Electronic Supplementary Information (ESI) for

One-step synthesis of quaternized silica nanoparticles with bacterial adhesion and aggregation properties for effective antibacterial and antibiofilm treatments

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Experimental section

Materials. Tetraethyl orthosilicate (TEOS) was bought from Shanghai Aladdin Biochemical Technology Co., Ltd. (China). Dimethyloctadecyl[3-(trimethoxysilyl)propyl]ammonium chloride (Si-QAC) solution (42 wt% in methanol) was obtained from Sigma-Aldrich (Shanghai, China). Dimethyl sulfoxide (DMSO) and 3-(4,5-dimethyl-2-thiazolyl)-2,5-diphenyl-2*H*-tetrazolium bromide (MTT) were purchased from Shanghai Aladdin Biochemical Technology Co., Ltd. (China). The LIVE/DEAD BacLight Bacterial Viability Kit was purchased from Thermo Fisher Scientific, Inc.. The TIANamp Bacteria DNA Kit was bought from Tiangen Biotech (Beijing) Co., Ltd. (China). Reactive Oxygen Species (ROS) Assay Kit was purchased from KeyGen Biotech Co., Ltd. (Nanjing, China). Dialysis membranes with different molecular weight cut-offs (MWCOs) were obtained from SpectrumLabs, Inc. (Rancho Dominguez, CA). All other chemicals were of analytical reagent grade and used without further purification. All solutions/suspensions were prepared with deionized water (18.2 MΩ cm) purified by a Milli-Q water-purification system (Billerica, MA, USA).

Characterization. The morphology of the products were characterized using a Zeiss Ultra Plus scanning electron microscope (Carl Zeiss, Germany). The size and morphology of TS4 were further characterized by transmission electron microscopy (TEM) using a Tecnai G2 20 transmission electron microscope (FEI Co., USA). The hydrodynamic diameters and zeta potentials were determined using a Nano ZS zetasizer instrument (Malvern Instruments, UK). Fourier transform infrared (FTIR) spectra were collected with a Nicolet iS50 FTIR spectrometer (Thermo Fisher Scientific Co., USA). The X-ray photoelectron spectroscopy (XPS) data were obtained using a Thermo ESCALAB 250Xi X-ray photoelectron spectrometer (Thermo Fisher Scientific Co., USA).

Culture of Bacterial Cells. For the culture of *Staphylococcus aureus* (*S. aureus*) bacteria, a single colony of the *S. aureus* bacteria was picked from the lysogeny broth

(LB) agar plate and cultured in fresh LB medium at 37 °C under shaking at 150 rpm. The same procedure was applied for the culture of *Escherichia coli* (*E. coli*).

Preparation of Biofilms. For the preparation of *S. aureus* bacterial biofilms, the *S. aureus* bacteria were cultured at 37 °C for 12 h under shaking (150 rpm). Then, the bacterial suspension was diluted to an OD_{600} of ~0.2 with 10% LB medium and was incubated in the 96-well plates for the biofilm formation under static condition at 37 °C for 72 h. During the biofilm culture process, half of the culture medium was replaced by an equal volume of fresh 10% LB medium every 24 h. Before the specific biofilm experiments, the mature biofilms were washed with sterile phosphate-buffered saline (PBS, pH 7.4) to remove the planktonic bacterial cells.

Live/Dead Staining Assay of *S. aureus* Bacteria and Biofilms. The live/dead staining experiments were performed by using the Live/Dead BacLight Bacterial Viability Kit (containing SYTO 9 and propidium iodide, PI). First, *S. aureus* cells (OD_{600}: ~0.5) were collected after centrifugation (7500 rpm, 5 min). Then the bacteria were resuspended in 1 mL of DI water containing 100 μg mL^{-1} TS4 and incubated at 150 rpm for different time periods in a shaker at room temperature (~28 °C). Then, the untreated (control) and treated bacterial cells were further stained with the green-fluorescent nucleic acid dye (SYTO 9, Ex = 488 nm) and the red-fluorescent nucleic acid dye (PI, Ex = 552 nm) for 15 min. Finally, the samples were separately imaged using a confocal microscope (TCS SP8, Leica, Germany).

