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Metal-phenolic networks Acted as a Novel Bio-filler of a Barrier Membrane to Improve Guided Bone Regeneration via Manipulating Osteoimmunomodulation

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1 Experimental section

1. 1 Cell culture

Rat bone mesenchymal stem cells (BMSCs) and a murine-derived macrophage cell line (RAW264.7) were applied in this study. BMSCs were isolated from tibias and fibulae of SD rats and then cultured in α-MEM containing 10% fetal bovine serum (FBS) and 1% (v/v) penicillin/streptomycin at 37 ℃ with a 5% CO_2 atmosphere. RAW264.7 cells were purchased from Procell Life Science & Technology Co. Ltd. in Wuhan and cultured in DMEM containing normal FBS and penicillin/streptomycin at 37 ℃ with a 5% CO_2 atmosphere.

1.2 The effects of Sr concentration on cells proliferation

Both BMSCs and RAW264.7 cells were respectively cultured for 1, 3, 5 days in the medium supplemented with different concentrations of $SrCl_2$ at a density of 3×10^3 cells/well in a 96-well-plate. Then the medium was replaced by medium containing 10% CCK8-reagent (KeyGEN BioTECH, Nanjing, China) and incubated for 30 minutes in the dark. After incubation, absorbance of the supernatant at 450 nm wavelength was tested.

1.3 The effects of Sr concentration on osteogenic differentiation

To investigate the osteogenic function of different doses of Sr2+, qualitative and quantitative alkaline phosphatase (ALP) activity and the expression of osteogenesis-related genes of BMSCs were tested. Qualitative alkaline phosphatase (ALP) activity was measured using a 5-bromo-4-chloro-3-ndolyl phosphate/p-nitro blue tetrazolium (BCIP/NBT) ALP colour development kit (Beyotime Co, Shanghai, China) at a density

of 2×10^4 cells/well in a 24-well-plate. After culturing in osteogenic medium for 7 days, the cells were fixed with 4% paraformaldehyde, rinsed with PBS, stained with the kit, and observed under an optical microscope. Also, quantitative ALP activity was measured by Alkaline phosphatase assay kit (Nanjing Jiancheng Bioengineering Institue, Nanjing, China). After incubation for 7 days, the cells were collected and lysed on ice. Then, the lysates were collected for further test using the kit under the construction of the manufacture. To investigate the expression of osteogenesis-related genes, BMSCs were cultured in a 6-well-plate at a density of 2×10^5 cells/well for 7 days. Total RNA was extracted from the BMSCs by the Trizol method (Thermo Fisher Scientific, USA). Complementary DNA was synthesized from 1 μg total RNA with a HiScript Q Select RT SuperMix for qPCR kit (Nanjing Vazyme Biotech Co., Ltd. China). Quantitative gene expression analysis of osteocalcin (OCN), osteopontin (OPN) and runt-related transcription factor 2 (RUNX2) was carried out by RT-PCR with the SYBR Premix Ex Taq system (Takara Biotechnology, Japan).

1.4 The effects of Sr concentration on immunomodulatory function

To test the immunomodulatory function of different concentration of Sr^{2+}, RT-PCR was conducted to assess the expression of the inflammatory-related genes (IL-10, IL-1β, Il-6) and macrophage phenotype-related genes (CD206) of RAW264.7 cells after co-cultured with medium supplemented with different doses of $SrCl_2$ for 1 day.

