Supporting Information

Increased Recruitment of Endogenous Stem Cells and Chondrogenic Differentiation by a Composite Scaffold Containing Bone Marrow Homing Peptide for Cartilage Regeneration

Jiaju Lu[1*], Xuezhen Shen[2*], Xun Sun[2], Heyong Yin[3], Shuhui Yang[1], Changfeng Lu[2], Yu Wang[2], Yifan Liu[1], Yingqi Huang[1], Zijin Yang[1], Xianqi Dong[1], Chenhao Wang[1], Quanyi Guo[2], Lingyun Zhao[1], Xiaodan Sun[1], Shibi Lu[2], Antonios G. Mikos[4], Jiang Peng[2✉], and Xiumei Wang[1✉]

1. State Key Laboratory of New Ceramics and Fine Processing, Key Laboratory of Advanced Materials of Ministry of Education, School of Materials Science and Engineering, Tsinghua University, Beijing 100084, China
2. Institute of Orthopedics, Chinese PLA General Hospital, Beijing 100853, China
3. Experimental Surgery and Regenerative Medicine, Department of Surgery, Ludwig-Maximilians-University, Munich 80336, Germany
4. Department of Bioengineering, Bioscience Research Collaborative, Rice University, Texas 77030, USA

* Contributed equally to this work

✉ Corresponding author: Xiumei Wang, E-mail: wxm@mail.tsinghua.edu.cn, Phone: 010-62782966. Fax: 86-10-62771160. Jiang Peng, pengjiang301@126.com

Table S1 Primer sequences used for quantitative RT-PCR

Gene	Primer sequence	GeneBank accession no.
Col2	5′ –CACGCTCAAGTCCCTCAACA- 3′	XM_002723438.1
	5′ –TCTATCCAGTAGTCACCGCTCT- 3′	
Col1	5′ –GCCACCTGCCAGTCTTTACA- 3′	NM_001195668.1
	5′ –CCATCATCACCATCTCTGCCT- 3′	
Aggrecan	5′ –GGAGGAGCAGGAGTTTGTCAA- 3′	XM_002723376.1
	5′ –TGTCCATCCGACCAGCGAAA- 3′	
Sox9	5′ –GCGGAGGAAGTCGGTGAAGAAT- 3′	XM_002719499
	5′ –AAGATGGCGTTGGGCGAGAT- 3′	
GAPDH	5′ –GAAGAAGGTGGTGAAGCAGG- 3′	NM_001082253.1
	5′ –CACTGTTGAAGTCGCAGGAG- 3′	

Table S2 Primer sequences used for MSCs characterization

Gene	Primer sequence	Size (bp)	GeneBank accession no.
CD29	5′ –CCCCATCGACCTCTACTACCT- 3′ 5′ –TCACCCTCCTCATCTCATTCA- 3′	107	XM_002721189
CD34	5′ –AGAACTTTCCAGCATGTTCCAGTTTATG- 3′ 5′ –GGCTTGCCACATCTTGCTCGGTGA- 3′	95	XM_002717543
CD44	5′ –AGGTTTGGTGGAAGACCTGG- 3′ 5′ –CTTCCTCCTCTGCCATGAGT- 3′	162	XM_002709048.3
CD45	5′ –AGGTAGTAGATGTTTTCCAAGTAGTGA- 3′ 5′ –ACTTGTCCATTCTGGGCAGGGTAG- 3′	130	XM_002717662
CD90	5′ –ATTTGCTTAGGGCTTATCCTTGTG- 3′ 5′ –CTTGCATCTGGGTCTTGAAGTG- 3′	241	XM_002722718
CD105	5′-CAGCGTTGCGTCCTTCGTGG-3′ 5′-CGGGCTGCACCTGTTCTTCG-3′	130	XM_002722985

Table S3. ICRS macroscopic evaluation of cartilage repair

Criteria	Appearance	Points
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 <1 mm	3
	3/4th of graft integrated, 1/4 with a notable border > 1 mm width	2
	1/2 of graft integrated with surrounding cartilage, 1/2 with a notable border >1 mm	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 cracs	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

Table S4. Wakitani cartilage repair scoring system

Criteria	Appearance	Points
Cell morphology	Hyaline cartilage	0
	Mostly hyaline cartilage	1
	Mostly fibrocartilage	2
	Mostly non-cartilage	3
	Non-cartilage only	4
Matrix staining (metachromasia)	Normal (compared with host adjacent cartilage)	0
	Slightly reduced 1	1
	Markedly reduced 2	2
	No metachromatic stain	3
Surface regularity (total smooth area compared with entire area of cartilage defect)	Smooth (> 3/4) 0	0
	Moderate (> 1/2–3/4) 1	1
	Irregular (1/4–1/2) 2	2
	Severely irregular (< 1/4)	3
Thickness of cartilage (compared with that of surrounding cartilage)	> 2/3	0
	1/3–2/3	1
	< 1/3	2
Integration of donor with host adjacent cartilage	Both edges integrated	0
	One end intergrated	1
	Neither edge integrated	2

