Supporting Information

Mussel-inspired nanostructures potentiate the immunomodulatory properties and angiogenesis of mesenchymal stem cells

Tao Li,[1†], Hongshi Ma,[1†] Hongzhi Ma,[2†] Zhenjiang Ma,[1] Lei Qiang,[3] Zezheng Yang,[1] Xiaoxiao Yang,[1] Xiaojun Zhou,[1] Kerong Dai,[1*] Jinwu Wang.[1*]*

1. Shanghai Key Laboratory of Orthopaedic Implant, Department of Orthopaedic Surgery, Shanghai Ninth People's Hospital, Shanghai Jiao Tong University School of Medicine, Shanghai 200011, P.R. of China.

2. Department of Radiation Oncology, Hunan Cancer Hospital and The Affiliated Cancer Hospital of Xiangya School of Medicine, Central South University, Changsha, Hunan, 410006, China.

3. Southwest Jiaotong University College of Medicine, No.111, North Section, 2nd Ring Road, Chengdu, Sichuan, 610031, China.

† Tao Li, Hongshi Ma and Hongzhi Ma equally contributed to this work.

* Kerong Dai, Jinwu Wang and Xiaojun Zhou are correspondent authors.

Tel: +86 15800577039 / +86 13301773680

Fax: +86 (21) 23271699

E-mail:

Jinwu_wang@163.com, Daikr@163.com, xjz362@163.com.

Supplementary experimental section

Histological analysis and immunofluorescence staining

Rats were anesthetized and killed at days 7 or 14 with the dermal specimens of the skin defect harvested for histological analysis. The collected tissues were fixed with 10% formaldehyde for overnight and then embedded in paraffin. For Hematoxylin and Eosin (H&E) and Masson trichrome staining, sections with the 5 μm-thickness were prepared and mounted on slides before imaging. For immunohistochemical staining, nonspecific binding was blocked by incubating the specimen with 5% goat serum for 0.5 h, and the sections were decanted and immersed into the primary antibodies at 4 °C overnight. Immunofluorescence stain of CD31 was performed with primary CD31 antibody (mouse anti-rat CD31, ab119339, Abcam). Then the sections were added with secondary antibodies (goat anti-mouse Alexa Fluor-488, ab150113, Abcam) at 37 ℃ for 2 h. To evaluate the polarization of macrophages, primary antibodies of the pan-macrophage marker CD68 (mouse anti-rat CD68, ab201340, Abcam), and the M2 macrophage marker CD206 (rabbit anti-rat CD206, ab64693, Abcam) were incubated with specimen at 4 ℃ overnight. After washing with PBS, secondary antibodies (goat anti-mouse Alexa Fluor-594, ab150116, Abcam) and goat anti-rabbit Alexa Fluor-488 were added to specimen for 1 h at 37℃. The nuclei of the cells were stained by DAPI, and fluorescence mounting medium (AR1109, Boster Bio) for confocal microscopy imaging was applied to cover the slides. The cell number of different macrophage phenotype was calculated using ImageJ software (National Institutes of Health).

Supplementary figures

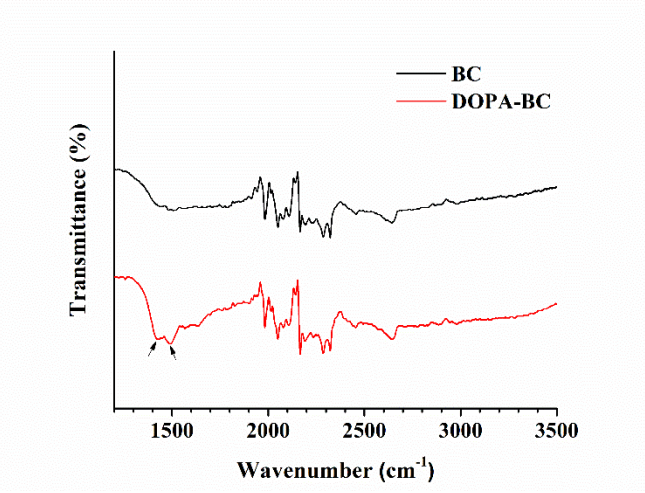

Fig. S1. FTIR spectra of BC and DOPA-BC scaffolds.

