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Construction of a 3D-printed polycaprolactone scaffold functionalized with extracellular matrix and an MSC-affinity peptide for articular cartilage repair

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Abstract

Cartilage damage has emerged as a major clinical challenge due to the limited intrinsic regenerative capacity of articular cartilage. Current cartilage repair strategies often rely on exogenous cells and growth factors to promote tissue regeneration; however, their clinical translation is hindered by high costs, complex manufacturing processes, and potential immunogenicity. Thus, the development of high-performance acellular tissue-engineering scaffolds has attracted increasing attention. In this study, a biomimetic ternary composite scaffold was engineered consisting of polycaprolactone (PCL), a bone marrow mesenchymal stem cell-affinity peptide, and cartilage extracellular matrix (ECM) to facilitate enhanced cartilage regeneration through synergistic multifunctional effects. PCL scaffolds with well-defined porous architectures and favorable mechanical properties were fabricated using three-dimensional printing. Following surface functionalization with the mesenchymal stem cell (MSC)-affinity peptide E7, the scaffolds were further coated with decellularized cartilage ECM. This biomimetic design enhances cell adhesion, selectively recruits endogenous MSCs, and provides the structural support required for tissue regeneration. Moreover, the bioactive components and biochemical cues retained within the ECM promote chondrogenic differentiation. *In vitro* studies demonstrated that E7-modified PCL (E7-PCL) significantly enhanced MSC adhesion and recruitment. The ECM/E7-PCL scaffold further promoted MSC adhesion, proliferation, chondrogenic differentiation, and extracellular matrix deposition while maintaining favorable biocompatibility and mechanical integrity. *In vivo*, implantation of the ECM/E7-PCL scaffold resulted in markedly improved cartilage repair compared with ECM/PCL scaffolds and untreated controls. Overall, these findings from *in vitro* and rabbit *in vivo* experiments indicate that ECM/E7-PCL represents a promising acellular tissue-engineering candidate for cartilage defect regeneration, warranting

further validation in larger and more clinically relevant models.

Keywords: Articular cartilage repair; Bone marrow mesenchymal stem cell-targeting peptide; Three-dimensional printing; Peptide-modified polycaprolactone; Cartilage tissue engineering

1. Introduction

Articular cartilage defects are common orthopedic disorders that frequently progress to degenerative osteoarthritis, chronic joint pain, functional impairment, and progressive tissue deterioration,[1-3] However, effective cartilage regeneration remains a major challenge because articular cartilage is avascular, aneural, and possesses a limited intrinsic capacity for self-repair.[4,5] Current clinical treatments, including microfracture, autologous chondrocyte implantation (ACI), matrix-assisted ACI, and osteochondral autograft transfer, provide varying degrees of symptomatic relief and tissue repair but remain limited by inconsistent long-term outcomes, high costs, and suboptimal patient satisfaction.[6] Hence, tissue-engineering approaches incorporating biomimetic scaffolds, bioactive molecules, and stem cell-based therapies have been extensively investigated as alternatives for cartilage regeneration.[6]

Among these strategies, the recruitment of endogenous reparative cells has emerged as a particularly attractive approach for in situ cartilage regeneration.[7] The microfracture procedure, which stimulates the release of stem and progenitor cells from the bone marrow cavity, is widely used clinically to promote cartilage repair.[7] Nevertheless, the limited retention, migration, and survival of recruited cells at the defect site often result in unsatisfactory regenerative outcomes.[8,9] To address these challenges, bone marrow mesenchymal stem cell (BMSC)-affinity peptides have been developed as a promising means of enhancing endogenous BMSC recruitment. These short functional peptides specifically recognize and bind to BMSCs, enabling their selective recruitment, capture, and retention at injury sites without the need for exogenous cell transplantation, complex cell manipulation, or ethical concerns. Through specific ligand–receptor interactions, BMSC-affinity peptides facilitate the in-situ recruitment, immobilization, and functional regulation of endogenous stem cells, thereby maximizing the intrinsic regenerative potential of host tissues. Because cartilage defects encountered in clinical practice often exhibit irregular geometries, there is a growing demand for tissue-engineered scaffolds that can be customized to match defect morphology while achieving stable integration with the surrounding native cartilage.[10,11]

Three-dimensional (3D) printing has emerged as a powerful fabrication technology in regenerative medicine, enabling the production of scaffolds with precisely controlled architectures, pore structures, and mechanical properties.[12,13] Polycaprolactone (PCL) is one of the most widely used materials for 3D-printed scaffolds owing to its excellent processability, favorable biocompatibility, and suitable degradation profile. However, the hydrophobic surface and lack of bioactive cell-recognition motifs in pure PCL limit cell adhesion, proliferation, and chondrogenic differentiation, thereby limiting its effectiveness in cartilage repair.[14] To address these challenges, various surface-functionalization strategies have been explored. For example, the incorporation of cartilage-derived extracellular matrix (ECM) components into PCL scaffolds has been shown to significantly enhance BMSC chondrogenesis and cartilage-specific matrix formation.[15,16]

In addition, stem cell-targeted recruitment strategies have gained increasing attention. Functional peptides such as arginine–glycine–aspartic acid and tyrosine–isoleucine–glycine–serine–arginine can improve cell–material interactions, while BMSC-specific affinity peptides, including E7 and P19, identified through phage-display screening, exhibit strong binding affinity toward BMSC surface receptors and effectively recruit endogenous stem cells to sites of injury.[17,18] These approaches eliminate the need for *ex vivo* cell expansion and transplantation, offering a minimally invasive strategy for cartilage regeneration.

Cartilage ECM, composed primarily of type II collagen, proteoglycans, and matrix-bound growth factors, provides a highly specialized microenvironment that supports chondrogenesis, matrix remodeling, and tissue integration. Hence, the incorporation of cartilage-derived ECM into tissue-engineering scaffolds has emerged as a promising biomimetic strategy for cartilage regeneration.[19] Unlike synthetic biomaterials, which often lack essential biological signals, native ECM closely replicates the biochemical and structural characteristics of the cartilage niche, thereby enhancing cell recruitment, chondrogenic differentiation, and functional matrix deposition. When processed using

techniques such as decellularization and lyophilization, cartilage ECM can retain key bioactive components while preserving favorable biocompatibility and mechanical properties. ECM-functionalized scaffolds have been shown to promote cartilage-specific matrix synthesis, reduce immunogenicity, and facilitate long-term tissue integration.[20] Furthermore, the intrinsic anti-angiogenic properties of cartilage ECM help preserve the avascular environment required for hyaline cartilage formation, thereby minimizing the risk of undesirable endochondral ossification.

Although previous studies have incorporated either ECM components or bioactive peptides into PCL scaffolds, most existing approaches provide only partial functionality and fail to achieve effective synergistic functionality.[21,22] Current composite scaffold designs primarily focus on enhancing cell–material interactions while often overlooking the integration of mechanical support, targeted stem-cell recruitment, and biochemical induction. For example, the incorporation of ECM can improve the biological activity of PCL scaffolds but does not fully overcome the poor cell adhesion associated with the hydrophobic PCL surface. Conversely, the grafting of nonspecific adhesion peptides, such as arginine–glycine–aspartic acid, enhances cellular attachment but lacks selectivity for BMSCs, thereby limiting efficient endogenous stem-cell recruitment.

Furthermore, many existing fabrication strategies rely on physical blending or simple surface coating, which may lead to heterogeneous distribution, rapid loss of bioactive components, and inadequate long-term stability. More importantly, the ability to actively direct endogenous BMSC homing, retention, and chondrogenic differentiation through rational biomaterial design remains a major challenge in cartilage tissue engineering.[23] Therefore, there is an urgent need to develop multifunctional scaffolds capable of integrating mechanical support, selective stem-cell recruitment, and biomimetic biochemical signaling within a single platform.[24]

To address these challenges, a biomimetic ternary composite scaffold was developed based on a novel "mechanical skeleton–targeting peptide–natural matrix" design concept. The scaffold consists of a 3D-printed PCL framework, a BMSC-targeting peptide, and cartilage-derived ECM. First, a porous PCL scaffold was fabricated using 3D printing to provide structural integrity and mechanical support. A previously validated BMSC-targeting peptide was then covalently immobilized onto the scaffold surface to facilitate the selective recruitment and retention of endogenous stem cells. Subsequently, decellularized cartilage-derived ECM was uniformly integrated into the scaffold through cross-linking, creating a biomimetic microenvironment enriched with native biochemical cues. We hypothesized that this multifunctional scaffold would promote cartilage regeneration through a synergistic mechanism involving sustained mechanical support, targeted BMSC recruitment, and ECM-mediated chondrogenic induction. Accordingly, the present study aimed to evaluate the ability of this composite scaffold to enhance articular cartilage repair, activate endogenous regenerative potential, and promote the formation and integration of hyaline cartilage-like tissue without the need for exogenous cell transplantation (Figure 1).

2. Materials and methods

2.1. Preparation of decellularized cartilage extracellular matrix

Articular cartilage was isolated from fresh porcine hind-knee joints (n = 20). The cartilage tissue was frozen in liquid nitrogen and pulverized into a fine powder. Decellularization was performed according to a previously reported protocol with minor modifications.[14] Briefly, cartilage powder was treated with 2% sodium dodecyl sulfate (Solarbio, Beijing, China) at 4 °C for 4 h, followed by washing in phosphate-buffered saline (PBS; pH 7.4; Solarbio, Beijing, China) for 1 h. The samples were subsequently incubated in DNase I solution (200 U/mL; Sigma-Aldrich, USA) for 4 h to remove residual nucleic acids. After the second PBS wash for 1 h, one complete decellularization cycle was completed.[20] The procedure was repeated until a fully decellularized ECM was obtained. The decellularized ECM was then digested in pepsin solution (0.25 mg/mL) at 37 °C for 24 h, rinsed thoroughly with ultrapure water, and prepared as a gel. The ECM gel was frozen at −80 °C and subsequently lyophilized to obtain ECM powder. The resulting powder was sterilized by irradiation and stored at −20 °C until further use.