2 Supplementary Figure

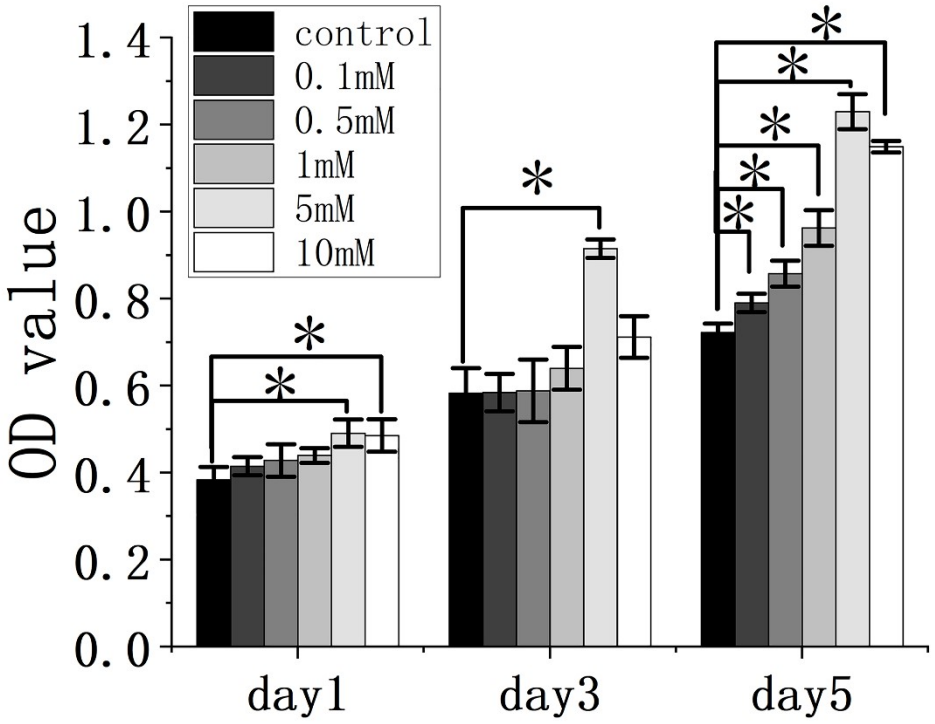

Fig. S1. The result of CCK8 test of BMSCs after co-culturing with different doses of Sr^{2+} at day 1, 3, 5. Significant differences are indicated as □(P□0.05).