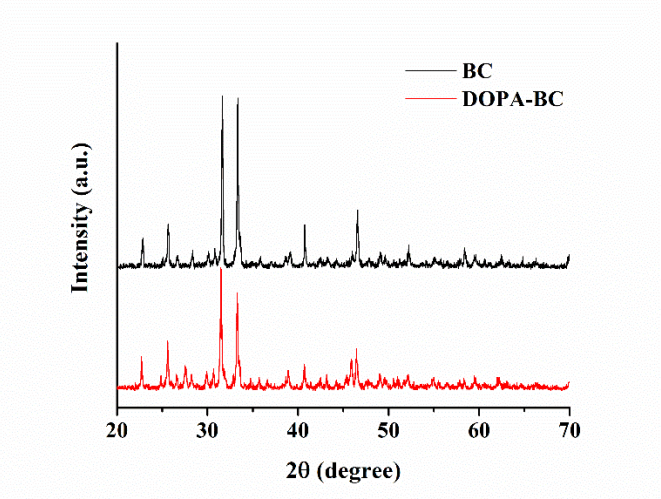

Fig. S2. The XRD spectra of BC and DOPA-BC scaffolds.

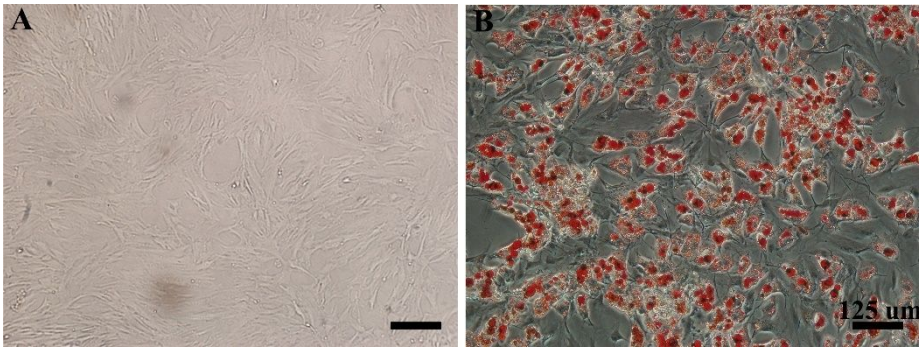

Fig. S3. (A) The morphology of Ad-MSCs under inverted microscope at passage 3.

(B) Oil red O staining after adipogenic induction for 3 weeks.

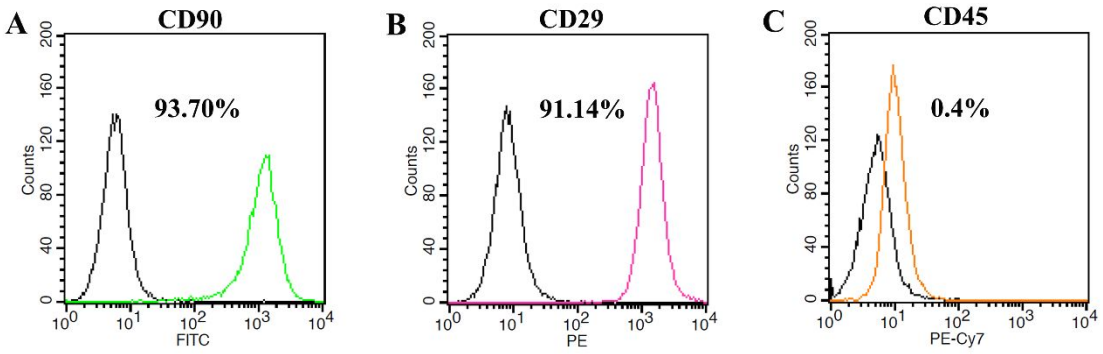

Fig. S4. Surface markers of Ad-MSCs at passage 3 determined using flow cytometry, (A) CD90, (B) CD29 and (C) CD45.