Supporting Information

Temperature-controlled reversible exposure and hiding of antimicrobial peptides on an implant for killing bacteria at room temperature and improving biocompatibility in vivo

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1. Results and discussion

1.1 The N1s high-resolution spectra of each step

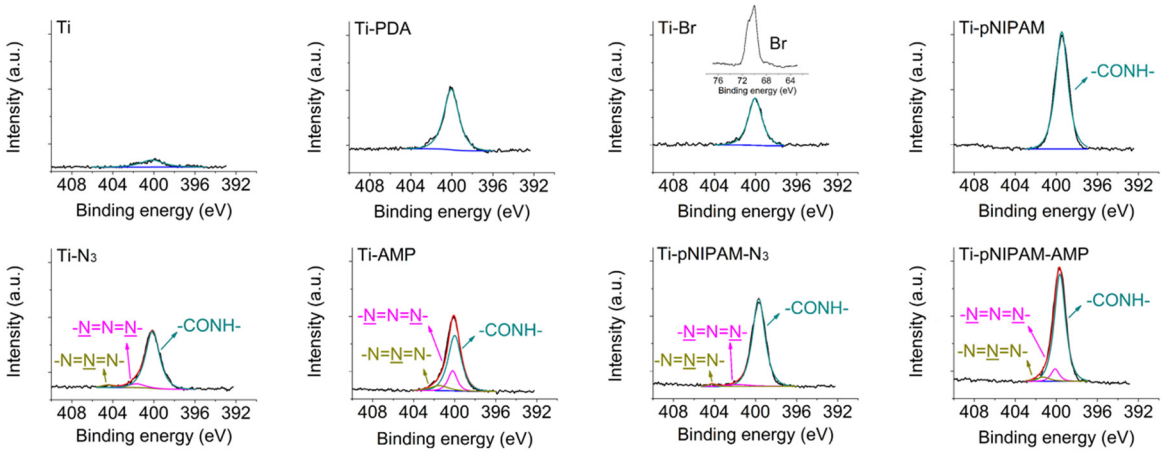

Figure S1. The N1s high-resolution spectra of indicated surfaces.

The N element percentage on Ti-PDA was increased to 7.55%, indicating that Ti-PDA was prepared successfully. After initiator treatment, Br element appeared on Ti-Br and the N element percentage was decreased to 5.47%. This is expected as the initiator contained Br element but without N element. The increased N element percentage of Ti-N_3 compared to Ti-Br (increased to 7.78%) illustrated that the click reaction site was introduced through the reaction between Ti-Br and NaN_3. After integration of AMP, the ratio of peak area between the peaks at 400.2 eV and 401.5 eV is 2:1,[1] confirming that AMPs were grafted to Ti to form Ti-AMP by the click chemistry through -C=C- in the AMPs and N_3 on the Ti-N_3. The N element percentage was increased to 9.66% after the formation of Ti-AMP. pNIPAM brush was formed as the N element percentage was increased on Ti-pNIPAM compared to that on Ti-Br (increased from 5.47 % to 12.42%), indicating Ti-pNIPAM was prepared. The N element percentage on Ti-NIPAM-N3 was decreased to 9.63% after NaN_3 treatment, probably due to the PDA dissolution in DMF. But the existence of $–N_3$ group, reflected by the peaks at 404.5

eV (–N=N=N−) and 401.7 eV (–N=N=N−) with an intensity ratio of 2:1, proved that Ti-pNIPAM-N_3 was prepared[2-3]. Meanwhile, the existence of the -N=N-N- and the increased N content (from 9.63% to 11.88%) illustrate that Ti-pNIPAM-AMP was prepared successfully. The C:N ratio of Ti-AMP, Ti-pNIPAM, Ti-pNIPAM-AMP were analyzed from the XPS spectra (Table S1).

Table S1 The ratio of C, N element on the indicated surfaces.

