

Supplementary Information

All-in-one asymmetric porous membrane enables full protection in guided bone regeneration

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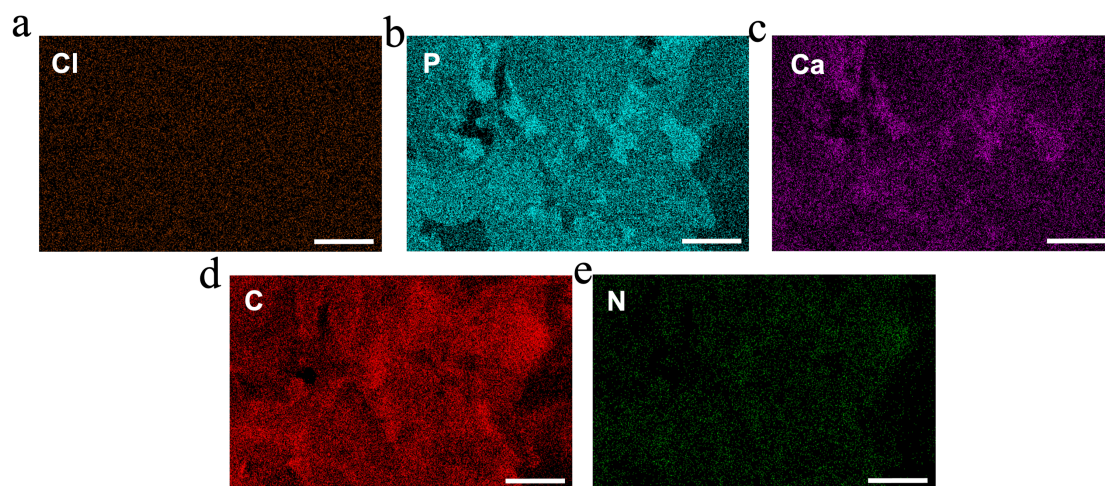
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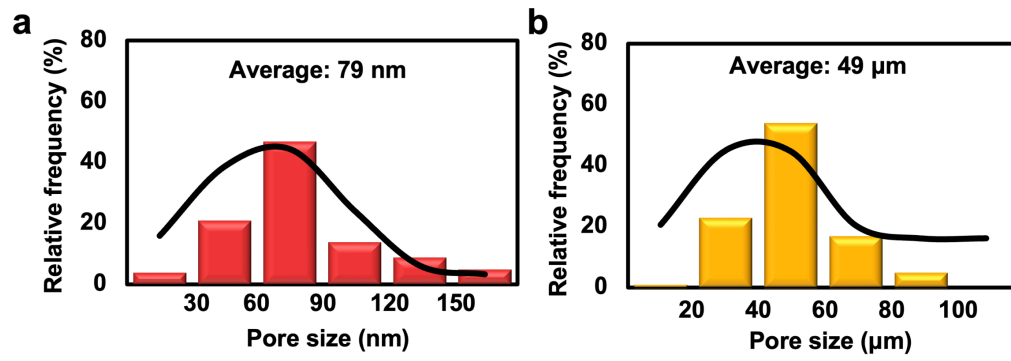
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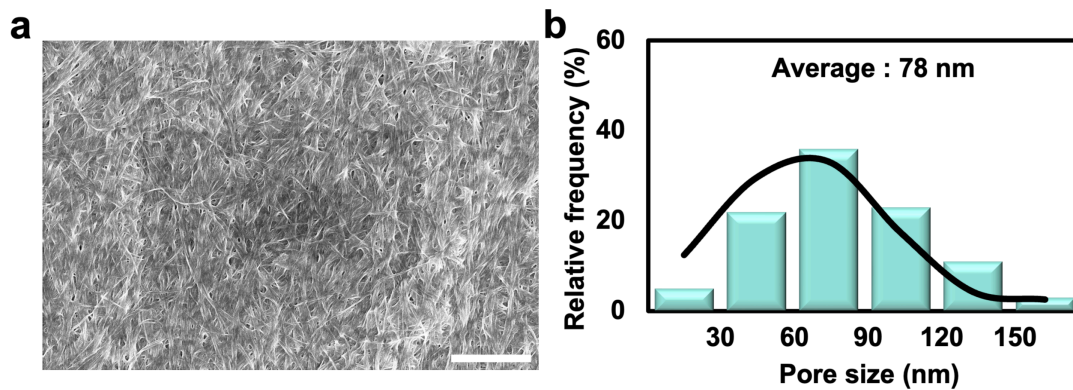


Supplementary Fig. 1 Elemental distributions of BC-g-NCl/CS-HAP membrane.