2.2. Preparation of polycaprolactone scaffolds

Polycaprolactone (Shanghai Yuanye Bio-Technology Co., Ltd., China) was used to fabricate 3D-printed scaffolds. Scaffold models were designed using SolidWorks 2016 (SolidWorks Corporation, USA) with a thickness of 2.5 mm, a pore size of 0.9 mm, and a filament laydown angle of 90°. The digital models were imported into a fused deposition modeling 3D printer, and porous PCL scaffolds with interconnected architectures were fabricated using optimized printing parameters. Following fabrication, all scaffolds were sterilized with ethylene oxide and stored at room temperature until use.[22]

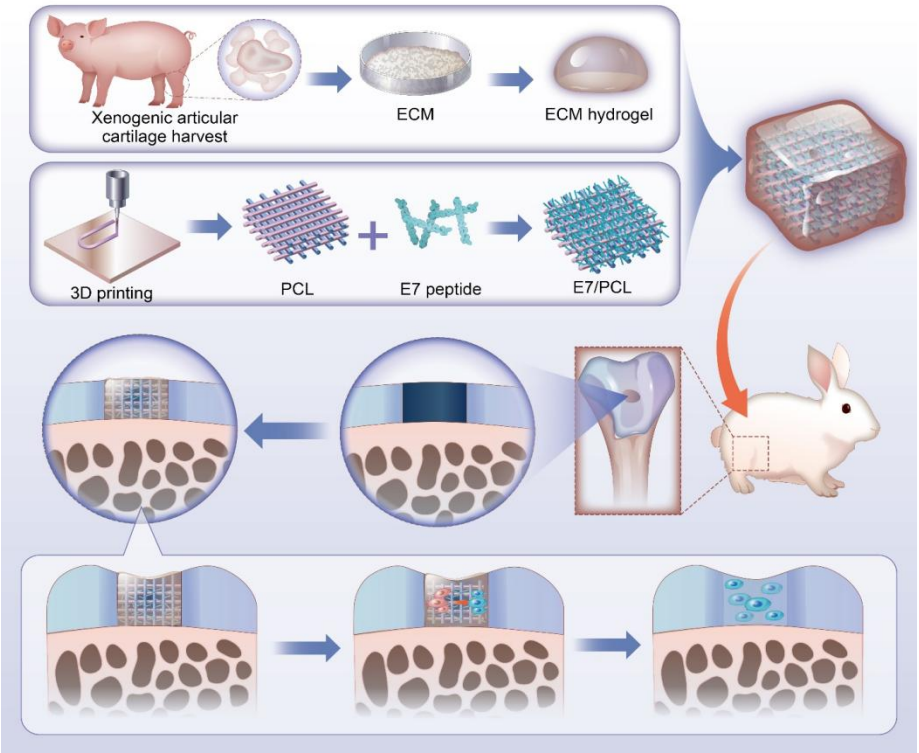

Figure 1. Schematic illustration of the 3D scaffold fabrication process and its application in cartilage tissue engineering
Abbreviations: 3D: Three-dimensional; ECM: Extracellular matrix; PCL: Polycaprolactone.

2.3. Surface functionalization of polycaprolactone scaffolds with E7 peptide

The BMSC-affinity peptide E7 (sequence: EPLQLKM; molecular weight: 858 Da) was commercially synthesized and purified to >95% purity (Scilight Peptide Inc., China). Surface functionalization was performed using sulfosuccinimidyl 4-(N-maleimidomethyl) cyclohexane-1-carboxylate (Thermo Fisher Scientific, USA) as a heterobifunctional cross-linker.

Briefly, prefabricated PCL scaffolds were immersed in 10 wt% 1,6-hexanediamine dissolved in isopropanol and incubated at 37 °C for 2 h to introduce surface amine groups. The scaffolds were then thoroughly rinsed with ultrapure water and washed with activation buffer (0.1 M PBS containing 0.15 M sodium hydroxide, pH 7.2). Subsequently, the scaffolds were incubated in sulfosuccinimidyl 4-(N-maleimidomethyl) cyclohexane-1-carboxylate solution (2 mg/mL in PBS) for 1 h at room temperature. After activation, the scaffolds were washed with conjugation buffer (0.1 M PBS containing 0.1 M ethylenediaminetetraacetic acid, pH 7.0) and incubated overnight at 4 °C in E7 peptide solution (2 mg/mL in conjugation buffer). The peptide-functionalized scaffolds were washed three times with ultrapure water to remove unbound peptide and stored at 4 °C until further use.

2.4. Preparation of ECM/E7-PCL composite scaffolds

Extracellular matrix powder was dispersed in ultrapure water at a concentration of 20 mg/mL and homogenized using a high-speed homogenizer until a uniform suspension was obtained. The E7-modified PCL (E7-PCL) scaffolds were immersed in the ECM suspension and centrifuged at 8,000× *g* for 5 min to facilitate ECM infiltration and deposition throughout the scaffold structure. The scaffolds were subsequently frozen at −80 °C for 2 h and lyophilized. To stabilize the ECM layer, the freeze-dried scaffolds were immersed in 70% (v/v) ethanol for 24 h to induce cross-linking. The scaffolds were then lyophilized again to obtain the final ECM/E7-PCL composite scaffolds.[1,21]

2.5. Isolation, culture, and characterization of bone marrow mesenchymal stem cells

Bone marrow mesenchymal stem cells were isolated from one-month-old male New Zealand White rabbits (n = 4; each sample were isolated, cultured, and preserved

individually without pooling) using Percoll density-gradient centrifugation as previously described.[23] Briefly, bone marrow was aspirated from the tibial and femoral medullary cavities using a bone marrow aspiration needle and layered onto lymphocyte separation medium for density-gradient centrifugation. The mononuclear cell fraction enriched with mesenchymal stem cells (MSCs) was collected, diluted with PBS, washed twice, and centrifuged at 1,000× *g* for 10 min. The resulting cell pellet was resuspended in Dulbecco's Modified Eagle Medium/F12 medium (Gibco, Thermo Fisher Scientific, USA) supplemented with 10% fetal bovine serum (Gibco, USA) and 1% penicillin–streptomycin (100 U/mL penicillin and 100 μg/mL streptomycin; Macklin, China). Cells were seeded into culture flasks and maintained under standard culture conditions.

To verify their stem-cell characteristics, the isolated cells were subjected to trilineage differentiation assays, including adipogenic, osteogenic, and chondrogenic induction. In addition, flow cytometric analysis was performed to assess the expression of characteristic BMSC surface markers and confirm cell identity.

2.6. Scaffold characterization

2.6.1. Scanning electron microscopy

The scaffolds were characterized using scanning electron microscopy (SEM). The 3D-printed PCL, ECM/PCL, and ECM/E7-PCL scaffolds were freeze-dried and sputter-coated with gold prior to imaging. The surface morphology and microstructural features of the scaffolds were examined using a scanning electron microscope operated at an accelerating voltage of 15 kV (Sigma 300, Carl Zeiss AG, Oberkochen, Germany). The average fiber diameter was quantified using ImageJ software (version 1.54p, National Institutes of Health, Bethesda, USA).

2.6.2. Fourier-transform infrared spectroscopy

Fourier-transform infrared (FTIR) spectroscopy was performed to characterize the chemical composition of PCL and E7-PCL scaffolds. Spectra were acquired using a Nicolet Nexus 670 FTIR spectrometer (Thermo Fisher Scientific, USA) over a wavenumber range of 200–4,000 cm^{-1}, with a resolution of 2 cm^{-1}.

2.6.3. Pore size and porosity analysis

The pore morphology of the freeze-dried composite scaffolds was examined by SEM. Scaffold porosity was determined using the ethanol displacement method. Briefly, the initial volume of ethanol was recorded as V_0. After immersion of the scaffold in ethanol for 5 min, the total volume was recorded as V_1. Following removal of the scaffold, the remaining ethanol volume was recorded as V_2. The porosity of the scaffolds was calculated using **Equation 1**:

$$Porosity = \frac{V_0 - V_2}{V_1 - V_2} \times 100\% \quad (1)$$

All measurements were performed in triplicate.

2.6.4. Mechanical testing

Prior to mechanical testing, scaffolds were immersed in PBS at 37 °C for 1 h to achieve equilibrium hydration. Excess surface moisture was gently removed using filter paper. Cylindrical specimens (diameter = 5 mm; height = 4 mm) were subjected to uniaxial compression using a universal testing machine (Zwick Z020, ZwickRoell, Germany). Compression was applied at a displacement rate of 0.5 mm/min until a maximum strain of 10% was reached, followed by complete unloading. This loading–unloading cycle was repeated 2–5 times to precondition the samples. The compressive modulus was determined from the initial linear region of the stress–strain curve.[25] All measurements were performed in triplicate.

2.6.5. Water absorption capacity

The water absorption capacities of PCL, ECM/PCL, and ECM/E7-PCL scaffolds were evaluated. The initial dry weight of each scaffold was recorded as W_0. Samples were immersed in deionized water, and wet weights (W_t) were measured after 2, 4, 6, and 8 min. Prior to weighing, excess surface water was gently removed using filter paper. The water absorption rate was calculated using **Equation 2**:

$$Water\ absorption\ rate = \frac{W_t - W_0}{W_0} \times 100\% \quad (2)$$

where W_t is the wet weight at time t. All measurements were performed in triplicate.

