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Influence of Pore Architectures of Silk Fibroin/Collagen Composite Scaffolds on the Regeneration of Osteochondral Defects *in vivo*

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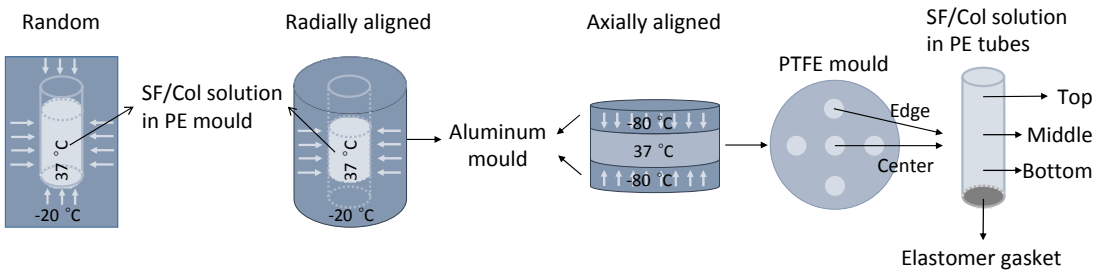

Fig. S1 Schematic illustration of mould structure for fabricating random SF/Col composite scaffold, radially aligned SF/Col composite scaffold, and axially aligned SF/Col composite scaffold. For the fabrication of the axially aligned scaffold, the PE moulds containing silk fibroin and collagen mixed solution was mounted into the central hole and edge holes of a larger PTFE molud, which was placed between two -80 °C precooled aluminum moulds to ensure temperature transduction in an axially directional manner.

Table S1. Primers and size of analyzed genes.

Gene	Primer Sequences (5'to3')	Size (bp)
PPIA	Forward: CAAACGGCTCCCAGTTCTTCATC	92
	Reverse: GCTCATGCCCTCTTTCACTCT	
aggrecan	Forward: GGAGGTCGTGGTGAAAGGTGTTG	157
	Reverse: CTGGTGGAAGCCATCCTCGTA	
SOX9	Forward: CAGCCTCTACTCCACCTTCACCT	152
	Reverse: CTCAAGGTCTGGTGAGCTGTGTG	
Col II	Forward: GACTGTCCTGTGCGACGACATAAT	134
	Reverse: GCCCCTTTGGTCCTGGTTTC	
Col X	Forward: GTGCATGTGAAAGGGACTCATGT	94
	Reverse: CCAGGTAGCCCTTAGCATATTCA	
Col I	Forward: GCGGTGGTTACGACTTTGGTT	140
	Reverse: AGTGAGGAGGGTCTCAATCTG	
IL-1β	Forward: CGCATCTCCTGCCAACCCTACA	71
	Reverse: GCTTCTCCAGAGCCACAACGACT	
IL-6	Forward: CGGTCAGAACACACCATCCTGT	80
	Reverse: GTGTCCTAACGCTCATCTTCCTAGT	
TNF-α	Forward: CGCCGTCTCCTACCCGAACA	144
	Reverse: GCCGGTCACCCTTCTCCAAC	

Table S2. Manufacturers and product codes of primary antibodies for WB test.

Product name	Manufacturer	Product code
Anti-aggrecan antibody	Thermo Fisher	MA3-16888
Anti-collagen I antibody	Abcam	ab6308
Anti-collagen II antibody	Novus Biologicals	NB600-844
Anti-collagen X antibody	Abcam	ab58632
Anti-IL-1β antibody	Arigo	ARG22429
Anti-IL-6 antibody	Arigo	ARG22430
Anti-TNF-α antibody	R&D Systems	MAB56701
Anti-GAPDH antibody	Abcam	Ab181602

