Supplementary information

Tao Li,[1,2#] Hongtao He,[3#] Zezheng Yang,[4#] Junjie Wang,[1] Yuxin Zhang,[5] Guangxu He,[1] Jun Huang[1], Deye Song,[1] Jiangdong Ni,[1] Xiaojun Zhou,[6*] Junfeng Zhu,[2*] Muliang Ding.[1*]

1. Department of Orthopaedics, The Second Xiangya Hospital, Central South University, Changsha, Hunan, 410011, P.R. China.

2. Department of Orthopaedics, Xinhua Hospital affiliated to Shanghai Jiaotong University School of Medicine, No.1665 Kongjiang Road, Shanghai, 200092, P. R. China.

3. The Third Ward of Department of Orthopedics, The Second Hospital of Dalian Medical University, No. 467, Zhongshan Road, Shahekou District, Dalian, Liaoning Province 116000, P. R. China.

4. Department of Orthopedics, The Fifth People's Hospital of Shanghai, Fudan University, Minhang District, Shanghai 200240, P. R. China.

5. Department of Rehabilitation Medicine, Shanghai Ninth People's Hospital Affiliated to Shanghai Jiao Tong University School of Medicine, Huangpu District, Shanghai 200011, China.

6. College of Chemistry, Chemical Engineering and Biotechnology; State Key Laboratory for Modification of Chemical Fibers and Polymer Materials, Donghua University, Shanghai 201620, P. R. China.

[#] These authors contributed equally to this work.

* Muliang Ding, Junfeng Zhu, and Xiaojun Zhou are corresponding authors.

Tel: +86 15800577039 / +86 13974865370

Fax: +86 (21) 23271698

Email address: dingmuliang@csu.edu.cn, zhujunfeng@xinhuamed.com.cn, xjz362@163.com.

Supplementary experimental section

Histological analysis and immunofluorescence staining

Mouse were anesthetized and killed at days 3, 7, and 14 with the implanted scaffolds and the surrounding tissue 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 and α-SMA were performed with primary CD31 antibody (mouse anti-mouse CD31, ab24590, Abcam) and α-SMA antibody (rabbit anti-mouse, ab32575, Abcam). Then the sections were added with secondary antibodies for CD31 (Goat anti-mouse Alexa Fluor-594, ab150120, Abcam) and α-SMA (goat anti-rabbit Alexa Fluor-488, ab150077, Abcam) at 37 ℃ for 2 h respectively. To evaluate the polarization of macrophages, primary antibodies of the pan-macrophage marker CD68 (rat anti-mouse CD68, ab53444, Abcam), and the M2 macrophage marker CD206 (rabbit anti-mouse CD206, ab209327, Abcam) were incubated with specimen at 4 ℃ overnight. After washing with PBS, secondary antibodies (Donkey anti-rat Alexa Fluor-647, ab150155, Abcam and goat anti-rabbit Alexa Fluor-488, ab150077) 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). To assess the infiltration and polarization of neutrophils, the specimens were deparaffinized and rehydrated, primary antibodies for immunohistochemistry against Ly6G (rabbit anti-mouse, ab238132, Abcam) and MPO (rabbit anti-mouse, ab208670, Abcam) were incubated with specimen at 4 ℃ overnight. After washed with PBS for three times, the nuclei of were stained by DAPI and the stained slides were imaged using a light microscope (Nikon).