	C (%)	N (%)	C:N
Ti-pNIPAM	73.02	12.42	5.88
Ti-AMP	62.27	9.66	6.44
Ti-pNIPAM-AMP	65.32	11.88	5.50
PDA (theoretical value)	72.73	9.09	8.00
pNIPAM (theoretical value)	75.00	12.50	6.00
AMP (theoretical value)	68.52	22.22	3.08

1.2. The surface mass density of grafted AMP

The QCM results (Figure S2) showed that the surface mass density of grafted AMP was increased with reaction times. After a mixture of ethanol and water (3:7,v:v) was introduced, only few physically adsorbed AMP molecules were washed away, indicating that the grafted AMP was very stable and the density was similar to the mass without rinsing. The density after reaction for 3 h was similar to the value for 3 h on the curve, about 641 ng/cm^2.

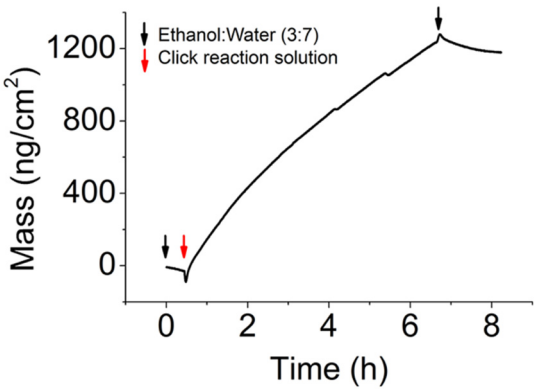

Figure S2. The surface mass density of grafted AMP on the Ti-pNIPAM-N_3 with the reaction times.

1.3 The MIC of AMPs

The MIC of AMPs was assessed and the results are shown in Figure S2. The MIC of HHC36 against *E.coli* and *S.aureus* is between 4-6 μM, and 6-8 μM, respectively. After N-terminal modification modification, PraHHC36 also displayed an excellent antimicrobial activity. The MIC of the PraHHC36 against *E.coli* and *S.aureus* is between 6-8 μM and 8-10 μM, which is a very low concentration. The effect on the growth of *E.coli* and *S.aureus* could been showed even at 2 μM.

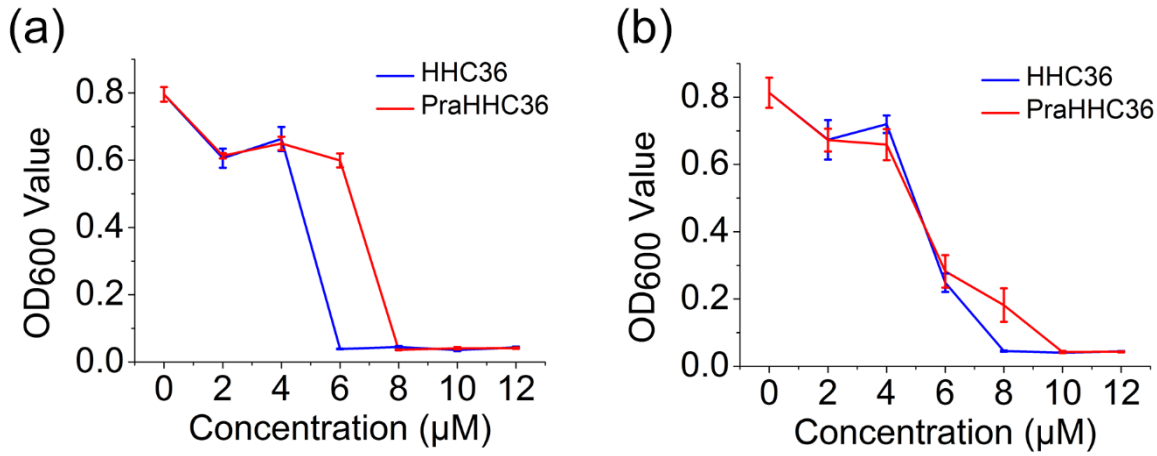

Figure S3. The antimicrobial activity of HHC36 and PraHHC36 against *E. coli* (a) and *S. aureus* (b).