2.7. Quantification of collagen and glycosaminoglycan contents in the extracellular matrix

The collagen content retained in the decellularized ECM was quantified using a Sircol™ Insoluble Collagen Assay Kit (catalog no.S2000, Biocolor, United Kingdom). Briefly, 20 mg of ECM powder was mixed with pepsin dissolved in 0.5 M acetic acid at a ratio of 1:50 (w/v) and incubated at 37 °C for 18 h, with vortexing every 30 min to facilitate digestion. The resulting suspension was transferred to a 2 mL centrifuge tube, and dissociation reagent was added at a ratio of 20:1 (w/v) relative to the ECM. Samples were subsequently incubated at 65 °C for 3 h with intermittent vortexing every 30 min. Following centrifugation at 12,000

rpm for 10 min, the supernatant was collected for analysis. A standard curve was generated using hydrolyzed bovine collagen standards. Samples and standards were added to a 96-well plate, and absorbance was measured at 550 nm using a microplate reader (BIO-DL, Shanghai, China). Collagen concentrations were calculated from the standard curve. All measurements were performed in triplicate.

The glycosaminoglycan (GAG) content of the ECM was determined using the dimethylmethylene blue assay.[24] Briefly, approximately 50 mg of each sample was digested in 1 mL of papain digestion buffer (100 mM sodium phosphate, 10 mM ethylenediaminetetraacetic acid, 10 mM cysteine-hydrochloric acid, pH 6.5) containing 125 μg/mL papain. Samples were incubated at 60 °C for 24–72 h until complete digestion was obtained. The digested samples were centrifuged at 10,000× *g* for 5 min, and 100 μL of the supernatant was mixed with 3 mL of dimethylmethylene blue reagent (Sigma-Aldrich, USA). Absorbance was measured at 480 nm using an ultraviolet-visible spectrophotometer (Purkinje General, Beijing, China). GAG concentrations were determined from a standard calibration curve and expressed as micrograms of GAG per milligram of dry ECM.

2.8. Cytotoxicity and cell proliferation assays

To evaluate the biocompatibility of the scaffolds, BMSCs were seeded onto ECM/PCL and ECM/E7-PCL composite scaffolds at a density of 5×10^5 cells per scaffold. After allowing 3 h for cell attachment, the cell-seeded scaffolds were cultured in Dulbecco's Modified Eagle Medium supplemented with 10% fetal bovine serum and 1% penicillin–streptomycin. BMSCs cultured under standard conditions without scaffolds served as the control group. Cell viability and proliferation were assessed after 1, 3, and 5 days of culture using a live/dead viability assay and a Cell Counting Kit-8 (CCK-8; catalog no. CK04, Dojindo Laboratories, Japan), respectively. For the CCK-8 assay, absorbance was measured at 450 nm using a microplate reader.

2.9. Quantitative reverse transcription polymerase chain reaction

To evaluate chondrogenic differentiation, second-passage BMSCs were seeded onto E7-PCL and ECM/E7-PCL composite scaffolds at a density of 2×10^7 cells per scaffold according to a previously reported protocol.[26] Following 4 h of cell attachment, the constructs were cultured in standard growth medium. BMSCs cultured under standard conditions without scaffolds served as the control group. After 7 and 14 days of culture, total RNA was extracted using TRIzol reagent (TaKaRa, Japan) according to the manufacturer's instructions.

Complementary DNA was synthesized and subjected to quantitative reverse transcription–polymerase chain reaction (qRT-PCR) using a LightCycler® 480 II system (Roche Diagnostics Ltd., China). The expression levels of cartilage-related genes, including *SOX9*, *COL2A1*, *PRG4*, and *ACAN*, were analyzed. *GAPDH* was used as the internal reference gene. Relative gene expression levels were calculated using the $2^{-\Delta\Delta Ct}$ method. Primer sequences are listed in Table 1. The PCR cycling conditions consisted of an initial denaturation step at 95 °C for 5 min, followed by 35 cycles of denaturation at 95 °C for 15 s, annealing at 55 °C for 30 s, and extension at 72 °C for 30 s.

2.10. Immunofluorescence staining

To detect the expression of cartilage-specific proteins, BMSCs were seeded onto the composite scaffolds (1×10^7 cells/mL) and cultured in chondrogenic differentiation medium for 21 days. The constructs were gently washed with PBS, fixed with 4% paraformaldehyde for 30 min, permeabilized with 0.5% Triton X-100 (Sigma-Aldrich, USA) for 15 min, and blocked with 5% bovine serum albumin (Gibco, Thermo Fisher Scientific, USA) for 1 h at room temperature. Samples were subsequently incubated overnight at 4 °C with primary antibodies against *PRG4* (MABT400, Merck, Germany) and aggrecan (MA3-16888, Invitrogen, USA) at a final concentration of 6 μg/mL.[27] After washing with PBS, the constructs were incubated

Table 1. Primer sequences

Gene	Primer sequence	
	Forward (5'–3')	Reverse (5'–3')
ACAN	TCGAGGACAGCGAGGCC	TCGAGGGTGTAGCGTGTAGAGA
COL2A1	AGCCTGGTGTCATGGGTTTC	GTCCCTTCTCACCAGCTTTGC
SOX9	CCCCAACAGATCGCCTACAGT	GAGTTCTGGTGGTCGGTGTAGTC
PRG4	TTGCTCCTGGTGCTGCTG	GCTGATGGTGGTGATGGTG

with a Cy3-conjugated goat anti-mouse IgG secondary antibody (AB97035, Abcam, United Kingdom) for 1 h at room temperature. The actin cytoskeleton was stained with fluorescein isothiocyanate–phalloidin (Shanghai Biotics Co., Ltd., China), and cell nuclei were counterstained with 4',6-diamidino-2-phenylindole (Invitrogen, USA). Fluorescence images were acquired using a confocal laser scanning microscope (Sigma 300, Carl Zeiss AG, Germany).

2.11. *In vivo* animal study

Twenty-four healthy male New Zealand White rabbits (4 months old, 2.5–3.0 kg) were used in this study. All experimental procedures were approved by the Animal Care and Use Committee of Beijing Vital River Laboratory Animal Technology Co., Ltd. (Approval No. AWE2022060305). Animals were randomly assigned to three groups (n = 8 per group): control, ECM/PCL, and ECM/E7-PCL. General anesthesia was induced by intravenous injection of 3% pentobarbital sodium (30 mg/kg) via the marginal ear vein. The right knee joint was exposed through a medial parapatellar approach, and a full-thickness osteochondral defect (4 mm in diameter × 4 mm in depth) was created in the center of the femoral trochlear groove. Subsequently, 3–4 microfracture holes (1 mm in diameter and 5 mm in depth) were created in the subchondral bone with a 1-mm Kirschner wire. Composite scaffolds were press-fitted into the defects in the ECM/PCL and ECM/E7-PCL groups, whereas defects in the control group were left untreated. Animals were sacrificed at 6 and 12 weeks postoperatively, and repaired tissues were harvested for evaluation. Cartilage repair outcomes were assessed using the International Cartilage Regeneration and Joint Preservation Society (ICRS) scoring system.[28]

2.12. Gross observation, magnetic resonance imaging, and histological assessment

Macroscopic evaluation of cartilage repair was independently performed by two blinded observers using the ICRS macroscopic assessment scale. Magnetic resonance imaging (MRI) was conducted to assess tissue regeneration within the defect area and the restoration of the articular surface. MRI parameters were as follows: zoom factor = 1.58, field strength = 3.0 T, slice thickness = 0.5 mm, repetition time = 26.0 ms, and echo time = 4.9 ms.[29] Following MRI examination, osteochondral specimens were harvested, fixed, decalcified, embedded, and sectioned for histological analysis. Tissue sections were subjected to hematoxylin and eosin, Safranin O/Fast Green, toluidine blue, and Van Gieson staining to evaluate matrix deposition and tissue morphology. Collagen-specific staining was also performed to assess the quality of regenerated cartilage.

2.13. Statistical analysis

All quantitative data are presented as mean ± standard deviation. Statistical analyses were performed using SPSS version 20.0 (IBM Corporation, USA). Comparisons among multiple groups were conducted using one-way analysis of variance (ANOVA), followed by Tukey's post hoc test for pairwise comparisons. Differences were considered statistically significant at $p < 0.05$.

3. Results

3.1. Morphology of the composite scaffolds

The fabrication process of the E7-PCL scaffold is illustrated in Figure 2. Representative images of the 3D-printed PCL scaffold are shown in Figure 3, demonstrating a well-defined architecture with interconnected porous networks generated using the selected printing parameters. SEM observations and FTIR analysis demonstrated the presence of ECM and E7 peptide-associated features on the PCL scaffolds (Figures A1 and 4). The resulting ECM/E7-PCL composite scaffold exhibited a biphasic structure in which both the PCL framework and ECM layer retained their characteristic morphological features. No obvious interfacial separation or structural discontinuity was observed between the two phases, indicating effective integration between the PCL framework and ECM layer. Such structural continuity suggests the potential of the scaffold to maintain structural integrity during tissue generation.

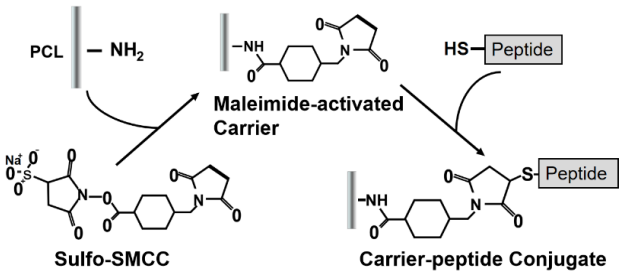

Figure 2. Schematic illustration of the covalent conjugation of fluorescein isothiocyanate-labeled peptides onto PCL scaffolds using sulfo-SMCC as the cross-linker between the fluorescein isothiocyanate-tagged peptide and the electrospun PCL fibers
Abbreviations: PCL: Polycaprolactone; Sulfo-SMCC: Sulfosuccinimidyl 4-(N-maleimidomethyl) cyclohexane-1-carboxylate.