Supplementary figures:

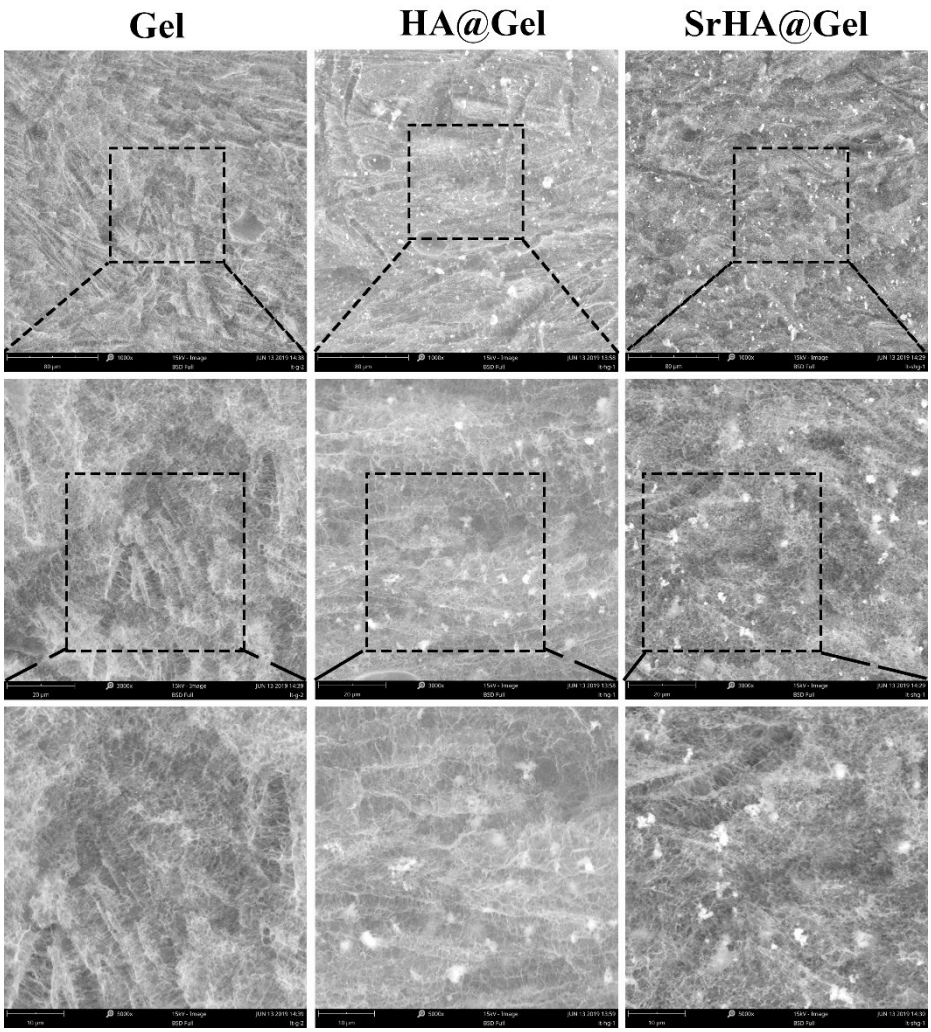

Figure.S1. The SEM analysis of Gel, HA@Gel, and SrHA@Gel scaffolds.

