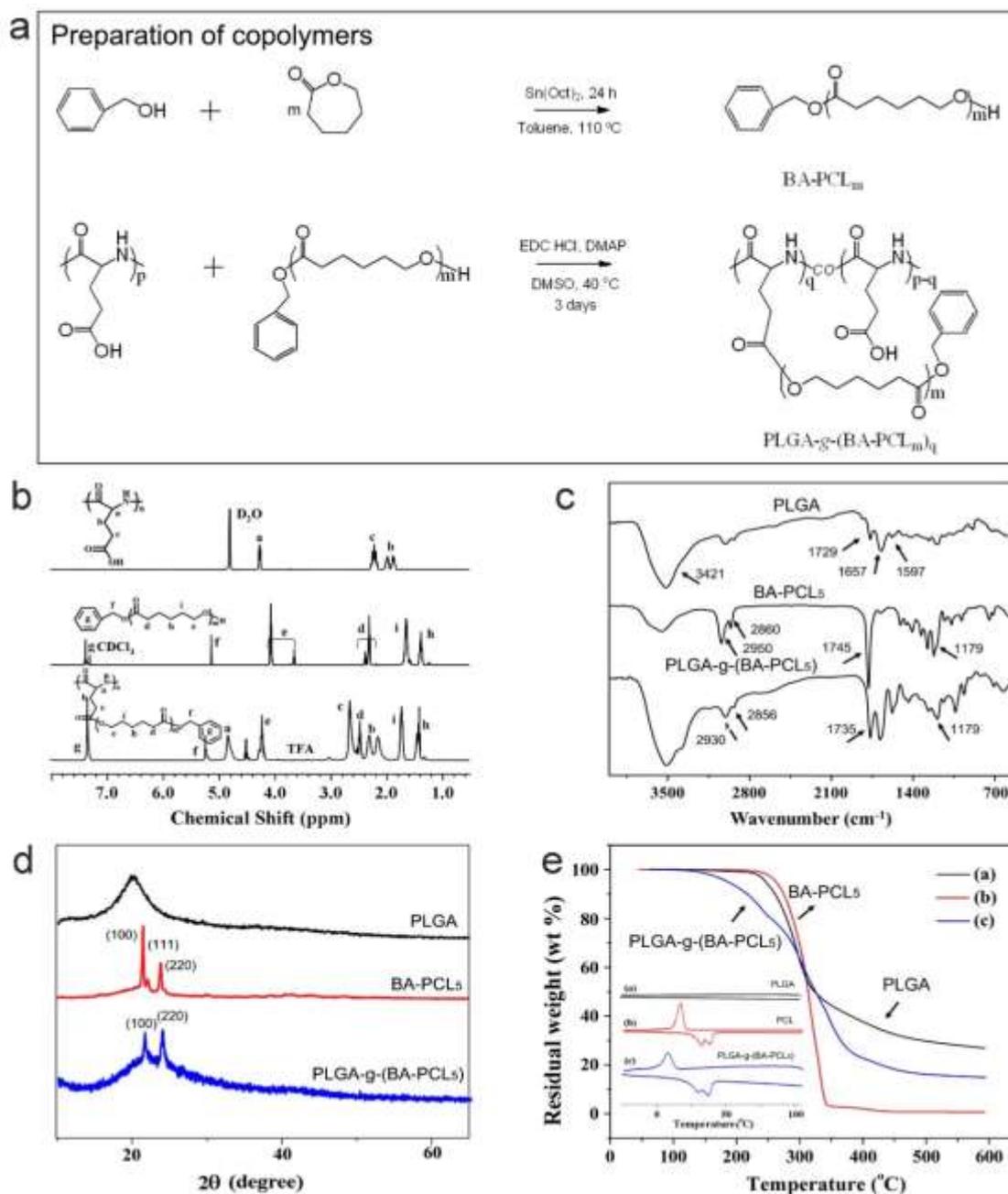


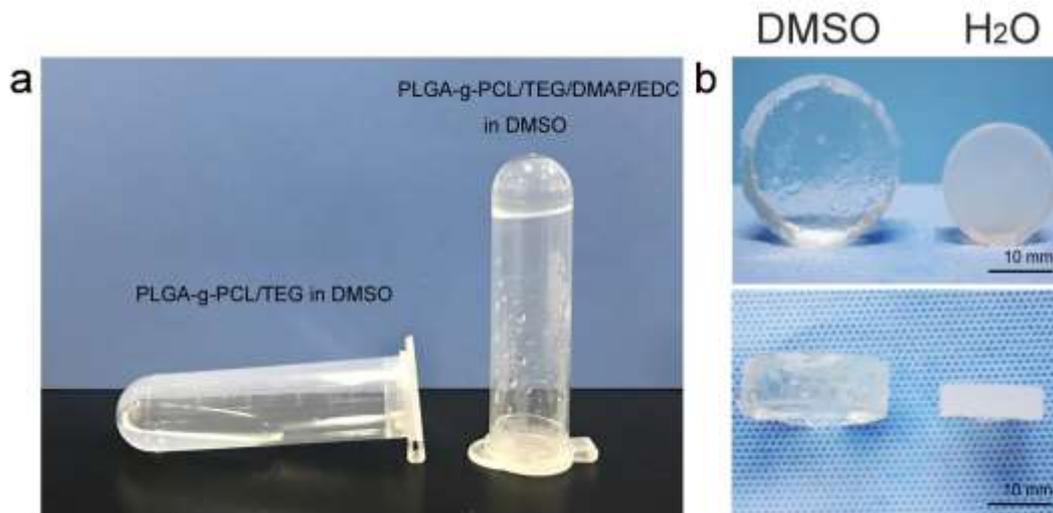
Supplementary Information

In-situ formation of hydrophobic clusters to enhance mechanical performance of biodegradable poly (L-glutamic acid)/poly ( $\epsilon$ -caprolactone) hydrogel towards meniscus tissue engineering†

