODₜₜₜₜₜₜ and the absorbance of cells cultured for 48 h was taken as ODₜₜₜₜₜₜ.

2.7. Cell adhesion

The phalloidine/DAPI staining was performed to investigate cell adhesion. The HUVECs were passaged, and the cell suspension was dropped onto the surface of samples, while the blank group was passaged onto the glass bottom dishes with no hydrogel. The cells were rinsed in PBS and fixed in 4% paraformaldehyde for 10 min after 48 h. The cells were stained with phalloidine and 4′,6-diamidino-2-phenylindole (DAPI) after being treated with 0.1% Triton X-100, and the morphology of the cells was studied using a laser scanning confocal microscope.

2.8. In vivo study

We used the 40-20 PVA/dECM hydrogels with excellent mechanical properties and well cytocompatibility as experimental group. All animal researches were carried out in accordance with the Ethics Committee of Drum Tower Hospital Affiliated to Nanjing University’s Medical School (Nanjing, China) legislation and guidelines, as well as the Institutional Animal Care and Use Committee recommendations.

A total of 24 female New Zealand white rabbits with a bodyweight of 2.5 kg were used in the present study and randomly divided into four groups (PVA/dECM hydrogel group, control group, blank group, and sham group; n = 6 rabbits per each group). The surgery was carried out under general anesthesia with a 0.2 mL/kg intramuscular injection of xylazine HCl (Jilin Huamu Animal Health Products Co., Ltd., Jilin, China). Knee surgery was carried out under sterile conditions. Briefly, a lateral parapatellar incision was made and the lateral meniscus was disclosed. A 3-mm full-thickness circular defect was created in the central part of lateral meniscus using sharp blades (Figure S1). The defects in the hydrogel group were implanted with pre-fabricated PVA/dECM hydrogel and control hydrogel, and the defects in the blank group remained blank. Only the articular cavity was opened in the sham group. All the hydrogels used for implantation were from the same batch. The lateral collateral ligament was securely sutured after the lateral meniscus was reduced, the joint capsule was repaired with 3-0 nylon sutures, and the skin was closed with 1-0 nylon sutures. After surgery, the rabbits were returned to their cages and permitted to resume full weight-bearing activities. To prevent infection, penicillin was administered intramuscularly for 3 days following surgery. At week 12 after surgery, all animals were slaughtered to assess meniscus regeneration and cartilage preservation.

2.9. Macroscopic evaluation

The meniscus, femoral condyle, and tibial plateaus were photographed and the evaluation of gross morphology was performed. The meniscus defect repair was compared using ImageJ software (National Institutes of Health, Bethesda, MD, USA). Color, integrity, shape, and smoothness were assessed on the surface of the cartilage worn zone. The quality of the repair was noted. The macroscopic appearance of the restored tissue was evaluated using the International
Cartilage Repair Society’s (ICRS) macroscopic grading system[26]. Table S1 lists the grading criteria for the score, which describes cartilage wear after meniscus defects.

2.10. Micro-magnetic resonance imaging
At week 12 post-operation, the complete knee joint structure was assessed by micro-magnetic resonance imaging (micro-MRI). Micro-MRI of the knee joints was performed using a 9.4T Bruker Biospec 94/20 USR Micro-MRI system (Bruker, Bremen, Germany) with a dedicated knee coil (Siemens Munich, Germany). The sagittal micro-MRI was acquired using the fat-suppressed proton-density weighted turbo spin-echo sequences (Field of view, 62 mm; echo time, 6.82 ms; repetition time, 1027 ms; thickness, 1 mm). The micro-MRI findings were viewed using an Onis Digital Imaging and Communications in Medicine Viewer (ver. 2.5; Digital Core Co., Ltd., Tokyo, Japan).

2.11. Histological evaluation
The femoral condyle and tibial plateaus were decalcified with 15% EDTA for 28 days after the collected samples were fixed with 10% formalin for 24 h. After that, all of the samples were soaked in paraffin and sliced into 5-mm thick slices. Hematoxylin and eosin (H&E), toluidine blue, Safranin O, and collagen II were used to stain these sections. The tissue integrity and staining of the meniscus were compared to assess the histology results. The O’Driscoll rating system[27] was used to evaluate the cartilage histology data, and the scoring criteria are listed in Table S2[27]. A microscope with a charge-coupled device camera was used to examine all sections (Olympus).

2.12. Statistical analysis
Three investigators were blinded to grouping and assessed the macroscopic and histological data. SPSS 19.0 software (IBM Corp., Armonk, NY, USA) and IGOR Pro 6.12 software were used for statistical analysis and exponential curve fitting (WaveMetrics Inc., Portland, OR, USA). The results were analyzed using an unpaired Student’s t-test and provided as mean standard deviation. Results with $P < 0.05$ was considered statistically significant.

