Supplementary Materials

Hydrogel preparation

Synthesis of Silk Fibroin (SF) solution: SF was prepared according to a previous method, with slight modification of the extraction procedure.[1] Briefly, after *Bombyx mori* silk cocoons were cut into pieces, they were boiled for 30 min in 0.5% (m/v) Na_2CO_3 solution and rinsed with deionized water to extract any impurities. The degummed SF solution was dissolved in 9.3 M LiBr solution. The LiBr was removed from the solution using dialysis cassettes with a 8–14-kDa molecular-weight cutoff for 3 days. The final concentration of SF solution was 6% (w/v), determined from the dry weight.

Synthesis of cellulose nanocrystals (CNCs): CNCs were prepared by the acid hydrolysis of microcrystalline cellulose (MCC), as reported in a previous study.[2] MCC (10 g) was hydrolyzed with 65 wt% sulfuric acid at 55 °C for 5 h. A five-fold volume of deionized water was added to the flask to terminate the reaction. The suspension was then dialyzed against distilled water to produce a neutral aqueous solution and freeze-dried to a white powder.

Synthesis of ultrasmall superparamagnetic iron oxide (USPIO): USPIO nanoparticles were synthesized with a modified hydrothermal approach.[3] Briefly, 1.789 g of $FeCl_2 \cdot 4H_2O$ and 1.5 g of polyethylene glycol were each dissolved in 15 ml of deionized water and then mixed together to produce a homogeneous suspension. Then 2.5% $NH_3 \cdot H_2O$ (17 mL) was added dropwise to the whole mixture, followed by 3% H_2O_2 (5 mL) and 50 mL of deionized water. The pH was adjusted to 11 with dilute NaOH solution. The solution was then transferred to a thermal water kettle and reacted for 1 h

at 60 °C. The USPIO nanoparticles were rinsed several times with distilled water and separated from the solution with an external magnetic field.

Isolation and culture of bone-marrow mesenchymal stem cells (BMSCs)

All animal experiments were approved by the Medical Ethics Committee on Animal Care at Southern Medical University. BMSCs were isolated from 4-week-old New Zealand white rabbits and expanded by density gradient centrifugation according to a previous description.[4] Briefly, 4–5 mL of bone marrow was aspirated from the tibia with a 16-gauge needle rinsed in heparin. The mononuclear cells (MNCs) were separated with Ficoll–Paque centrifugation at 400 ×*g* for 20 min. The MNCs were expanded in growth medium containing α-MEM, 10% (v/v) FBS, and 1% (v/v) penicillin–streptomycin at 37 °C under 5% CO_2. Nonadherent cells were removed after incubation for 48 h. At 80%–90% confluence, the BMSCs were serially passagedand passage 3 was used for all experiments. To induce chondrogenesis *in vitro*, the hydrogels were seeded with 50 μL of the suspension (BMSCs density, 2×10^7 mL^{-1}). The chondrogenic medium was carefully changed every 3 days.

Cytotoxicity

The cytotoxicity of the prepared hydrogels [CNC/SF with 0%−0.6% (w/w) USPIO] was evaluated with a Cell Counting Kit-8 at 450 nm on days 1, 4, 7, and 10 after seeding. BMSCs were seeded on a 48-well plate with 2×10^3 cells per well. The hydrogels of the different experimental groups were placed into the appropriate wells, and BMSCs cultured in wells without hydrogel were used as the control group. At each indicated point, the optical density was measured with a SpectraMax® M5 multi-mode microplate

reader (Molecular Devices, USA).

Biochemical analysis

Glycosaminoglycan (GAG) measurement: A 1,9-dimethylmethylene blue (DMMB) dye binding assay was used to evaluate the GAG content of the non-labeled and 0.1% (w/w) USPIO-labeled BMSC-encapsulating hydrogels *in vitro*. The specimens were collected at days 7, 14 and 28, and digested with papain solution (1 mg mL^{-1} papain in 0.1 M phosphate buffer with 5 mM L-cysteine hydrochloride and 5 mM EDTA) for 18 h at 60 °C. After centrifugation at 1000 ×*g* for 15min, 20 μL of supernatant from each specimen was mixed with 200 μL of DMMB dye (16 mg of DMMB in 1 L of water containing 3.04 g of glycine, 2.37 g of NaCl, and 95 mL of 0.1 M hydrochloric acid) for 30 min at 37 °C. The GAG content was standardized using shark chondroitin 6-sulfate. The DNA content was measured with Hoechst 33258 diluent and normalized to a certified calf thymus DNA standard. Absorbance and fluorescence values were immediately determined after the incubation time using a SpectraMax® M5 multi-mode microplate reader (Molecular Devices).

