Supplementary Material

Construction of a layer-by-layer self-assembly rosemary acid delivery system on the surface of CFRPEEK implants for enhanced anti-inflammatory and osseointegration activities

Shanshan Zhao[1], Xingyu Zhou[1], Junbo Dang[1], Yilong Wang[1], Junhui Jiang[1], Tianhao Zhao[2], Dahui Sun[2], Chen Chen[3], Xin Dai[3], Yan Liu[3], Mei Zhang[1*]

1. *Key Laboratory of High Performance Plastics, Ministry of Education, College of Chemistry, Jilin University, Changchun 130012, P. R. China;*

2. *Norman Bethune First Hospital, Jilin University, Changchun 130021, P. R. China*

3. *Jilin Province Guoda Bioengineering Co., Ltd, Changchun 130000, P. R. China*

**Corresponding author: zhangmei@jlu.edu.cn*

Experimental methods of zeta potential and CCK-8 in the exploration of the optimal number of LBL layers for the surface modification

The zeta potential of SCPP/CC_n (n = 1, 3, 5, 10, 15, or 20) was evaluated in a 0.001 M potassium chloride mobile electrolyte solution using the 35 × 15 × 1.5 mm^3 samples placed in an adjustable gap cell. HCl solution (0.1 M) and NaOH solution (0.1 M) were used to adjust the pH of the electrolyte to 5.0.

Cell Counting Kit-8 (CCK-8, Beyotime, China) was utilized for the cell proliferation assay. Cells were seeded on the surface of SCPP/CC_n (n = 1, 3, 5, 10, 15, or 20) with 1×10^4 cells/well in 48-well plates. At the preset times (1, 4, and 7 days), cell medium was removed and 10% CCK-8 reagent contained in cell medium was added. After culturing at 37℃ for 2 h, the OD value was determined at 450 nm.

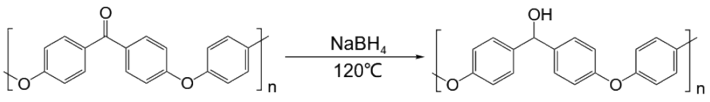

Fig.S1. The reduction reaction formula of SCP.

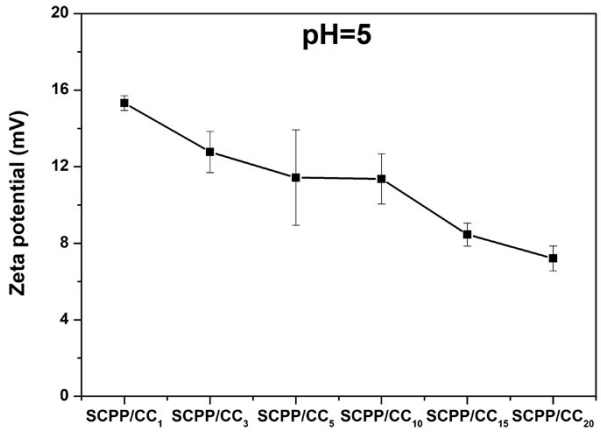

Fig.S2. Zeta potential of SCPP/CC_n (n = 1, 3, 5, 10, 15, or 20).

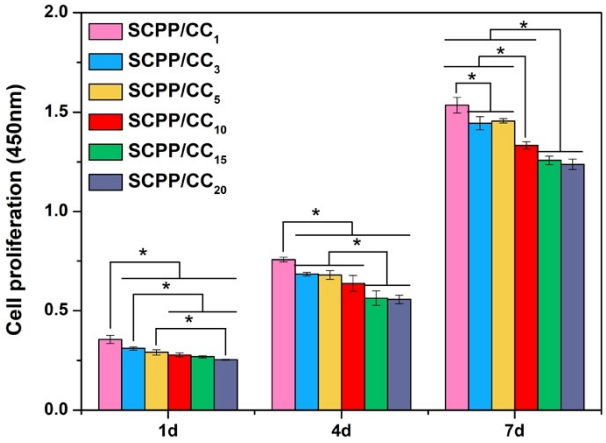

Fig.S3. The proliferation of BMSCs after 1, 4, and 7 days of incubation on SCPP/CC_n (n = 1, 3, 5, 10, 15, or 20). *$p < 0.05$.