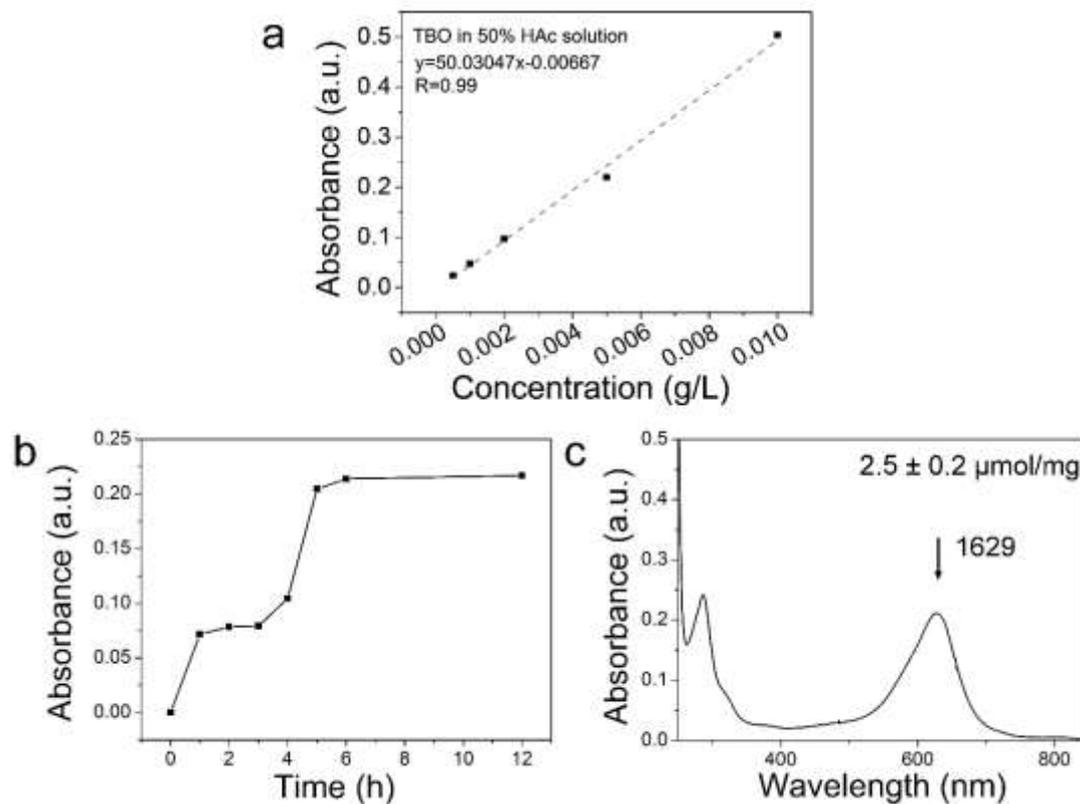
Kunxi Zhang,<sup>a</sup> Jie Wu,<sup>a</sup> Weijun Zhang,<sup>a</sup> Shifeng Yan,<sup>a</sup> Jianxun Ding,<sup>b</sup> Xuesi Chen,<sup>b</sup> Lei Cui<sup>\*c</sup> and Jingbo Yin<sup>\*a</sup>