High-resolution scanning electron microscopy verified the uniform immobilization of E7 peptides on the surface of surface-modified PCL scaffolds (Figure 5). Water contact angle measurements further demonstrated

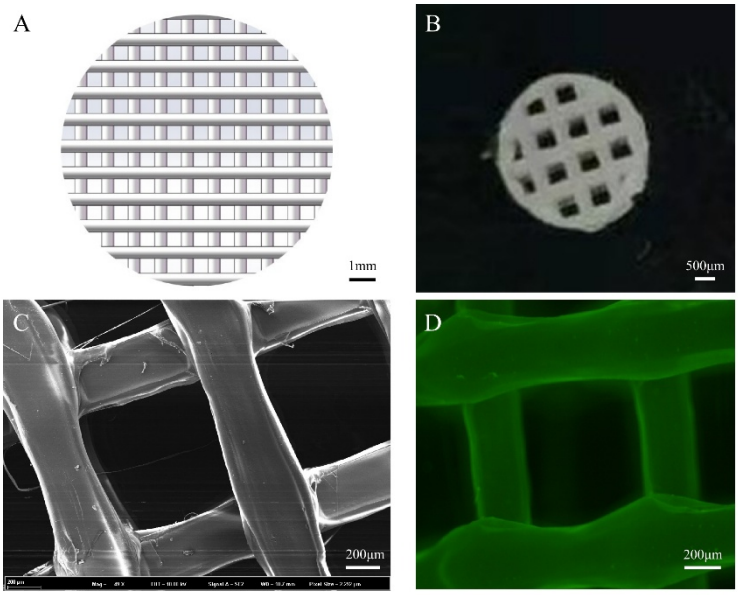

Figure 3. Characterization of 3D-printed PCL scaffolds. (A) 3D structural design (scale bar = 1 mm). (B) Macroscopic image (scale bar = 500 μm). (C) Scanning electron micrographs of PCL fibers (scale bar = 200 μm; magnification = 50×). (D) Fluorescence microscopy image demonstrating successful conjugation of fluorescein isothiocyanate-labeled E7 peptide (scale bar = 200 μm; magnification = 10×).
Abbreviations: 3D: Three-dimensional; PCL: Polycaprolactone.

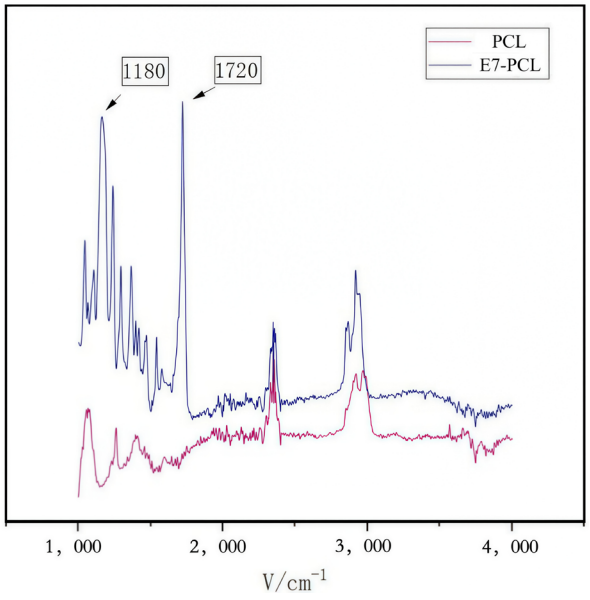

Figure 4. Fourier-transform infrared spectroscopy spectra of pure PCL and E7-PCL scaffolds
Abbreviation: PCL: Polycaprolactone.

that E7 peptide functionalization substantially improved the hydrophilicity of PCL substrates (Figure 5), which constructs a more favorable microenvironment for cell adhesion. Subsequent fluorescence imaging of seeded stem cells revealed markedly enhanced cell attachment on E7-grafted PCL constructs relative to pristine PCL counterparts (Figure 6), creating superior conditions for cellular proliferation.

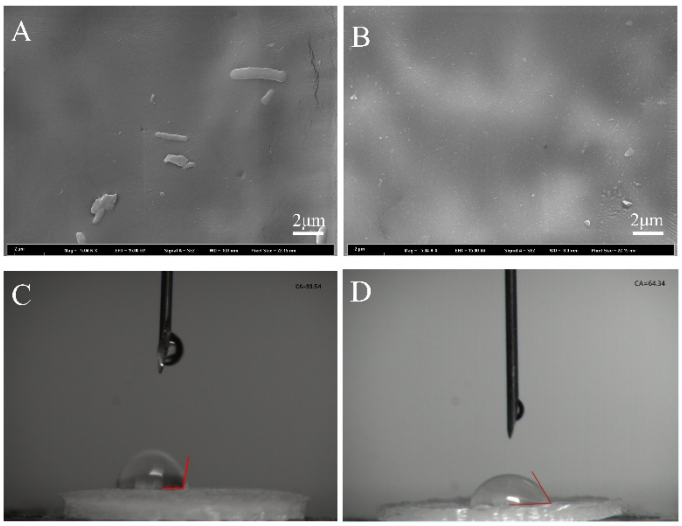

Figure 5. Surface morphology and hydrophilicity characterization. (A) SEM image of the pure PCL and scaffold surface (scale bar = 2 μm; magnification = 5,000×). (B) SEM image of the E7-PCL scaffold surface (scale bar = 2 μm; magnification = 5,000×). (C) Water contact angle of the pure PCL scaffold. (D) Water contact angle of the E7-PCL scaffold.
Abbreviations: PCL: Polycaprolactone; SEM: Scanning electron microscopy.

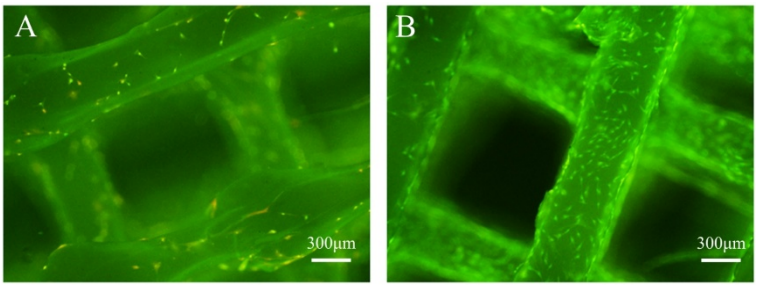

Figure 6. Cytoskeleton fluorescence staining of bone marrow mesenchymal stem cells cultured on scaffolds (fluorescein isothiocyanate–phalloidin staining; scale bar = 300 μm; magnification = 10×): (A) Pure PCL scaffold and (B) E7-PCL scaffold
Abbreviation: PCL: Polycaprolactone.

3.2. Characterization of the composite scaffolds

Macroscopic observation revealed that the ECM/E7-PCL composite scaffold possessed a uniform white appearance and a highly porous 3D structure (Figure 7). SEM analysis further demonstrated the presence of interconnected pores distributed throughout the scaffold (Figure 8A). Consistently, fluorescence imaging of BMSCs cultured within the scaffold revealed homogeneous cellular distribution and infiltration across the porous network (Figure 8B). The highly interconnected architecture may support nutrient transport, cellular infiltration, waste exchange, and ECM deposition, all of which are essential for successful tissue regeneration. Previous studies have shown that scaffold pore size can influence stem-cell fate by modulating cellular morphology and the local microenvironment. Smaller pores tend to favor chondrogenic differentiation by promoting cell aggregation and creating a relatively hypoxic niche, whereas larger pores are generally more conducive to vascularization and osteogenic differentiation.[30,31] Accordingly, the pore architecture of the present scaffold provided a structural environment suitable for cartilage tissue engineering applications. To evaluate the quality of the decellularized ECM, residual DNA, type II collagen, and GAG contents were quantified.

As shown in Figure 9A–9C, the decellularization process effectively removed cellular components while preserving key cartilage-specific ECM constituents, including type II collagen and GAGs. These results indicate that the prepared ECM retained its essential biological

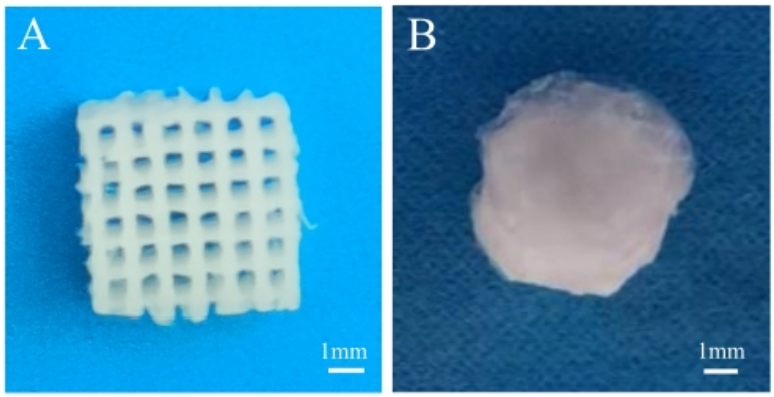

Figure 7. Macroscopic images of the (A) pure PCL scaffold and (B) ECM/E7-PCL composite scaffold (scale bar = 1 mm)
Abbreviations: ECM: Extracellular matrix; PCL: Polycaprolactone.