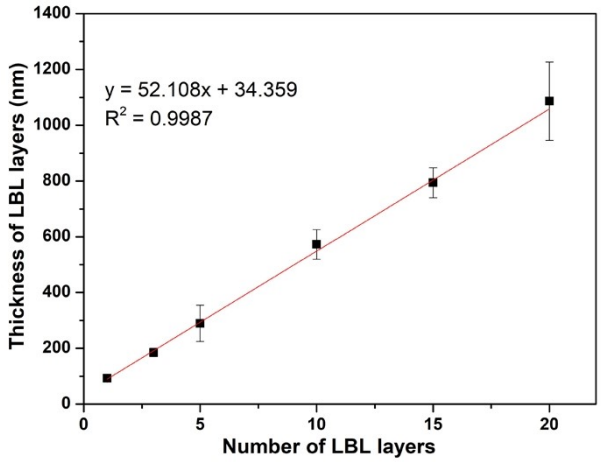

Fig.S4. Fitting diagram of the thickness and number of LBL layers.

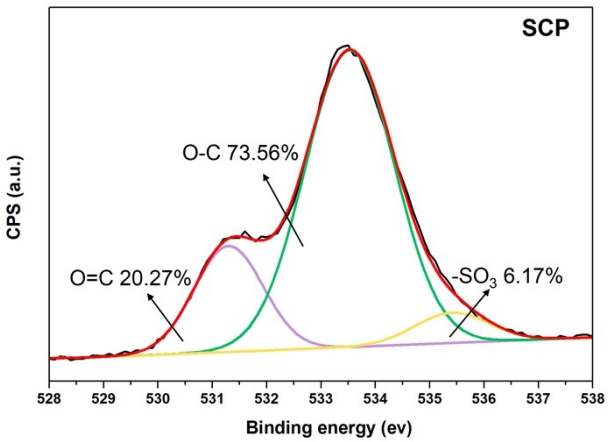

Fig.S5. O1s high-resolution XPS spectra of SCP.

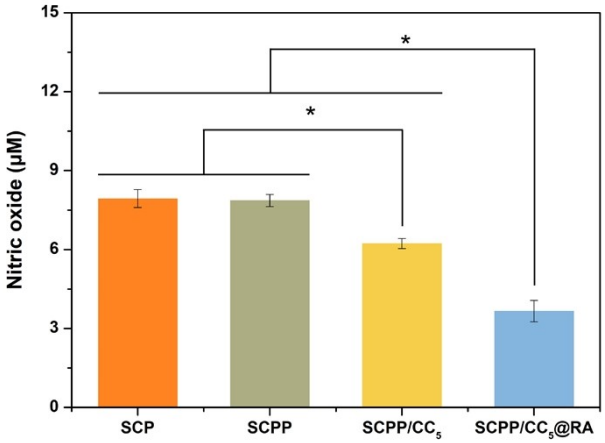

Fig.S6. NO release level of RAW 264.7 cells on various specimens.

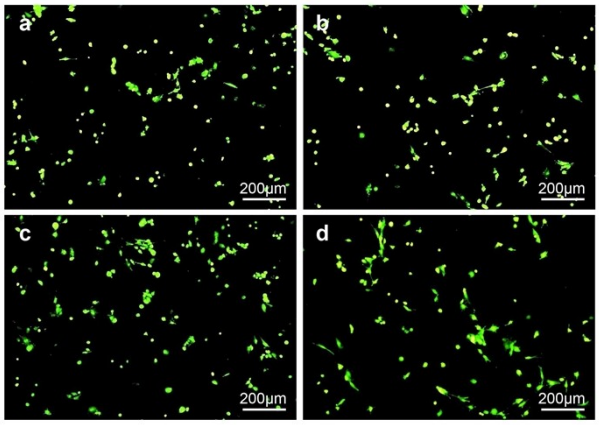

Fig.S7. Live/Dead fluorescent staining images (scale bar = 200 μm) of BMSCs (green represents living cells). (a) SCP, (b) SCPP, (c) SCPP/CC_5, and (d) SCPP/CC_5@RA.