3. Results
3.1. Characterization of the PVA/dECM hydrogel
As shown in Figure S2, the bio-ink was a light yellow and viscous liquid after configuration. The bio-ink was irradiated by blue light to form hydrogel that was a white opaque hydrogel. The hydrogel was dipped into sodium hydroxide to obtain pale-yellow translucent hydrogel, and the freezing/thawing process was repeated. Finally, the white opaque hydrogel was obtained after soaking calcium chloride. SEM was performed to investigate the microstructure of the hydrogels and the natural meniscus. As illustrated in Figure 1A-C and Figure S3, the 40/20 PVA/dECM hydrogel showed a reticulated pore structure similar to the natural meniscus. The pore size of hydrogel was about 20 μm in diameter, which well simulated the structure of natural meniscus. However, the control group showed the small number of holes with different sizes. According to Figure S4, the PVA/dECM hydrogel and control group had a similar content of surface element to the natural meniscus, including carbon, oxygen, chlorine, and calcium. The content of sodium in the PVA/dECM hydrogel was more than that in the natural meniscus, which caused by soaking in the sodium hydroxide.

Furthermore, the dynamic properties of the hydrogels were studied in a detailed manner using a rheometer at 20°C. The results of strain-sweep and the frequency-sweep tests confirmed that this hydrogel had obvious colloidal characteristics, which were higher according to the increased storage modulus ($G'$) and loss modulus ($G''$). The results of destroy-recovery experiment showed the well structural stability of hydrogel (Figure 1D-I, Figure S5-S7). Moreover, almost the same dynamic response was observed at 37°C, suggesting that the performance of PVA/dECM hydrogels will not be affected by temperature in vivo.

3.2. Compressibility of the PVA/dECM hydrogel
We quantitatively measured the compressive properties of the hydrogels by standard mechanical tests. The typical stress-strain curves from compression-crack testing of PVA/dECM hydrogel and control hydrogel are presented in Figure 2A and Figure S8. The 40-20 PVA/dECM hydrogel showed the best mechanical properties compared with other groups. The control hydrogel was broken when compressed to 70%. The PVA/dECM hydrogel indicated an excellent load bearing capacity and a distinct improvement. The Young’s modulus of control group was 0.29 MPa and the stress limitation was 2.9 MPa. Meanwhile, the Young’s modulus of control group was 0.29 MPa and the stress limitation was 1.0 MPa, which were lower than those in the 40-20 PVA/dECM hydrogel group. The PVA/dECM hydrogel exhibited significantly higher values of Young’s modulus, fracture strain and stress compared with those in the control group.

The energy dissipation of hydrogels was also tested by compression-relaxation cycles. As displayed in Figure 2B and Figure S9, an obvious energy dissipation...
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was observed in the 20-20 PVA/dECM hydrogel group, and the energy dissipation became smaller with the extension of sodium hydroxide soaking time. The control group showed a similar energy dissipation with the 40-20 in the uniaxial compression-relaxation curves. The reason of energy dissipation may be related to the change of the PVA crystalline domains and breaking the ionic bonds.

The fast recovery capability of the hydrogel was tested by applying continuous compression-relaxation cycles under 50% strain to the same hydrogels for 20 cycles without any time interval between two cycles (Figure 2C-F and Figure S10), and the trend of maximum stress and energy dissipation in each cycle is summarized in Figure 2G-H and Figure S11-S12. According to the results, the relative maximum stress of each cycle slightly decreased and remained at N80% after continuous compression-relaxation for 20 cycles. Similarly, the energy dissipation was attenuated under compression-relaxation cycles and remained at N20% after 20 cycles. The control group showed a greater reduction of maximum stress and energy dissipation. There results suggested that the PVA/dECM hydrogel kept an excellent elasticity and a fast recovery ability. The introduction of PVA increased the elasticity and the long-term mechanical properties of hydrogels. The addition of PVA can obviously improve the stress-relaxation behavior of the bio-ink (Figure 2I and Figure S13). The relaxation time was affected by the cycles of freezing/thawing or the alkaline treatment time (Figure S14). The reason for this phenomenon may be the diversity of crystallization or water content caused by the abovementioned physical operation.

Taken together, these results demonstrated that the PVA/dECM hydrogel exhibited excellent and reliable mechanical properties. Moreover, the fast recovery and stress bearing capabilities of the designed PVA/dECM hydrogel can mimic other mechanical properties of natural meniscus. Thus, the PVA/dECM hydrogel can be regarded as a substantial candidate for meniscus regeneration.