1.4. The residual Cu ion and Bromide ion analysis of Ti-pNIPAM-AMP

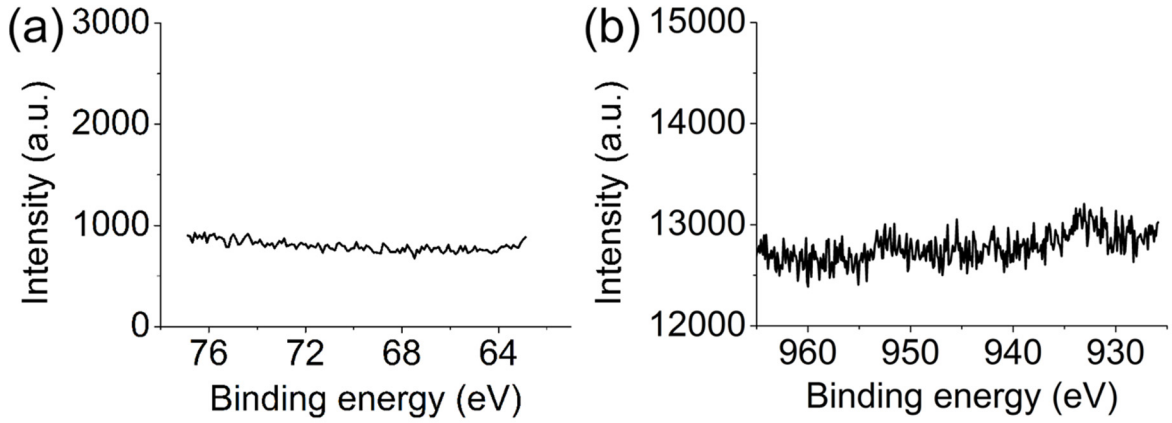

Figure S4. The Br3d (a) and Cu2p (b) high-resolution spectra of Ti-pNIPAM-AMP.

Considering the antimicrobial activity of Br ion and Cu ion, the residual Cu ion and Bromide ion of Ti-pNIPAM-AMP were analyzed with XPS methods (Figure S4). There were no residual Cu ion and Bromide ion on Ti-pNIPAM-AMP since no Cu and Bromide peaks were observed on Ti-pNIPAM-AMP. The results could prove that the antimicrobial activity of Ti-pNIPAM-AMP was contributed from AMP rather than Cu ion and Bromide ion.

1.5. LIVE/DEAD image of different surfaces

Only Ti-pNIPAM-AMP presented strong red fluorescence when the temperature was changed to 25 °C (Figure S5), which showed that the antimicrobial activity of Ti-pNIPAM-AMP was responsive to the temperature and the high antimicrobial activity was displayed at room temperature.