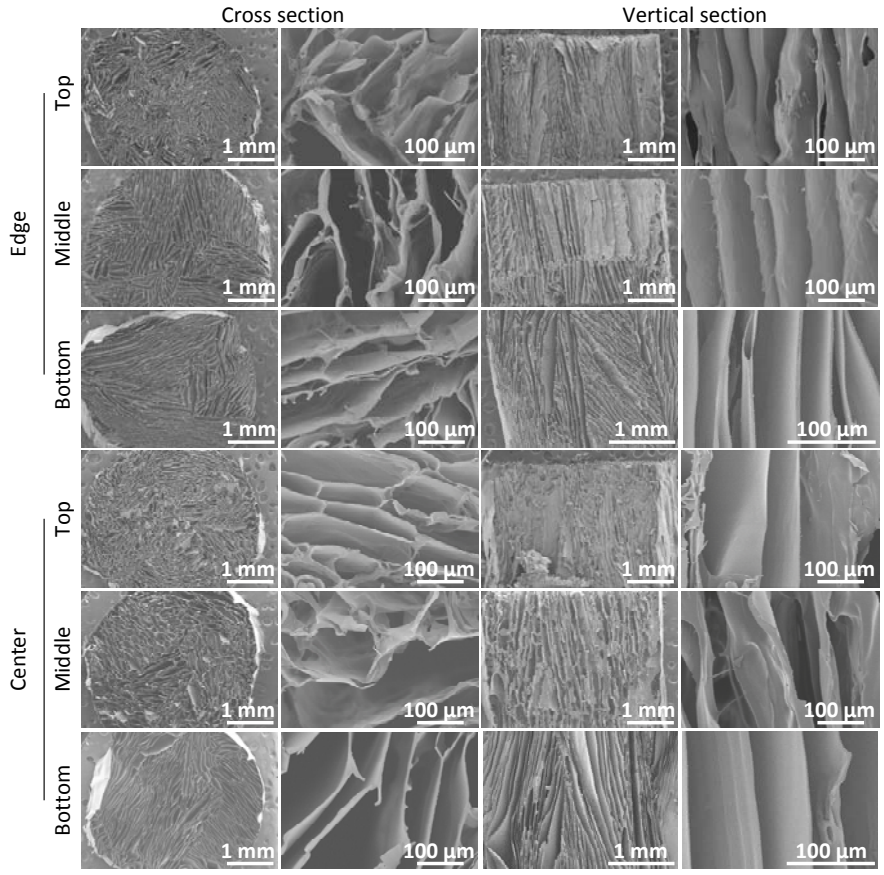

Fig. S2 SEM images showing the morphology of axially aligned scaffold in cross section and vertical section at top, middle and bottom of PE tubes, which were placed in the edge and center of PTFE mould, respectively.

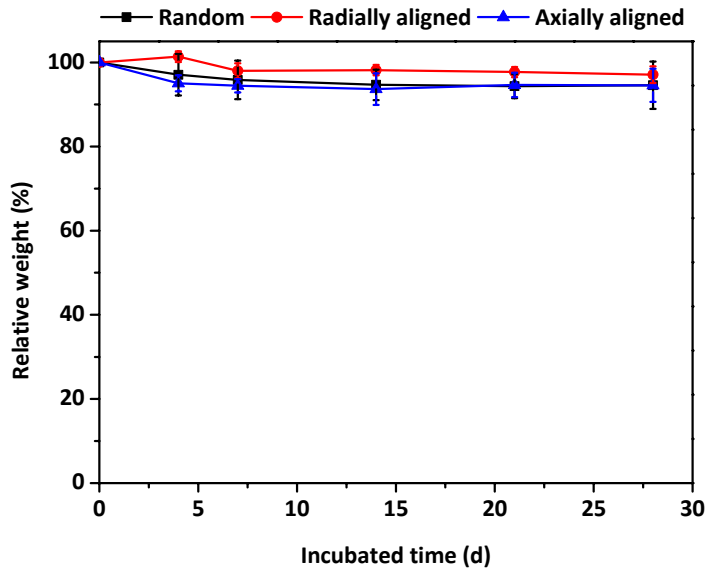

Fig. S3 Degradation behaviors of the SF/Col composite scaffolds in PBS at 37 ºC (n=4).

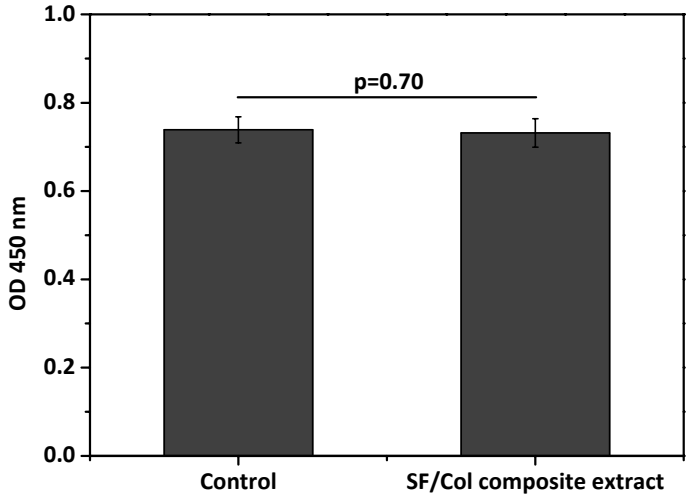

Fig. S4 Cytoviability of BMSCs being cultured in SF/Col composite-extracted medium for 1 d using complete DMEM as control (n=6).