3.3. 3D printing test

According to the measurement of the 3D reconstruction model, the longitudinal axis of meniscus was 9.20 mm and the horizontal axis was 25.12 mm. The dimension of the 3D printed scaffold can precisely reproduce this size. A microscope could clearly show that the pore size (~1000 µm) and filament diameter (~400 µm) were uniform (Figure 3A). The semi-quantitative analysis revealed that there was no significant difference between the dimension of the original model and the 3D printed scaffold (Figure 3B). In addition, the scaffold exhibited an outstanding deformation capability. It could quickly
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3.4. Biocompatibility test

HUVECs were cultured on the surface of various hydrogels at the same initial concentration to assess the biocompatibility of the materials. The results of the Calcein/PI staining and phalloidine/DAPI staining showed an obvious adhesion morphology and a large number of living cells in the PVA/dECM hydrogel group and control group (Figure 4 and Figure S15-S16). As shown in Figure S17, although the cell viability of hydrogel groups decreased after 48 h of culture, there are still a large number of living cells on the hydrogel surface. Alkali treatment and freeze-thaw cycle had little effect on cell compatibility. Overall, the above-mentioned results revealed that PVA/dECM hydrogel group had a promising biocompatibility, and it would be appropriate for cell adhesion and proliferation, which could be used as tissue engineering material for constructing and repairing meniscus.

3.5. Evaluation of meniscus regeneration

After 12 weeks of implantation, the meniscus defects were repaired eminently by PVA/dECM hydrogels. Immunological rejection or synovial proliferation was not observed in any of these groups. The defective region of the PVA/dECM hydrogel group was covered by glossy and smooth meniscus, which was close to the natural meniscus. In the control and blank groups, the defects were filled with regenerated tissues, which were irregular, depressive, and clearly distinguishable from the surrounding meniscus (Figure 5A). The ImageJ software showed that the regeneration area of the PVA/dECM meniscus was significantly larger than that in the control and blank groups (Table 1). Meanwhile, the contact surface between femur and tibial plateau in the PVA/dECM hydrogel group was smooth without roughness. Obvious cartilage defects were observed in the contact surface between femur and tibial plateau in the control and blank groups. The macroscopic scores are presented in Table 1. The PVA/dECM hydrogel group demonstrated a significant increase in all the assessments. In the ICRS scoring system, the cartilage in

recover the original shape after folding (Figure 3C) and stretching (Figure 3D).

Figure 2. Compressive mechanical properties of PVA/dECM hydrogels. (A) Uniaxial stress-strain curves under compression until 80% of height. (B) Uniaxial compression-relaxation curves of PVA/dECM hydrogel group and control group. The compression-relaxation cycles of the same gel for 20 consecutive cycles without any time interval between two cycles, including control (C), 20-20 (D), 40-20 (E), and 120-20 (F) PVA/dECM hydrogel groups. (G) The normalized maximum compressive stress of the hydrogels in the 20 cycles. (H) The dissipation energy of the hydrogels in the 20 cycles. (I) The stress-relaxation of the hydrogels. The strain of each loading step increased by 10% in the range of 0 – 50%.
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the PVA/dECM hydrogel group showed a minor damage and the same height level with the surrounding cartilage. The overall assessment was roughly normal for the PVA/dECM hydrogel group (mean score was 10 ± 1.08), and abnormal for the control and blank groups (mean scores were 7 ± 1.16 and 6 ± 1.50, respectively). The well filled meniscus was observed in the PVA/dECM hydrogel group using micro-MRI, and the defective and displaced meniscus could be visualized in the control and blank groups (Figure 5B).

According to the results of the H&E staining (Figure 6), the defects in the PVA/dECM group were filled by the regenerated tissue that was intact and uniform. Obvious gap and defects were observed in the control and blank groups. The meniscus ECM of the regenerated region showed a strong staining with toluidine blue and Safranin O in the PVA/dECM group, indicating the active regeneration of meniscus. The cells in the defected area retained the morphology and were interweaved in the PVA/dECM group, which was similar to that in the natural meniscus. However, no healing was found in the control and blank groups, in which irregularly shaped cells were found in the defected area (Figures 7 and 8). The staining of collagen II on ECM was pronounced and homogeneous in the PVA/dECM group, which indicated that the meniscus has been well regenerated (Figure 9).

The cartilage in PVA/dECM group showed a smooth structure similar to natural cartilage from H&E staining. The results of staining with toluidine blue, Safranin O, and collagen II in the PVA/dECM group showed the cartilage matrix strong staining and the uniform cell distribution. Immature chondrocytes arranged parallel to the surface of cartilage and mature chondrocytes were distributed in groups in cartilage lacunae, which was similar to natural cartilage. These results showed the well cartilage protection in the PVA/dECM hydrogel group. Compared with the PVA/dECM group, the color of ECM in the control and blank groups was dimmed, and an obvious gap could be observed. The arrangement of immature chondrocytes had no orderly pattern, and the number of cartilage lacunae was reduced. In the O’Driscoll scoring system, the cellular morphology, Safranin O staining, structural integrity, cartilage thickness, and cellularity were used to evaluate the cartilage conditions and the PVA/dECM hydrogel group showed the best performance. The mean scores for the PVA/dECM hydrogel, control, and blank groups were 20.33 ± 2.15, 16.33 ± 1.89, and 14.66 ± 2.67, respectively (Table 1).