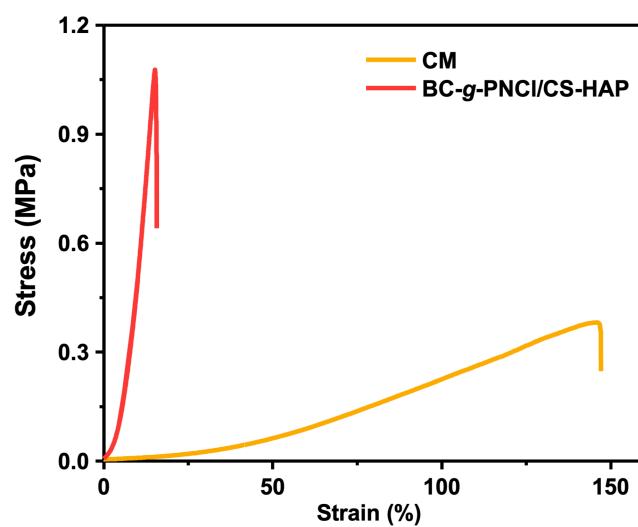
a Elemental mapping image of Cl (**a**) in the BC-g-NCl layer (scale bar = 2 μm), showing Cl element was evenly introduced in BC-g-NCl layer. **b-e** Elemental mapping images of P (**b**), Ca (**c**), C (**d**) and N (**e**) in the CS-HAP layer (scale bars = 10 μm), showing HAP particles and CS were well composited in CS-HAP layer. Observation was repeated three times independently, yielding similar results.



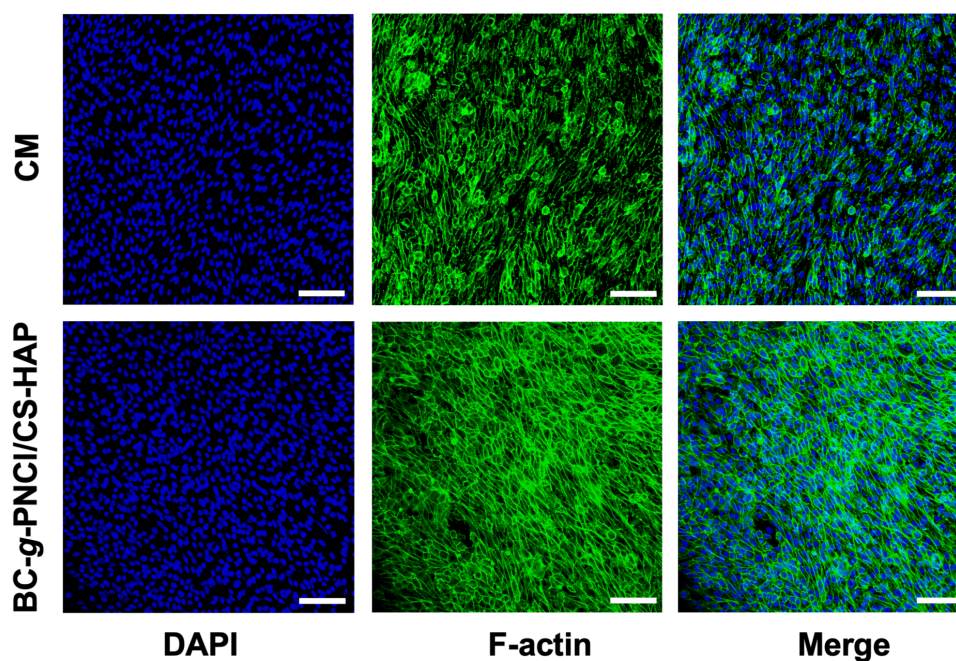
Supplementary Fig. 2 Pore size distributions of the dense and loose layers. a, b Pore size distributions of BC-g-PNCl dense layer **(a)** and CS-HAP loose layer **(b)** of BC-g-PNCl/CS-HAP according to the analyses of SEM images in Fig. 2e, f.



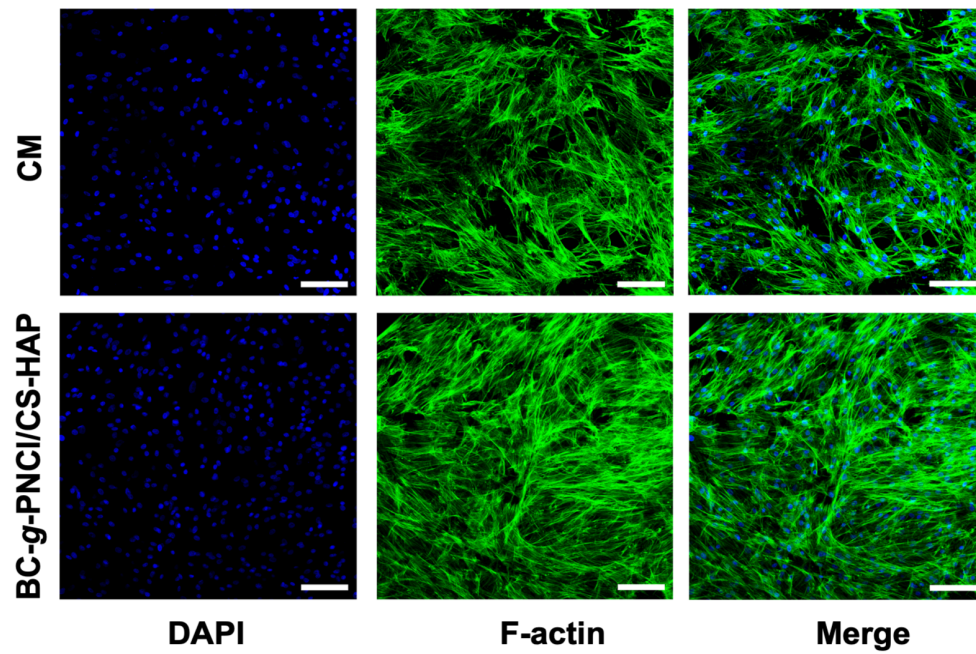
Supplementary Fig. 3 Surface morphology and pore size distribution of BC. a, b SEM image **(a)** (scale bar = 2 μm) and corresponding pore size distribution **(b)** of BC. SEM observation was repeated three times independently, yielding similar results.



