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Supporting Information

The Promotion of Antimicrobial Activity on Silicon Substrates by A "Click" Immobilized Short Peptide

Lin Wang, [a, b] Junjian Chen, [a, b] Lin Shi, [a, b] Zhifeng Shi, [a, b] Li Ren, [#, b] and Yingjun Wang [*, a]

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Materials.

The silicon (100) wafer (5mm×5mm) was purchased from Shanghai institute of optics and fine mechanics. The antimicrobial peptide Tet213 tethering an EG_{11}-N3 group at the N-terminus, whose structure was shown in Scheme.1, was purchased from GL Biochem (Shanghai, China) Ltd. The 1,6-heptadiyne, azide-PEG3-biotin (biotin-azide), Tris[(1-benzyl-1H-1,2,3-triazol-4-yl)methyl]amine (ligand) and ascorbic acid sodium salt (Na-Asc) were got from Sigma, and L-Homopropargylglycine (HPG) as well as avidin-FITC were purchased from Invitrogen. The 3-azido-7-hydroxy-coumarin (coumarin-azide) were got from Atomole Scientific Co. Ltd. (Wuhan, China).

CuAAC reaction rate test.

To determine the best reaction condition, we test the CuAAC reaction rate in the solution with the coumarin-azide (0.1 mM) and HPG (0.1 mM), and a freshly prepared mixture of reagents for the click reaction, named "click solution", containing different concentration of $CuSO_4$, ligand and Na-Asc in PBS/DMSO (80/20 in volume) solution at room temperature. The CuAAC reaction rates were evaluated by a plate reader with excitation at 405nm and emission at 460 nm.

Preparation of alkyl-terminated Si (100) surface.

The Si wafer was cleaned with an aqueous fluoride solution (5% hydrofluoric acid, 2 min) at room temperature, and the surface was marked as ***surface 1***. Subsequently, the sample was immersed into the 1,6-heptadiyne in the nitrogen tank and reacted for 18 h under room light. The sample was then transferred to the atmosphere, and the functionalized surface (***surface 2*** in Scheme 1) was rinsed with ethanol before being either analyzed or further reacted with substituted azide species.

Click reaction on functionalized Si wafer.

To probe the presence of alkynyl groups on the ***surface 2***, we treated it with biotin-azide (0.1 mM) and a freshly prepared ***click solution 3***, containing $CuSO_4$ (100 μmol), ligand (200 μmol) and Na-Asc (5 mM) in PBS/DMSO (4:1 in volume) solution, for 40 min in the dark at room temperature. After the reaction and washing with PBS containing 20% DMSO and PBS with 5 mM EDTA, respectively, the

sample was stained with avidin-FITC (0.1 mg/mL) for 20 min at 4 ℃ in the dark, and washed again with PBS for 3 times. Then the sample was named as ***surface 3*** and observed with a Nikon eclipse 80i fluorescence microscope at FITC channel using a 10 X objective. A CoolSnap HQ2 camera (Photometrics, Tuscon, AZ) and NIS Elements software (Version 3.0, Nikon Instruments, Melville, NY) were used for image acquisition and analysis. The ***surface 1*** treated at the same condition was as the control group, which named as ***surface 3 control***.

To integrate the Tet213 peptide onto the silicon wafer via the click reaction, ***surface 2*** was immersed in the above ***click solution 3*** containing 0.1 mM Tet213-azide to replace the biotin-azide. After reaction for 40 min in the dark at 37 ℃, the samples were washed with PBS containing 20% DMSO and PBS with 5 mM EDTA, respectively, to remove the reagents. The resultant samples are named as ***surface 4*** (Scheme 1). The ***surface 1*** treated with the "click solution" containing Tet213 peptide at the same condition was as the control group (which abbreviated as ***surface 4 control***).

Characterization of the wafer.

X-ray Photoelectron Spectroscopy (XPS) was performed with a PHI 5700 X-ray photoelectron spectrometer equipped with a monochromatic Al Kα X-ray source (1486.7eV) at a takeoff angle (TOA) of 45° from the film surfaces. Atomic Force Microscopy (AFM) imaging of the surfaces was performed using a MultiMode Nanoscope IIIa AFM (Digital Instruments Inc., Santa Barbara, CA). Images were acquired in tapping mode using a silicon nitride cantilever (MikroMasch, San Jose, CA) with a resonance frequency of 132.9 kHz and a nominal force constant of 1.75 N/m.