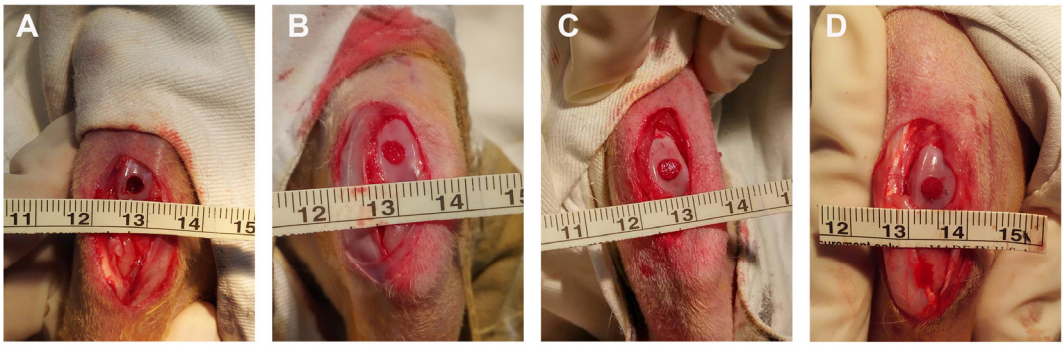

Figure S1. Animals were randomly divided into 4 groups (A-D: MF, ACM, ACM+RAD, and ACM+RAD/PFS groups) and defects of articular cartilage were filled with or without the scaffolds after microfracture surgery.

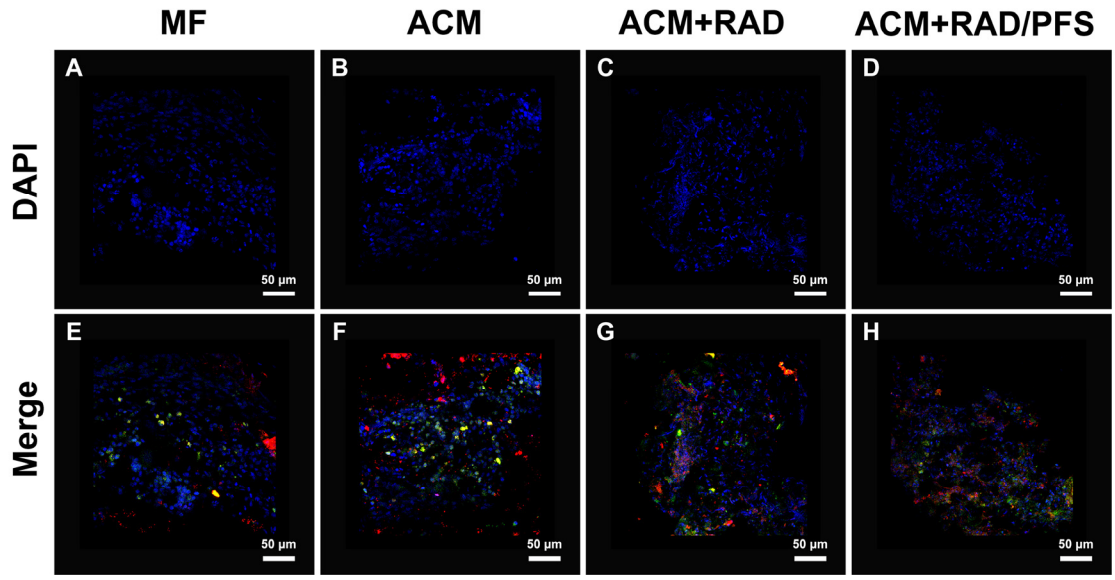

Figure S2. Representative maximum intensity projection images of confocal microscopy at 1 week after implantation for the different groups examined.

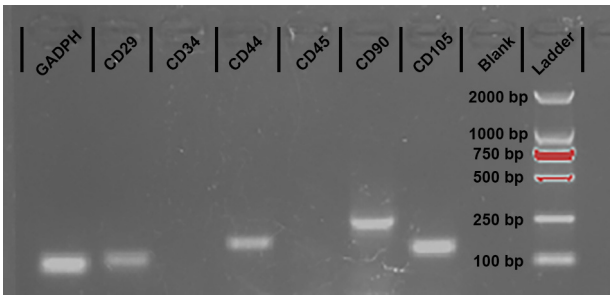

Figure S3. Gene expression of MSC-specific markers by agarose gel electrophoresis assay. The isolated MSCs at passages 2 were positive for CD29, CD44, CD90 and CD105, but negative for CD34, and CD45.