The cytoviability of BMSCs being incubated in the SF/Col composite-extracted medium was evaluated by CCK8 assay. The SF/Col composite scaffolds were cut into small pieces, and were immersed in 75 % v/v ethanol for more than 4 h for sterilization. The medium was then replaced with DMEM to remove residual ethanol for three times. 0.1 g SF/Col composite was further incubated in 1 mL medium at

37 °C for 24 h to obtain the extracted medium, which was supplemented with 10 % v/v FBS before use. The BMSCs resuspended in complete DMEM were seeded into 96-well plate, and cultured at 37 ºC and 5 % CO_2 atmosphere for 4 h to allow cell adhesion. The complete DMEM medium was replaced with the extracted medium from SF/Col composite scaffolds. The BMSCs cultured with complete DMEM were used as control. After 1 d of in vitro cell culture, 200 μL complete DMEM medium containing 10 % v/v CCK8 reagent was added, and the cells were continuously cultured for 2 h. The medium was collected to measure the OD value at 450 nm using a multiplate reader (Infinite M200PRO, Tecan, Switzerland).

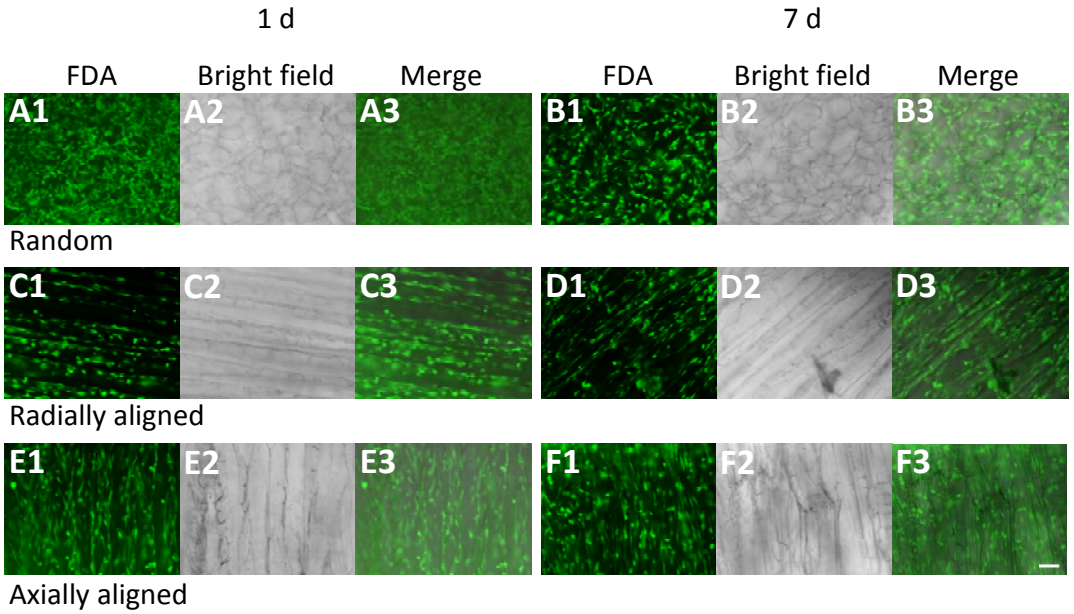

Fig. S5 Distribution of BMSCs in (A, B) random scaffold, (C, D) radially aligned and (E, F) axially aligned SF/Col composite scaffolds being cultured in 5 % CO_2 atmosphere at 37 ºC for (A, C, E) 1 d and (B, D, F) 7 d, respectively. (A3-F3) are merged images of fluorescence mode (A1-F1) and transmition mode images (A2-F2), respectively. Scale bar: 100 μm.