To evaluate the activities of the bacteria in the treated biofilms via the live/dead staining assay, the 72 h-old *S. aureus* biofilms were incubated with water (control) or 100 μg mL^{-1} TS4 suspension for 24 h. After that, the treated biofilms were subjected to the live/dead staining assay as mentioned above.

Scanning Electron Microscopy (SEM) Observation of Bacteria. The *S. aureus* and *E. coli* bacterial suspensions (OD_{600}: ~0.5) left untreated (control) and treated with 1 mg mL^{-1} TS4 were centrifuged at 6500 rpm to remove the supernatants and separately fixed with 2.5% glutaraldehyde in PBS (pH 7.4) at 4 °C overnight. The samples were then washed with PBS by centrifugation to remove the glutaraldehyde

and dehydrated using graded ethanol solutions (30, 50, 70, 80, 90, 95, and 100 vol%). For each dehydration step, the samples were separately resuspended in ethanol solutions, incubated for 15 min, and centrifuged at 7000 rpm for 5 min. Finally, the bacterial suspensions in 100% ethanol solutions were dropped onto a polished silicon wafer and allowed to dry at room temperature for SEM observation.

Determination of Intracellular Reactive Oxygen Species (ROS). The intracellular ROS concentration was tested using an ROS probe (2',7'-dichlorodihydrofluorescein diacetate, DCFH-DA). The bacterial cells were incubated with TS4 in water for 4 h. The bacterial samples treated with water were also prepared. Then, 10 mM of the DCFH-DA probe was separately added to the above bacterial suspensions at a volume ratio of 1 : 1000, and further incubated at 150 rpm and 37 °C for ~20 min. After that, the bacterial cells were transferred onto the glass slides to observe the ROS generation via confocal microscopic imaging. Besides, the fluorescence of the bacterial cells (which directly reflects the amount of ROS generated in the cells) was measured by a flow cytometer (NovoCyte 2070R, ACEA Biosciences Inc., USA).

DNA Extraction and Agarose Gel Electrophoresis Assay. The bacterial cells were left untreated (control) and treated with TS4 for 4 h. The DNA from the above bacterial samples was extracted by the TIANamp Bacteria DNA Kit according to the manufacturer's instructions. Then, a 6x loading buffer solution was separately added to the above DNA samples. After that, the DNA of each sample was separated on a 0.7 wt% native agarose gel plate. Meanwhile, the DNA Ladder DL10000 purchased from KeyGen Biotech Co., Ltd. (Nanjing, China) was set as the DNA marker. The agarose gel electrophoresis was allowed to run for ~45 min at a fixed voltage of 90 V at room temperature using an electrophoresis apparatus (Tanon EPS 300, Tanon Science & Technology Co., Ltd., Shanghai, China). Finally, the above agarose gel plate was imaged by a gel imaging system (Tanon-3500R, Tanon Science & Technology Co., Ltd., Shanghai, China).

MTT Assay. The normal liver L02 cells were cultured in Dulbecco's modified Eagle's medium (DMEM), seeded in 96-well plates at a density of 5×10^3 cells per

well and incubated at 37 °C under 5% CO_2 for 24 h. Then, the medium in each well was replaced with fresh medium (control) or fresh media containing different concentrations of TS4 and further incubated overnight. The original medium was aspirated to remove the dead cells, and the cell viability was determined by MTT assay via measuring the absorbance at 570 nm using the microplate reader. The cell viability was calculated by the following equation (1):

$$\text{Cell viability} = \frac{OD_{\text{sample}} - OD_{\text{blank}}}{OD_{\text{control}} - OD_{\text{blank}}} \times 100\% \quad (1)$$

where OD_{control}, OD_{blank}, and OD_{sample} represent the optical density (OD) values of the control group, blank group, and sample groups, respectively.