Antimicrobial assay

Bacterial culture

Staphylococcus Aureus (SA, strain ATCC 29213) and *Escherichia Coli* (E. coli, strain ATCC 15224) were purchased from VWR International, LLC. Single colony of the bacteria was inoculated in 5 ml of LB media overnight at 37 ℃ with shaking (150

rpm). After that, 1 ml of the bacterial suspension was inoculated in 50 ml of fresh LB media, respectively, which was incubated for 5 h with shaking (250 rpm) at 37 ºC to achieve mid-log phase growth.

Antimicrobial activity of the peptide in solution

The antimicrobial activity of Tet213 peptide in LB medium was tested by adding the peptide into 100 μl LB medium with the bacterial concentration of 10^6 cfu/mL. The concentrations of the peptide were shown in Table 2S. After cultured in the shaking bed (150 rpm) at 37 ºC for the indicated time, 10 μL of the bacterial solution was taken out and the viability of the bacteria was tested with the serial dilution method using agar plates (diluted to 10^0, 10^1, 10^2, 10^3 and 10^4 times).

Bacterial culture on the surfaces

The bacteria were resuspened to PBS with the concentration of 1.0×10^7 cfu/ml. Prior to seeding, the samples were placed into a 48-well culture plate. Then 10 μl of bacterial suspension (1.0×10^7 cfu/ml in PBS) was added onto each sample to fully cover the surface of substrate.

Antimicrobial activity of the surfaces

After cultured the bacterial on different surfaces for 2.5 h at 37 ºC, 490 μl of PBS was added into each well to dilute the bacterial solution. After mixed with the pipettor roughly, the samples as well as the PBS were transferred to a new 15 mL tube, ultrasonic for 3 min and votex for 3 min to detach the adhered bacteria. Then the bacterial solution was diluted to 10^0, 10^1 and 10^2 times, and 10 μL of the bacterial suspension was taken to evaluate the viability of bacteria by using agar plates.

Observation of bacterial morphology

After cultured the bacterial on different surfaces for 2.5 h at 37 ºC, the samples were rinsed three times with PBS and fixed with 2.5% glutaraldehyde in PBS for 12 h at 4 ºC. After that, the samples were washed with PBS for three times and dehydrated in graded ethanol (50% and 70% ethanol for 30 min, respectively; 80% and 90% ethanol for 15 min, respectively; 95% and 100% ethanol for 5 min, respectively). Finally, the samples were dried in air and gold sputtered, and observed with the scanning electron microscopy (SEM, NOVA NANOSEM, Netherland).

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Cell assay

The influence of the functionalized surfaces on cell viability was tested with the rat bone mesenchymal stem cells (rMSCs) as follows.

Cell culture and seeding

rMSCs were cultured in High glucose Dulbecco's modified Eagle's medium (H-DMEM) (Hyclone, Logan, Utah) containing 10% fetal bovine serum (FBS) and in a 5% CO_2 atmosphere at 37 ºC. Medium was replaced every third day. The adherent cells were allowed to reach about 80% confluence. Cells were passaged in culture and passage 3-5 (P3-P5) cells were used for the experiments.

All the silicon substrates used for cell test were sterilized with 75% ethanol for 2 h before treatment. After sterilization, all the preparation processes were in aseptic condition to integrate the Tet213 peptide. The samples were placed individually into the 48-well plates, and the rat bone mesenchymal stem cells (hMSCs) were added directly onto each sample (25000 cells in 10 μl media suspension per sample). After 2 h incubation, complete medium (L-DMEM containing 10% FBS) was slowly added to each well to cover the surface of the samples. They were cultured for 24 h before the test.

CCK-8 assay

After being cultured for 24 h, the biocompatibility of the materials was evaluated with CCK-8 assay. Briefly, at indicated time points, the samples were transferred to a new 48-well plate and washed three times with PBS. Then 200 μl complete medium containing 20 μl CCK-8 solution was added to each disk. After incubation for 2 h at 37ºC in dark, 100 μl of the CCK-8 solution was transferred to a new 96-well plate and the optical density (OD) value of the solution was measured with an ELISA plate reader (Varioskan Flash 3001, Thermo, Finland) at 450 nm wavelength.