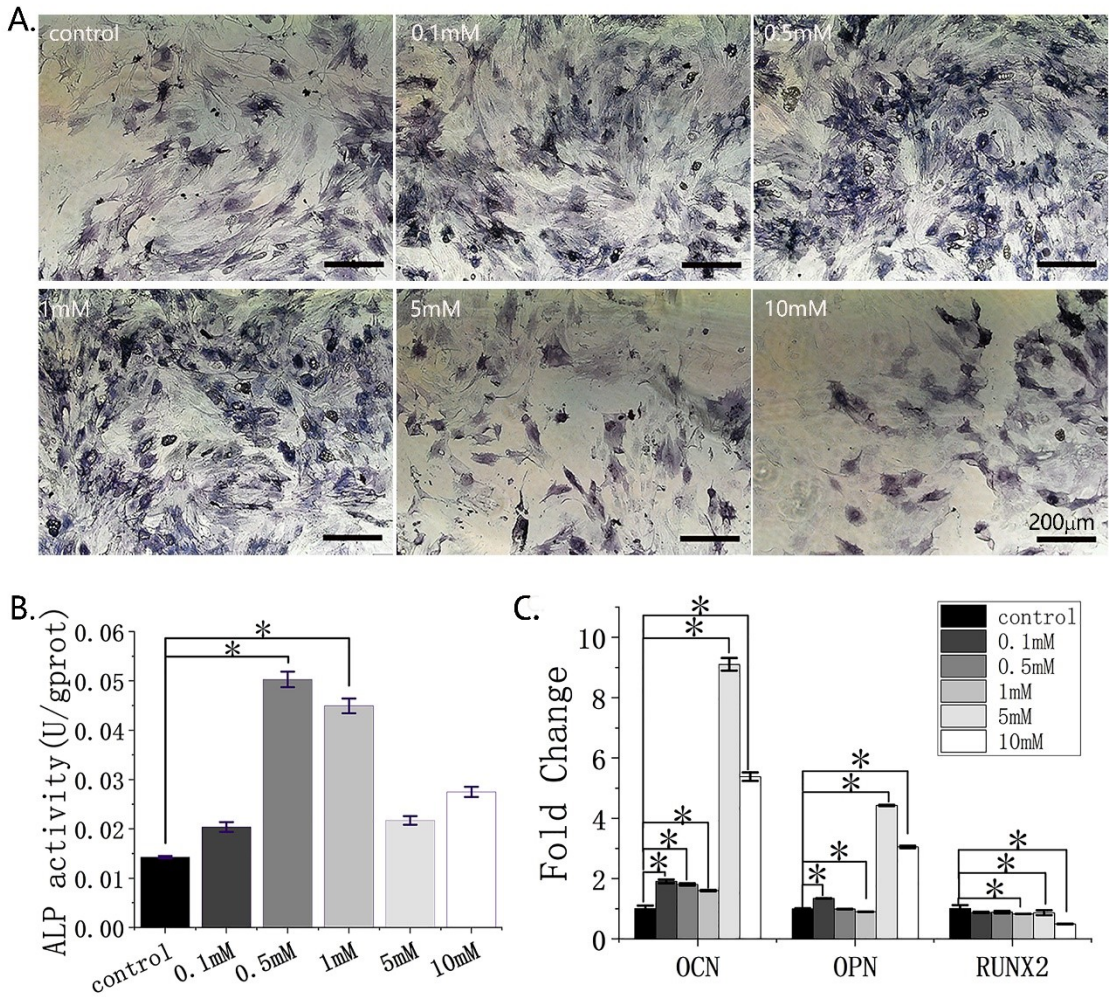

Fig. S2. (A) ALP staining of BMSCs cultured by the medium supplemented with different concentrations of Sr^{2+}. (B) Quantitative analysis of ALP activity of BMSCs cultured by the medium supplemented with different concentrations of Sr^{2+}. (C) Analysis of osteogenic-related genes expression of BMSCs after co-cultured with osteogenic medium containing different concentrations of Sr^{2+}. Significant differences are indicated as□(P□0.05).

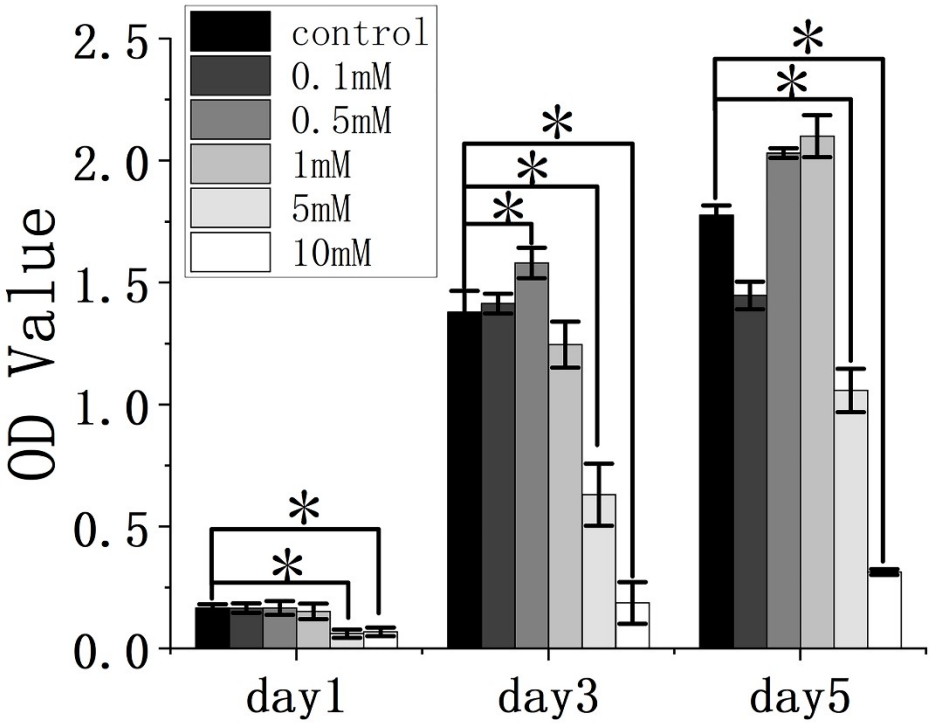

Fig. S3. The result of CCK8 test of RAW264.7 cells after co-culturing with different doses of Sr^{2+} at day 1, 3, 5. Significant differences are indicated as □(P□0.05).