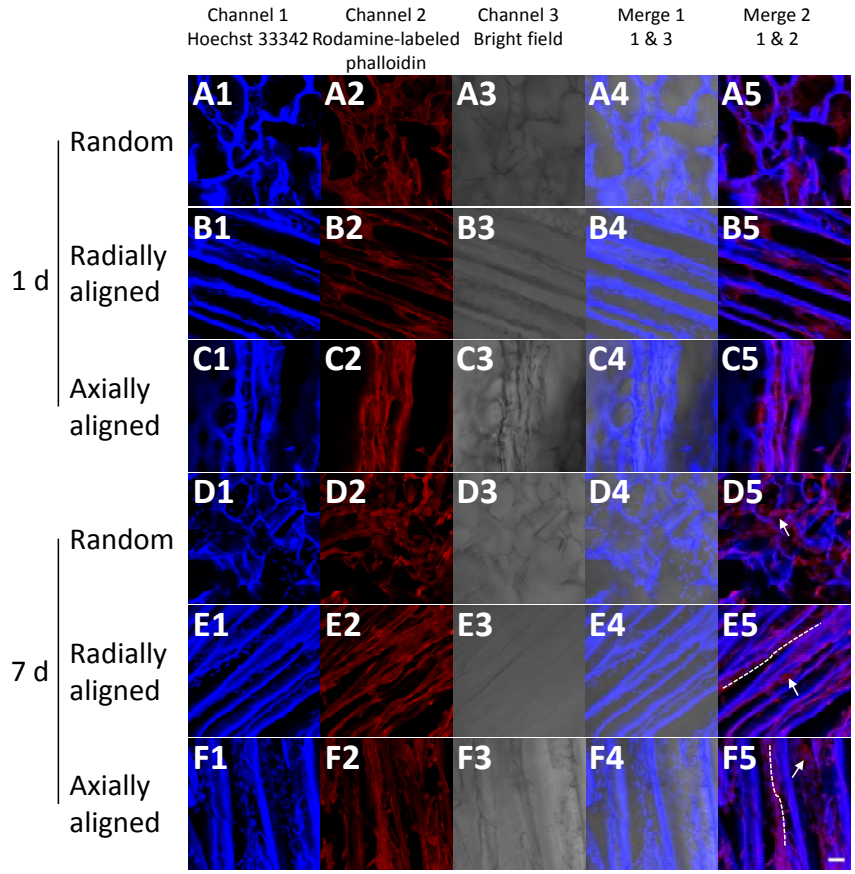

Fig. S6 CLSM images of BMSCs in (A, D) random, (B, E) radially aligned, and (C, F) axially aligned SF/Col composite scaffolds being cultured *in vitro* for (A, B, C) 1d and (D, E, F) 7 d at 37 °C and 5 % CO_2 atmosphere, respectively. Scale bar: 50 μm. The nuclei and cytoskeleton of BMSCs were stained with Hoechst 33342 (blue) and rhodamine-labeled phalloidin (red), respectively. The dyes, especially Hoechst 33342, were seriously adsorbed by the scaffolds. The white arrows in (A5-F5) indicate BMSCs. The white dashed lines in (E) and (F) indicate the arrangement of microfilaments of BMSCs on the aligned scaffolds.

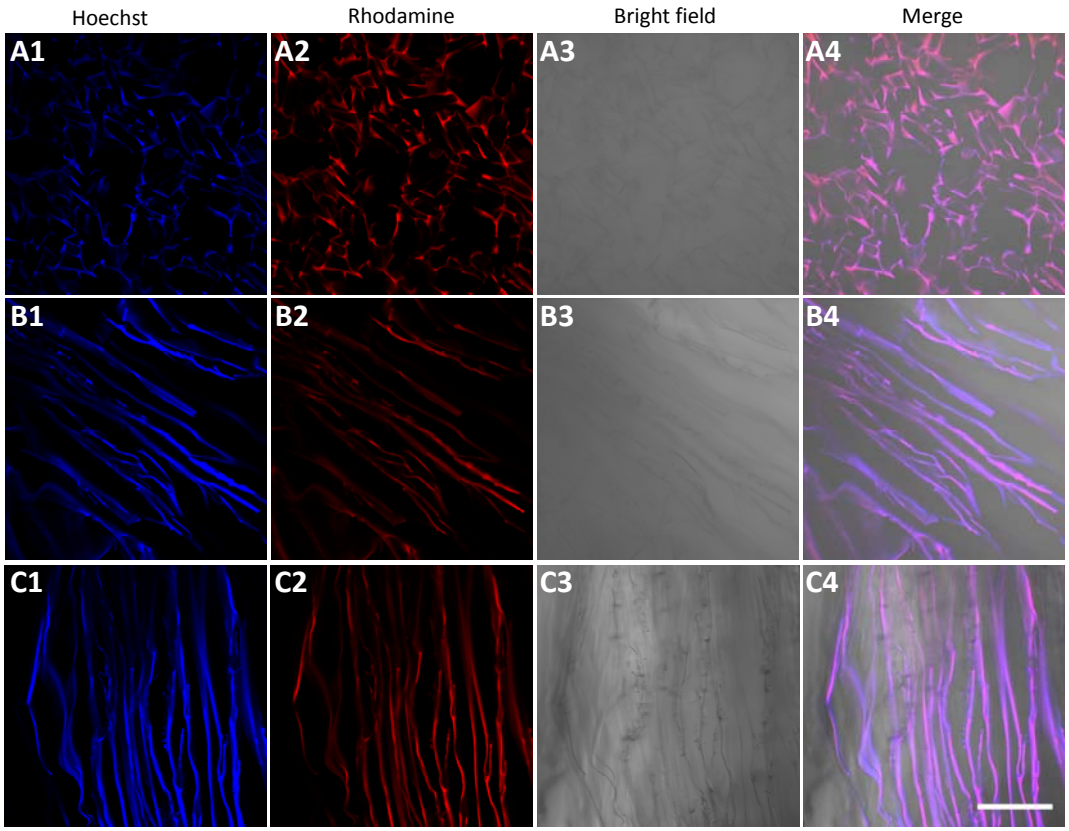

Fig. S7 CLSM images of (A) random, (B) radially aligned, and (C) axially aligned SF/Col composite scaffolds. Scale bar: 200 μm.