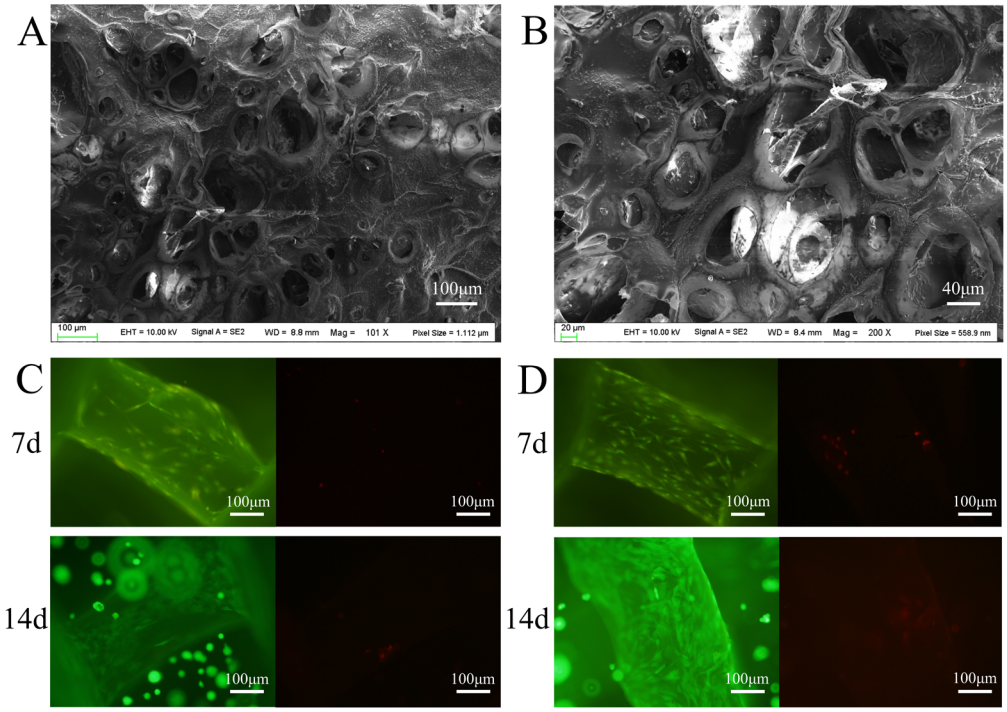

Figure 8. Surface morphology and cytocompatibility of the ECM/E7-PCL composite scaffold. (A,B) Scanning electron microscopy images showing the surface morphology of the ECM/E7-PCL composite scaffold (scale bar = 100 μm, magnification = 100×; scale bar = 20 μm, magnification = 200×). (C, D) Live/dead fluorescent staining images of bone marrow mesenchymal stem cells seeded on the (C) ECM/PCL and (D) ECM/E7-PCL scaffolds after 7 and 14 days of culture (scale bar = 100 μm; magnification = 10×).
Abbreviations: ECM: Extracellular matrix; PCL: Polycaprolactone.

composition and is therefore suitable for cartilage tissue-engineering applications.

Taken together, these findings demonstrate that the composite scaffold possesses a highly porous architecture, favorable structural characteristics, and a biologically active ECM component with preserved cartilage-associated biochemical properties.

3.3. Water absorption capacity of the composite scaffolds

The water absorption behavior of pure PCL and ECM/E7-PCL composite scaffolds is shown in Figure 9D. Water uptake is an important property of tissue-engineered scaffolds because it is associated with nutrient transport,

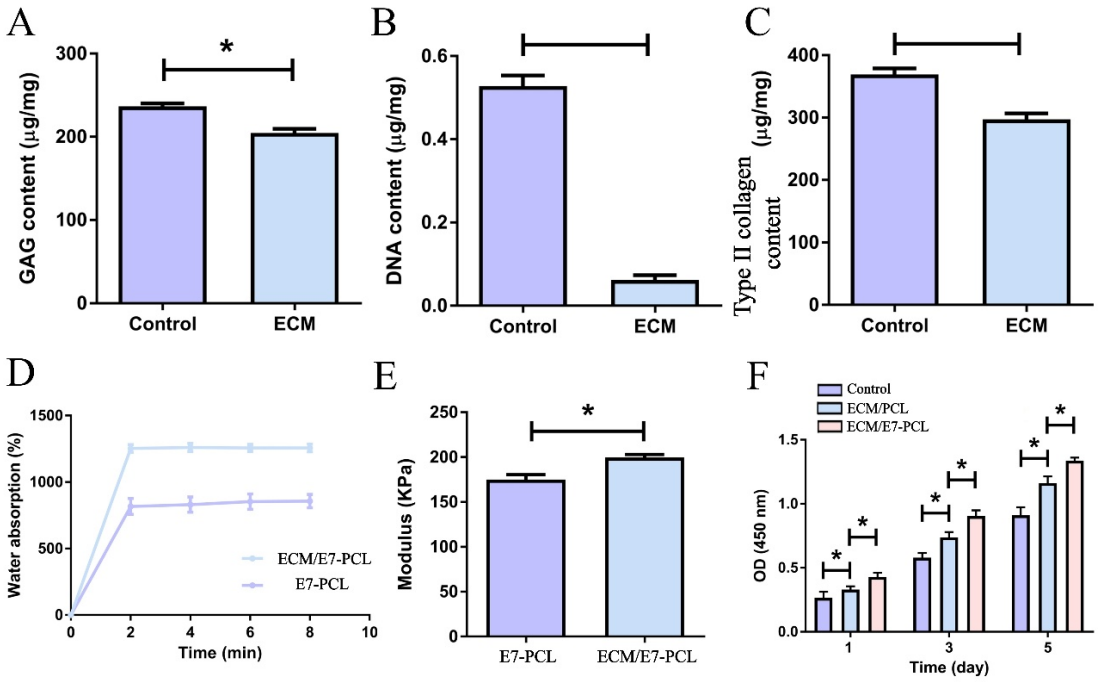

Figure 9. Biochemical characterization of the ECM and physicochemical properties of the scaffolds. (A) Quantification of GAG content in the ECM. (B) Assessment of residual DNA content in the ECM. (C) Type II collagen content in the ECM. (D) Water absorption kinetics of the pure PCL and ECM/E7-PCL scaffolds. (E) Compressive modulus of the pure PCL and ECM/E7-PCL scaffolds. (F) Cell Counting Kit-8 assay for cell proliferation of bone marrow mesenchymal stem cells cultured on the control, ECM/PCL, and ECM/E7-PCL scaffolds for 1, 3, and 5 days. *$p < 0.05$ indicates statistical significance.

Abbreviations: ECM: Extracellular matrix; GAG: Glycosaminoglycan; OD: Optical density; PCL: Polycaprolactone.

biomolecule retention, and cellular activity within the construct.[32] Compared with pure PCL, the ECM/E7-PCL scaffold exhibited significantly enhanced water absorption capacity ($p < 0.05$). The water absorption ratio of the ECM/E7-PCL scaffold increased rapidly over time and reached approximately 315% after 8 min of immersion, after which it remained relatively stable. Therefore, water absorption changes were recorded within 8 min to capture the rapid hydration phase of the scaffolds. In contrast, pure PCL displayed substantially lower water uptake. The enhanced hydrophilicity of the composite scaffold may be attributed to the incorporation of ECM components, which contain abundant hydrophilic functional groups capable of retaining water molecules. The improved water absorption capacity may provide a hydrated environment for cellular activity and matrix deposition, thereby facilitating cartilage regeneration.

3.4. Mechanical properties of the composite scaffolds

The compressive mechanical properties of pure PCL and ECM/E7-PCL scaffolds are presented in Figure 9E. Mechanical performance is a critical consideration in scaffold design because the mechanical microenvironment can influence stem-cell behavior and tissue regeneration. Compression testing demonstrated that the composite scaffold maintained favorable mechanical strength despite the incorporation of ECM. The PCL component likely contributed to structural stability and load-bearing capacity, whereas the ECM component provided additional biological functionality without substantially compromising the overall integrity of the scaffold. The resulting mechanical properties suggest that the scaffold may provide adequate structural support for defect repair while maintaining a microenvironment conducive to cellular activity. Previous studies have shown that substrate stiffness can regulate mesenchymal stem-cell differentiation through mechanotransduction pathways YAP/TAZ. In the present scaffold system, the mechanically robust PCL framework may contribute to structural support and subchondral bone repair, whereas the ECM-rich microenvironment provides biological cues favorable for chondrogenic differentiation and cartilage-specific matrix synthesis.

3.5. Cell survival, adhesion, and recruitment on composite scaffolds

To evaluate the cytocompatibility of the composite scaffolds, BMSCs were seeded onto ECM/PCL and ECM/E7-PCL scaffolds and cultured for up to five days. Live/dead staining (Figure 8B) and CCK-8 assays demonstrated high cell viability and sustained proliferation in both groups throughout the culture period (Figure 9F), indicating that neither scaffold exhibited obvious cytotoxicity. Although no significant differences in overall cell viability were observed between groups, the ECM/E7-PCL scaffold consistently showed enhanced initial cell attachment compared with the ECM/PCL scaffold. Transwell migration assays further demonstrated that the E7-modified scaffold enhanced BMSC migration under identical culture conditions (Figure 10). In addition, fluorescence imaging revealed that BMSCs were uniformly distributed throughout the porous scaffold architecture and exhibited normal cellular morphology.

3.6. *In vitro* chondrogenic differentiation assessed by immunofluorescence and qRT-PCR

The ability of the composite scaffolds to promote chondrogenic differentiation was evaluated by immunofluorescence staining and qRT-PCR. Immunofluorescence analysis demonstrated positive expression of superficial zone protein and aggrecan in both the ECM/PCL and ECM/E7-PCL groups (Figure 11A and 11B). Notably, the ECM/E7-PCL scaffold exhibited stronger fluorescence intensity for both markers, suggesting increased expression of chondrogenic markers upon incorporation of the E7 peptide and cartilage-derived ECM. Consistent with the immunofluorescence results, qRT-PCR analysis revealed substantial upregulation of cartilage-related genes, including *SOX9*, *PRG4*, and *ACAN*, in the scaffold-treated groups compared with the control group (Figures 11C–11E and 12A–12C). Gene expression levels were normalized to those of control BMSCs at day 0. Both the ECM/PCL and ECM/E7-PCL groups exhibited a time-dependent increase in chondrogenic gene expression during the culture period. The expression of key chondrogenic markers progressively increased and reached maximal levels at day 14. Subsequently, expression levels gradually declined by day 21, indicating a temporal change in chondrogenic marker expression during culture.