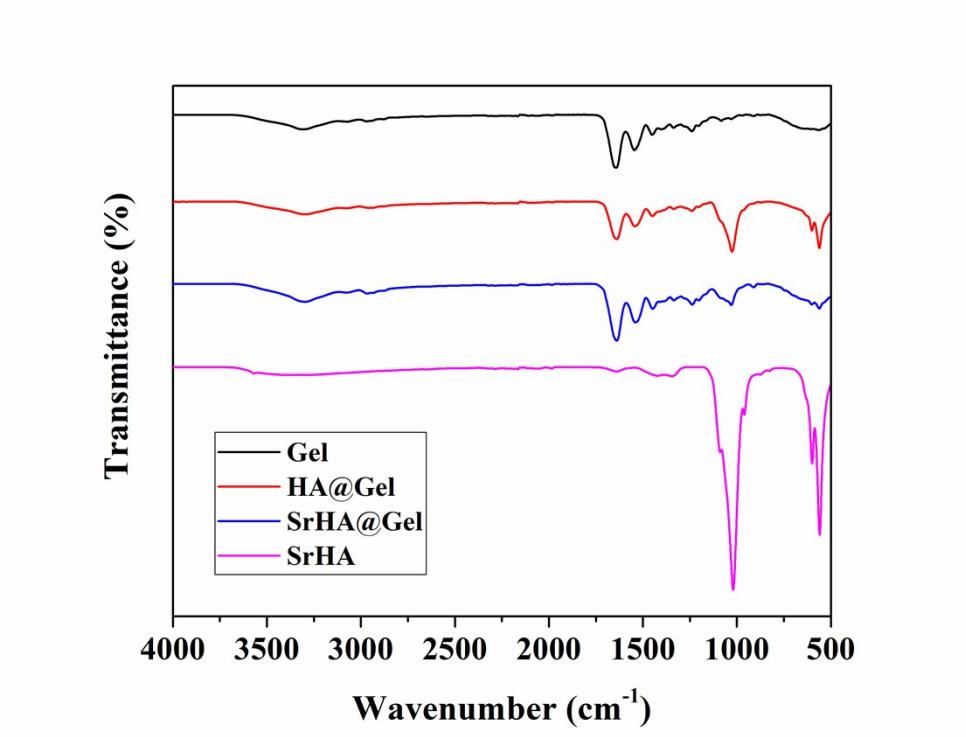

Figure.S2. The FTIR analysis of scaffolds Gel, HA@Gel, SrHA@Gel, and pure SrHA nanoparticles.

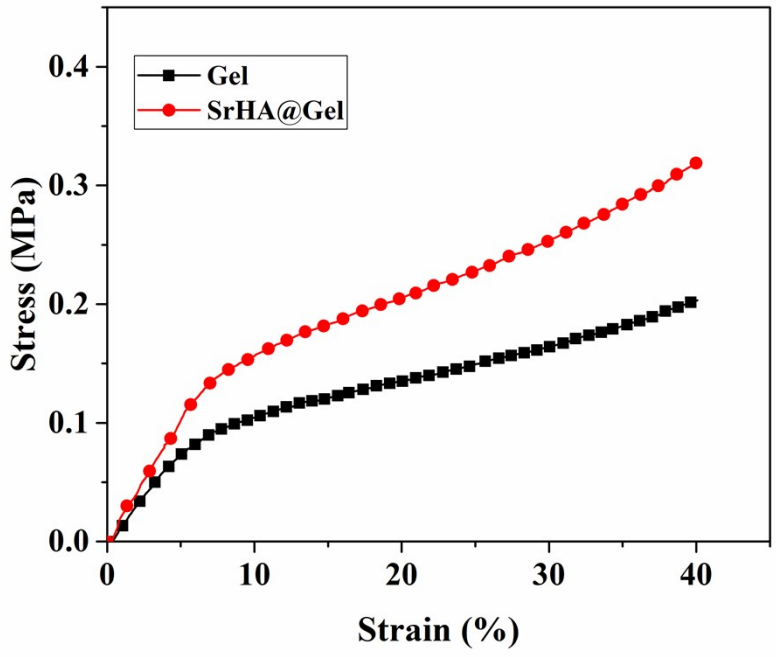

Figure.S3. The stress-strain curve of scaffolds Gel and SrHA@Gel.

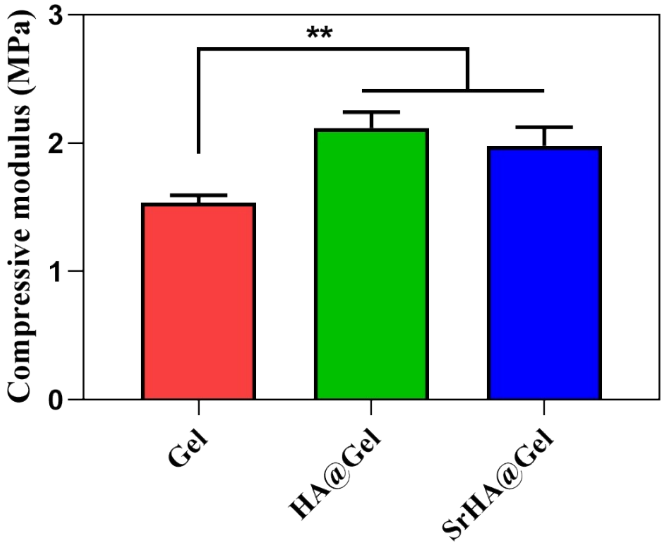

Figure.S4. The Young's modulus of scaffolds Gel, HA@Gel, and SrHA@Gel.