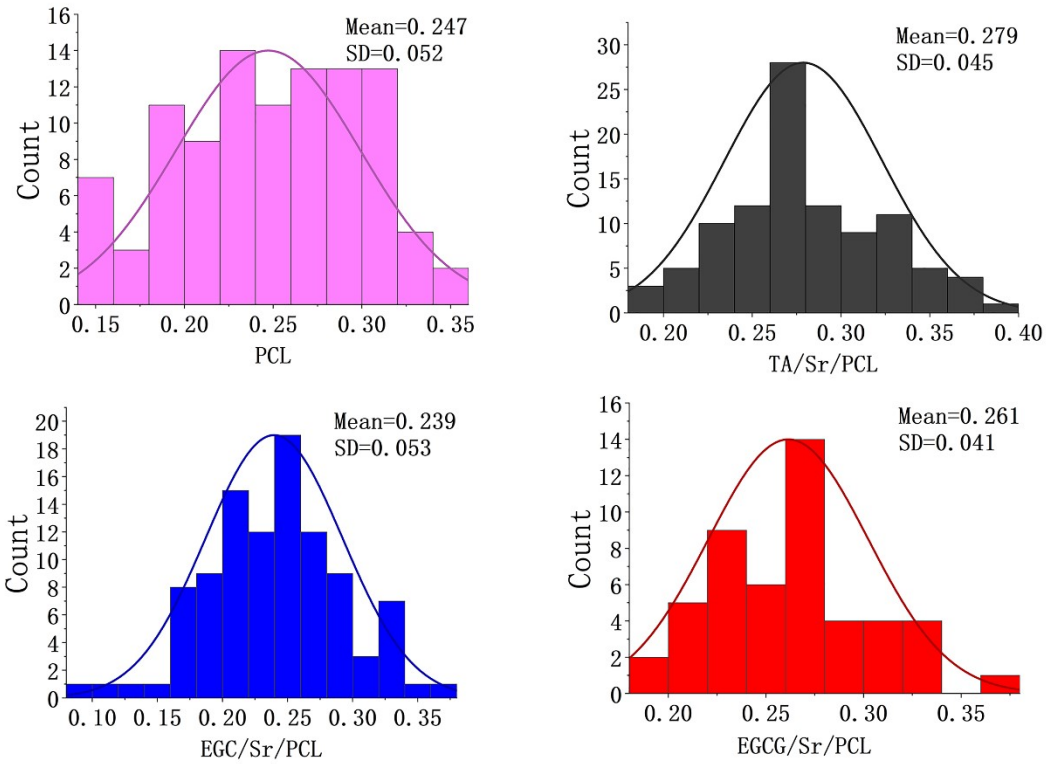

Fig. S4. The diameter of the nanofibers of different nanofibrous membranes. The diameter was measured in μm.