Statistics

The antimicrobial assay and cell assay were repeated at least three times and the results were expressed as means ± standard deviations. Statistical significance was calculated using the SPSS 17.0 statistical software. Statistical significance was defined as $p<0.001$.

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Table 1S. The composition of each ***click solution*** to test the CuAAC reaction rate

	Cu^{2+} (μM)	Ligand (μM)	Na-Asc (mM)
Click solution 1	50	100	5
Click solution 2	50	100	10
Click solution 3	100	200	5
Click solution 4	100	200	10
Click solution 5	200	400	5
Click solution 6	200	400	10
Control group	100	0	5

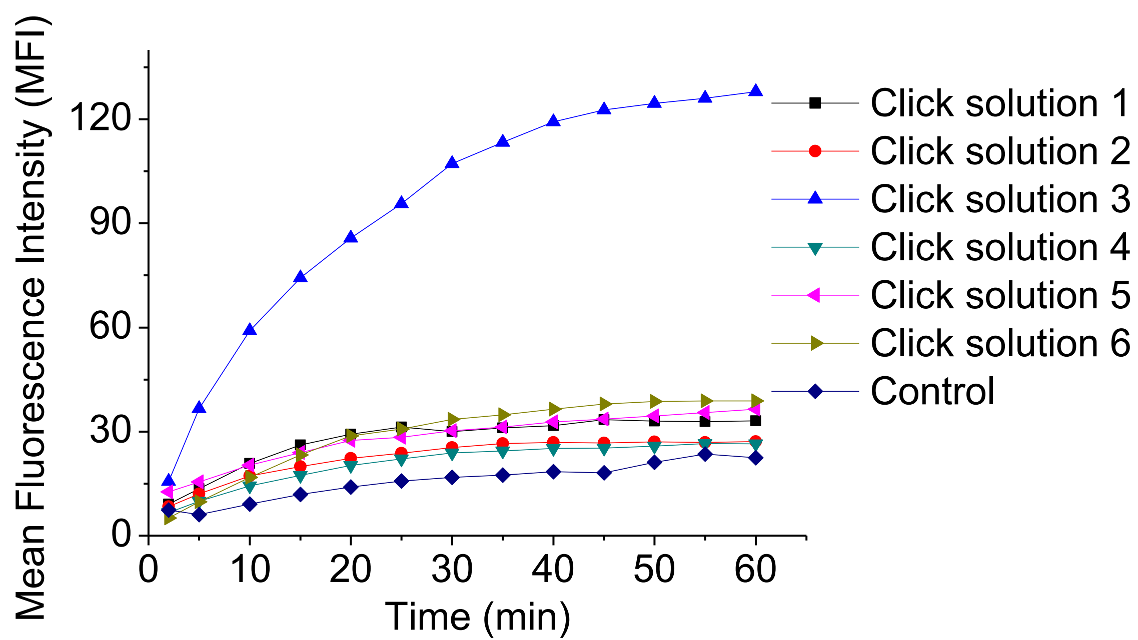

Fig. 1S The CuAAC reaction rate in different ***click solution***.

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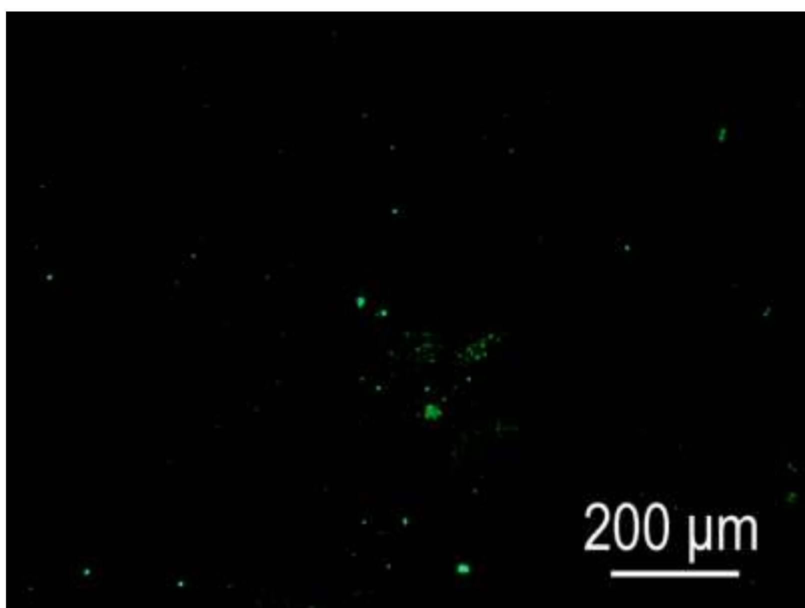

Fig. 2S The fluorescent image of the ***surface 2*** clicked in the ***click solution 1*** containing 50 μM $CuSO_4$, 100 μM ligand, 5 mM Na-Asc and 0.1 mM biotin-azide. The sample was stained with avidin-FITC (0.1mg/mL) for 20 min at 4 ˚C and observed under FITC channel.