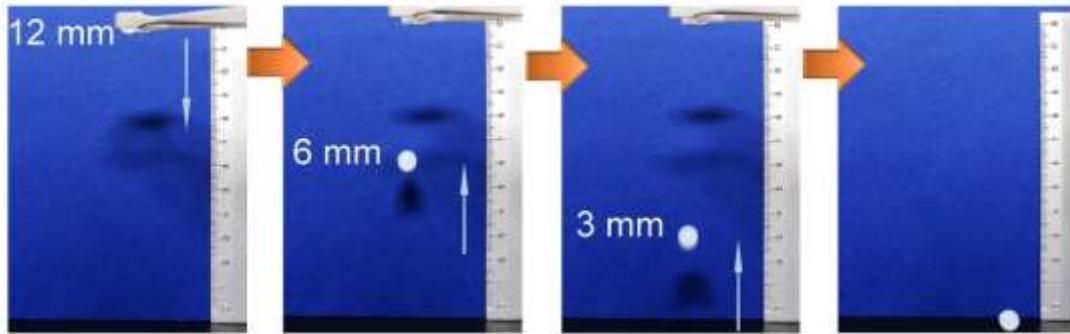
**Figure S1:** Synthesis and characterization of oligo-PCL<sub>5</sub> and PLGA-g-(PCL<sub>5</sub>)<sub>3</sub>. a) synthesis of oligo-PCL<sub>5</sub> and PLGA-g-(PCL<sub>5</sub>)<sub>3</sub>; b) <sup>1</sup>H NMR; c) FT-IR; d) XRD; e) TGA and DSC tests of oligo-PCL<sub>5</sub> and PLGA-g-(PCL<sub>5</sub>)<sub>3</sub>.



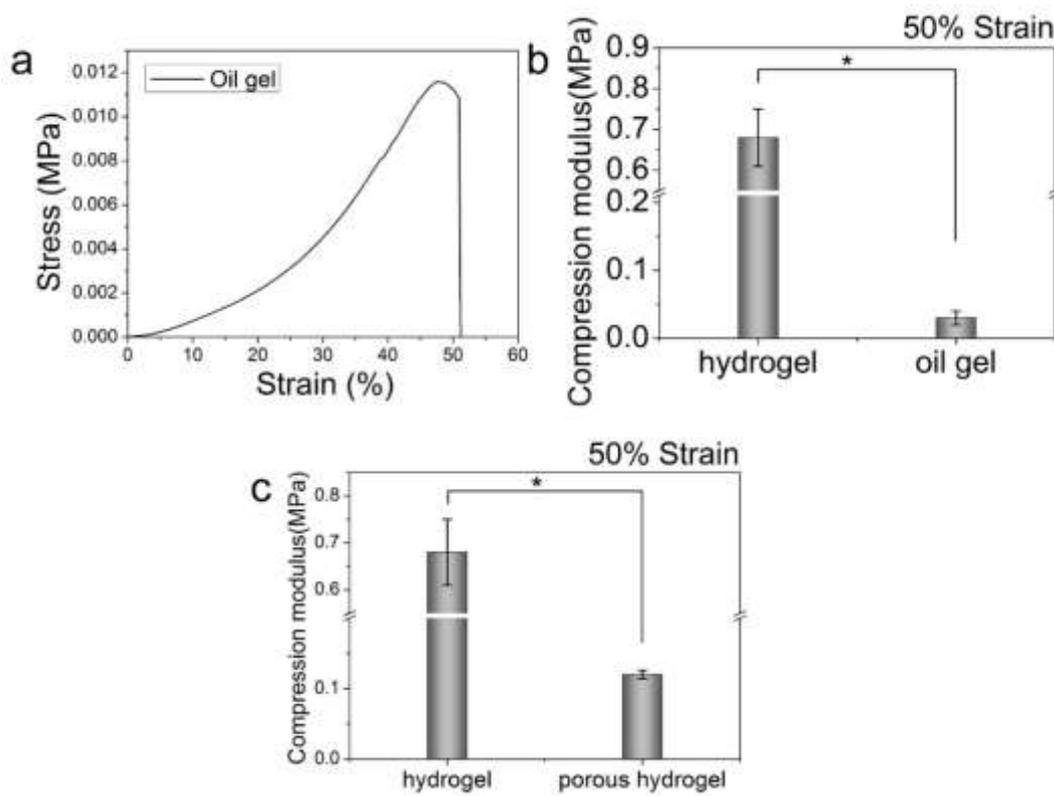
**Figure S2:** Gelation of PLGA-g-PCL oil gel (a); PLGA-g-PCL oil gel and hydrogel (b).