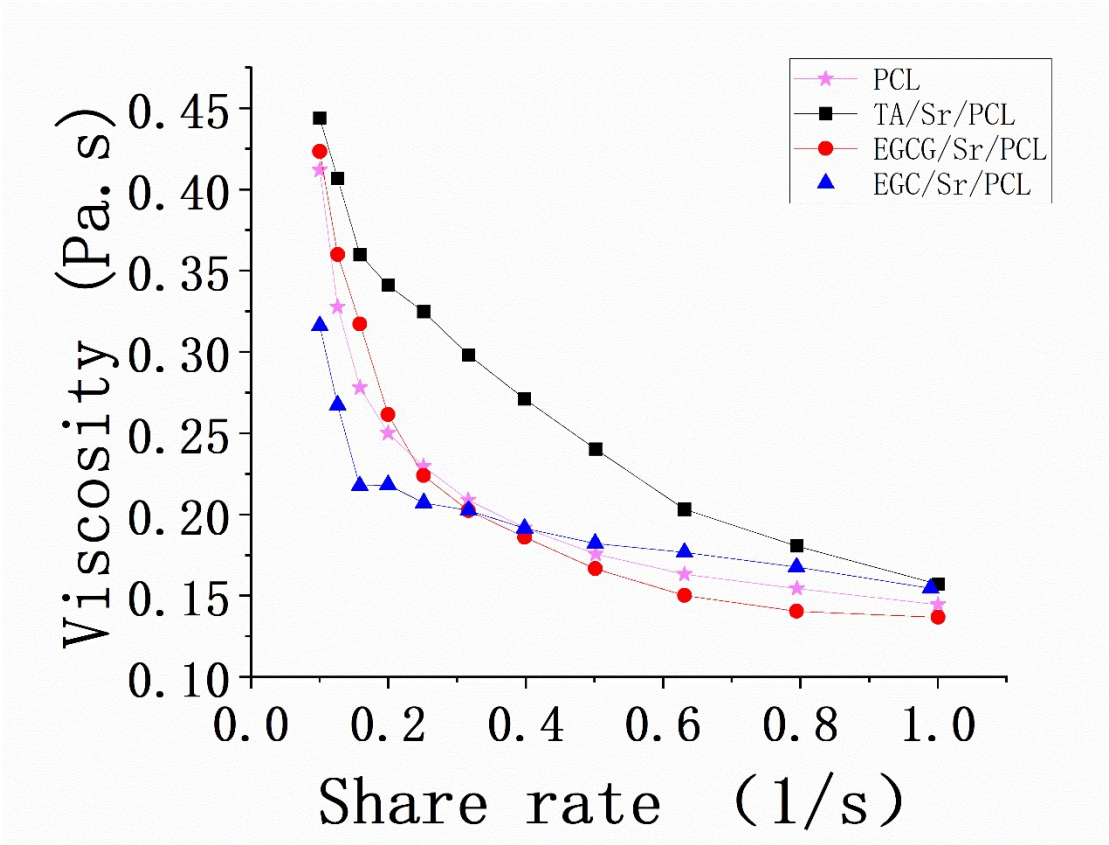

Fig. S5. The viscosity results of different electrospinning fluid.

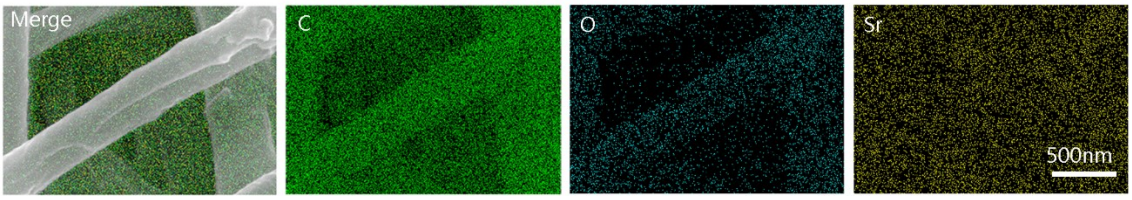

Fig. S6. EDS-mapping detection of smaller area of the modified membrane demonstrated that the incorporated Sr element was uniformly distributed.