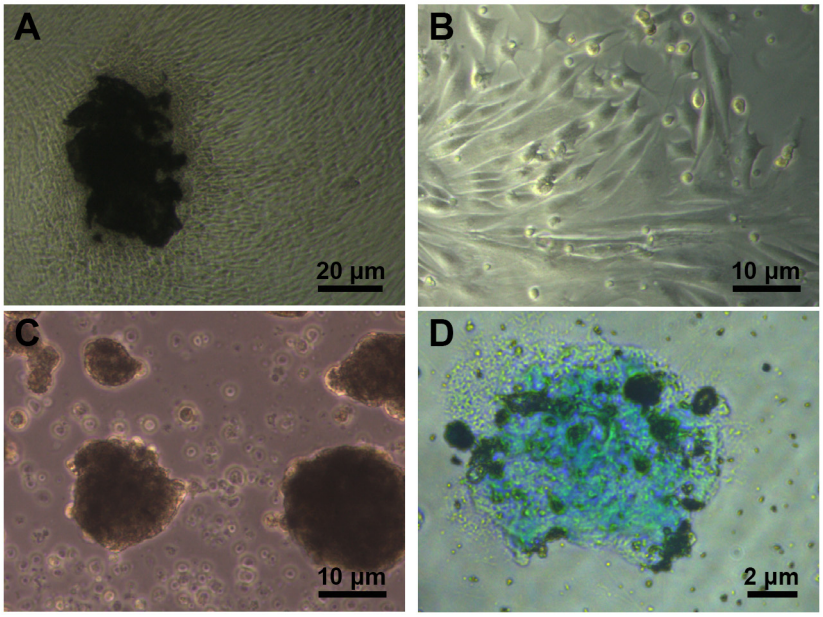

Figure S4. Isolation and identification of mesenchymal stem cells. (A) Primary adherent cells from tissue culture. (B) The morphology of isolated cells after 3-day culture. (C) The formation of multicellular spheroids from the isolated cells after 5 days of culture in a chondrogenic medium. (D) Differentiation into chondrocytes (Alcian blue staining) of isolated cells.

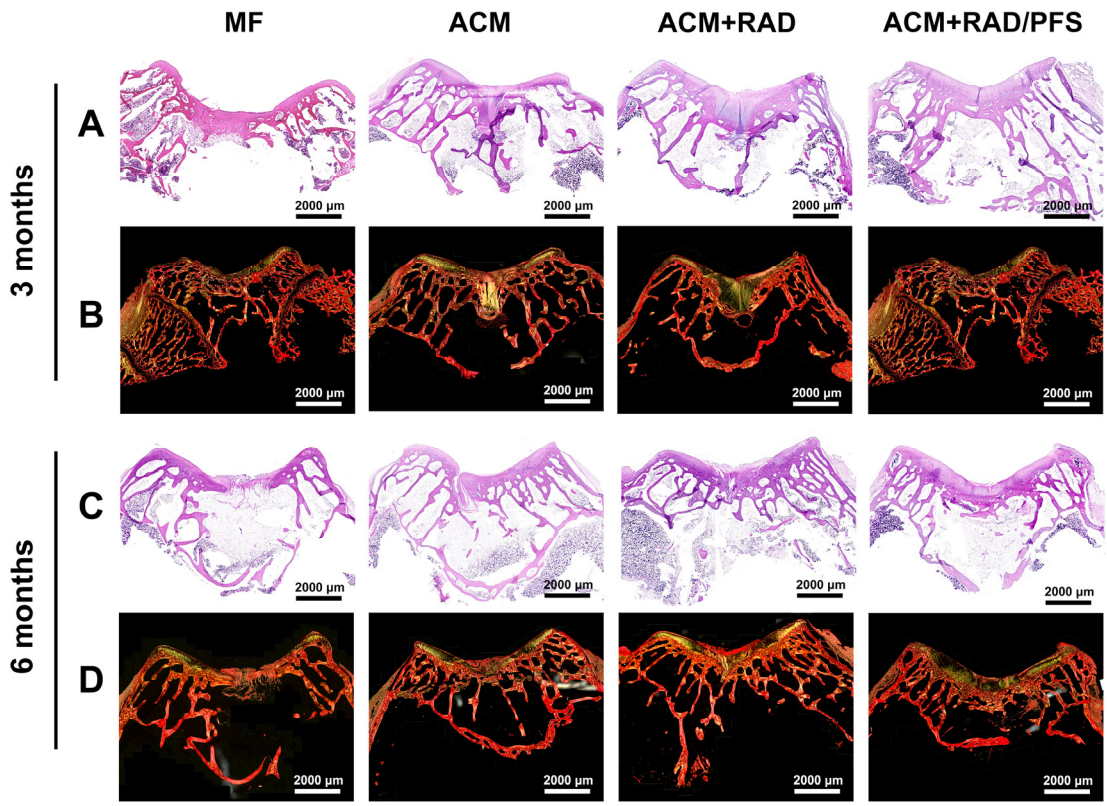

Figure S5. Representative images of H&E staining (A, C) and SR staining (B, D) for the osteochondral defects and repaired tissues at 3 and 6 months after surgery (n = 6).