3.7. *In vivo* assessment of chondrogenic regeneration

The cartilage repair performance of the composite scaffolds was assessed using gross observation, ICRS scoring, MRI, and histological analyses. At six weeks after implantation, defects in the untreated control group remained clearly visible, exhibiting distinct boundaries from the surrounding native cartilage and surface depression (Figure 13A and Table 2). In contrast, both scaffold-treated groups displayed newly formed cartilage-like tissue within the defect area. Quantitative ICRS assessment demonstrated significantly higher repair scores in the ECM/E7-PCL group than in the ECM/PCL and control groups ($p < 0.05$; Figure 13B), indicating improved early-stage cartilage repair outcomes. MRI findings were consistent with the macroscopic observations (Figure 13C). At six weeks, the ECM/E7-PCL group exhibited more extensive defect filling and greater tissue thickness than the ECM/PCL and control groups. The regenerated tissue in the ECM/E7-PCL group also displayed imaging characteristics more closely resembling those of native cartilage. At 12 weeks, defects

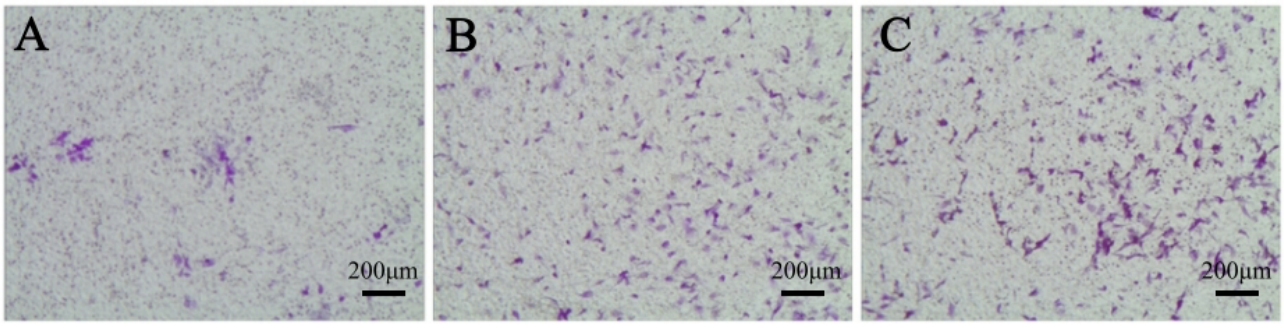

Figure 10. Optical microscopy images of the Transwell migration assay showing the migration of bone marrow mesenchymal stem cells after 24 h of incubation in the (A) control, (B) ECM/PCL, and (C) ECM/E7-PCL groups (scale bar = 200 μm; magnification = 10×)
Abbreviations: ECM: Extracellular matrix; PCL: Polycaprolactone.

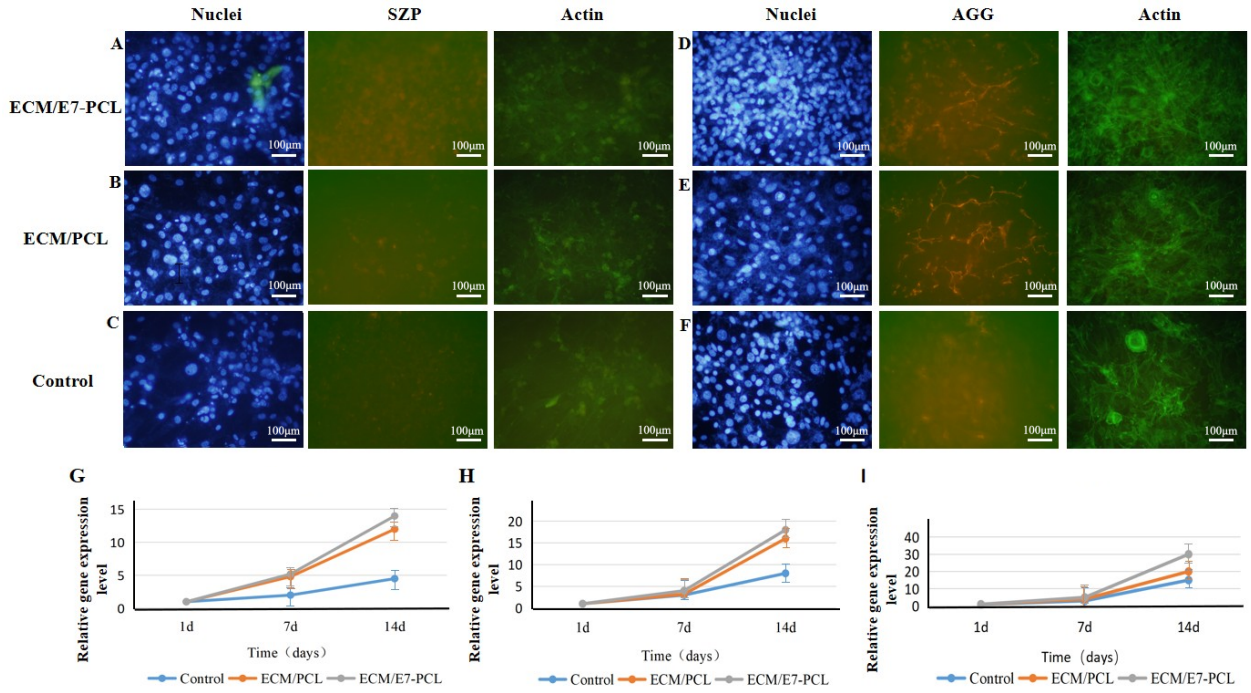

Figure 11. *In vitro* chondrogenic differentiation of bone marrow mesenchymal stem cells seeded on composite scaffolds. (A–C) Immunofluorescence staining of superficial zone protein (SZP); (D–F) Immunofluorescence staining of aggrecan (AGG) after 14 days of chondrogenic induction. (A,D): ECM/E7-PCL group; (B,E): ECM/PCL group; (C,F): Control group (scale bar = 100 μm; 20× magnification). (G–I) Quantitative analysis illustrating time-course changes in chondrogenic gene expression: (G) Chondrogenic markers *SOX9*, (H) *COL2A1*, and (I) *ACAN* of cells cultured on various scaffolds at culture days 1, 7 and 14.
Abbreviations: AGG: Aggrecan; ECM: Extracellular matrix; PCL: Polycaprolactone; SZP: Superficial zone protein.

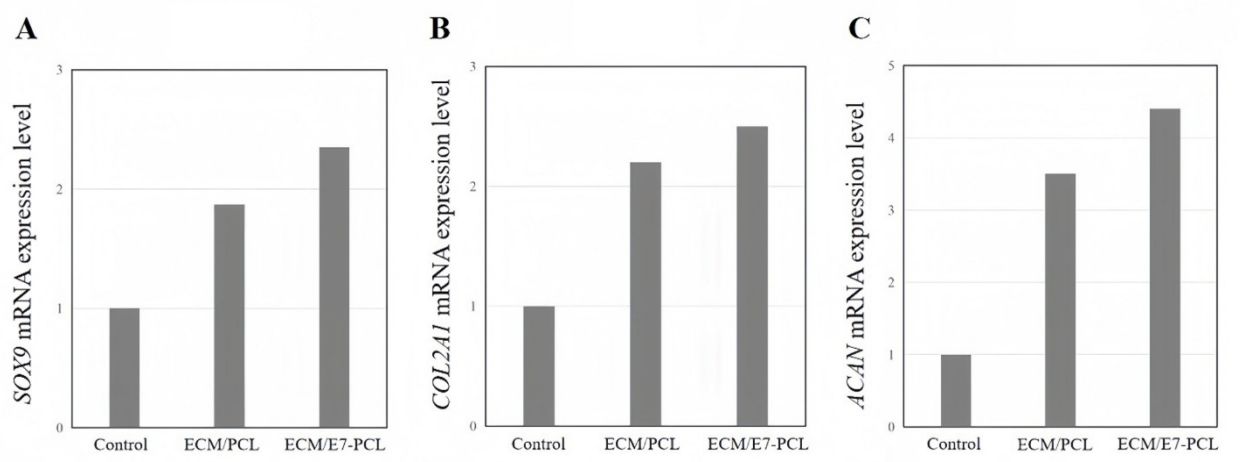

Figure 12. Relative mRNA expression levels of chondrogenic markers, (A) *SOX9;* (B) *COL2A1;* and (C) *ACAN* were determined by quantitative reverse transcription polymerase chain reaction. The mRNA expression levels were normalized to the reference gene *GAPDH* using the $2^{-\Delta\Delta Ct}$ method, and the expression level of the control group was set as the reference value (normalized to 1). All quantitative data are presented as mean ± standard error of the mean with four biological replicates ($n = 4$). Statistical significance was evaluated using one-way ANOVA followed by Tukey's multiple comparison test.
Abbreviations: ECM: Extracellular matrix; PCL: Polycaprolactone.