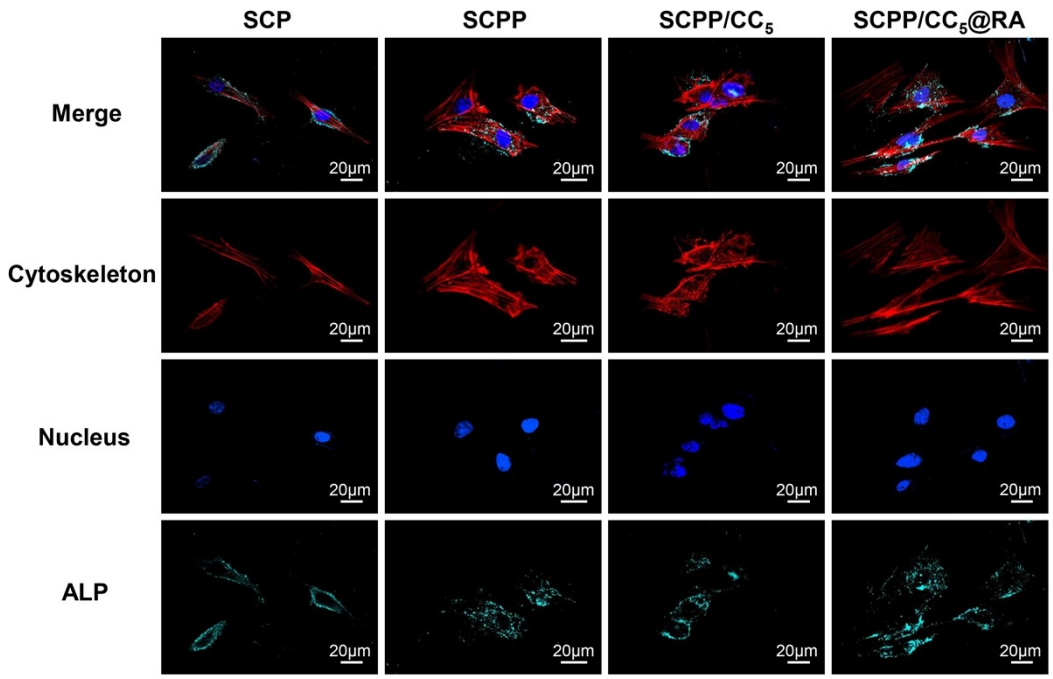

Fig.S8. Representative immunofluorescence staining images (scale bar = 20 μm) of ALP after culturing for 7 days on different sample surfaces (cyan: ALP; red: cytoskeleton; blue: nucleus).