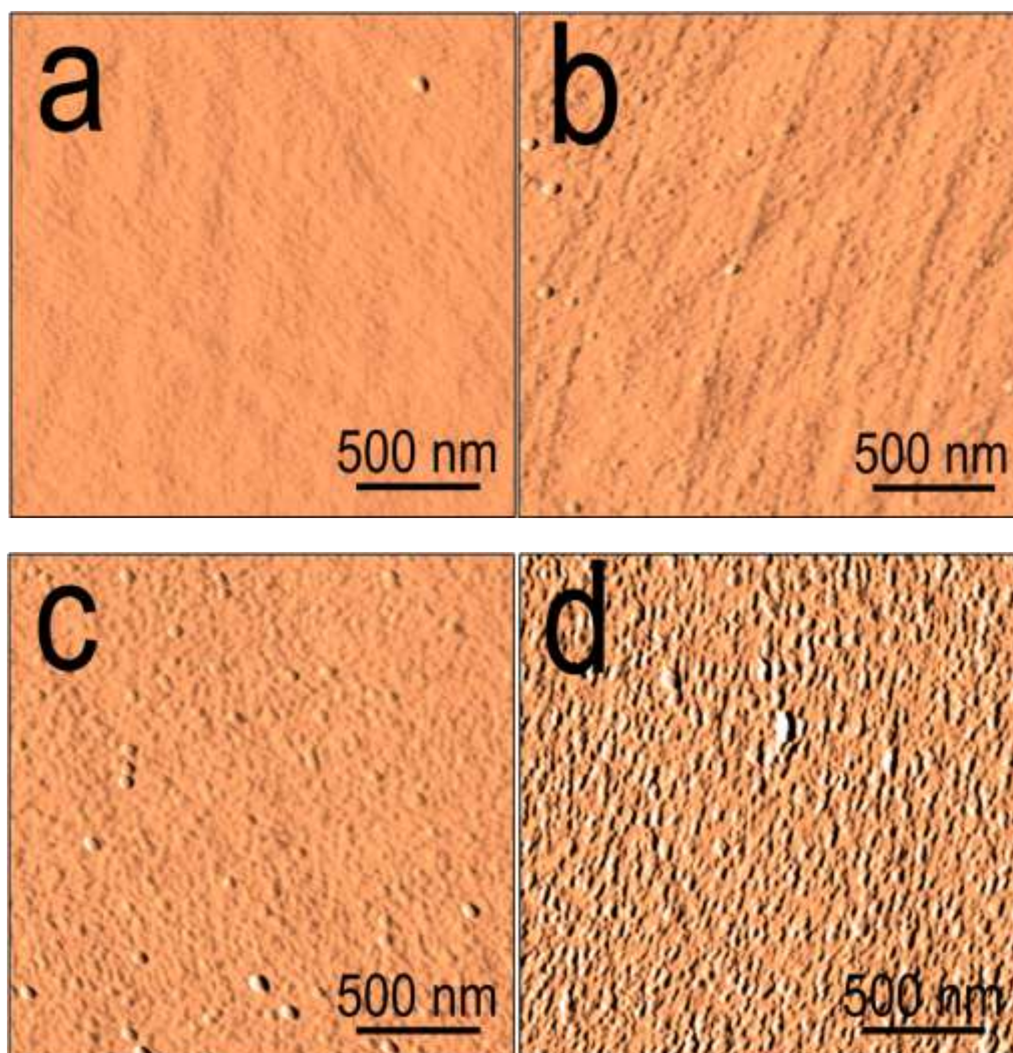

Fig. 3S The AFM image of the ***surface 1*** (a), ***surface 2*** (b), ***surface 4 control*** (c) and ***surface 4*** (d).