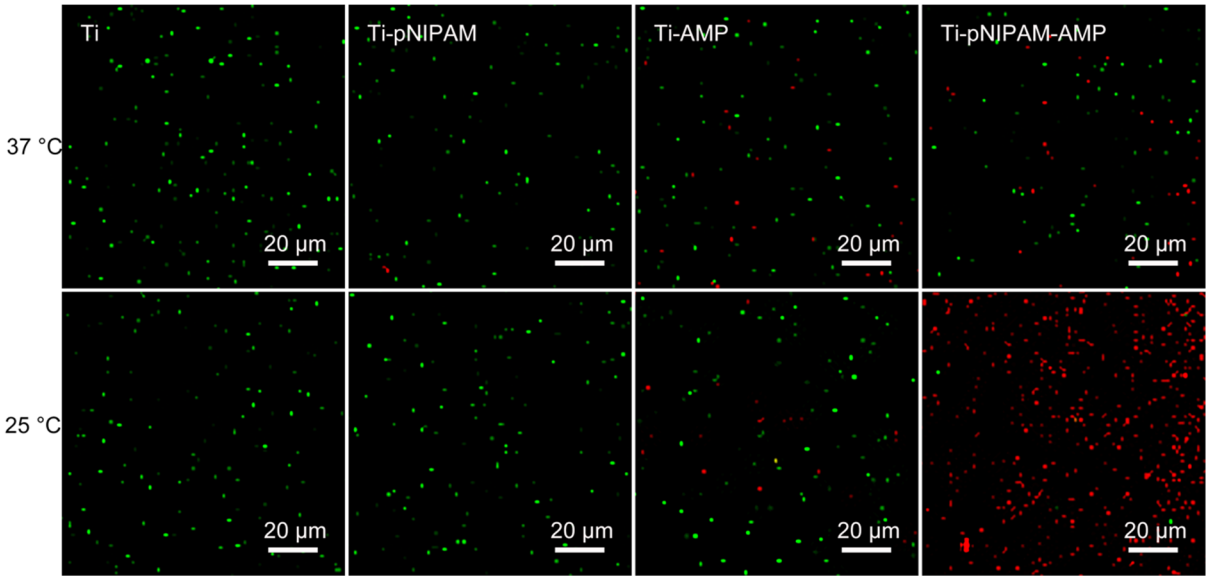

Figure S5. LIVE/DEAD images of different surfaces after incubation against *E.coli* for 2 h at 37 and 25 °C.

1.6. The Biocompatibility of AMPs

The BMSCs were used to evaluate the biocompatibility of HHC36 and PraHHC36 and the results are shown in Figure S6. The OD value of HHC36 and PraHHC36 was 0.86 and 0.81 times compared to that of blank, which indicated the low cytotoxicity of HHC36 and PraHHC36. However, mBMSCs were spread well on every group as stretched cytoskeleton and round nucleus were observed, indicating that the low cytotoxicity of HHC36 and PraHHC36 did no had much impact on the morphology of BMSCs.