Figure 3. 3D printing test. (A) Schematic diagram of upper meniscus model of 3D printer and the printing hydrogel meniscus. Uniform holes can be observed under a microscope. (B) Meniscus model size on the computer and the actual size. (C) Folding of the printed meniscus and recovery. (D) Stretching of the printed meniscus and recovery.
4. Discussion

Meniscal injury is common in clinical practice, and the treatment strategy for meniscal injury is mainly to remove the injured part. Tibiofemoral contact pressure increased in follow-up research when the medial meniscus was gradually resected to resemble meniscectomy[28]. Excision of the damaged meniscus is obviously not the most ideal surgical method, and it is urgent to put forward a reasonable repair scheme. In the present study, we designed a hybrid bio-ink for 3D printing of meniscal scaffolds. To improve both the mechanical and biological properties, the naturally derived components and synthetic polymers were induced in the bio-ink. PVA chains created crystalline domains, alginate chains formed ionic networks, PEGDA chains generated covalent link networks, and the dECM provided the bioactive components required for meniscus tissue regeneration. The results were admirable, in which the Young’s modulus reached 0.49 MPa and the stress limitation was 2.9 MPa. Meanwhile, the PVA/dECM hydrogel scaffold printed by bio-ink showed an excellent biocompatibility in vitro and a great meniscus regeneration ability in vivo. Thus, the PVA/dECM hydrogel scaffold can be regarded as a potential solution in the future meniscus tissue engineering methods and therapeutic strategies for meniscal injury.

In situations of severe meniscal injuries that cannot be treated with suturing or partial resection, meniscal allograft transplantation is used. In the aim of preserving/restoring the functioning of the knee in severe or irreparable meniscal injuries, meniscal allograft transplantation has been regularly employed as a state-of-the-art procedure. However, the possible complications of meniscal transplantation include re-rupture, graft shrinkage, and extrusion[29]. A previous study showed that treatment failure occurred in 9.9% of patients receiving the Actifit polyurethane meniscal scaffold at a mean follow-up time of 40 months and in 6.7% of patients receiving collagen meniscal implant at a mean follow-up time of 44 months[30]. The current meniscal scaffolds have not shown satisfactory results in their long-term effectiveness, none have displayed chondroprotective effect[20,31]. The weak repair ability of meniscus and insufficient mechanical strength of repair materials may be the causes of the above-mentioned situation. Mechanical stimuli

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Figure 4. (A) The results of Calcein/PI staining (×40 magnification; scale bar: 200 μm). (B) The results of phalloidine/DAPI staining (×100 magnification; scale bar: 100 μm).
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have been shown to be a key regulator of proper tissue shape when used in physiological settings. Meanwhile, multiple in vivo and in vitro investigations have revealed that mechanical variables play a key role in the meniscus’s health, degeneration, and regeneration[32,33]. The PVA/dECM hydrogel scaffold showed a promising mechanical strength. The PVA forms stable crystalline domains by a high concentration of hydroxyl ion and repeated freezing/thawing cycles. Physically crosslinked PVA biomaterials with excellent mechanical capabilities, low water content, damage resistance, and shape memory polymer properties may be made using this technique. It was also discovered that when water was added to the created PVA hydrogel, 90% of the plastic deformation due to extension was recovered, resulting in a significant contraction force capable of lifting items 1100 times their weight[17]. The PVA/dECM hydrogel showed a promising compression performance, and it was not broken when compressed to 80%. The Young’s modulus reached 0.49 MPa, and the stress limitation was 2.9 MPa. An excellent compression ability allows hydrogels to keep intact in meniscal repair. The mechanical environment has a big impact on cellular functions such cell adhesion, cell tension, and cytoskeleton architecture, and it even has a long-term stability influence. According to Zhang et al., cyclic hydrostatic compress force therapy stimulates proliferation of isolated primary meniscus fibrochondrocytes through increasing integrin 51 expression[34]. An appropriate mechanical environment plays an important role in the regeneration and repair of meniscus. Meanwhile, excellent mechanical properties are conducive to the protection of cartilage, which reduced friction between cartilage surfaces.