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Table 2S. The composition of each group to test the antimicrobial activity of the peptides with/without EG12-azide under different concentrations in LB medium.

Group	Tet213 (μmol)	Tet213 with azide (μmol)
Control group	0	0
AMP 10	10	0
AMP 20	20	0
AMP 50	50	0
AMP 100	100	0
AMP-azide 10	0	10
AMP-azide 20	0	20
AMP-azide 50	0	50
AMP-azide 100	0	100

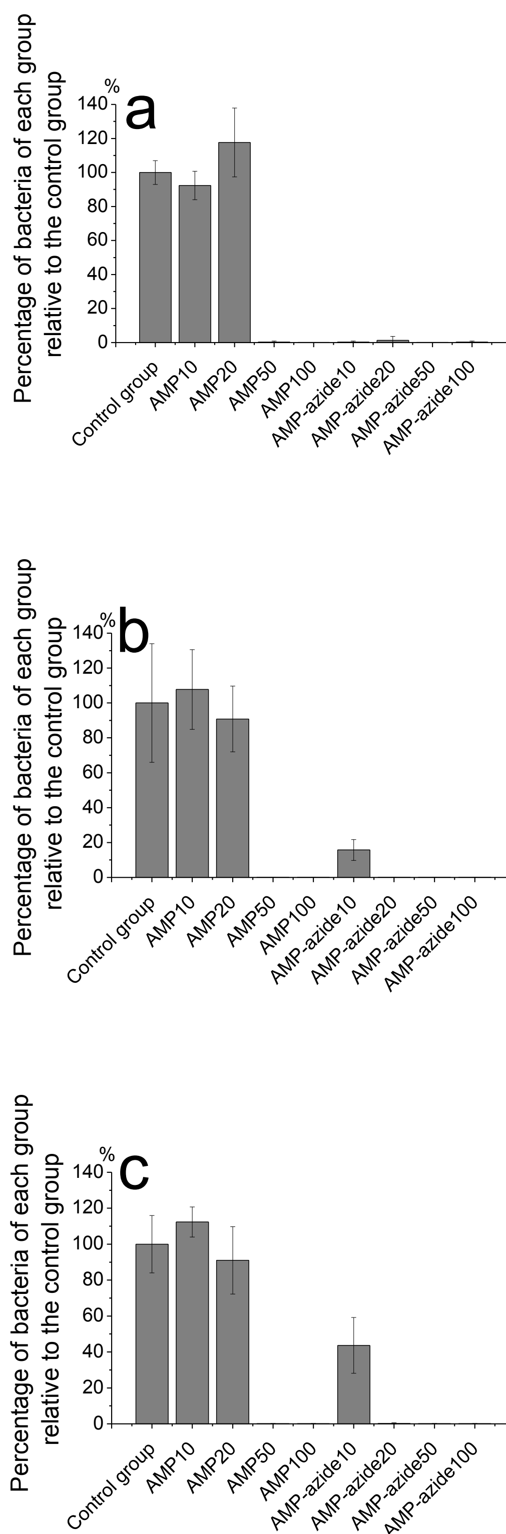

Fig. 4S The antimicrobial activity of the peptides with/without EG12-azide against *S. Aureus* under different concentration after 3 (a), 6 (b) and 12 (c) hours (n=3).