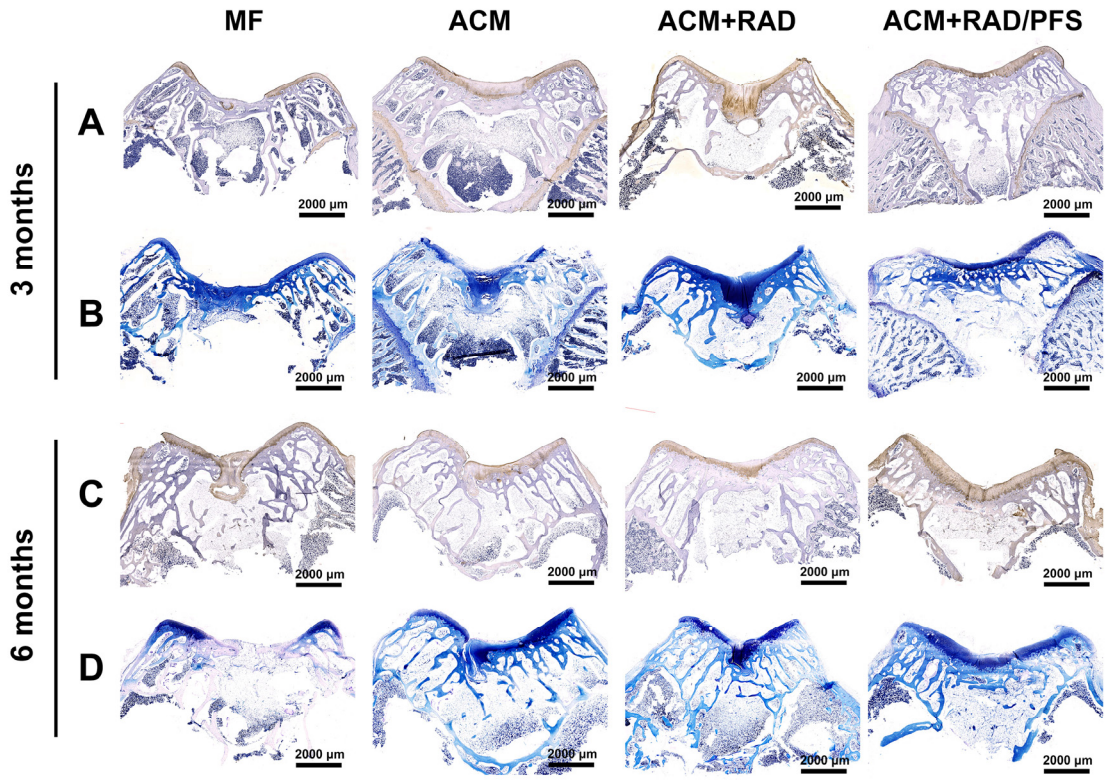

Figure S6. Representative images of Col2 staining (A, C) and TB staining (B, D) for the osteochondral defects and repaired tissues at 3 and 6 months after surgery (n = 6).

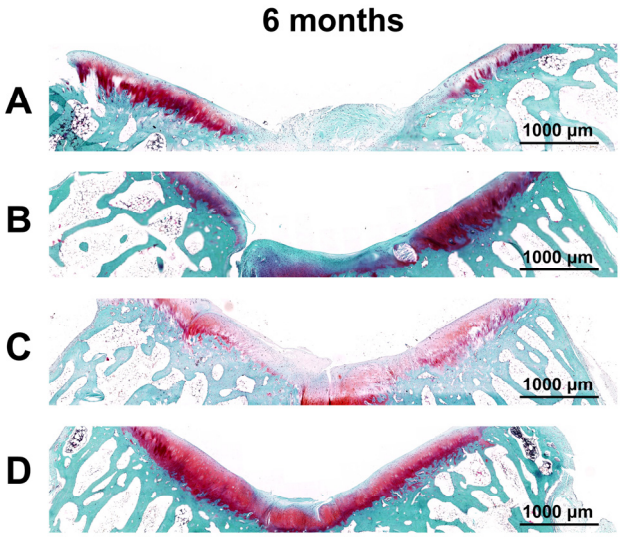

Figure S7. Representative images of Safranin O/Fast Green staining for the osteochondral defects and repaired tissues at 6 months after surgery (A-D: MF, ACM, ACM+RAD, and ACM+RAD/PFS groups, n = 6).