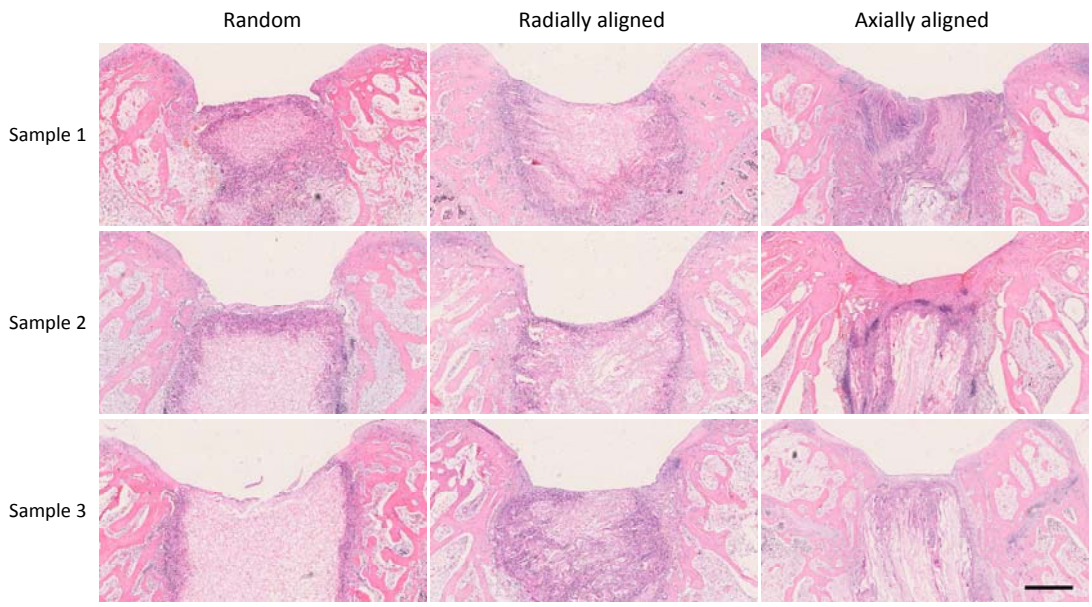

Fig. S8 Animal experiment samples implanted with random, radially aligned, and axially aligned SF/Col composite scaffolds at 6 w of post-surgery evaluated by histological H&E staining. Scale bar: 1 mm.