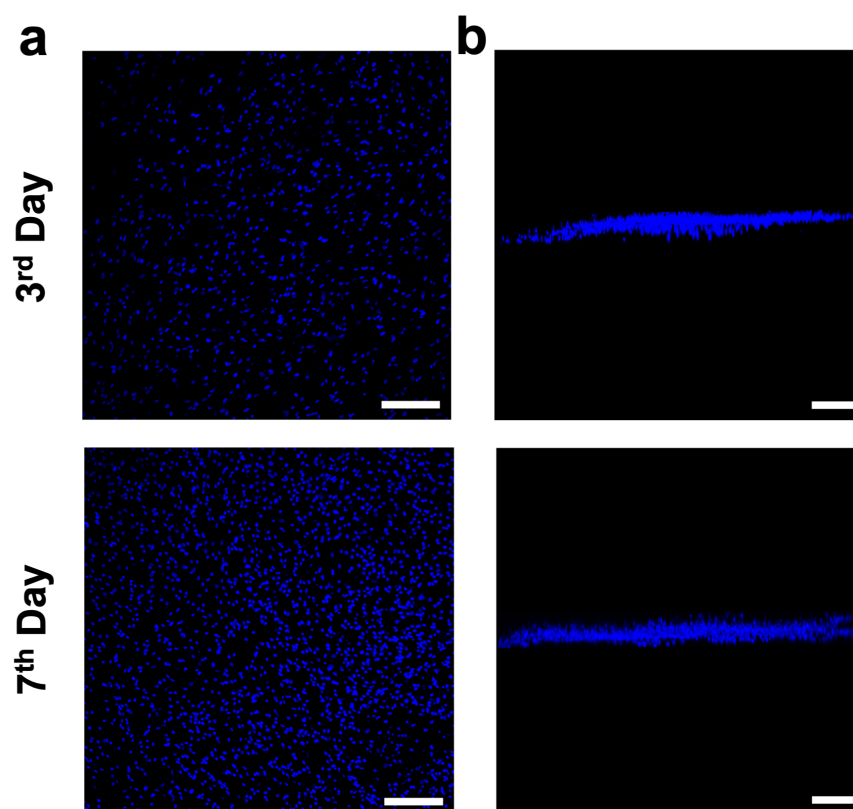
Supplementary Fig. 4 Tensile stress-strain curves of CM and BC-*g*-PNCl/CS-HAP in the wet state.



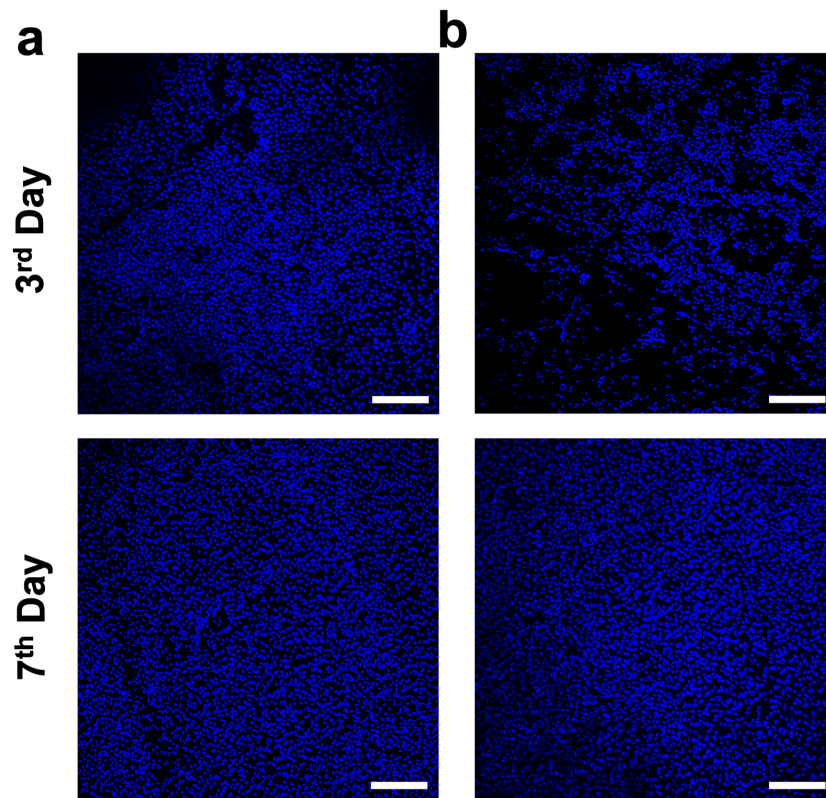
Supplementary Fig. 5 Morphology, extension, and viability of L929s. Morphology of L929s cultured on dense layer of CM and BC-g-PNCl layer of BC-g-PNCl/CS-HAP at the 7th day (green for F-actin, blue for cell nucleus, scale bars = 100 μm). Fluorescent observation was repeated three times independently, yielding similar results.