Cartilage-specific gene expression analysis: Gene expression differences between the non-labeled and 0.1% (w/w) USPIO-labeled hydrogel constructs loaded with BMSCs were analyzed with quantitative real-time reverse transcription–polymerase chain reaction on days 7, 14, and 28. Total RNA was isolated with TRIzol Reagent, quantified with the 260/280 absorbance ratio on a NanoDrop ND1000 Spectrophotometer (Thermo-Fisher Scientific, Massachusetts, USA) and reverse-transcribed to cDNA with PrimeScript™ RT Master Mix. The cDNA was used as the rt–qPCR template with

SYBR® Premix Ex Taq™ in the LightCycler®480 Real Time PCR System (LightCycler®480, Roche, Switzerland) to compare the expression of different genes. Collagen II, aggrecan, Sox9, and collagen I were the target genes and glyceraldehyde 3-phosphate dehydrogenase (GAPDH) was the internal control. The specific primers are listed in Table S2. Gene expression levels were calculated with the ΔΔCt method.

Rabbit cartilage defect model

All animal procedures were approved by the Institutional Animal Care and Use Committee of Nanfang Hospital, Southern Medical University. Thirty-two New Zealand White rabbits (2.0–2.5 kg, Guangdong Medical Laboratory Animal Center, Guangdong, China) were used for the *in vivo* experiment and were randomly divided into four groups (four knees per group): non-labeled hydrogel, 0.1% (w/w) USPIO-labeled hydrogel, non-labeled hydrogel with BMSCs, and 0.1% (w/w) USPIO-labeled hydrogel with BMSCs. The BMSCs-loaded (2×10^7 mL^{-1}) constructs were precultured in chondrogenic medium for 2 weeks before implantation. After the lateral dislocation of the patellar and medial parapatellar arthrotomy, bilateral critical partial-thickness osteochondral defects (3.5 mm in diameter and 3 mm in depth) were created centrally in the trochlear groove without blood exudation, using an electric drill under general anesthesia. Sterile isotonic saline (0.9%) was added dropwise to the defect during drilling to avoid heat damage. The corresponding hydrogels or cell–hydrogel constructs were implanted at the defect sites. The rabbits were injected with penicillin to prevent infection and allowed to move freely after waking.

International cartilage repair society (ICRS) macroscopic scores for repaired

cartilage

Gross images of the harvested femur condyles were taken to evaluate cartilage regeneration in weeks 8 and 12, and the ICRS macroscopic scoring system was used as described in Table S3.[5] All regeneration was scored by three independent observers blinded to the experimental groups.

References

1. Bhardwaj N, Nguyen QT, Chen AC, Kaplan DL, Sah RL, Kundu SC. Potential of 3-D tissue constructs engineered from bovine chondrocytes/silk fibroin-chitosan for in vitro cartilage tissue engineering. Biomaterials. 2011; 32: 5773-81.

2. Mo Y, Guo R, Liu J, Lan Y, Zhang Y, Xue W, et al. Preparation and properties of PLGA nanofiber membranes reinforced with cellulose nanocrystals. Colloids Surf B Biointerfaces. 2015; 132: 177-84.

3. Mertens ME, Frese J, Bolukbas DA, Hrdlicka L, Golombek S, Koch S, et al. FMN-coated fluorescent USPIO for cell labeling and non-invasive MR imaging in tissue engineering. Theranostics. 2014; 4: 1002-13.

4. Kadiyala S, Young RG, Thiede MA, Bruder SP. Culture expanded canine mesenchymal stem cells possess osteochondrogenic potential in vivo and in vitro. Cell Transplant. 1997; 6: 125-34.