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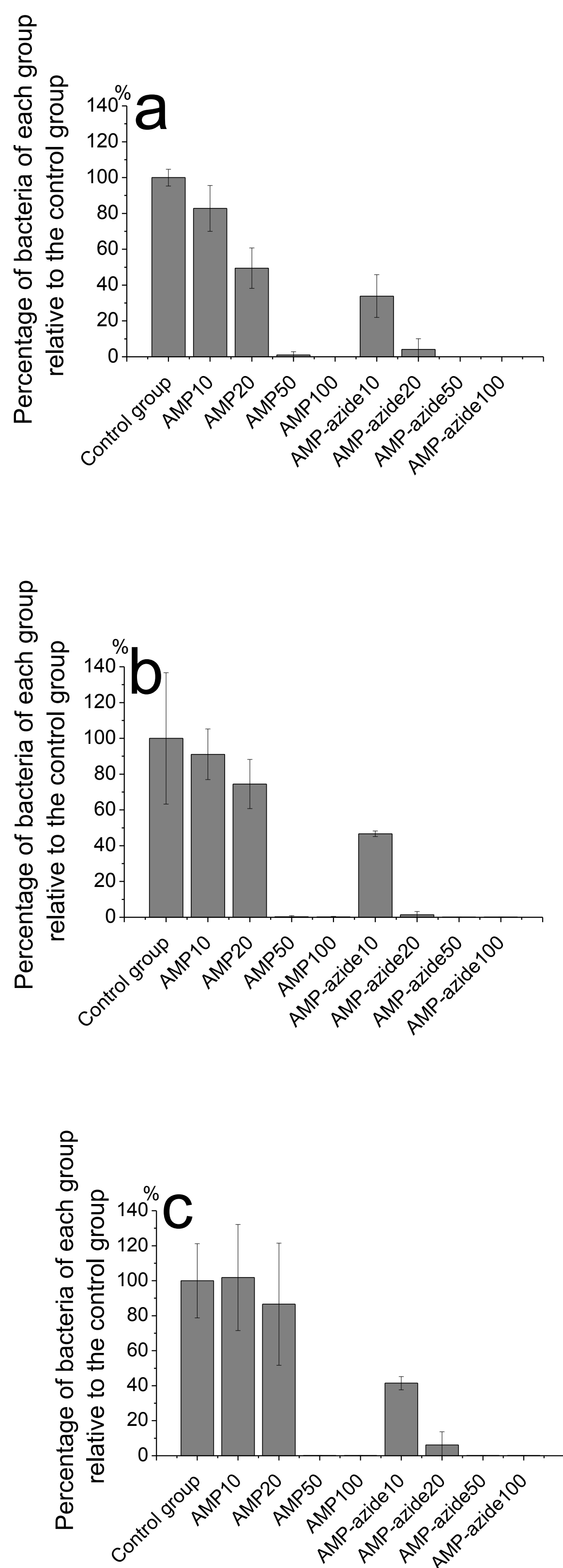

Fig. 5S The antimicrobial activity of the peptides with/without EG12-azide against *E. coli* under different concentration after 3 (a), 6 (b) and 12 (c) hours (n=3).

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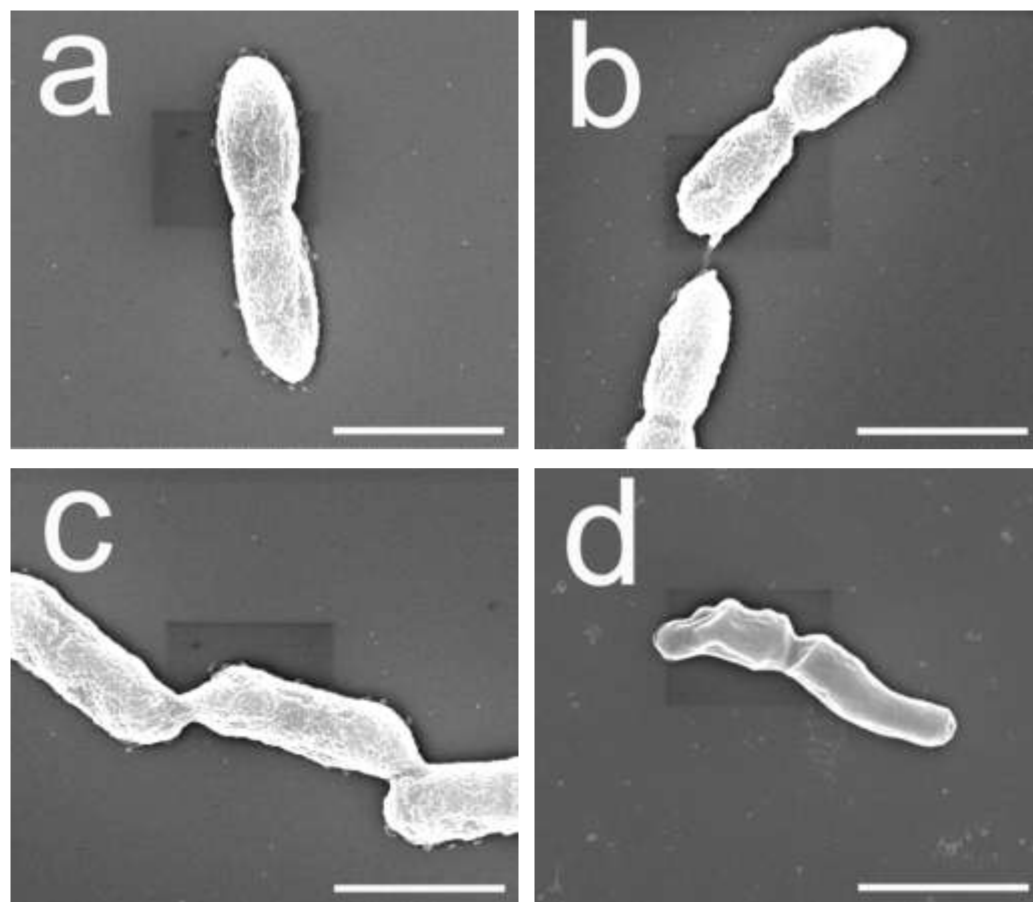