Table 1. The area of meniscal repair and the total scores of ICRS and O’Driscoll.

| Group       | Area of meniscal repair (%) | Total ICRS score | Total O’Driscoll score |
|-------------|-----------------------------|------------------|------------------------|
| PVA/dECM    | 92.37±3.68                  | 10±1.08          | 20.33±2.15             |
| Control     | 60.94±6.52                  | 7±1.16           | 16.33±1.89             |
| Blank       | 45.23±7.32                  | 6±1.50           | 14.66±2.67             |

Figure 5. (A) The general view of the regenerated meniscus and worn cartilage surface. The blue dotted line showed the regeneration of meniscus and the wear of cartilage on the surface of femoral and tibial plateau. (B) The micro-MRI of the rabbit knee after 12 weeks. The red arrow indicates the regeneration site of meniscus defect.
A photocrosslinked technique was utilized to 3D print the mixture of sodium alginate and PEGDA\textsuperscript{[35,36]}. It can not only form hydrogel quickly, but also possesses satisfactory mechanical properties, which can be used as an effective supplement to PVA network. PEGDA and bioactive glass nanoparticles containing copper and sodium alginate were used to create a nanocomposite scaffold, according to Li et al. The scaffold exhibited the great biomimetic elastomeric mechanical properties, with a high compressive strength of 6.1 kPa\textsuperscript{[35]}. The design of a double network provides a high-pressure resistance and a fast recovery for the PVA/dECM hydrogel. An excellent elasticity can simulate the biological functions of meniscus and play the role of buffering pressure. Furthermore, we discovered in this study that adding sodium alginate and PEGDA to bio-ink resulted in high printability. Zhang et al. suggested bioenergetics and bone regeneration using 3D-printed double-network alginate hydrogels containing polyphosphate. The pre-gel combining sodium alginate and PEGDA exhibited higher 3D printing performance than typical hydrogels for manufacturing complex scaffolds for tissue regeneration.

\textbf{Figure 6.} Histological results of the H&E staining (×200 magnification; scale bar: 50 μm). Histology and gross morphology at upper right corner (×40 magnification; scale bar: 500 μm).

\textbf{Figure 7.} Histological results of the toluidine blue staining (×200 magnification; scale bar: 50 μm). Histology and gross morphology at upper right corner (×40 magnification; scale bar: 500 μm).
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Hydrogels can be appropriately printed by bio-ink according to different types of injury, which is highly appropriate for severe or irregular defects of meniscus. The printed meniscus showed a uniform pore size under SEM, in which the pore size was similar to that of natural meniscus. A homogeneous void structure can promote cell interactions, diffusion, and nutrient exchange with surrounding tissues, as well as integration with host tissue in the meniscal repair. A uniform pore size is also advantageous to the surface modification and drug loading of scaffolds. Bochyńska et al. wanted to see if modifying the tissue surface with collagenase and adding transforming growth factor-β3 might enhance the number of cells in meniscus wounds that were healed using tissue adhesives based on isocyanate-terminated block copolymers. The regulated release of collagenase and platelet-derived growth factor-AB in the defective meniscus enhances cellularity at the interface and inside the scaffold, as well as integration with the surrounding tissue, according to Qu et al. In the future, treatment of PVA/dECM hydrogel surface by different methods can further promote the regeneration of meniscus. In additional, the engineering. Hydrogels can be appropriately printed by bio-ink according to different types of injury, which is highly appropriate for severe or irregular defects of meniscus. The printed meniscus showed a uniform pore size under SEM, in which the pore size was similar to that of natural meniscus. A homogeneous void structure can promote cell interactions, diffusion, and nutrient exchange with surrounding tissues, as well as integration with host tissue in the meniscal repair. A uniform pore size is also advantageous to the surface modification and drug loading of scaffolds. Bochyńska et al. wanted to see if modifying the tissue surface with collagenase and adding transforming growth factor-β3 might enhance the number of cells in meniscus wounds that were healed using tissue adhesives based on isocyanate-terminated block copolymers. The regulated release of collagenase and platelet-derived growth factor-AB in the defective meniscus enhances cellularity at the interface and inside the scaffold, as well as integration with the surrounding tissue, according to Qu et al. In the future, treatment of PVA/dECM hydrogel surface by different methods can further promote the regeneration of meniscus. In additional, the

![Figure 8](image1.png) ![Figure 9](image2.png)
cartilage, drug-loaded injectable hydrogels may promote cartilage repair and reduce the incidence of OA.

The dECM is one of the commonly used materials for meniscal repair, which has shown a great potential in the previous studies. The dECM components (e.g., collagen, proteoglycans, and elastin molecules) are naturally derived. The retention of dECM is vital for the bioactivity to accommodate tissue specificity and to modulate immune response. However, the mechanical properties of dECM are about 200 – 500 kPa, which is far from the requirements of meniscal repair. The introduction of PVA and other crosslinking agents can enhance the crosslinking of hydrogels and maintain the long-term stability. The PVA/dECM hydrogel showed good meniscus regeneration and cartilage protection in vivo. Cell-based tissue engineering represents a promising management for repair and regeneration of meniscus. The regenerated fibrochondrocytes are closely arranged, secreting collagen fibers in the cell matrix. The regenerated meniscus has a good protective effect on the contact surface between femur and tibia, while subchondral bone remains intact. The application of PVA/dECM hydrogel might alter the long-term poor prognosis of cartilage.