5. Li X, Ding J, Zhang Z, Yang M, Yu J, Wang J, et al. Kartogenin-Incorporated Thermogel Supports Stem Cells for Significant Cartilage Regeneration. ACS Appl Mater Interfaces. 2016; 8: 5148-59.

Figure Captions

Figure S1. Morphology of nanoparticles and hydrogel fabrication. (A) TEM of USPIO. Scale bar indicates 100 μm. (B) TEM of CNC. Scale bar indicates 20 μm. (C) Sol–gel transition process at room temperature.

Figure S2. Extracellular matrix formation and cartilage-specific gene expression in the non-labeled and USPIO-labeled hydrogels. (A) GAG contents during *in vitro* chondrogenesis on days 7, 14, and 28 in non-labeled and 0.1% (w/w) USPIO-labeled hydrogels. (B) Expression of genes encoding collagen II, aggrecan, Sox9, and collagen I during *in vitro* chondrogenesis on days 7, 14, and 28 in non-labeled and 0.1% (w/w) USPIO-labeled hydrogels. Data are presented as means ± SD (n = 3, *p < 0.05, **p < 0.01 ***p < 0.001).

Figure S3. ICRS macroscopic evaluation of the repaired cartilage in week 8 (A) and week 12 (B). Data are presented as means ± SD (n = 4, *p < 0.05, **P < 0.01 ***p < 0.001).