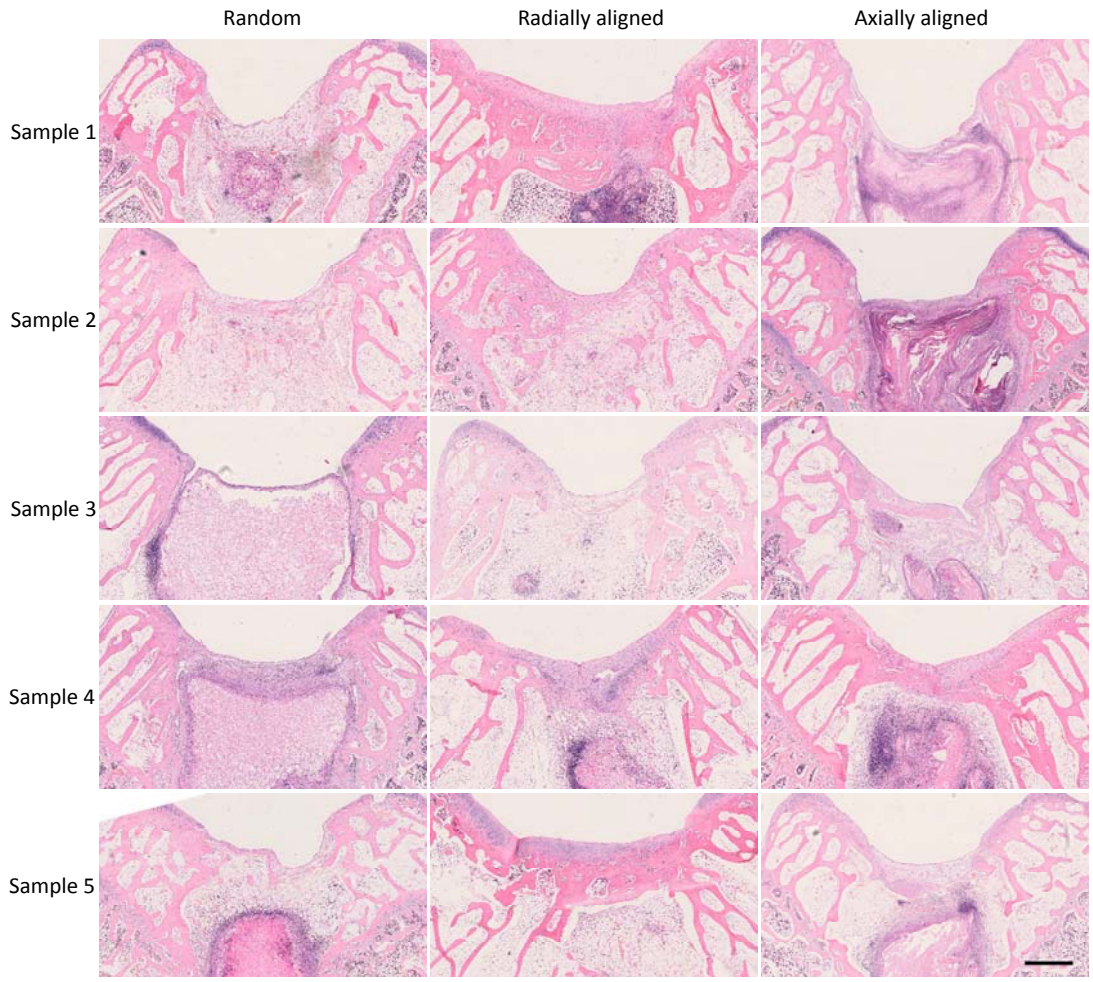

Fig. S9 Animal experiment samples implanted with random, radially aligned, and axially aligned SF/Col composite scaffolds at 12 w of post-surgery evaluated by histological H&E staining. Scale bar: 1 mm.