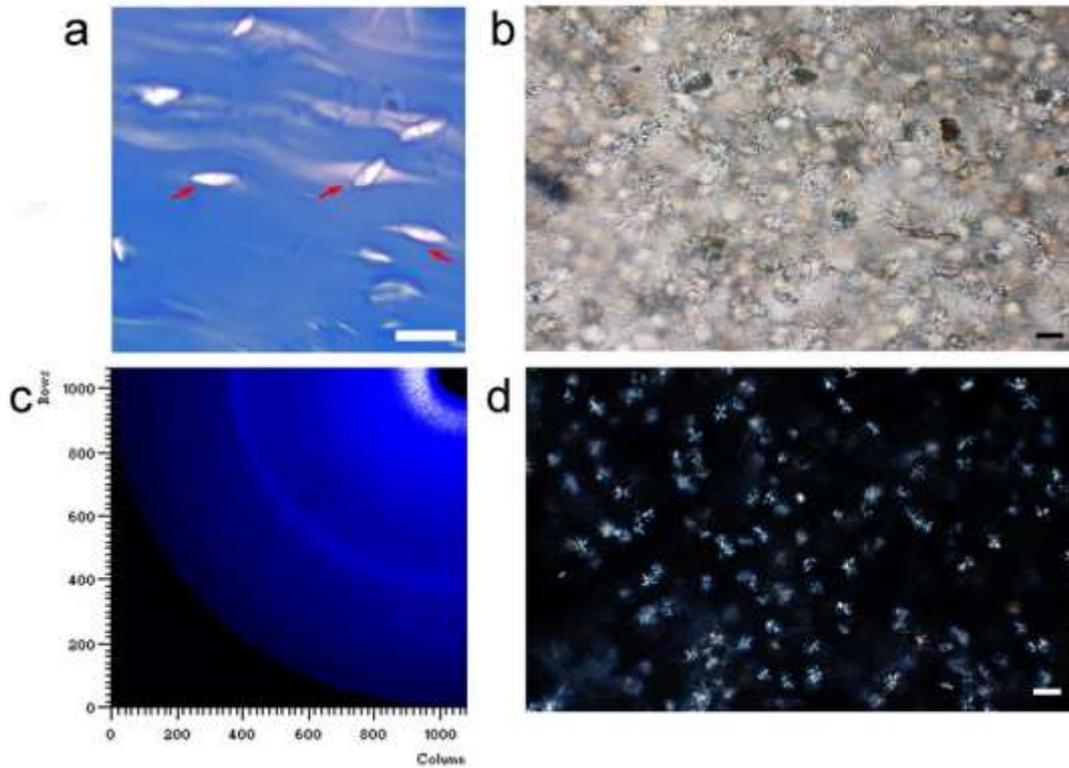
**Figure S3:** (a) Standard curve of TBO solution; (b) Adsorption kinetics; (c) Ultraviolet absorption curve, and the amount of TBO adsorption.  $N = 5$ . According to reference, we used toluidine blue O (TBO) to quantify the content of carboxyl groups, so to calculate the cross-linking degree. The amount of adsorbed TBO was  $2.5 \pm 0.2 \mu\text{mol/mg}$  hydrogel. By calculation, the cross-linking degree was  $56\% \pm 4\%$ , if both the hydroxyl groups of TEG were involved in cross-linking reaction.<sup>[1]</sup>