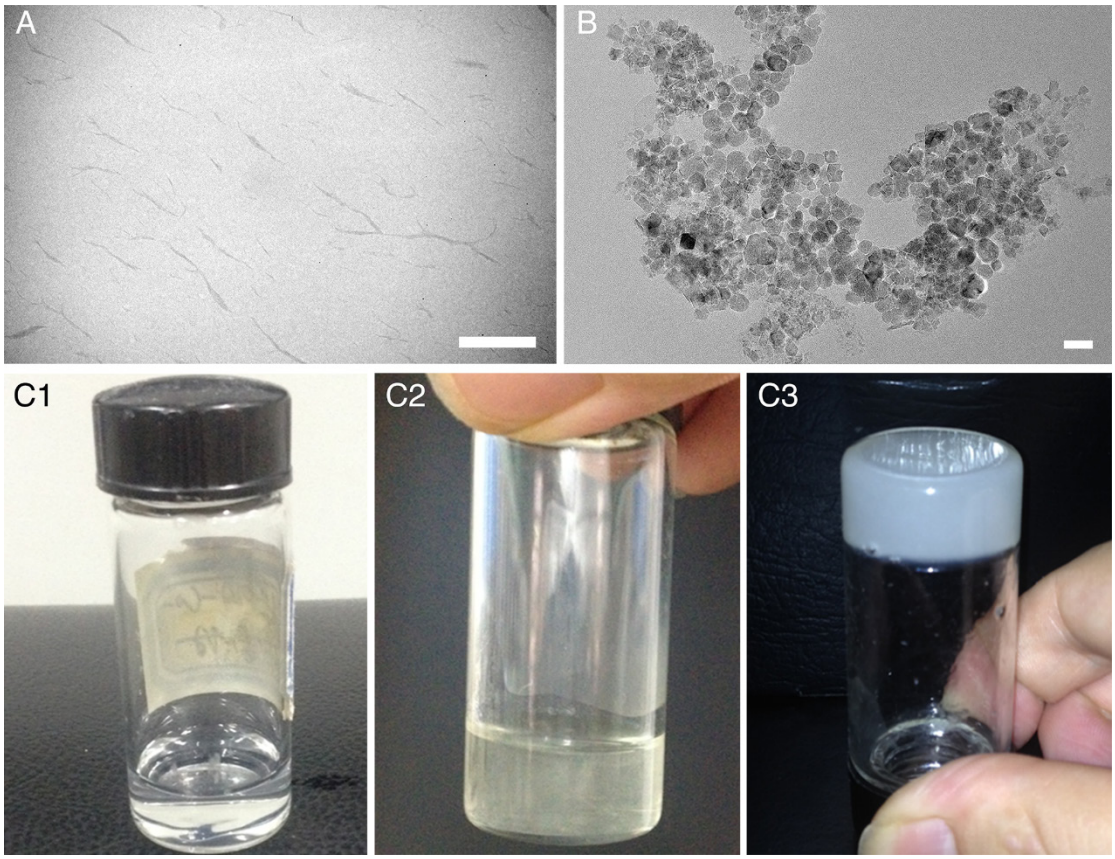

Figure S1

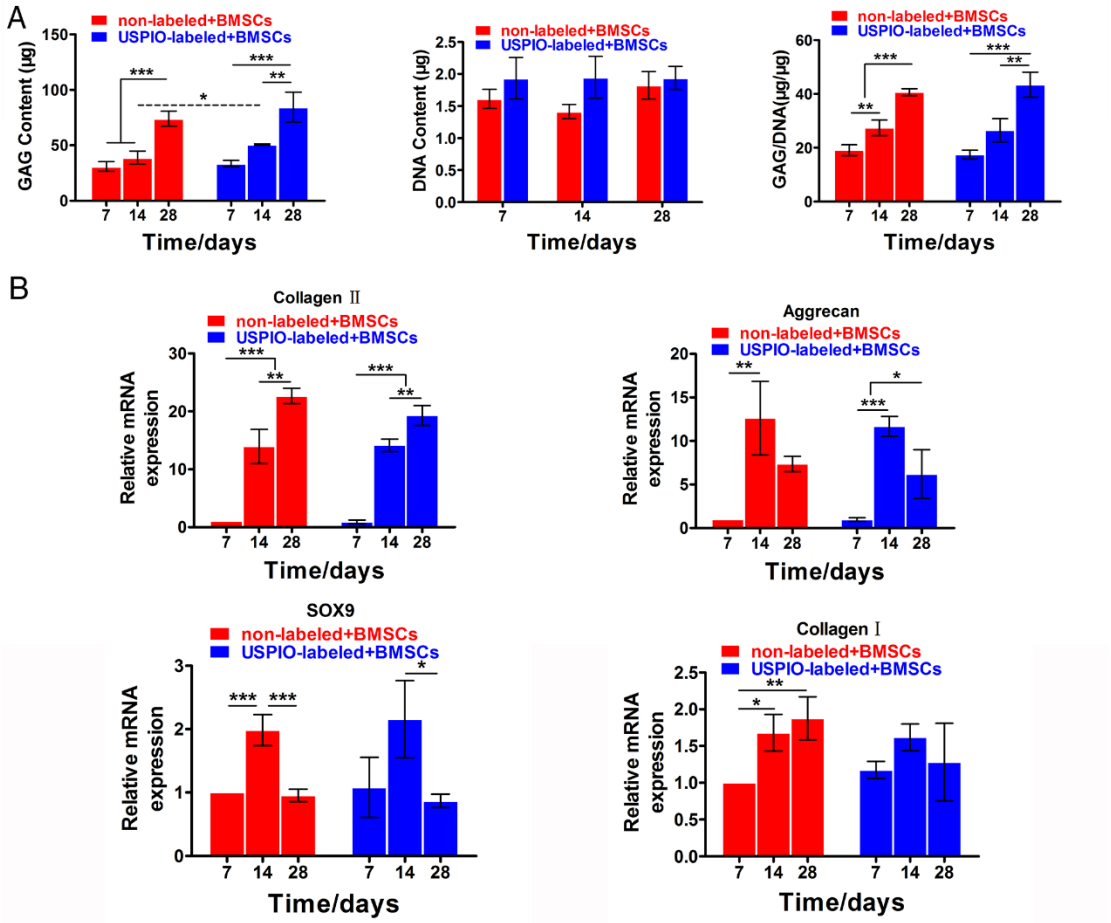
A
non-labeled+BMSCs
USPIO-labeled+BMSCs
GAG Content (μg)
DNA Content (μg)
GAG/DNA(μg/μg)
Time/days
B
Collagen Ⅱ
Aggrecan
SOX9
Collagen Ⅰ
Relative mRNA expression
Time/days