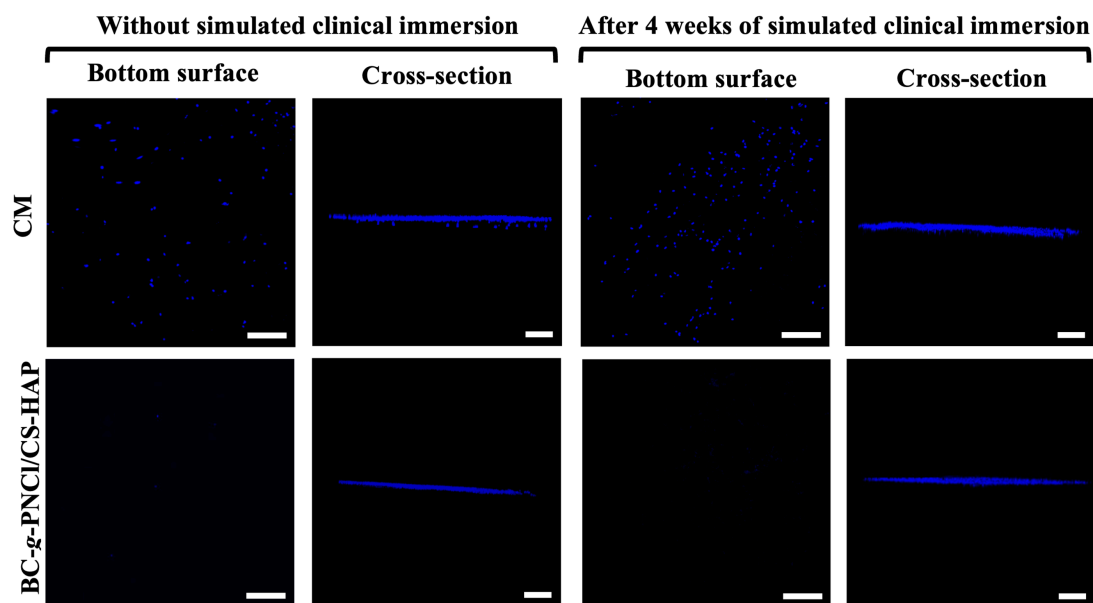
Supplementary Fig. 6 Morphology, extension, and viability of BMSCs. Morphology of BMSCs cultured on loose layer of CM and CS-HAP layer of BC-g-PNCI/CS-HAP at the 7th day (green for F-actin, blue for cell nucleus, scale bars =100 μm). Fluorescent observation was repeated three times independently, yielding similar results.



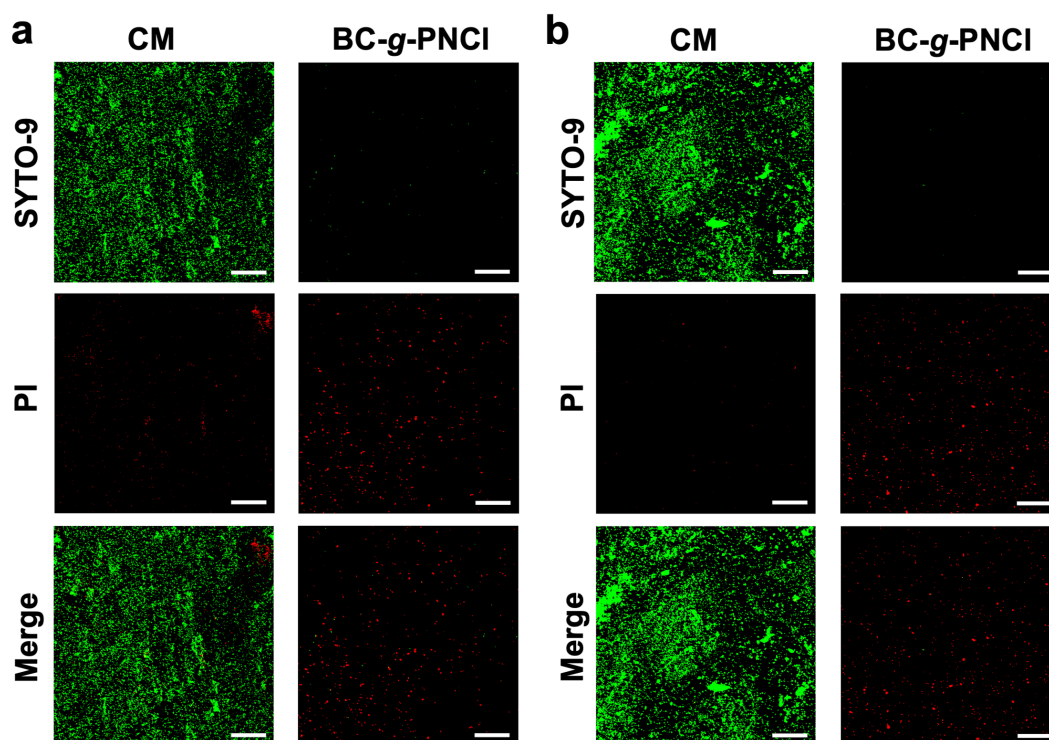
Supplementary Fig. 7 Proliferation and distribution of BMSCs. **a, b** Top-view (**a**) and cross-section (**b**) fluorescent images exhibiting the proliferation area and depth of BMSCs cultured on CS-HAP layer of BC-g-PNCI/CS-HAP at the 3rd and 7th days, respectively (blue for cell nucleus, scale bars = 200 μm). Fluorescent observation was repeated three times independently, yielding similar results.