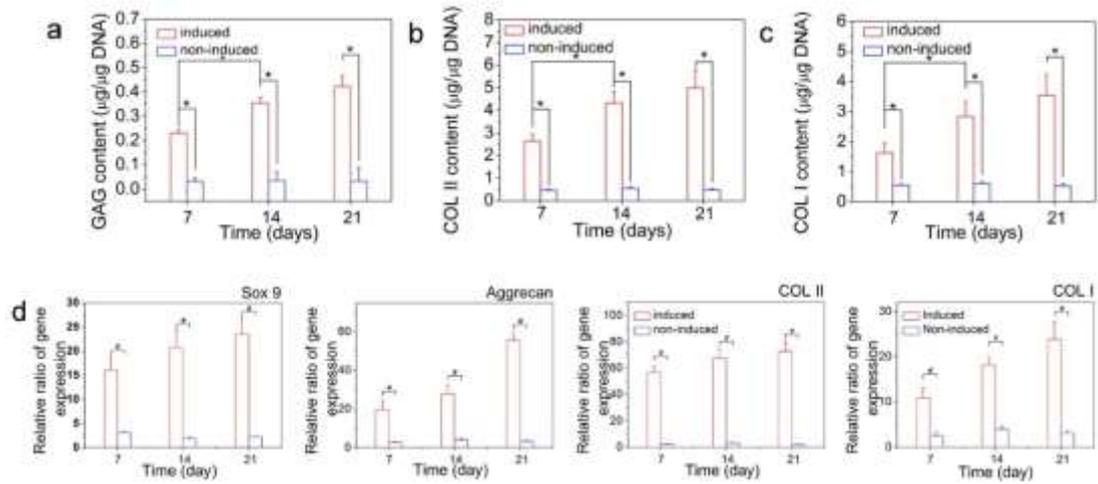
**Figure S4:** The PLGA-g-(PCL<sub>5</sub>)<sub>3</sub> hydrogel was fabricated into sphere with the diameter of 6 mm to undergo free-falls to show the elasticity.



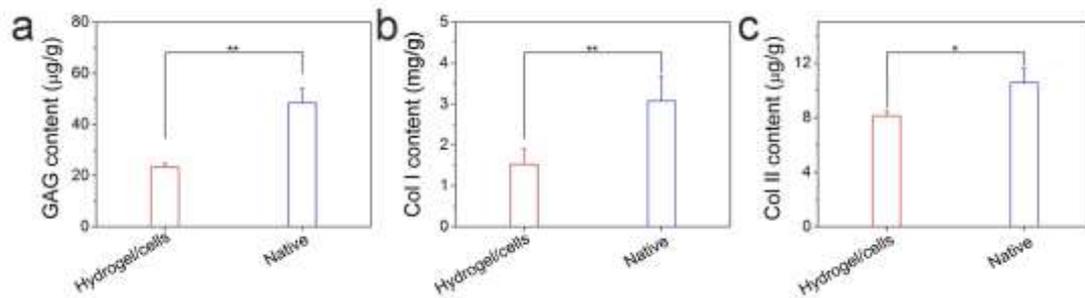
**Figure S5:** The mechanical analysis of pre-PCL cluster formed gel (PLGA-g-(PCL<sub>5</sub>) oil gel) (a), and the comparison of compression moduli between hydrogel and oil gel (b), as well as the comparison of compression moduli between hydrogel and porous hydrogel at the strain of 50% (c). \*p<0.01. N = 5.



**Figure S6:** Recognition of PCL clusters inside the hydrogels. a) TBO staining to illustrate the colorless regions were PCL clusters; b) Observation of PCL clusters in PLGA-g-(PCL<sub>15</sub>)<sub>3</sub> hydrogel by phase contrast microscope; c) 2D WAXS pattern of PLGA-g-(PCL<sub>15</sub>)<sub>3</sub> hydrogel; d) Representative image from polarizing microscope to observe the crystallization of PCL clusters in PLGA-g-(PCL<sub>15</sub>)<sub>3</sub> hydrogel. N=5. The Debye-Scherrer ring was only observed in PLGA-g-(PCL<sub>15</sub>)<sub>3</sub> hydrogel in WAXS pattern. PCL with longer chain in hydrogel may be more effective to form crystallization. Moreover, image from polarizing microscope showed significant crystal structure in PLGA-g-(PCL<sub>15</sub>)<sub>3</sub> hydrogel. Besides, we also used toluidine blue O (TBO), which could interacted with carboxyl groups in hydrogel, to stain the PLGA-g-(PCL<sub>5</sub>)<sub>3</sub> hydrogel sections. The PCL clusters inside the hydrogel sections cannot be stained positively. (bar scale: 10 μm for a; 20 μm for b,d)