Figure S2

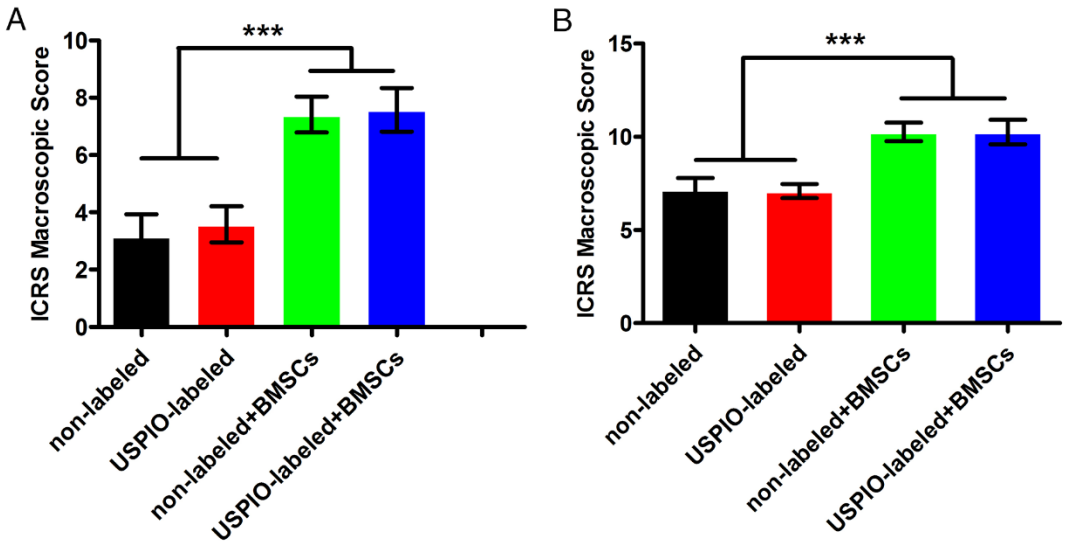
A
ICRS Macroscopic Score
10
8
6
4
2
0
***
non-labeled
USPIO-labeled
non-labeled+BMSCs
USPIO-labeled+BMSCs
B
ICRS Macroscopic Score
15
10
5
0
***
non-labeled
USPIO-labeled
non-labeled+BMSCs
USPIO-labeled+BMSCs

Figure S3

Table S1 USPIO contents and pore sizes of non-labeled and USPIO-labeled CNC/SF hydrogels.

	Fe (μg)	USPIO % (w/w)	Pore diameter (μm)
non-labeled	0	0	82.3±21.1
	13.12	0.1	81.1±25.0
	28.70	0.2	81.3±26.0
USPIO-labeled	41.35	0.3	78.3±21.7
	52.08	0.4	83.9±23.0
	67.90	0.6	85.1±22.4

Table S2 Primers for rt-qPCR.

Genes	Forward primer (5'->3')	Reverse primer (5'->3')
Collagen II	ACAGTCTTGCCCCACTTACCG	GCTCCCAGAACATCACCTACC
Aggrecan	CTCCAGAAACCAGGTCAGGGA	GGTCCACCATTCGGCATAACT
Sox9	GCTGTTTCTTCGGTCACTTTG	CAGCCTCTACTCCACCTTCAC
Collagen I	GAGATGAATGCAACGGCAAAA	CACCCCAGAAACAGACGACAA
GAPDH	TGGGATGGAAACTGTGAAGAG	TTTGGCTACAGCAACAGGGTG

Table S3 ICRS macroscopic evaluation of cartilage repair.

Characteristic	Grading	Score
Degree of defect repair	In level with surrounding cartilage	4
	75% repair of defect depth	3
	50% repair of defect depth	2
	25% repair of defect depth	1
	0% repair of defect depth	0
Integration to border zone	Complete integration with surrounding cartilage	4
	Demarcating border <1mm	3
	3/4 of graft integrated with surrounding	2
	With a notable border >1mm width and 1/2 of graft integrated with surrounding	1
	From no contact to 1/4 of graft integrated with surrounding cartilage	0
Macroscopic appearance	Intact smooth surface	4
	Fibrillated surface	3
	Small, scattered fissures or cracks	2
	Several, small or few but large fissures	1
	Total degeneration of grafted area	0
Overall repair assessment	Grade I: normal	12
	Grade II: nearly normal	11-8
	Grade III: abnormal	7-4
	Grade IV: severely abnormal	3-1