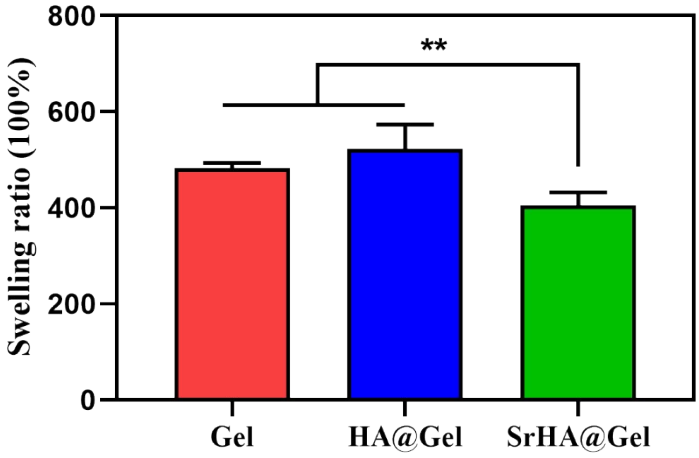

Figure.S5. The swelling ratio of scaffolds Gel, HA@Gel, and SrHA@Gel.

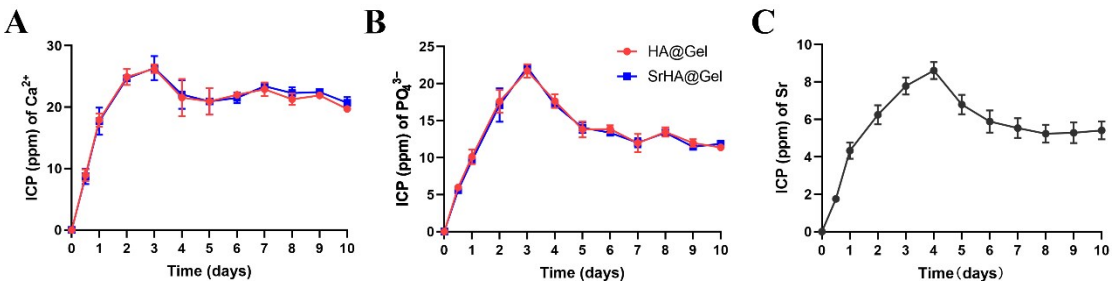

Figure.S6. The ICP analysis of different ions released from scaffolds. (A) The

releasement of Ca^{2+}, (B) PO_4^{3-} from HA@Gel and SrHA@Gel scaffolds. (C) The releasement of Sr from SrHA@Gel scaffolds.

