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Sodium butyrate-modified sulfonated polyetheretherketone modulates macrophage behavior and shows enhanced the antibacterial and osteogenic functions during implant-associated infections

Chao Yang[1, a], Liping Ouyang[1, b, c], Wei Wang[a], Baohui Chen[b], Wei Liu[a], Xiangwei Yuan[a], Yao Luo[a], Tao Cheng[a], Kelvin W. K. Yeung [d], Xuanyong Liu [b, *], Xianlong Zhang[a, *]

[a] Department of Orthopedics, Shanghai Jiao Tong University Affiliated Sixth People's Hospital, Shanghai 200233, China

[b] State Key Laboratory of High-Performance Ceramics and Superfine Microstructure, Shanghai Institute of Ceramics, Chinese Academy of Sciences, Shanghai 200050, China

[c] Center of Materials Science and Optoelectronics Engineering, University of Chinese Academy of Sciences, Beijing 100049, P. R. China

[d] Department of Orthopaedics and Traumatology, The University of Hong Kong, Hong Kong, P. R. China

[*] Corresponding Authors:

Xianlong Zhang. E-mail: dr_zhangxianlong@163.com

Xuanyong Liu. E-mail: xyliu@mail.sic.ac.cn

The samples were polished and ultrasonically cleaned in acetone, ethanol, and ultra-pure water in sequence. The porous structure on the PEEK surface was fabricated using a sulfonation technique and the residual sulfur was removed by hydrothermal treatment at 120 °C for 4 h. The surface microstructure was observed by SEM and the results are shown in Figure S1. Compared with the relatively flat surface of PEEK; a porous microstructure was fabricated on the sulfonated PEEK surface.

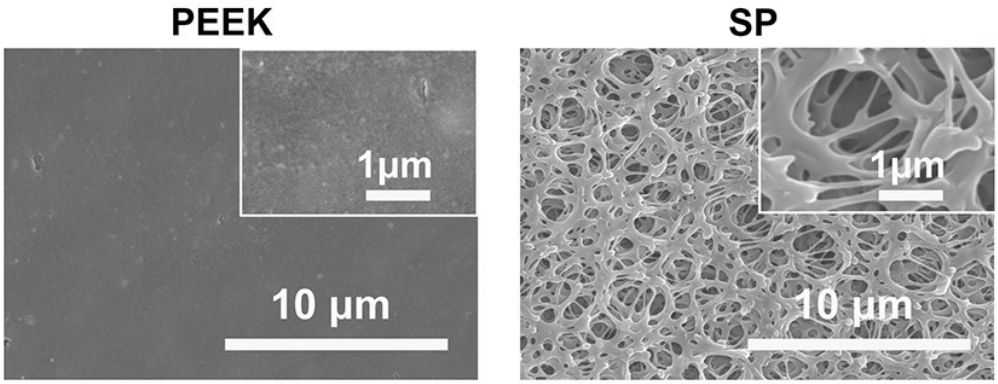

Figure S1. Surface morphologies of PEEK and SP observed by SEM at low and high magnification.

Bacteria counting was used to quantify the number of viable bacteria, and SEM was used to observe the state and the microstructure of bacteria. The *S. aureus* and *E. coli* on the agar plates is

demonstrated in Figure S2a. The amount of *E. coli* on the SP-B2 and SP-B3 decreased to approximately 82%, and 91%, respectively (Figure S2b), whereas the amount of *S. aureus* on the SP-B2 and SP-B3 decreased by nearly 98%, and 100%, respectively (Figure S2c). The samples loaded with SB exhibited excellent properties against *S. aureus* and *E. coli*. Moreover, the antibacterial rate of SP against *S. aureus* was decreased to 75%. The observations of SEM were presented in Figure S2d. Compared with the control group, the amounts of *S. aureus* and *E. coli* were significantly reduced. The distorted and broken morphology of bacteria was observed, both *S. aureus* and *E. coli*, indicating that bacteria adhesion and viability on SB loading groups were suppressed (especially SP-B2 and SP-B3). The observations of SEM were consistent with the result obtained by SPM.