These findings imply that the PVA/dECM bio-ink has high printability and that the bio-ink hydrogel may mimic the mechanical function of a natural meniscus, which could be useful in preventing meniscal degeneration after an initial lesion. Because the meniscus is so important in preventing OA, such protective effects on the matrix composition inside the meniscal body might have major therapeutic ramifications, especially for younger patients who are at a high risk of developing OA at an early-age.

Several limitations of the present study should be pointed out. First, the mechanism of stress-induced meniscus tissue regeneration was not explored. As there are multiple cellular signaling pathways, their roles in meniscal repair are worthy of study. In the next study, cellular assays will be conducted to further verify our findings. In addition, it will be attempted to explore the application of different organizations. The excellent mechanical properties are not only conductive to meniscal repair, but also are appropriate for other elastic tissues (e.g., cartilage and muscles).

5. Conclusion

In summary, the bio-ink had a promising printability and the PVA/dECM hydrogel showed excellent mechanical properties and cell compatibility through freezing/thawing and alkaline treatment. The combination of sodium alginate and PEGDA enhanced the printability of bio-ink and improved the strength of PVA network. In addition, the PVA/dECM hydrogel showed a similar surface structure to natural meniscus and provided enough stiffness and meniscus ECM composition. Such hydrogels exhibited excellent elasticity, toughness, fast recovery, and stress unloading properties. Excellent meniscus regeneration and cartilage protection could be verified in vivo. The proposed bio-ink will play an effective role in meniscal repair.

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Conflict of interest

The authors declare that there is no conflict of interest.

Author contributions

Conceptualization: Biao Cheng, Lan Li, Qing Jiang Methodology: Jianhao Huang, Jing Jin, Cunmei Xie Formal analysis: Bin Xue, Jiancheng Lai Writing-original draft: Jingwei Lu, Jianhao Huang Writing-review and editing: Lan Li.

References

1. Walker PS, Erkman MJ, 1975, The Role of the Menisci in Force Transmission Across the Knee. Clin Orthop Relat Res, 109:184–92. 
https://doi.org/1097/00003086-197506000-00027

2. Gear MW, 1967, The Late Results of Meniscectomy. Br J Surg, 54:270–72. 
https://doi.org/1002/bjs.1800540406

3. Allen PR, Denham RA, Swan AV, 1984, Late Degenerative Changes after Meniscectomy. Factors Affecting the Knee after Operation. J Bone Joint Surg Br, 66:666–71. 
https://doi.org/1302/0301-620x.66b5.6548755

4. Jackson JP, 1968, Degenerative Changes in the Knee after Meniscectomy. Br Med J, 2:525–27. 
https://doi.org/1136/bmj.2.5604.525

5. Lotke PA, Lefkoe RT, Ecker ML, 1981, Late results following medial meniscectomy in an older population. J Bone Joint Surg Am, 63:115–9.

6. Noble J, 1975, Clinical Features of the Degenerate Meniscus with the Results of Meniscectomy. Br J Surg, 62:977–81. 
https://doi.org/1002/bjs.1800621213

7. Johnson RJ, Kettlekamp DB, Clark W, et al., 1974, Factors Effecting Late Results after Meniscectomy. J Bone Joint Surg
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8. Noble J, Erat K, 1980, In Defence of the Meniscus. A Prospective Study of 200 Meniscectomy Patients. J Bone Joint Surg Br, 62:7–11. https://doi.org/10.1302/0301-620x.62b1.7351438

9. Roos H, Laurén M, Adalberth T, et al., 1998, Knee Osteoarthritis After Meniscectomy: Prevalence of Radiographic Changes after Twenty-One Years, Compared with Matched Controls. Arthritis Rheum, 41:687–93. https://doi.org/10.1002/1529-0131(199804)41:4<687:Aid-art16>3.0.Co;2-2

10. Wang X, Ding Y, Li H, et al., 2021, Advances in Electrospun Scaffolds for Meniscus Tissue Engineering and Regeneration. J Biomed Mater Res B Appl Biomater, 110:923–49. https://doi.org/10.1002/jbmb.34952

11. Azhim A, Ono T, Fukui Y, et al., 2013, Preparation of Decellularized Meniscal Scaffolds Using Sonication Treatment for Tissue Engineering. Annu Int Conf IEEE Eng Med Biol Soc, 2013:6953–6. https://doi.org/10.1109/embc.2013.6611157