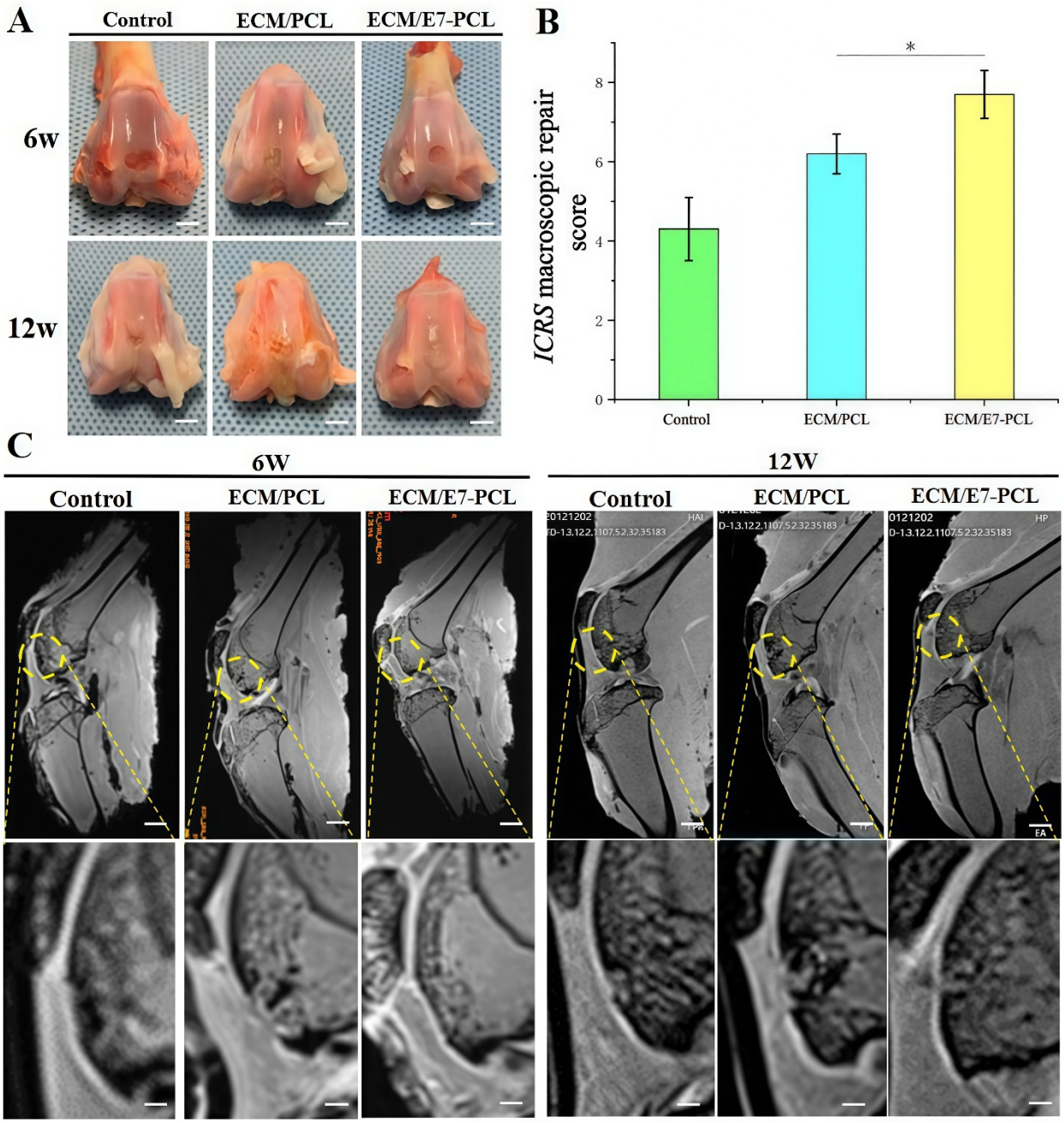

Figure 13. Gross observation, ICRS scoring, and MRI evaluation of cartilage repair at 6 and 12 weeks postoperatively. (A) Macroscopic appearance of repaired cartilage in the control, ECM/PCL, and ECM/E7-PCL groups at 6 and 12 weeks (scale bar = 5 mm). (B) ICRS macroscopic scores of cartilage repair in each group at 12 weeks. (C) MRI images showing the evaluation of repaired cartilage at 6 and 12 weeks. Yellow dashed circles indicate the defect sites (scale bar = 5 mm for full-field images; scale bar = 2 mm for magnified images). *$p < 0.05$ indicates statistical significance.
Abbreviations: ECM: Extracellular matrix; ICRS: International Cartilage Regeneration and Joint Preservation Society; MRI: Magnetic resonance imaging; PCL: Polycaprolactone.

Table 2. Macroscopic and histological evaluation scores of femoral trochlear cartilage defect repair in control, ECM/PCL, and ECM/E7-PCL groups

Group	ICRS score	O'Driscoll score
Control group at 6W	2.83 ±.037	3.67 ±.197
Control group at 12W	3.33 ±.047	5.50 ±.096
ECM/PCL group at 6W	4.65 ±.053	5.75 ±.154
ECM/PCL group at 12W	7.23 ±.066	9.62 ±.085
ECM/E7-PCL group at 6W	8.00 ±.081	16.17 ±.157
ECM/E7-PCL group at 12W	10.67 ±.075	21.83 ±.186

Abbreviations: ECM: Extracellular matrix; ICRS: International Cartilage Regeneration and Joint Preservation Society; O'Driscoll score: O'Driscoll Cartilage Repair Scoring System; PCL: Polycaprolactone.

in the control group remained readily identifiable and were predominantly filled with fibrous or fibrocartilaginous repair tissue. In contrast, both scaffold-treated groups showed substantial restoration of the articular surface.

Although a slight surface depression remained in the ECM/PCL group, the ECM/E7-PCL group exhibited nearly complete restoration of the defect region with a smooth and continuous articular surface. Histological analyses further demonstrated the enhanced cartilage repair characteristics of the ECM/E7-PCL scaffold (Figure 14). At six weeks, the ECM/E7-PCL group displayed more abundant and thicker cartilage-like tissue formation than the ECM/PCL group (Figure 14A). By 12 weeks, the defect area in the ECM/E7-PCL group was almost completely filled with cartilage-like tissue exhibiting characteristics similar to native articular cartilage (Figure 14B). The interface between regenerated and surrounding cartilage was largely indistinguishable, and a well-defined tidemark was observed, indicating successful integration with the underlying subchondral bone.

4. Discussion

In the present study, we developed a biomimetic ternary composite scaffold consisting of cartilage-derived ECM, an E7 BMSC-affinity peptide, and a 3D-printed PCL framework for the repair of articular cartilage defects. The scaffold was systematically characterized in terms of its physicochemical properties, cytocompatibility, and cartilage-regenerative capacity both *in vitro* and *in vivo*. Our findings demonstrate that the integration of these three functional components creates a synergistic regenerative platform that combines mechanical support, targeted stem-cell recruitment, and biomimetic biochemical signaling. This multifunctional design addresses several key limitations of conventional scaffold systems and provides a promising strategy for cell-free cartilage tissue engineering.

Polycaprolactone has been widely used as a structural material for cartilage tissue engineering owing to its excellent processability, favorable biocompatibility, and tunable mechanical properties. However, the hydrophobic nature of PCL and its lack of intrinsic biological recognition sites limit cellular attachment, migration, and differentiation. Consequently, pure PCL scaffolds often exhibit insufficient bioactivity to effectively support cartilage regeneration, resulting in the formation of fibrocartilaginous repair tissue rather than mechanically competent hyaline cartilage-like tissue.[33,34] To address these limitations, we introduced E7 affinity peptides and cartilage-derived ECM into the PCL scaffold. Covalent immobilization of the E7 peptide significantly enhanced BMSC adhesion and recruitment, while ECM incorporation provided a biologically relevant microenvironment enriched with cartilage-specific matrix components, including collagens and GAGs. The *in vitro* findings support this synergistic effect. Compared with unmodified PCL, the composite scaffold promoted greater cell attachment and proliferation and significantly enhanced the expression of chondrogenic markers, including *COL2A1*, *ACAN*, and *SOX9*. These findings are consistent with previous studies demonstrating that affinity-peptide functionalization can improve stem-cell targeting and retention while preserving the chondrogenic phenotype.[26] Moreover, the ECM component provided structural and biochemical cues that closely mimic the native cartilage microenvironment, thereby promoting cartilage-associated matrix synthesis and differentiation-related responses.[35] However, these *in vitro* findings cannot be directly extrapolated to the complex *in vivo* joint microenvironment.

Rather than acting independently, the peptide and ECM components appear to function cooperatively: E7-mediated recruitment increases the availability of reparative cells at the defect site, whereas the ECM

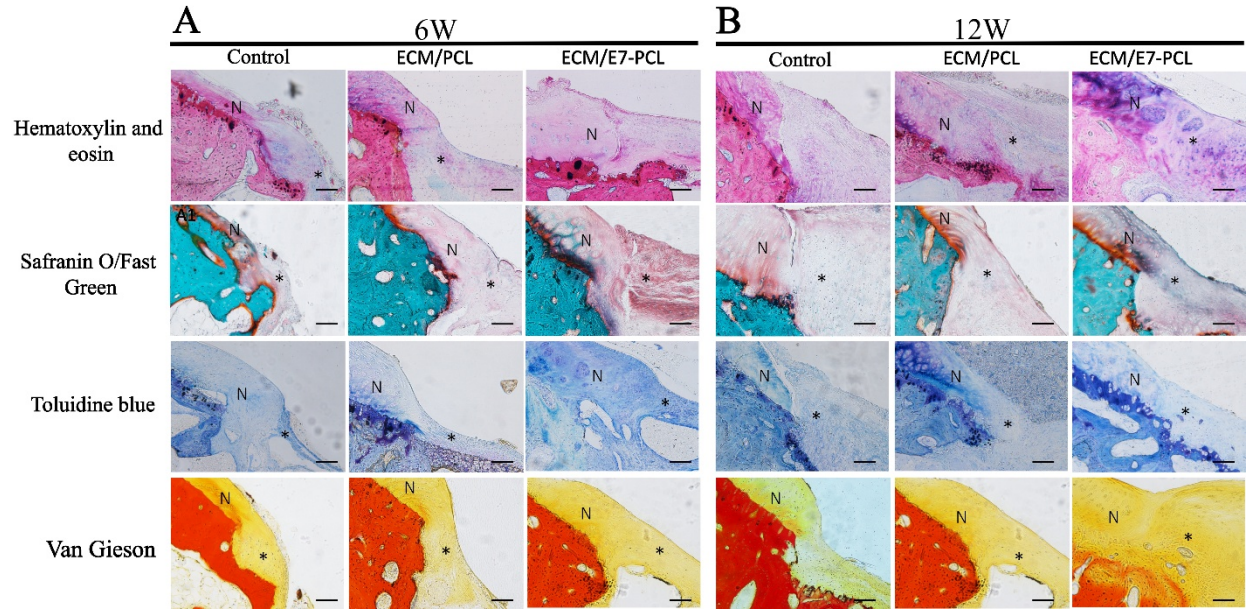

Figure 14. Representative histological images of the repaired cartilage defect regions in the control, ECM/PCL, and ECM/E7-PCL groups at (A) 6 weeks and (B) 12 weeks postoperatively, obtained using hematoxylin and eosin, Safranin O/Fast Green, toluidine blue, and aggrecan immunostaining. "N" indicates native cartilage, and asterisks (*) denote the original defect sites (scale bar = 200 μm; magnification = 10×).
Abbreviations: ECM: Extracellular matrix; PCL: Polycaprolactone