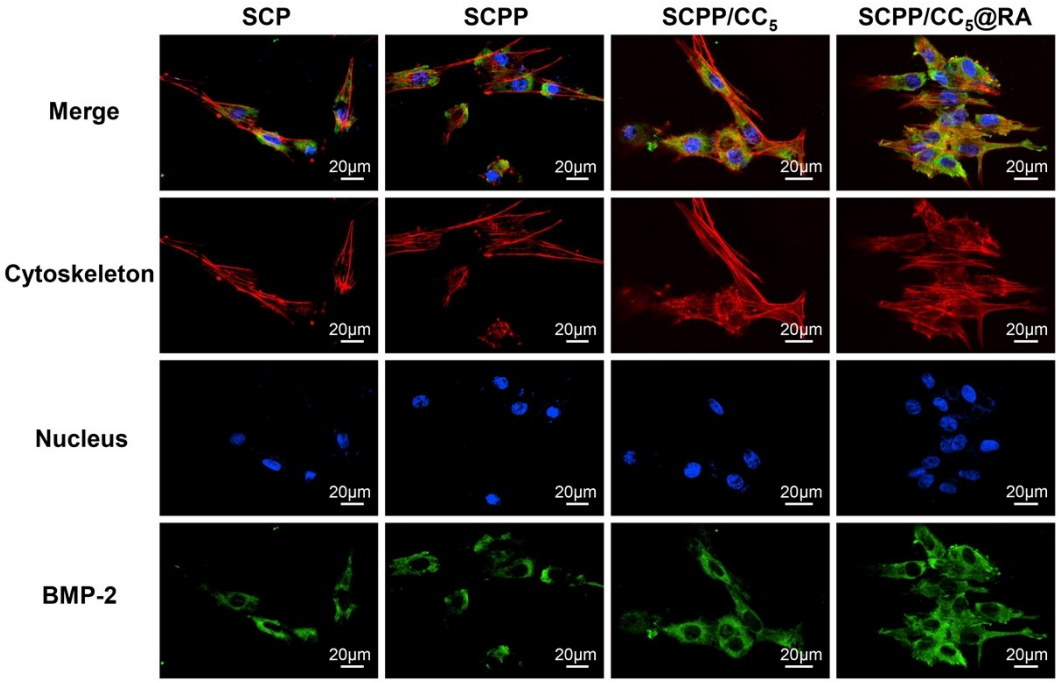

Fig.S9. Representative immunofluorescence staining images (scale bar = 20 μm) of BMP-2 after culturing for 7 days on different sample surfaces (green: BMP-2; red:

cytoskeleton; blue: nucleus).

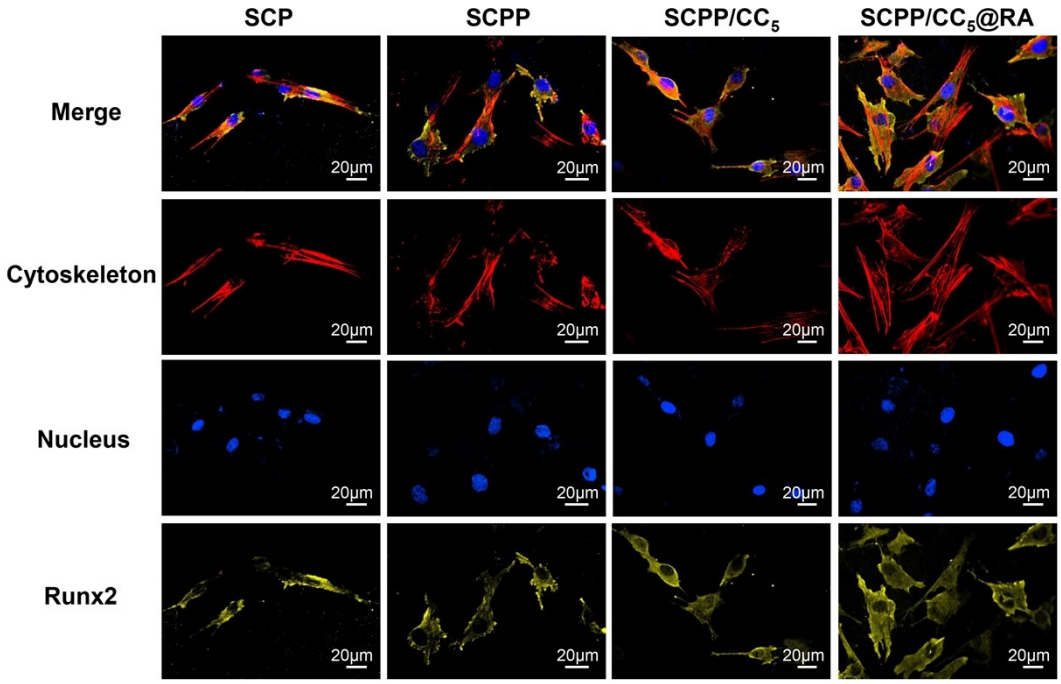

Fig.S10. Representative immunofluorescence staining images (scale bar = 20 μm) of Runx2 after culturing for 7 days on different sample surfaces (yellow: Runx2; red: cytoskeleton; blue: nucleus).