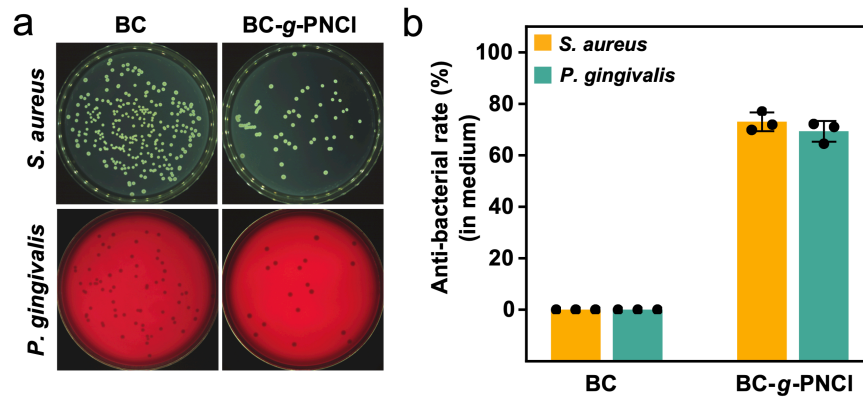
Supplementary Fig. 8 Proliferation and distribution of L929s. Top-view fluorescent images exhibiting the proliferation area of L929s cultured on dense layer of CM **(a)** and BC-g-PNCl layer of BC-g-PNCl/CS-HAP **(b)** at the 3rd and 7th days, respectively (blue for cell nucleus, scale bars = 200 μm). Fluorescent observation was repeated three times independently, yielding similar results.



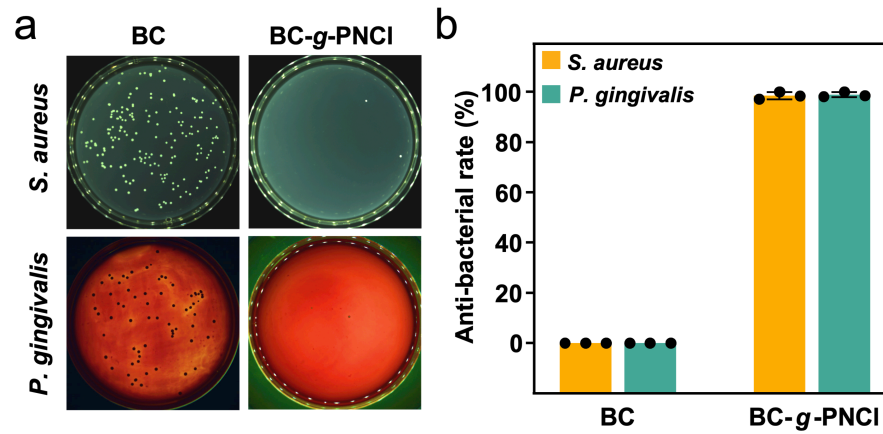
Supplementary Fig. 9 Barrier function against L929s. Fluorescent images of penetrated cells on the bottom surfaces and penetration depths after 3 days for CM and BC-g-PNCl/CS-HAP, exhibiting their barrier functions against L929s before and after simulated clinical immersion for 4 weeks (blue for cellular nucleus, scale bars = 200 μm). Fluorescent observation was repeated three times independently, yielding similar results.