12. Sandmann GH, Eichhorn S, Vogt S, et al., 2009, Generation and Characterization of a Human Acellular Meniscus Scaffold for Tissue Engineering. J Biomed Mater Res A, 91:567–74. https://doi.org/10.1002/jbm.a.32269

13. Shimomura K, Rothrauff BB, Tuan RS, 2017, Region-Specific Effect of the Decellularized Meniscus Extracellular Matrix on Mesenchymal Stem Cell-Based Meniscus Tissue Engineering. Am J Sports Med, 45:604–11. https://doi.org/10.1177/0363546516674184

14. Yang Y, Cheng Y, Peng S, et al., 2021, Microstructure Evolution and Texture Tailoring of Reduced Graphene Oxide Reinforced Zn Scaffold. Bioact Mater, 6:1230–41. https://doi.org/10.1016/j.bioactmat.2020.10.017

15. Yang Y, Cheng Y, Yang M, et al., 2022, Semicoherent Strengthen Graphene/Zinc Scaffolds. Mater Today Nano, 17:100163. https://doi.org/10.1016/j.mtnano.2021.100163

16. Wu J, Ding Q, Dutta A, et al., 2015, An Injectable Extracellular MatrixDerived Hydrogel for Meniscus Repair and Regeneration. Acta Biomater, 16:49–59. https://doi.org/10.1016/j.actbio.2015.01.027

17. Darabi MA, Khosrozadeh A, Wang Y, et al., 2020, An Alkaline Based Method for Generating Crystalline, Strong, and Shape Memory Polyvinyl Alcohol Biomaterials. Adv Sci (Weinh), 7:1902740. https://doi.org/10.1002/advs.201902740

18. Huang X, Guan N, Li Q, 2021, A Marine-Derived Anti-inflammatory Scaffold for Accelerating Skin Repair in Diabetic Mice. Mar Drugs, 19:496. https://doi.org/10.3390/md19090496

19. Thankam FG, Diaz C, Chandra I, et al., 2021, Hybrid Interpenetrating Hydrogel Network Favoring the Bidirectional Migration of Tenocytes for Rotator Cuff Tendon Regeneration. J Biomed Mater Res B Appl Biomater, 110:467–77. https://doi.org/10.1002/jbmb.34924

20. Rajagopal K, Dutt V, Balakumar B, et al., 2021, Long-Term Evaluation of Allogenic Chondrocyte-Loaded PVA-PCL IPN Scaffolds for Articular Cartilage Repair in Rabbits. Indian J Orthop, 55:853–60. https://doi.org/10.1007/s43465-020-00290-5

21. Parameswaran-Thankam A, Al-Anbaky Q, Al-Karakooly Z, et al., 2018, Fabrication and characterization of hydroxypropyl guar-poly (vinyl alcohol)-nano hydroxyapatite composite hydrogels for bone tissue engineering. J Biomater Sci Polym Ed, 29:2083–105. https://doi.org/10.1080/09205063.2018.1494437

22. Sung J, Lee DG, Lee S, et al., 2020, Crosslinking Dynamics and Gelation Characteristics of Photo- and Thermally Polymerized Poly(Ethylene Glycol) Hydrogels. Materials (Basel), 13:3277. https://doi.org/10.3390/ma13153277

23. Peppas NA, Keys KB, Torres-Lugo M, et al., 1999, Poly(ethylene glycol)-containing hydrogels in drug delivery. J Control Release, 62:81–7. https://doi.org/10.1016/S0168-3659(99)00027-9

24. Rehman SR, Augustine R, Zahid AA, et al., 2019, Reduced Graphene Oxide Incorporated GelMA Hydrogel Promotes Angiogenesis for Wound Healing Applications. Int J Nanomed, 14:9603–17. https://doi.org/10.2147/ijn.S218120

25. Sigen A, Zeng M, Johnson M, et al., 2020, Green Synthetic Approach for Photo-Cross-Linkable Methacryloyl Hyaluronic Acid with a Tailored Substitution Degree. Biomacromolecules, 21:2229–35. https://doi.org/10.1021/acs.biomac.0c00196

26. van den Borne MP, Raimakers NJ, Vanlauwe J, et al., 2007, International Cartilage Repair Society (ICRS) and Osswestry Macroscopic Cartilage Evaluation Scores Validated for Use in Autologous Chondrocyte Implantation (ACI) and Microfracture. Osteoarthritis Cartilage, 15:1397–402. https://doi.org/10.1016/j.joca.2007.05.005

27. O’Driscoll SW, Keeley FW, Salter RB, 1988, Durability of Regenerated Articular Cartilage Produced by Free
Autogenous Periosteal Grafts in Major Full-thickness Defects in Joint Surfaces under the Influence of Continuous Passive Motion. A Follow-up Report at One Year. *J Bone Joint Surg Am*, 70:595–606.