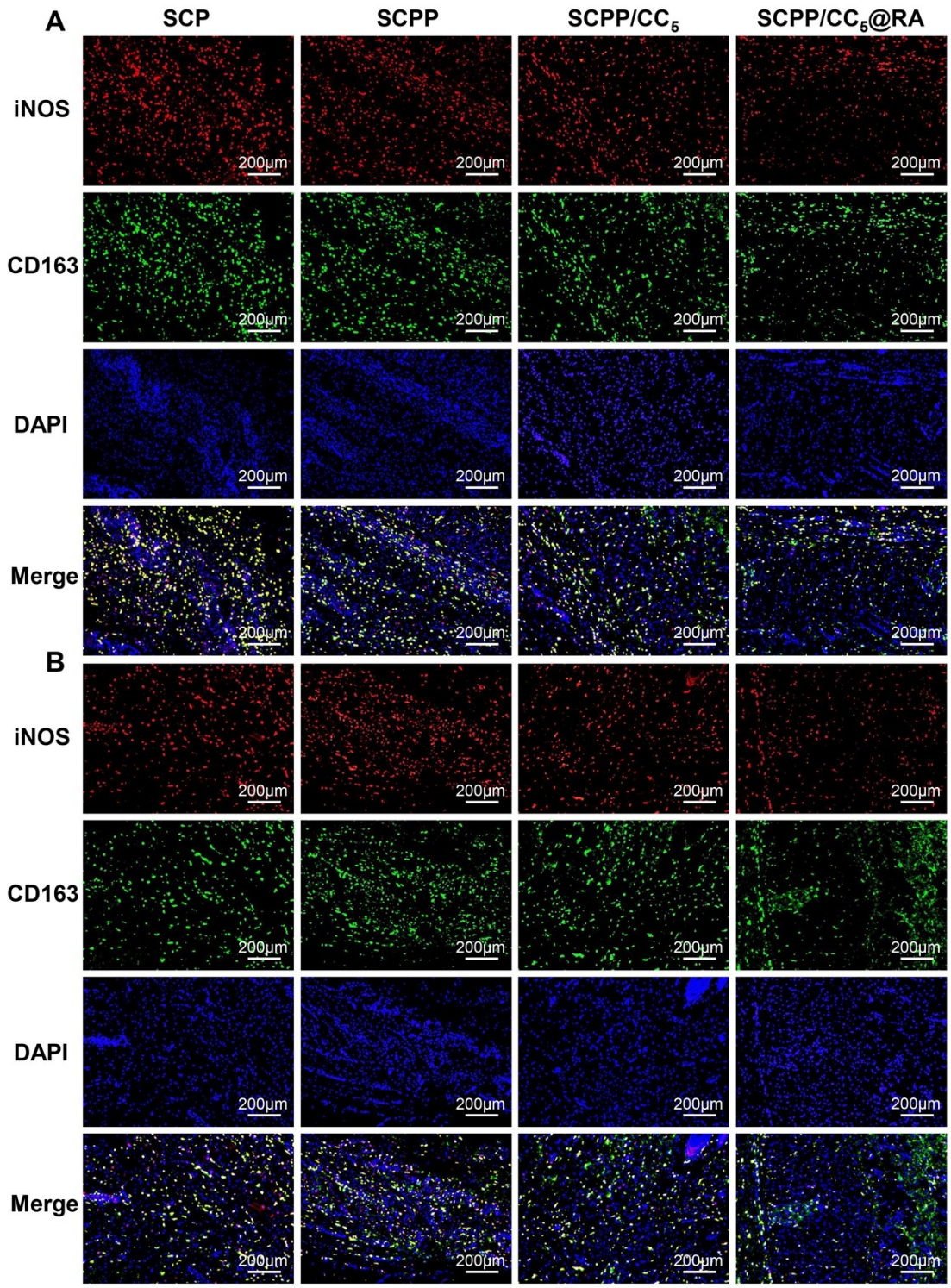

Fig.S11. Immunofluorescence staining images (scale bar = 200 μm) of tissues around implants after 3 days (A) and 7 days (B) of implantation. (Red represents iNOS; green represents CD163; blue represents DAPI).