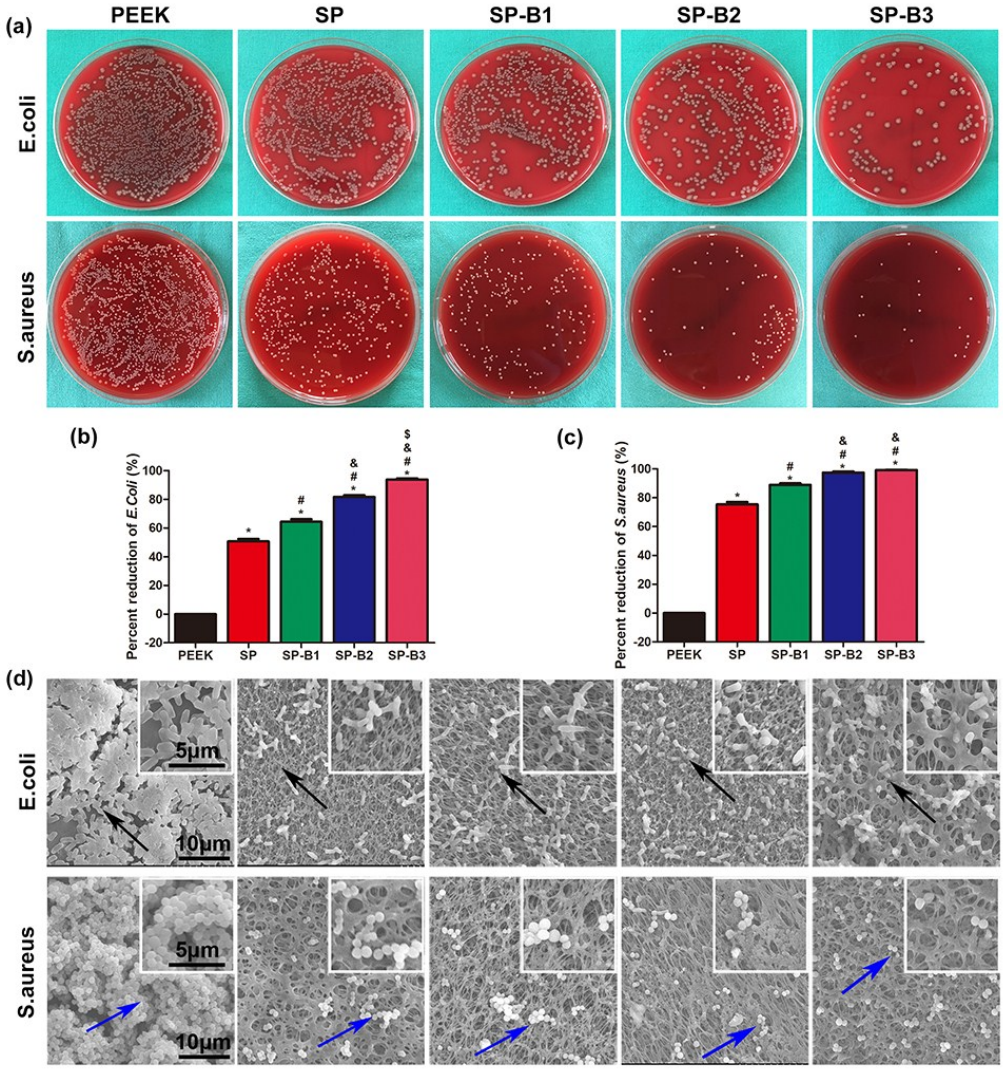

Figure S2. Antibacterial efficacy against E. coli and S. aureus *in vitro*.

The time-related proliferation of RAW264.7 were evaluated using CCK-8 assay and the result was

presented in Figure S3. After incubation for 1 day, there were no statistically significant differences between the five groups. After culturing for 4 days, RAW264.7 cells on the surface of SP-B2 group was higher than that on the other samples, and experimental groups possessed higher cell proliferation. As the culture time was extended to 7 days, the trend in RAW264.7 cell proliferation was as follows: PEEK < SP < SP-B3 < SP-B1 < SP-B2, which indicates better proliferation of RAW264.7 cells on SP-SB samples (especially SP-B2).

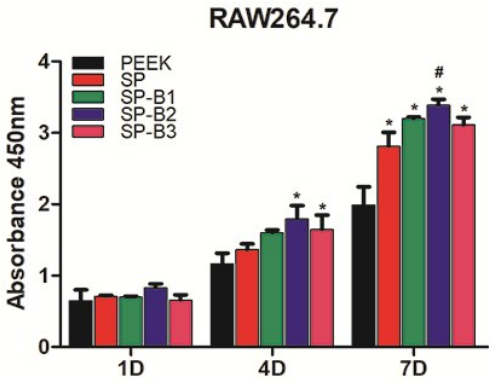

Figure S3. CCK-8 results of RAW264.7 cultured on various samples for 1, 4 and 7 days.