28. Willinger L, Lang JJ, Berthold D, *et al.*, 2020, Varus Alignment Aggravates Tibiofemoral Contact Pressure Rise after Sequential Medial Meniscus Resection. *Knee Surg Sports Traumatol Arthrosc*, 28:1055–63. https://doi.org/10.1007/s00167-019-05654-5

29. Lee SR, Kim JG, Nam SW, 2012, The Tips and Pitfalls of Meniscus Allograft Transplantation. *Knee Surg Relat Res*, 24:137–45. https://doi.org/10.1007/s00167-012-243.137

30. Houck DA, Kraeutler MJ, Belk JW, *et al.*, 2018, Similar Clinical Outcomes Following Collagen or Polyurethane Meniscal Scaffold Implantation: A Systematic Review. *Knee Surg Sports Traumatol Arthrosc*, 26:2259–69. https://doi.org/10.1007/s00167-018-4838-1

31. Rongen JJ, van Tienen TG, van Bochove B, *et al.*, 2014, Biomaterials in Search of a Meniscus Substitute. *Biomaterials*, 35:3527–40. https://doi.org/10.1016/j.biomaterials.2014.01.017

32. McNulty AL, Guilak F, 2015, Mechanobiology of the Meniscus. *J Biomech*, 48:1469–78. https://doi.org/10.1016/j.jbiomech.2015.02.008

33. Yy A, Yc A, My A, *et al.*, 2021, Semicoherent Strengthens Graphene/Zinc Scaffolds. *Mater Today Nano*, 17:100163. https://doi.org/10.1016/j.mtnano.2021.100163

34. Zhang Y, Wang F, Bao L, *et al.*, 2019, Cyclic hydrostatic Compress Force Regulates Apoptosis of Meniscus Fibrochondrocytes via Integrin Alpha5Beta1. *Physiol Res*, 68:639–49. https://doi.org/10.33549/physiolres.934088

35. Li Y, Xu T, Tu Z, *et al.*, 2020, Bioactive Antibacterial Silica-based Nanocomposites Hydrogel Scaffolds with High Angiogenesis for Promoting Diabetic Wound Healing and Skin Repair. *Theranostics*, 10:4929–43. https://doi.org/10.7150/thno.41839

36. Zhou W, Zhang H, Liu Y, *et al.*, 2020, Sodium Alginate-polyethylene Glycol Diacrylate Based Double Network Fiber: Rheological Properties of Fiber Forming Solution with Semi-interpenetrating Network Structure. *Int J Biol Macromol*, 142:535–44. https://doi.org/10.1016/j.ijbiomac.2019.09.125

37. Zhang M, Qian T, Deng Z, *et al.*, 2021, 3D printed Double-network Alginate Hydrogels Containing Polyphosphate for Bioenergetics and Bone Regeneration. *Int J Biol Macromol*, 188:639–48. https://doi.org/10.1016/j.ijbiomac.2021.08.066

38. Li H, Wang X, Liu J, *et al.*, 2021, Nanofiber Configuration Affects Biological Performance of Decellularized Meniscus Extracellular Matrix Incorporated Electrospun Scaffolds. *Biomater*, 16:065013. https://doi.org/10.1088/1748-605X/ac28a5

39. Bochyńska AI, Hannink G, Verhoeven R, *et al.*, 2017, The Effect of Tissue Surface Modification with Collagenase and Addition of TGF-β3 on the Healing Potential of Meniscal Tears Repaired with Tissue Glues *In Vitro*. *J Mater Sci Mater Med*, 28:22. https://doi.org/10.1007/s10856-016-5832-0

40. Qu F, Holloway JL, Esterhai JL, *et al.*, 2017, Programmed Biomolecule Delivery to Enable and Direct Cell Migration for Connective Tissue Repair. *Nat Commun*, 8:1780. https://doi.org/10.1038/s41467-017-01955-w

41. Pabbruwe MB, Kafienah W, Tarlton JF, *et al.*, 2010, Repair of Meniscal Cartilage White Zone Tears Using a Stem Cell/Collagen-Scaffold Implant. *Biomaterials*, 31:2583–91. https://doi.org/10.1016/j.biomaterials.2009.12.023

42. Hadidi P, Athanasiou KA, 2013, Enhancing the Mechanical Properties of Engineered Tissue through Matrix Remodeling Via the Signaling Phospholipid Lysophosphatidic Acid. *Biochem Biophys Res Commun*, 433:133–8. https://doi.org/10.1016/j.bbrc.2013.02.048

43. Koski JA, Ibarra C, Rodeo SA, *et al.*, 2000, Meniscal Injury and Repair: Clinical Status. *Orthop Clin North Am*, 31:419–36. https://doi.org/10.1016/s0030-5898(05)070161-9

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