provides the biological signals required to direct their differentiation toward a chondrogenic lineage. Among the various MSC populations investigated for cartilage regeneration, BMSCs remain one of the most extensively studied owing to their robust proliferative capacity, multilineage differentiation potential, and accessibility. Increasing attention has therefore been directed toward strategies that recruit endogenous BMSCs rather than relying on *ex vivo* cell expansion and transplantation.[36] Although endogenous BMSCs can migrate toward sites of injury, their regenerative contribution is often limited by poor cell retention, insufficient homing efficiency, and an unfavorable local microenvironment. As a result, endogenous cell recruitment represents an attractive alternative to conventional cell-based therapies because it reduces manufacturing complexity, lowers treatment costs, minimizes immunological concerns, and avoids regulatory challenges associated with cell transplantation.[27] The success of such approaches depends largely on the ability of biomaterials to actively recruit, retain, and regulate endogenous progenitor cells. Accordingly, the development of scaffolds that simultaneously provide structural support and biological guidance represents an important direction in cartilage tissue engineering.[28]

The structural characteristics of the scaffold also contributed substantially to its regenerative performance. An interconnected porous architecture is essential for facilitating nutrient transport, waste removal, cell migration, and matrix deposition within engineered cartilage constructs.[34] The 3D-printed PCL framework used in this study maintained a well-defined porous structure and adequate compressive strength, thereby providing mechanical stability during the early stages of tissue repair.[37] Meanwhile, incorporation of cartilage-derived ECM increased scaffold hydrophilicity and water-retention capacity, creating a more favorable microenvironment for cell survival and matrix production.[38] The enhanced water absorption observed in the ECM/E7-PCL scaffold may further promote the retention of endogenous cells and bioactive molecules within the defect site. Importantly, the addition of E7 affinity peptides significantly improved BMSC recruitment compared with the ECM/PCL scaffold alone, suggesting that selective cell targeting is a critical determinant of scaffold performance. Notably, although E7 affinity peptides significantly enhanced BMSC recruitment *in vitro*, whether this effect translates into improved endogenous stem cell homing and retention within cartilage defects *in vivo* remains to be established.

In vivo evaluation demonstrated that, by 12 weeks postoperatively, defects treated with the ECM/E7-PCL composite scaffold were largely filled with cartilage-like

tissue exhibiting hyaline cartilage. Histological analyses revealed significantly higher repair scores in the ECM/E7-PCL group than in the control and ECM/PCL groups, and the regenerated tissue exhibited continuous integration with the surrounding native cartilage without evidence of adverse inflammatory responses. In the *in vivo* experiments, to better preserve the structural integrity of the regenerated cartilage, histological staining was conducted using resin-embedded hard tissue sections, in which the regenerated region was hardened with resin prior to staining. Compared with conventional paraffin sections, hard tissue sections have inherent limitations in staining contrast and detail presentation. Nevertheless, the staining results still provide valid histological evidence to support that the composite scaffold is conducive to cartilage tissue regeneration and repair. Future studies should further optimize the tissue decalcification, embedding, and staining procedures to improve staining quality and more clearly visualize the components and structure of the regenerated tissue.

Consistent findings from gross observation, MRI, and histological assessments collectively indicated that the ECM/E7-PCL scaffold achieved enhanced cartilage repair outcomes compared with both untreated defects and ECM/PCL scaffolds. It should be noted that these *in vivo* findings are limited to young, healthy rabbits, and the repair efficacy of the scaffold in aged osteoarthritis pathological models has not been evaluated. Notably, although the regenerated cartilage exhibited similarities with native articular cartilage, morphological differences remained compared with intact hyaline cartilage. This discrepancy is most likely associated with the observation duration, as the formation of fully mature hyaline cartilage requires extended periods of regeneration and tissue remodeling, which aligns with the inherently limited regenerative capacity of articular cartilage. These results suggest that the synergistic combination of mechanical support, targeted stem-cell recruitment, and ECM-derived biological cues may help overcome several limitations associated with spontaneous cartilage healing and improve tissue regeneration quality.[39] Unlike conventional cell-based therapies that require exogenous cell isolation, expansion, and transplantation, the present strategy aims to utilize stem-cell recruitment and regulation. This approach may reduce many of the challenges associated with cell manufacturing, including contamination risks, phenotypic instability during *in vitro* expansion, immunological concerns, and regulatory complexity. Consequently, endogenous regeneration-based therapies may offer advantages in terms of procedural simplicity, scalability, and clinical feasibility.[40]

Despite these encouraging findings, several limitations should be acknowledged. Although the E7 affinity peptide significantly enhanced endogenous stem-cell recruitment, its grafting density, spatial distribution, and long-term stability were not systematically investigated. Future studies should optimize peptide immobilization strategies and clarify how peptide density and surface presentation influence biological performance. In addition, while the decellularized cartilage ECM retained key matrix components, further standardization of the decellularization and purification procedures is required to improve batch-to-batch consistency and ensure rigorous control of residual nucleic acids and antigenicity. From a translational perspective, the rabbit osteochondral defect model cannot fully reproduce the mechanical loading conditions, defect dimensions, and long-term repair processes of human joints. Moreover, although the PCL framework provided adequate structural support in the present study, its mechanical properties may be insufficient for high-load-bearing applications, highlighting the need for composite or hybrid biomaterials with improved mechanical performance while preserving favorable bioactivity. Methodologically, long-term scaffold degradation, degradation-associated immune responses, and the dynamic processes of cell behavior and tissue remodeling were not systematically monitored, limiting the evaluation of long-term biosafety and regenerative mechanisms. Furthermore, matrix characterization relied primarily on biochemical assays and semi-quantitative histological analyses, whereas advanced techniques such as spatial proteomics or Raman spectroscopy were not employed to assess the spatial heterogeneity and compositional maturation of regenerated cartilage. Finally, the biological performance of the scaffold was evaluated only using rabbit BMSCs under standard culture conditions without multicellular co-culture systems or osteoarthritis-related inflammatory stimulation, leaving its regenerative potential in the complex pathological joint microenvironment to be further established. Collectively, addressing these limitations will facilitate the optimization of scaffold design and provide a stronger foundation for future preclinical development and potential clinical translation.

5. Conclusion

In this study, we developed a biomimetic composite scaffold comprising a 3D-printed PCL framework, an E7 BMSC-affinity peptide, and cartilage-derived ECM for articular cartilage regeneration. Functionalization with the E7 peptide enhanced the biological activity of the scaffold and significantly improved its ability to recruit and retain BMSCs. The incorporation of cartilage-derived ECM further provided a biomimetic microenvironment that

supported cell adhesion, chondrogenic differentiation, and ECM deposition. The resulting composite scaffold exhibited favorable mechanical properties, excellent biocompatibility, and enhanced regenerative potential both *in vitro* and *in vivo*. Importantly, implantation of the ECM/E7-PCL scaffold promoted the formation of cartilage-like tissue with improved integration characteristics and achieved enhanced repair outcomes compared with control treatments. Within the scope of the current data and its inherent experimental limitations, these findings demonstrate that the combined strategy of mechanical stabilization, targeted endogenous stem-cell recruitment, and ECM-mediated biological induction represents a promising strategy for cartilage regeneration.

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Conflict of interest

The authors declare they have no competing interests.

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Formal analysis: Xinqiang Zhao
Investigation: Xinqiang Zhao
Methodology: Xinqiang Zhao
Project administration: Kun Ding
Resources: Yantao Zhao, Zhenggang Bi
Software: Xinqiang Zhao
Supervision: Yantao Zhao, Zhenggang Bi
Validation: Xinqiang Zhao
Visualization: Zhen Huang
Writing–original draft: Xinqiang Zhao
Writing–review & editing: Xiaoning Liu, Yantao Zhao, Zhenggang Bi
All authors have read and approved the final manuscript.

Ethics approval and consent to participate

This study was approved by the Animal Care and Use Committee of Beijing Vital River Laboratory Animal Technology Co., Ltd. (Approval No. AWE2022060305).

Consent for publication

Not applicable.

Availability of data

The datasets generated and/or analyzed during the current study are available from the corresponding author on reasonable request.

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Appendix

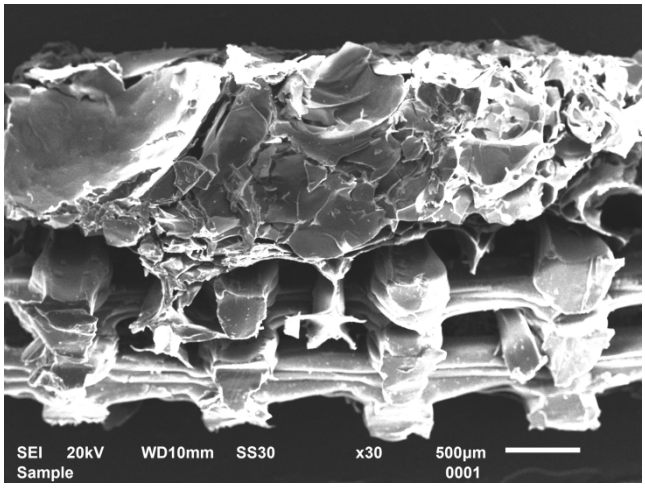

Figure A1. Representative scanning electron microscopy image showing the interfacial structure of the ECM/E7-PCL scaffold (scale bar = 500 μm; magnification = 30×). Abbreviations: ECM: Extracellular matrix; PCL: Polycaprolactone.