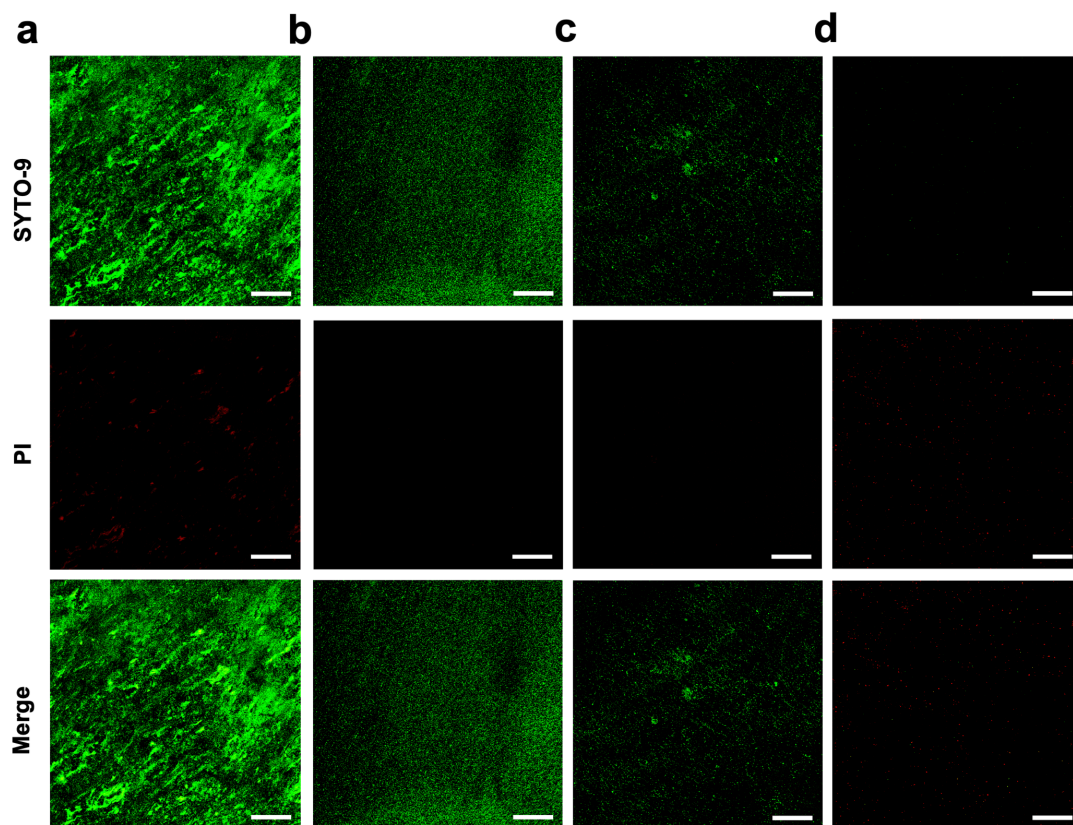
Supplementary Fig. 10 Anti-adhesion and anti-bacterial performances. **a, b** Fluorescent images exhibiting the live/dead bacterial distributions of *S. aureus* (**a**) and *P. gingivalis* (**b**) on the surfaces of CM and BC-g-PNCl. Only sporadic dead bacteria exist on the surface of BC-g-PNCl, while plenty of live bacteria are bred on the surface of CM (green for live bacteria, red for dead bacteria, scale bars = 50 μ m). Fluorescent observation was repeated three times independently, yielding similar results.



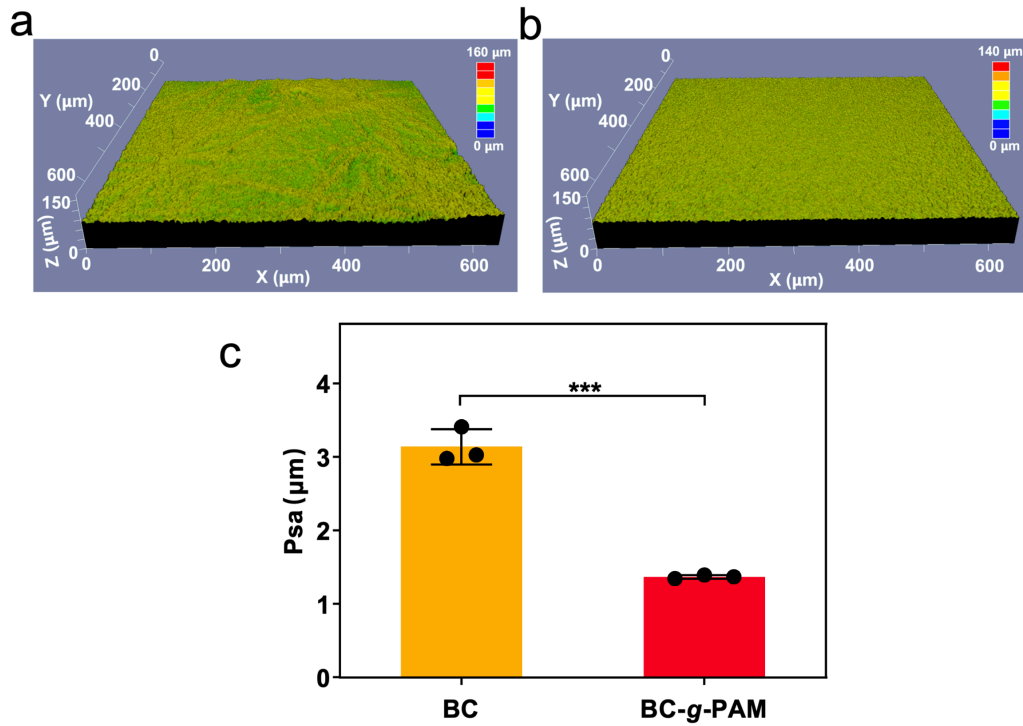
Supplementary Fig. 11 Release-killing ability. **a** Images of the bacterial colonies formed by *S. aureus* and *P. gingivalis* in the medium treated with BC and BC-g-PNCI. **b** Release-killing rates of BC and BC-g-PNCI against *S. aureus* and *P. gingivalis* according to **a** (n = 3 independent samples; error bars = SD; data are presented as mean values \pm SD).