**Figure S7:** Chondrogenic differentiation of ASCs in porous hydrogel. a-c) cartilage specific matrix deposition in the induced and non-induced groups; d) cartilage gene expression profiles of induced chondrogenic ASCs *in vitro*. \* $p < 0.05$ . N=5



**Figure S8:** Biochemical evaluation of the neo-tissue. a) GAG content; b) COL I content; c) COL II content of neo-tissue and native tissue. The quantitation of protein was normalized to the wet weight of the sample. \* $p < 0.05$ , \*\* $p < 0.01$ . N=5.

**Table S1. Synthesis of BA-PCL<sub>m</sub>**

No.	Products	Feed ratio ( $\epsilon$ -CL/BA)	DP <sup>a</sup>	Mn <sup>a</sup>	Mn <sup>b</sup>	PDI <sup>b</sup>
a1	BA-PCL <sub>5</sub>	5.0	8.6	1090	7705	1.04

<sup>a</sup> from <sup>1</sup>H NMR; <sup>b</sup> from GPC.

**Table S2. Synthesis of PLGA-g-(PCL<sub>m</sub>)<sub>p</sub>**

No.	Polymers	Feed ratio	Graft ratio <sup>a</sup>
P1-1	PLGA-g-(PCL <sub>5</sub> ) <sub>3</sub>	100 : 3 COOH/PCL (mol/mol)	3.6

<sup>a</sup> from <sup>1</sup>H NMR

**Table S3. Grading scale for gross appearance of neo-tissue [2]**

Description	Score
Implant integration	1-3
Implant position	1-3
Horn position	1-3

Shape	1-3
Implant presence of tears	1-3
Implant surface	1-3
Implant size	1-3
Tissue quality	1-3
Condition of the synovia	1-3
Minimum total score	9 (The best)

**Table S4. The gross evaluation scores of neo-tissue**

Description	ASCs/hydrogels [mean (range)]	Hydrogels [mean (range)]	Blank [mean (range)]
Integration	2 (1-3)	2.7 (2-3)	3.0 (3-3)
Implant position	1.4 (1-2)	2.8 (2-3)	2.8 (2-3)
Horn position	1.7 (1-3)	3.0 (3-3)	3.0 (3-3)
Shape	2.0 (1-3)	2.8 (2-3)	2.8 (2-3)
Tears	1.2 (1-2)	2.8 (2-3)	2.8 (2-3)
Surface	2.3 (2-3)	3.0 (3-3)	3.0 (3-3)
Size	2.3 (2-3)	3.0 (3-3)	3.0 (3-3)
Tissue	1.7 (1-2)	2.7 (2-3)	3.0 (3-3)
Synovial	1.7 (1-2)	2.7 (2-3)	2.8 (2-3)
Total score	16.3 (15-18)**	25.3 (24-26)	26.0 (25-27)

\*\*Statistically significant (\*\*p < 0.01)

**Table S5. Grading scale for gross appearance of the knee joint <sup>[3]</sup>**

Description	Grade
Anterior medial femoral condyle	0-4
Posterior medial femoral condyle	0-4
Anterior lateral femoral condyle	0-4
Posterior lateral femoral condyle	0-4
Patellar femoral groove	0-4
Patella articular surface	0-4
Medial tibial plateau	0-4
Lateral tibial plateau	0-4
Medial meniscus	0-4
Lateral meniscus	0-4
Osteophytes	0-4
Condyle groove junction	0-4
Minimum total score	0 (The best)

Grade 0: no observable gross changes;

Grade 1: intact surface with color changes or surface irregularities;

Grade 2: surface fibrillation or loss of cartilage;

Grade 3: exposed bone less than 10% of surface area;

Grade 4: greater than 10% of exposed bone.