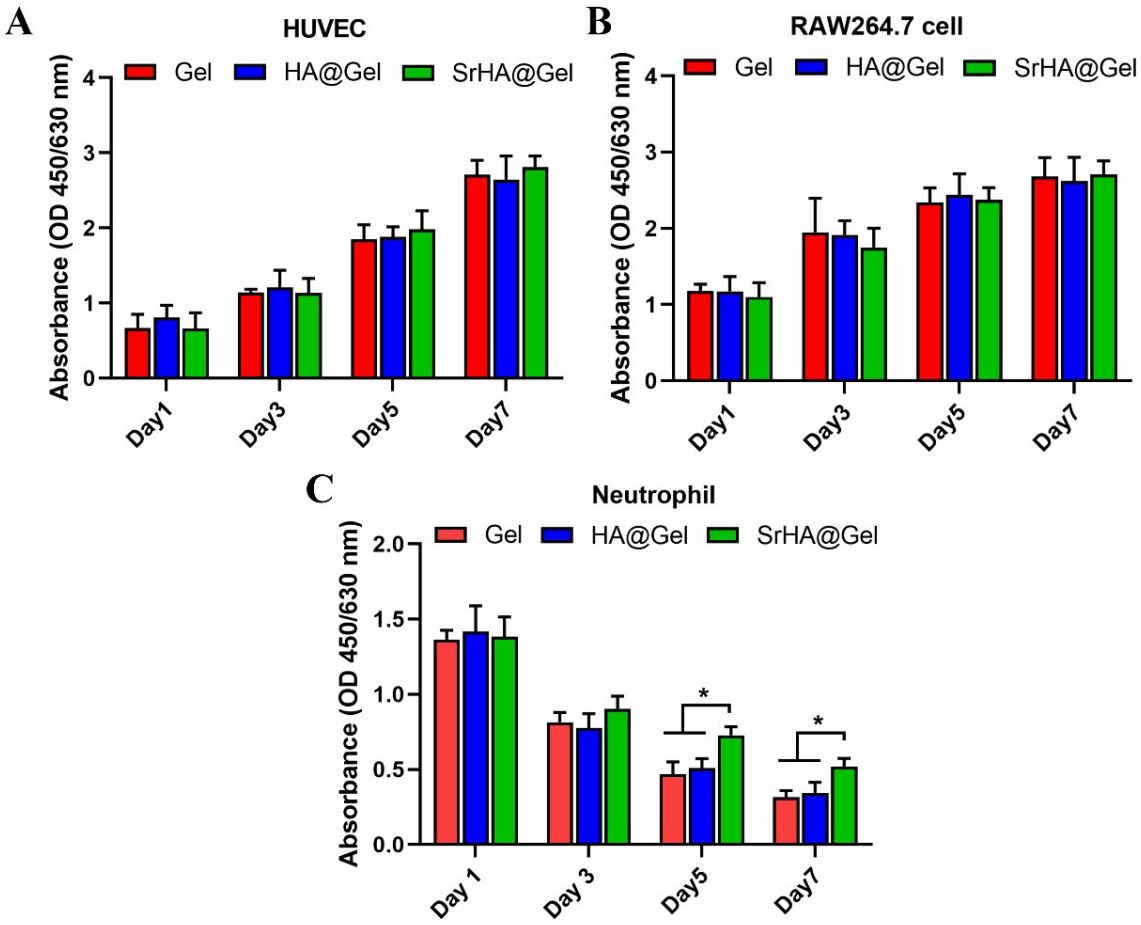

Figure.S7. The cell-proliferation rates of (A) HUVEC, (B) RAW264.7, and (C) neutrophils on Gel, HA@Gel, and SrHA@Gel scaffolds.

Coculture analysis

The harvested neutrophils were firstly polarized into N1 or N2 in vitro respectively, then these cells (5×10^4 cells/mL) were culture with RAW264.7 or HUVEC at the concentration of (5×10^4 cells/mL, 1:1) in DMEM culture medium. The proliferation of different coculture systems was evaluated by CCK-8 test as previously described at day 1, day 3, day 5, and day 7. The results (Figure.S8) showed no discrepancy between

each groups.