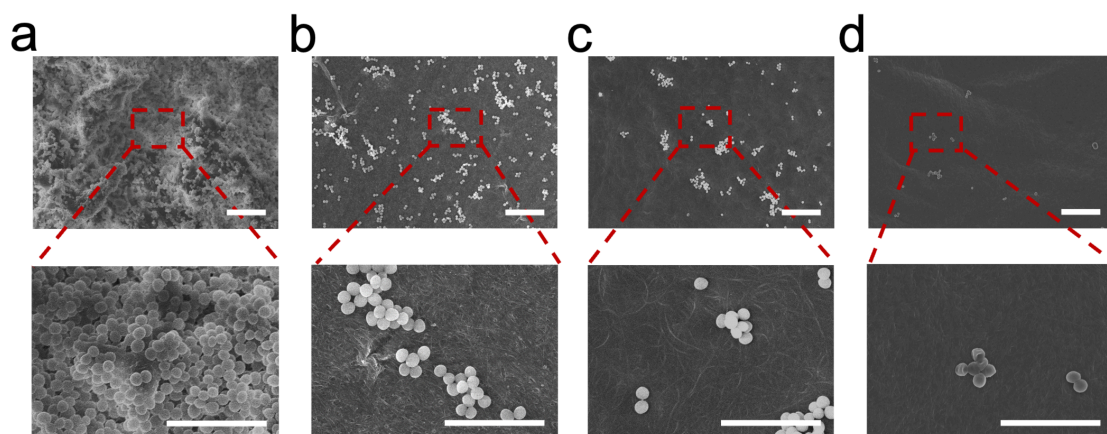
Supplementary Fig. 12 Contact-killing ability. **a** Images of the bacterial colonies formed by *S. aureus* and *P. gingivalis* contacted with BC and BC-g-PNCI. **b** Contact-killing rates against *S. aureus* and *P. gingivalis* contacted with BC and BC-g-PNCI according to **a** (n = 3 independent samples; error bars = SD; data are presented as mean values \pm SD).



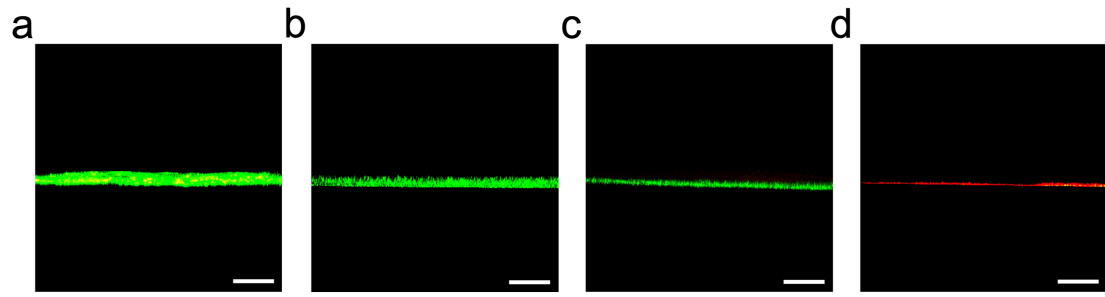
Supplementary Fig. 13 Anti-adhesion ability. Fluorescent images exhibiting bacterial adhesion of *S. aureus* on the surfaces of CM dense layer (**a**), BC (**b**), BC-g-PAM (**c**), and BC-g-PNCl (**d**) (green for live bacteria , red for dead bacteria , scale bars = 200 μ m). Fluorescent observation was repeated three times independently, yielding similar results.