**Table S6. The gross evaluation scores of knee joint**

Rabbit No.	ASCs/hydrogels	Hydrogels	Blank
1	3	7	17
2	2	8	16
3	1	9	18
4	1	5	15
5	3	7	14
6	3	9	17
Mean ± SD	2.2 ± 1.0**	7.5 ± 1.5**	16.2 ± 1.5**

\*\*Statistically significant (\*\*p < 0.01)

**Table S7. ICRS grade [4]**

Description	Grade
Cartilage without notable defects	ICRS 0
Cartilage with fibrillation and slight softening	ICRS 1a
Cartilage with superficial fissures and lacerations	ICRS 1b
Defect depth < 50% of the cartilage thickness	ICRS 2
Defect depth > 50% of the cartilage thickness	ICRS 3
Full-thickness osteochondral injuries	ICRS 4

**Table S8. The gross evaluation scores of femoral condyle and tibial plateau cartilage based on ICRS**

	ASCs/hydrogels [mean (range)]	Hydrogels [mean (range)]	Blank [mean (range)]
femoral condyle	1.2 (1-2) **	2.3 (2-3) **	3.1 (3-4) **
tibial plateau	0.7 (0-1) *	1.5 (0-2)	1.6 (1-2)

\*Statistically significant (\*p < 0.05, \*\*p < 0.01)

**Table S9. The histological grading scale for neo-tissue (Pauli's score) [2]**

Description	Points
<b>Size</b>	
Large;	0
Moderate;	1
Small;	2
Not observed	3
<b>Morphology of regenerated meniscus</b>	
C-shaped like normal meniscus;	0
Gradual C-shape;	1
Distinct shape from meniscus;	2
No appearance of meniscus	3
<b>Surface integrity</b>	
Smooth;	0
Slight fibrillation or slightly undulating;	1
Moderate fibrillation or markedly undulating;	2

Severe fibrillation or disruption	3
<b>Integration with native meniscus</b>	
Complete integration without detectable border;	0
Incomplete integration with detectable border;	1
Separated with narrow space;	2
Far from native meniscus	3
<b>Cellularity of meniscal cells</b>	
Normal cell distribution;	0
Almost normal cell distribution;	1
Hypercellularity or hypocellularity;	2
No meniscal cells	3
<b>Cell morphology</b>	
Similar to normal chondrocyte;	0
Round shape, but small or hyperplastic;	1
Mix of round cells and other shapes of cells;	2
No chondrocyte shaped cells	3
<b>Collagen fiber organization</b>	
Collagen fibers well organized, no separations or tears;	0
Collagen fibers moderately well organized, slight separations or tears;	1
Collagen fibers unorganized, moderate separations or tears;	2
Collagen fibers unorganized, severe separations or tears	3
<b>Matrix staining</b>	
Well stained like normal meniscus;	0
Moderately stained;	1
Slightly stained;	2
No stain	3

**Table S10. The histological grading result of neo-tissue (Pauli's score)**

	ASCs/hydrogels [mean (range)]	Hydrogels [mean (range)]	Blank [mean (range)]
Size	0.3 (0-1)	2.3 (2-3)	2.8 (2-3)
Morphology of regenerated meniscus	1.2 (1-2)	2.3 (2-3)	2.5 (2-3)
Surface integrity	1.2 (1-2)	3.0 (3-3)	3.0 (3-3)
Integration with native meniscus	1.0 (0-2)	2.3 (2-3)	3.0 (3-3)
Cellularity of meniscal cells	0.5 (0-1)	3.0 (3-3)	3.0 (3-3)
Cell morphology	1.2 (1-2)	3.0 (3-3)	3.0 (3-3)
Collagen fiber organization	1.2 (1-2)	3.0 (3-3)	3.0 (3-3)
Matrix straining	1.0 (0-2)	3.0 (3-3)	3.0 (3-3)
Mean ± SD	7.6 (6-8)**	21.9 (21-24)	23.3 (22-24)

\*Statistically significant (\*\*p < 0.01)

**Table S11. The histological grading for articular cartilage degeneration (Mankin score) [5]**

Description	Points
<b>Structure</b>	

Normal;	0
Surface irregularity;	1
Pannus and surface irregularity;	2
Clefts to transitional zone	3
Clefts to radial zone	4
Clefts to calcified zone	5
Complete disorganization	6
<b>Cells</b>	
Normal;	0
Diffuse hypercellularity;	1
Cloning;	2
Hypocellularity	3
<b>TB staining</b>	
Normal;	0
Slight reduction;	1
Moderate reduction;	2
Severe reduction	3
<b>Tidemark integrity</b>	
Intact	0
Crossed by blood vessel	1