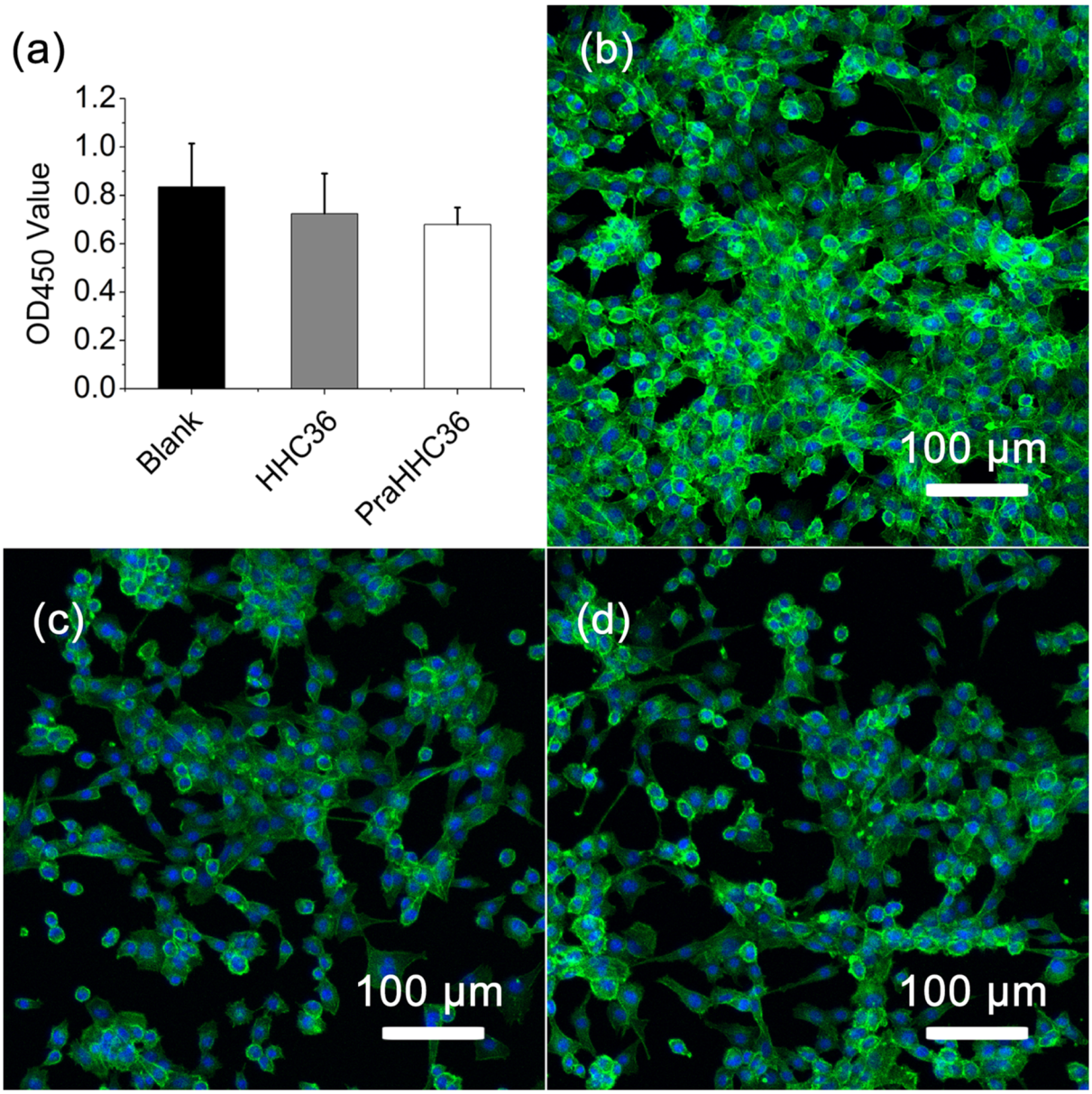

Figure S6. Cellular viability and morphology of BMSCs after incubation for 24 h at the MIC of HHC36 and PraHHC36. (a) CCK-8 assay; (b) Blank; (c) HHC36; (d) PraHHC36.

2. Experiment

2.1. The MIC of AMP

The bacteria were incubated as mention in the text 2.11. Antimicrobial assay and bacterial culture. The minimum inhibitory concentration (MIC) of AMP against *S.aureus* and *E. coli* was tested with the difference concentration AMP solution. Bacteria were diluted to 5×10^5 CFU / mL. Then, 90 μL bacteria suspension was added to per well in a 96-

well culture plate, followed with adding 10 μL difference difference concentration AMP solution (0,40,60,80,100,120 μM). Each concentration of AMP was prepared with 3 parallel samples. After incubation at 37 °C for 24 h, the optical density (OD) values at 600 nm were measured with an ELISA plate reader (Varioskan Flash 3001, Thermo, Finland). The smallest concentration that inhibits bacterial growth is defined as the minimum inhibitory concentration (MIC).

2.2. The biocompatibility of free AMPs

The BMSCs were incubated as mention in the text 2.12. Biocompatibility of the samples. The biocompatibility of free AMPs was tested at the concentration of the MIC in a 96 well culture plate with BMSCs (30,000 /mL). The cellular viability was evaluated with CCK-8 assay and the morphology of BMSCs was observed with CLSM.

CCK-8 assay. After incubation for 24 h, the medium was removed. Then, 200 μL DMEM with 10% FBS and 20 μL Cell Counting Kit-8 (CCK-8) reagent were mixed and added to the wells for incubation 1 h. The 100 μL medium was transferred to a new 96-well plate and the OD values at 450 nm were measured with an ELISA plate reader (Varioskan Flash 3001, Thermo, Finland).

The morphology of BMSCs. After incubation for 24 h, the cells were rinsed with PBS for 3 times, followed with adding 4% neutral formaldehyde at 4 ℃ overnight. After rinsed with PBS, F-Actin staining agent was immersed for 1 h at room temperature. Then, the cells were rinsed with PBS and were immersed 0.1% triton permeable for 10 min. After rinsed with PBS, the cells were stained with 4',6-diamidino-2-phenylindole (DAPI) stain for 5 min in the dark. Then, the cells were rinsed with PBS and were observed by CLSM (Leica TCS SP8, Germany).

2.3. The surface mass density of grafted AMP characterization with QCM

The surface mass density of grafted AMP was characterized with QCM-D9 (Q-Sense E4, Biolin, Sweden). Briefly, Ti-pNIPAM-N_3 was maintained a baseline in the mixed solution of ethanol and water (3:7, v:v). After a click reaction solution was introduced for 6 h, the mixture of ethanol and water was added to rinse the ungrafted AMP.

REFERENCE

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