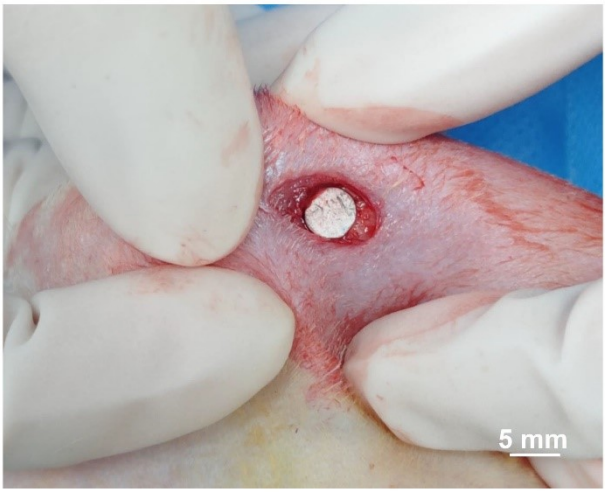

Fig.S12. Development of the rabbit tibial defect model (scale bar = 5 mm).

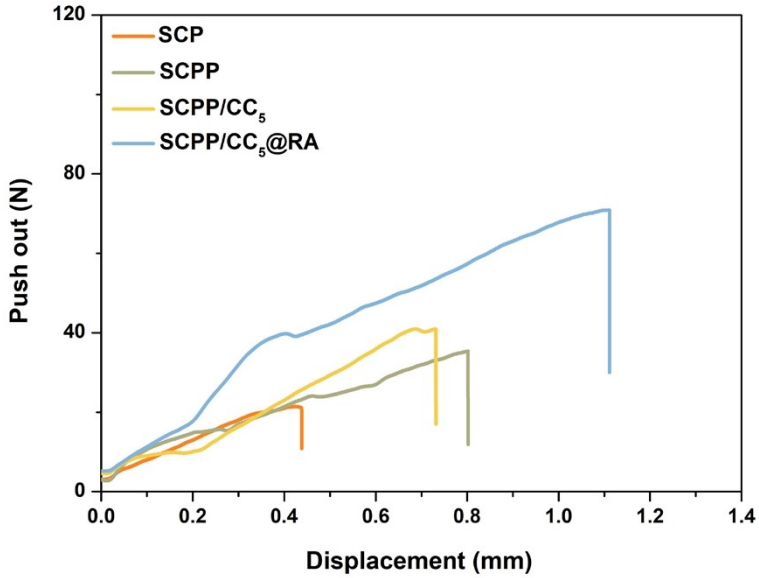

Fig.S13. The load-displacement curves of different implants after 4 weeks of implantation.

Table S1. The elemental composition of different sample surfaces determined by XPS.

Substrate	C%	O%	N%	S%
SCP	70.40	28.89	——	0.72
SCP-OH	70.21	29.58	——	0.21
SCPP	66.20	31.17	2.63	——
SCPP/CC_5	46.02	50.59	3.39	——
SCPP/CC_5@RA	41.21	55.05	3.74	——

Table S2. Primer pairs used to measure inflammation-related genes in RT-PCR.

Genes	Sequence (5′-3′)
iNOS	F: CCTTTGCTCATGACATCGACCA
	R: TCCTGCCCACTGAGTTCGT
IL-6	F: GACTTCCATCCAGTTGCCTT
	R: ATGTGTAATTAAGCCTCCGACT
IL-10	F: GAAGACAATAACTGCACCCACT
	R: AGTCGGTTAGCAGTATGTTGT
CD206	F: GTCATACCGTGTTGAACCTCT
	R: ACACAATCATTCCGTTCACCAG
GAPDH	F: GAACATCATCCCTGCATCCACT
	R: GATCCACGACGGACACATTGG

Table S3. Primer pairs used to measure osteogenesis-related genes in RT-PCR.

Genes	Sequence (5′-3′)
ALP	F: ATCGGAACAACCTGACTGACC
	R: CTGCCTCCTTCCACTAGCAA
BMP-2	F: ATTAGCAGGTCTTTGCACCA
	R: ACGCTTTTCTCGTTTGTGGA
Col-1	F: ATGCCATCAAGGTCTACTGCAA
	R: GAACCTTCGCTTCCATACTCG
Runx2	F: GGCAGCACGCTATTAAATCCAA
	R: GACTCATCCATTCTGCCGCTA
GAPDH	F: TATGACTCTACCCACGGCAAG
	R: ATACTCAGCACCAGCATCACC