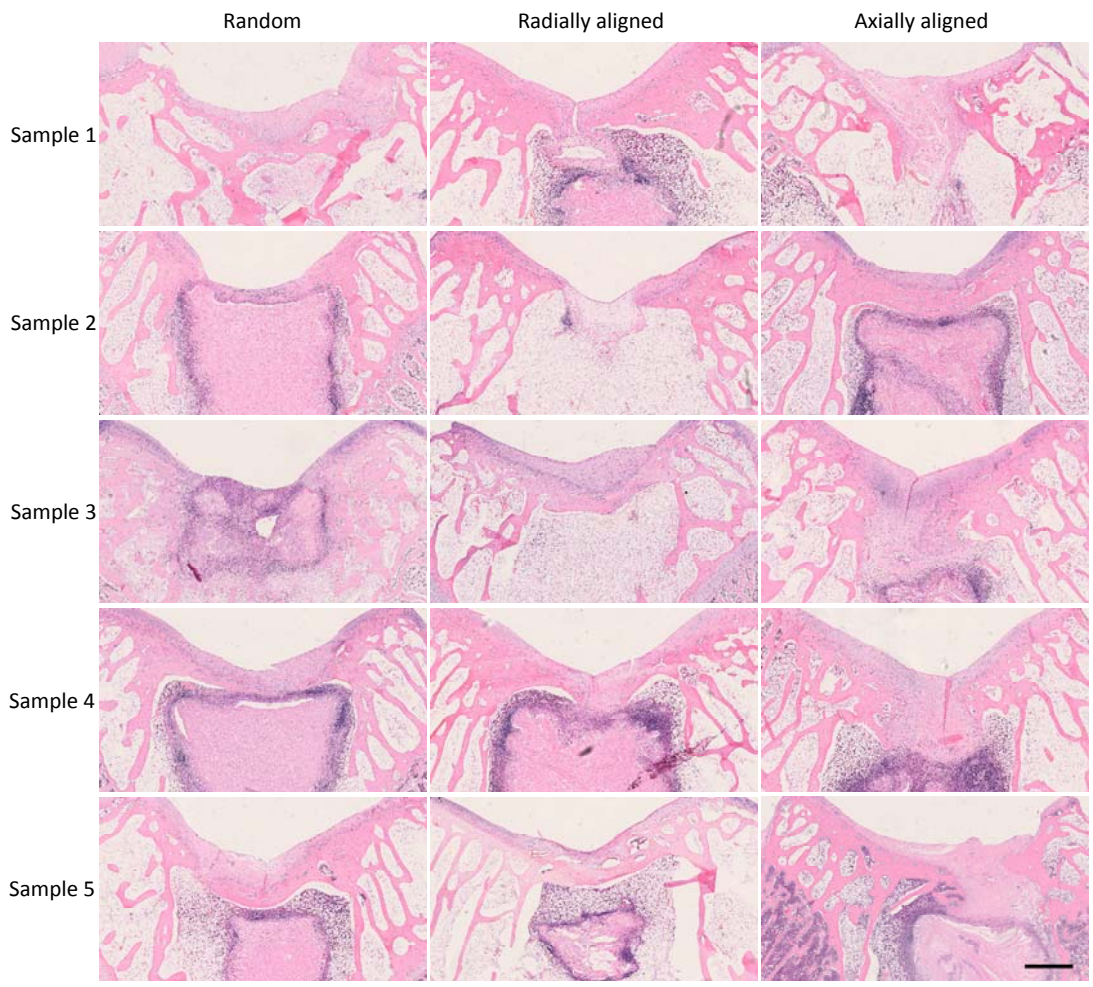

Fig. S10 Animal experiment samples implanted with random, radially aligned, and axially aligned SF/Col composite scaffolds at 18 w of post-surgery evaluated by histological H&E staining. Scale bar: 1 mm.

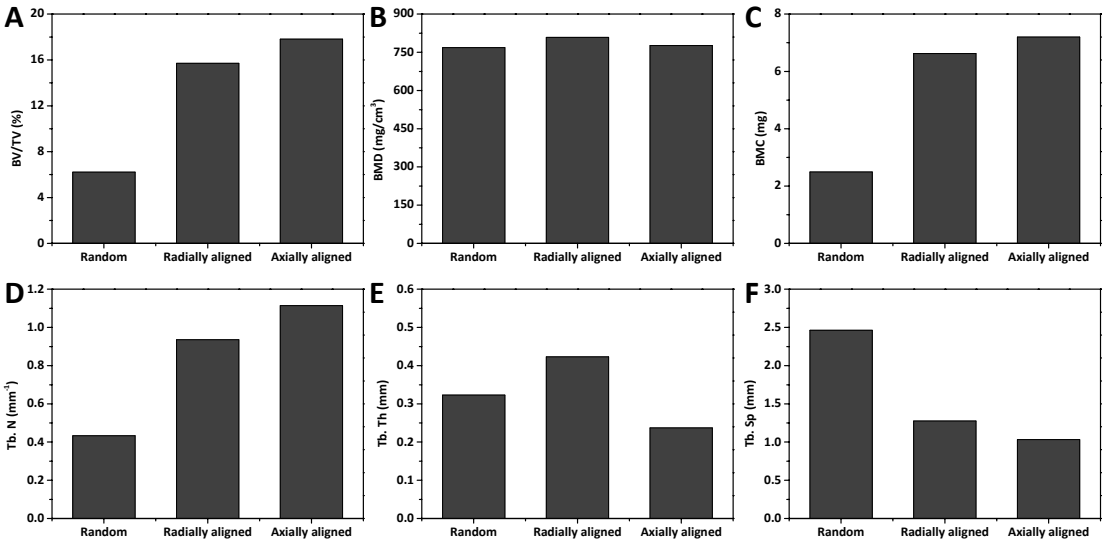

Fig. S11 Quantitative analysis of neo-subchondral bone within the osteochondral defects implanted with the three types of SF/Col composite scaffolds at 18 w of post-surgery by μ-CT.