**Table S12. The histological grading result of femoral condyle cartilage (Mankin score)**

<b>Femoral condyle</b>	<b>ASCs/hydrogels</b> <b>[mean (range)]</b>	<b>Hydrogels</b> <b>[mean (range)]</b>	<b>Blank</b> <b>[mean (range)]</b>
Structure	0.5 (0–1)	3.5 (3–4)	5.3 (4–6)
Cells	0.5 (0–1)	1.5 (1–2)	2.3 (2–3)
TB staining	0.3 (0–1)	2.0 (1–3)	3.3 (2–4)
Tidemark integrity	0.3 (0–1)	0.3 (0–1)	0.3 (0–1)
Mean ± SD	1.7 (0–1) **	7.5 (5–8) **	10.2 (9–11) **

\*Statistically significant (\*\*p < 0.01)

**Table S13. The histological grading result of tibial plateau cartilage (Mankin score)**

<b>Tibial plateau</b>	<b>ASCs/hydrogels</b> <b>[mean (range)]</b>	<b>Hydrogels</b> <b>[mean (range)]</b>	<b>Blank</b> <b>[mean (range)]</b>
Structure	0.1 (0–1)	1.3 (0–2)	1.5 (0–2)
Cells	0.1 (0–1)	0.3 (0–1)	0.5 (0–1)
TB staining	0.1 (0–1)	0.5 (0–1)	0.5 (0–1)
Tidemark integrity	0 (0–0)	0 (0–0)	0 (0–0)
Mean ± SD	0.3 (0–1) **	2.2 (1–3)	2.5 (1–3)

\*Statistically significant (\*\*p < 0.01)

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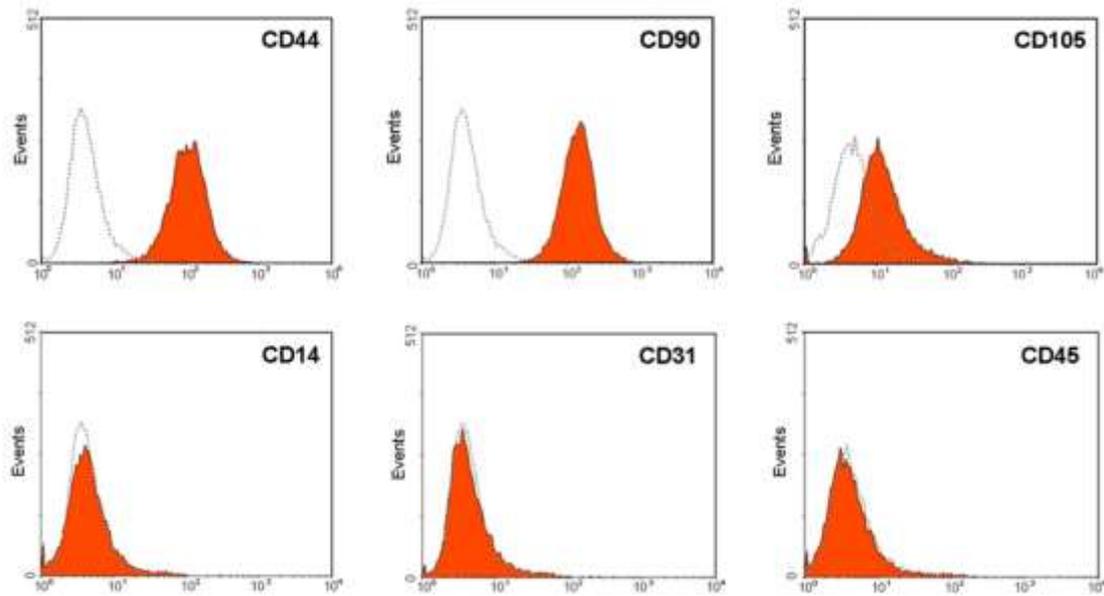
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### Results of flow cytometry

According to the results of flow cytometry, the expression of CD44, CD90 and CD105 was positive. At the same time, the expression of CD14, CD31 and CD45 was negative, indicating the absence of the contamination of hematopoietic system and endothelial cells.



CD antigen	Percentage of expression (%)	CD antigen	Percentage of expression (%)
CD44	91.25±0.92	CD14	3.45±0.75
CD90	92.76±1.05	CD31	2.72±1.17
CD105	25.88±0.84	CD45	1.42±0.52