Fig. 6S The morphology of *E. coli* on different surfaces: (a) on ***surface 1***; (b) on ***surface 2***; (c) on ***surface 4 control*** and (d) on ***surface 4***. The scale denotes 1 μm.

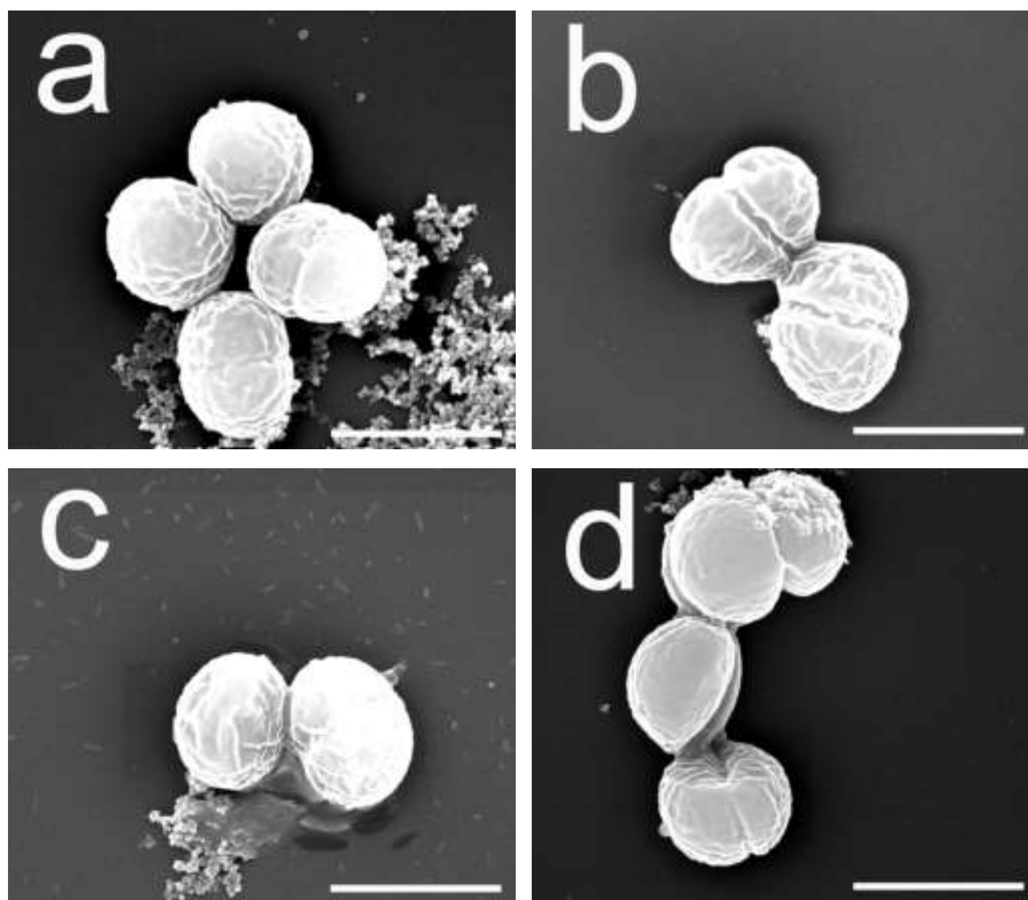

Fig. 7S The morphology of *S. aureus* on different surfaces: (a) on ***surface 1***; (b) on ***surface 2***; (c) on ***surface 4 control*** and (d) on ***surface 4***. The scale denotes 1 μm.