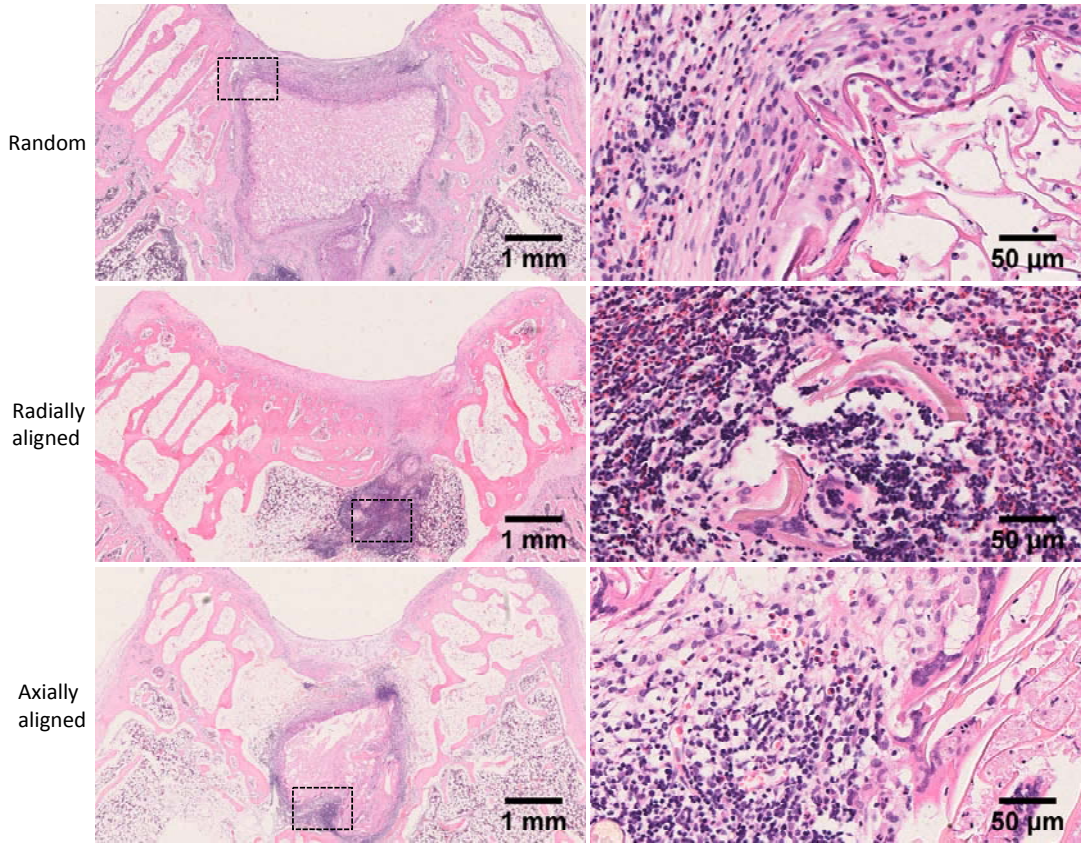

Fig. S12 Histological H&E analysis of osteochondral defects implanted with the SF/Col composite scaffolds at 12 w of post-surgery.

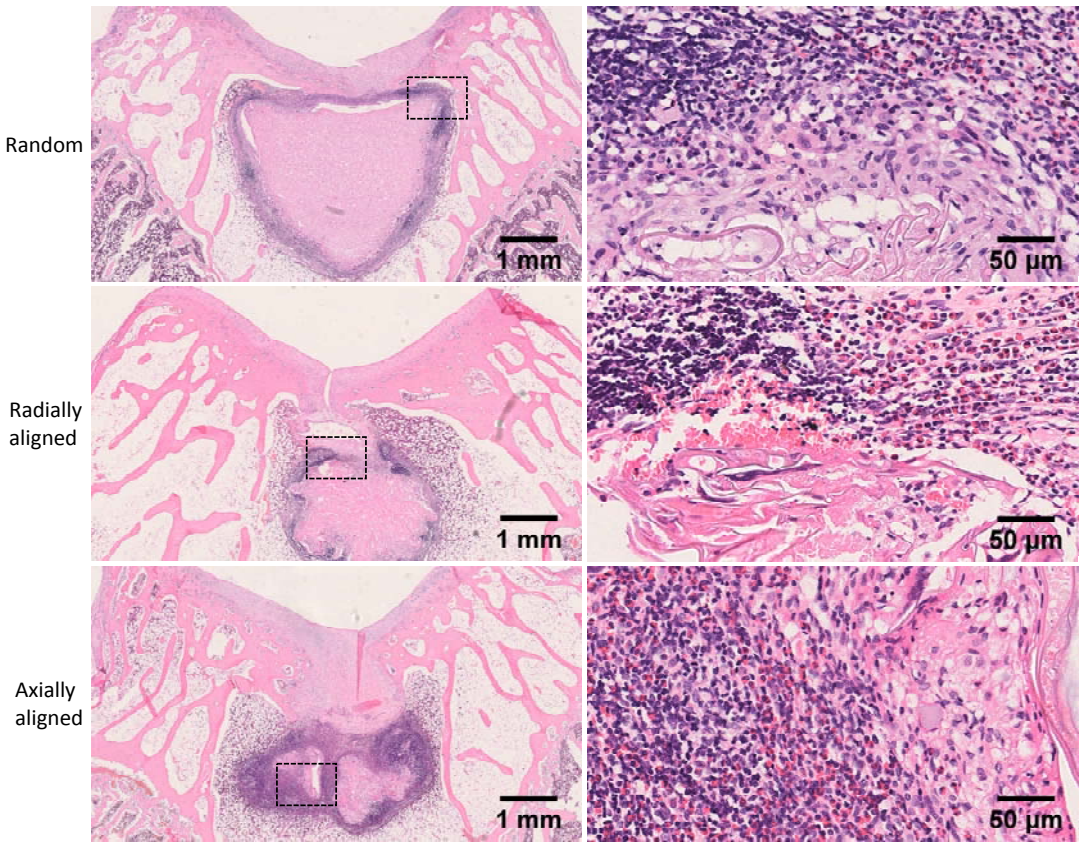

Fig. S13 Histological H&E analysis of osteochondral defects implanted with the SF/Col composite scaffolds at 18 w of post-surgery.