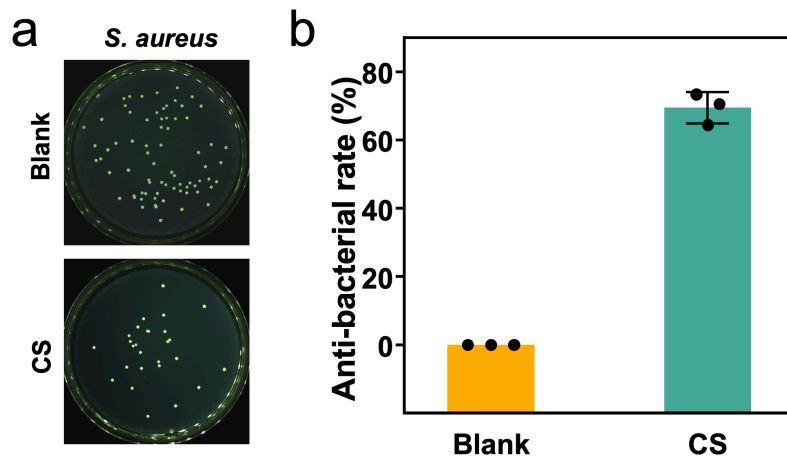
Supplementary Fig. 14 Surface roughness of BC and BC-g-PAM. a, b Roughness of BC (**a**) and BC-g-PAM (**b**). **c** Roughness analysis between BC and BC-g-PAM ($n = 3$ independent samples; Student's t test; *** two-tailed $p = 0.0002$; error bars = SD; data are presented as mean values \pm SD).



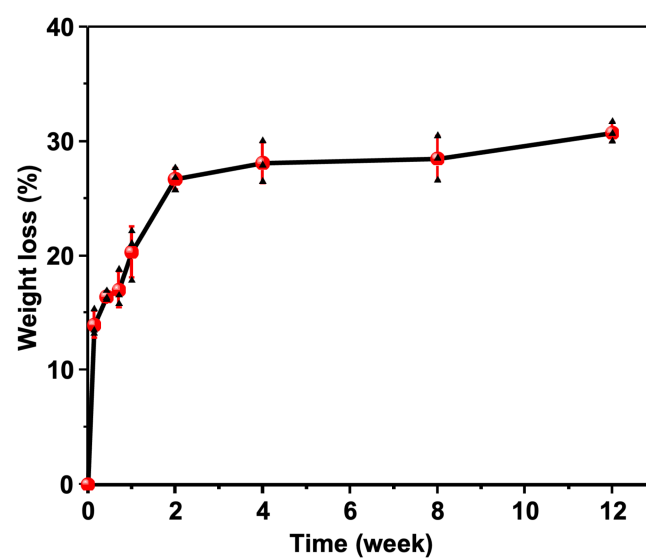
Supplementary Fig. 15 Anti-adhesion and anti-biofilm capacities. **a-d** SEM images exhibiting the bacterial adhesion situation of *S. aureus* on the surfaces of CM (**a**), BC (**b**), BC-g-PAM (**c**), and BC-g-PNCl (**d**). The number of bacteria on the surfaces of the CM dense layer, BC, BC-g-PAM, and BC-g-PNCl is gradually reduced (upper scale bars = 10 μm , lower scale bars = 5 μm) . SEM observation was repeated three times independently, yielding similar results.



Supplementary Fig. 16 Anti-biofilm capacities. Three-dimensional fluorescent images exhibiting the bacterial adhesion situation of *S. aureus* on the surfaces of CM (a), BC (b), BC-g-PAM (c), and BC-g-PNCI (d). The depth of biofilm on the surfaces of the CM dense layer, BC, BC-g-PAM, and BC-g-PNCI is gradually reduced (green for live bacteria, red for dead bacteria, scale bars = 200 μm). Fluorescent observation was repeated three times independently, yielding similar results.



Supplementary Fig. 17 Anti-bacterial ability of CS. **a** Images of the bacterial colonies formed by *S. aureus* in the medium in the blank group and CS group. **b** Anti-bacterial rate of CS against *S. aureus* according to **a** (n = 3 independent samples; error bars = SD; data are presented as mean values \pm SD).



Supplementary Fig. 18 Degradation of BC-g-PNCI/CS-HAP after incubation in PBS for 12 weeks (n = 3 independent samples; error bars = SD; data are presented as mean values \pm SD).

Supplementary Table 1 Primer sequences used for RT-qPCR

Genes	Primers
<i>ALP</i>	Forward: 5' - GCACAACATCAAGGACATCG - 3' Reversed: 5' - TCAGTTCTGTTCTTGGGGTACAT - 3'
<i>Col1</i>	Forward: 5' - GGTTTGGAGAGAGCATGACC - 3' Reversed: 5' - TTTGGGGAAATTGAGTTTGG - 3'
<i>RUNX2</i>	Forward: 5' - GCGGTGCAAACCTTTCTCCAG - 3' Reversed: 5' - TGCAGCCTTAAATGACTCGG - 3'
<i>OCN</i>	Forward: 5' - CCCAATTGTGACGAGCTAGC - 3' Reversed: 5' - CTGTGCCGTCCATACTTTCG - 3'
<i>GAPDH</i>	Forward: 5' - TCTCTGCTCCTCCCTGTTCT - 3' Reversed: 5' - CCGATACGGCCAAATCCGTT - 3'