The ALP activity of rBMSCs cultured on various samples was measured using an ALP kit and the

result was presented in Figure S4. After incubation for 14 day, the ALP activity of rBMSCs cultured on SP-B2 samples show significant differences compare to PEEK group, while the SP-B1 and SP-B3 groups have no significant differences compare to control group.

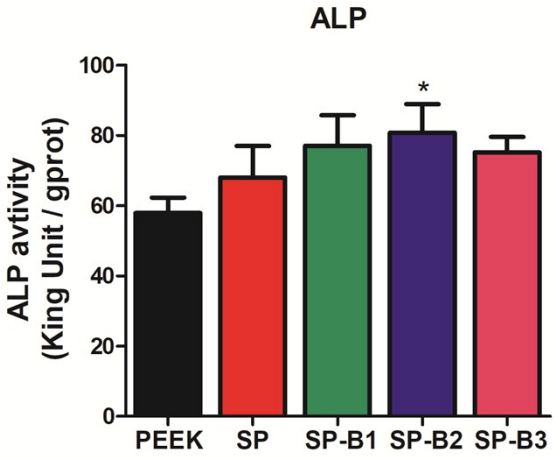

Figure S4. ALP activity of rBMSCs cultured on various samples for 14 days.

An in vivo mouse air pouch model was used evaluate the immune response of the samples. To

assess the inflammatory response and different macrophages phenotypes infiltrate the skin of the air pouch, we carried out histological staining and immunofluorescence staining of the skin sections (Figure S5). In contrast to PEEK and SP groups, SP-B2 attenuated the inflammatory reaction by decreasing the thickness of fibrous layer and cell infiltration (HE staining). As shown in immunofluorescence staining, more CCR7 (M1 marker) positive cells were observed in the PEEK and SP groups; in contrast, the SP-B2 group featured a higher proportion of Arg-1 (M2 marker) positive cells.

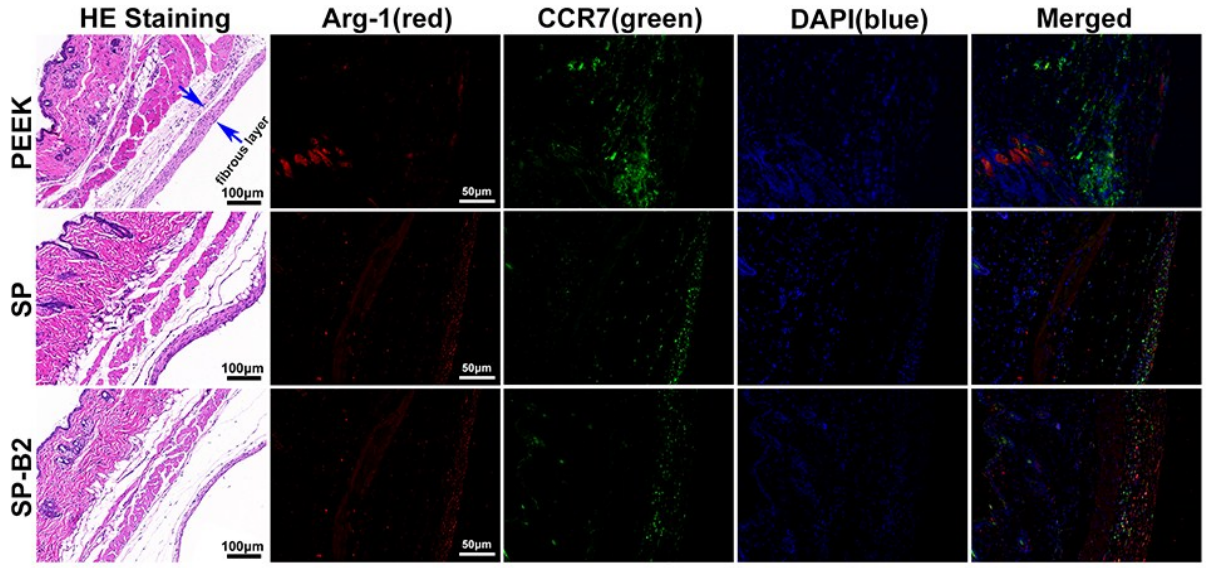

Figure S5. Histological analysis and immunofluorescence staining of air pouch tissues.