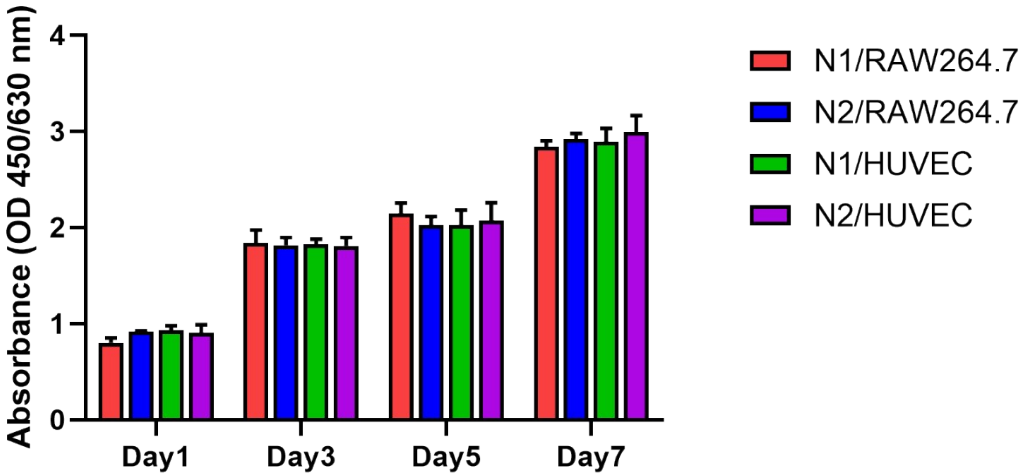

Figure.S8. The cell-proliferation rates of (A) HUVEC, (B) RAW264.7 with N1 and N2 neutrophils respectively.

Degradation analysis

Degradation was assessed at 1, 3, 5, 7, 14, and 21 days. The samples were weighed prior to incubation (Wi) in immersed in PBS (pH 7.4) containing 8 $X10^4$ U m1 lysozyme within a humidified incubator at 37℃. After 21 days, the samples were removed from the solution, gently blotted with filter paper to remove surface water, and immediately weighed again (Ws). The samples were next dehydrated in an oven for 48 hours to remove the remaining water, thereafter all of the samples were weighed (Wd) for a third time. Scaffolds degradation ratios were calculated with the following equation:

Degradation ratio =Wi -Wd /Wi X 100 %”

As shown in Figure. S9, the degradation rate of each group follows a first-order curve. The Gel group showed the highest degradation (a weight loss of 18.2 % at day 21). While the incorporation of HA or SrHA into Gel, the degradation ratio decreased to 15.9 % (HA@Gel group) and 15.4 % (SrHA@Gel group) at day 21.

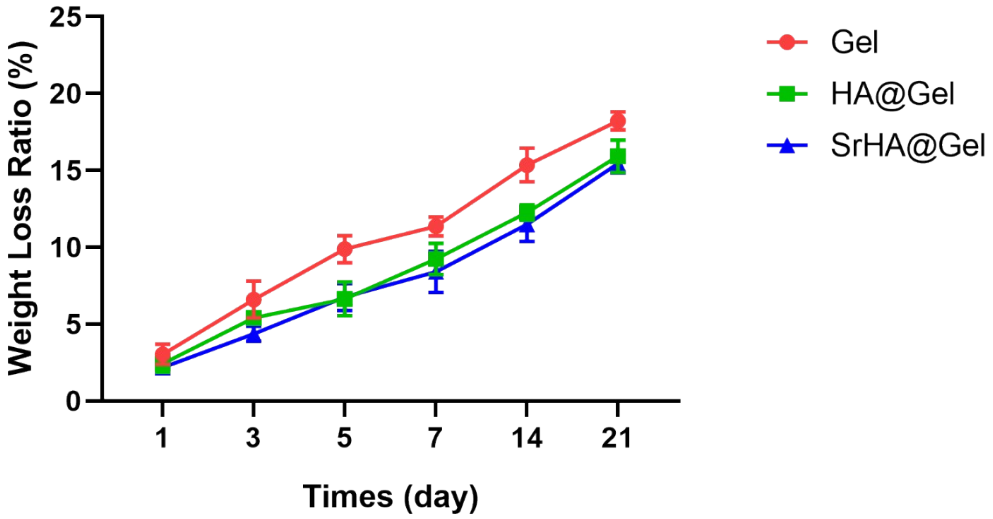

Figure. S9. Degradation ratio of Gel, HA@Gel, and SrHA@Gel scaffolds at different time points. (N = 3)