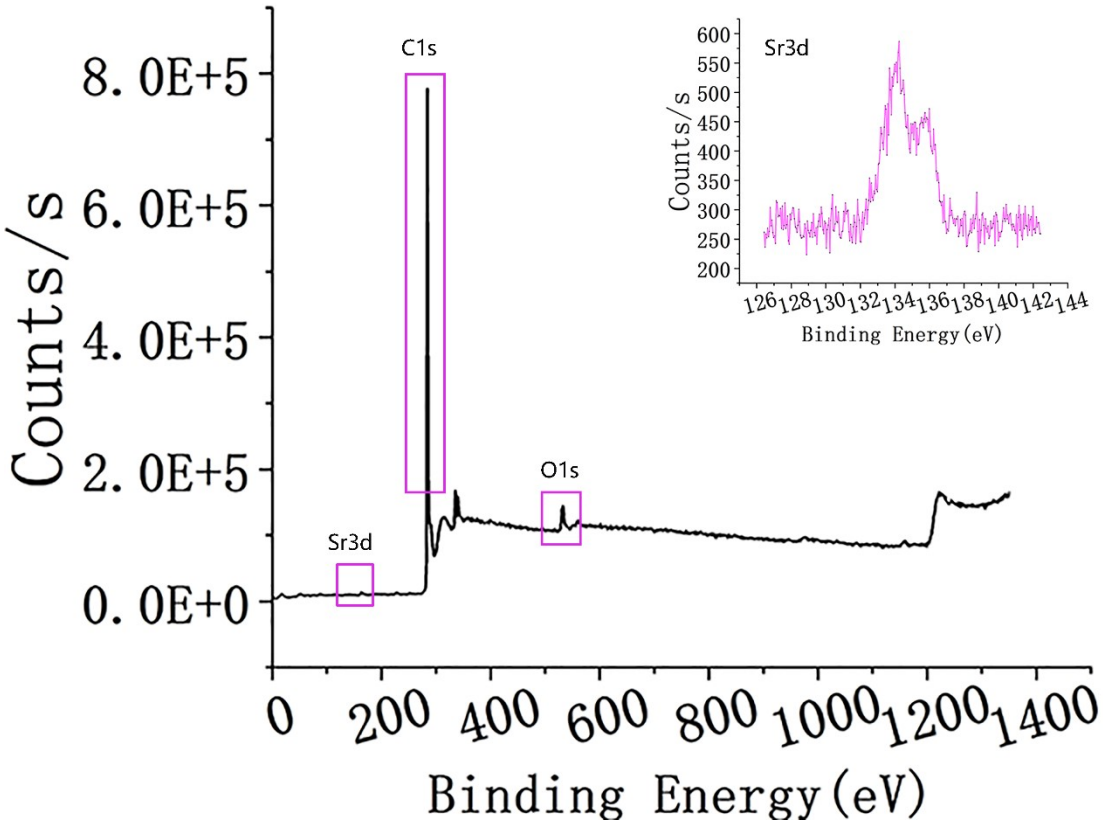

Fig. S7. XPS spectrum of TA-Sr-MPNs modified membrane.

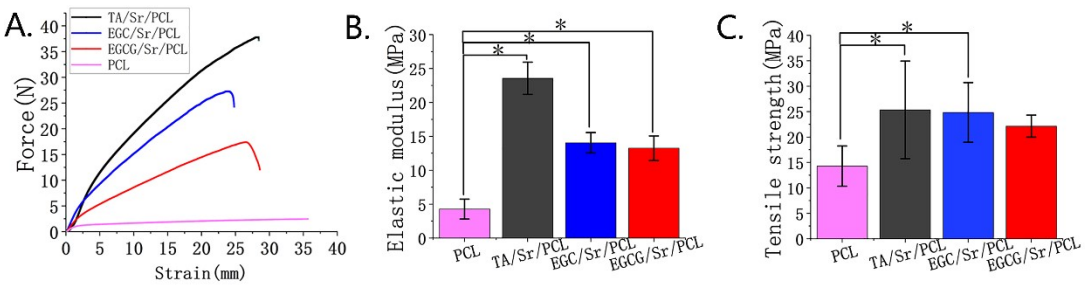

Fig. S8. (A) Force-strain curves and (B) The elastic modulus and (C) The tensile strength of polyphenols-Sr-MPNs membranes and pure PCL membrane showing mechanical performance of the membranes. Significant differences are indicated as □(P□0.05).

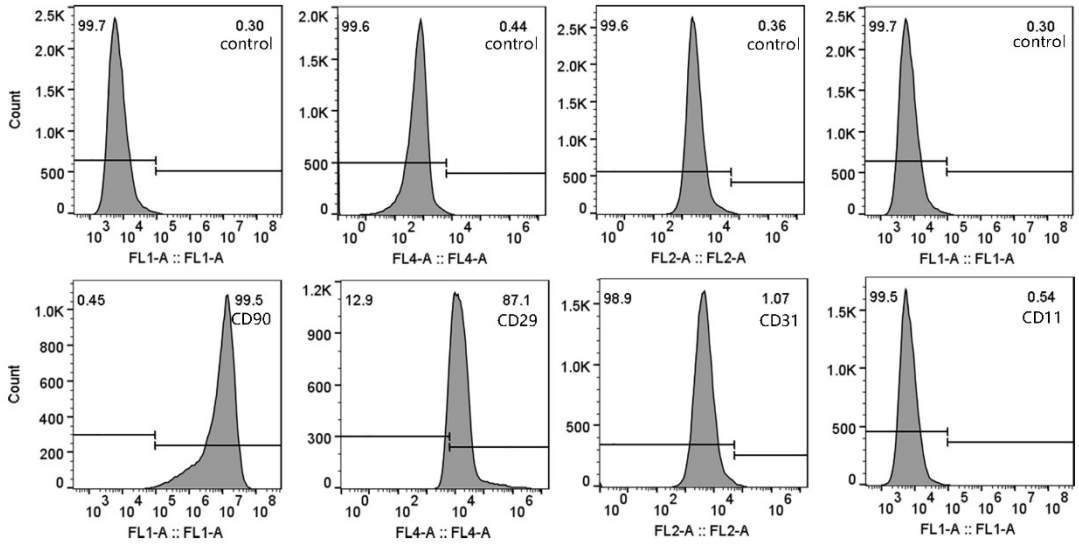

Fig. S9. Flow cytometric analysis of the obtained BMSCs which showed the cells marked by positive marker and negative marker.