Hemolysis Assay. To assess the blood compatibility of TS4, the hemolysis tests were carried out by incubating red blood cells (RBCs) with different concentrations of TS4 in PBS. The fresh mouse blood (2 mL) was collected from healthy mice. The blood cells were isolated via centrifugation at 1800 rpm for 10 min, and then washed and resuspended in PBS for several times. After that, 20 μL of the above cell suspension was separately added into each tested sample with a solution/suspension volume of 180 μL and incubated at room temperature for 1 h. The RBCs treated with PBS and water were set as the negative control and positive control, respectively. Following centrifugation, the supernatants were introduced into 96-well plates and the absorbance of each well at 570 nm was measured. The hemolysis assay was carried out in triplicate. The hemolysis rate (%) was calculated according to the following equation (2):

$$\text{Hemolysis} = \frac{OD_{\text{sample}} - OD_{\text{negative control}}}{OD_{\text{positive control}} - OD_{\text{negative control}}} \times 100\% \quad (2)$$

where $OD_{\text{positive control}}$, $OD_{\text{negative control}}$, and OD_{sample} represent the OD values of the positive control group, positive control group, and sample groups, respectively.

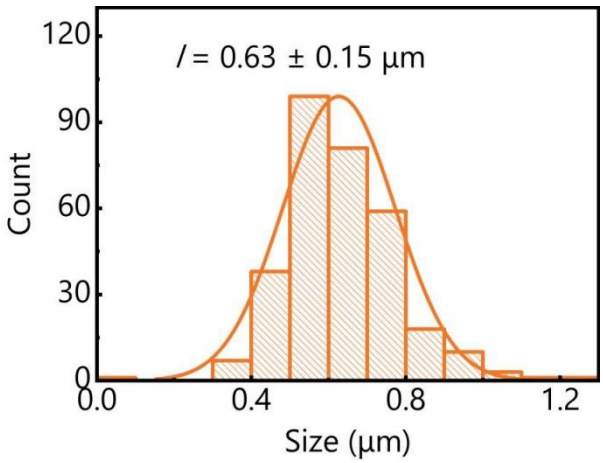

Fig. S1. Statistical results of the lengths (*l*) of TS0.5.

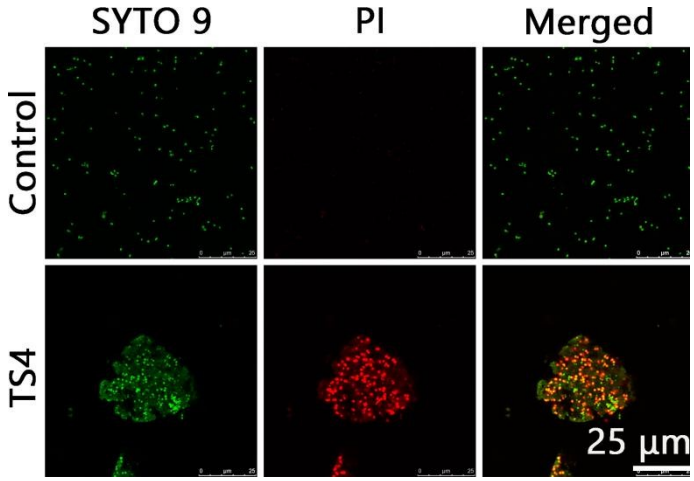

Fig. S2. Confocal microscopic images of the *S. aureus* cells that were left untreated (control) and treated with 100 μg mL^{-1} TS4 for 24 h. Before imaging, the bacteria were stained by the Live/Dead BacLight Bacterial Viability Kit containing SYTO 9 (which can stain live and dead cells green) and propidium iodide (PI, which can stain dead cells red).

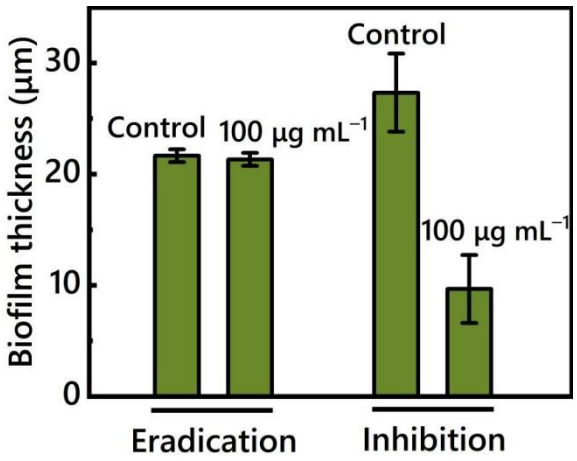

Fig. S3. Statistical results of the *S. aureus* biofilm thicknesses corresponding to Fig. 4b and c.

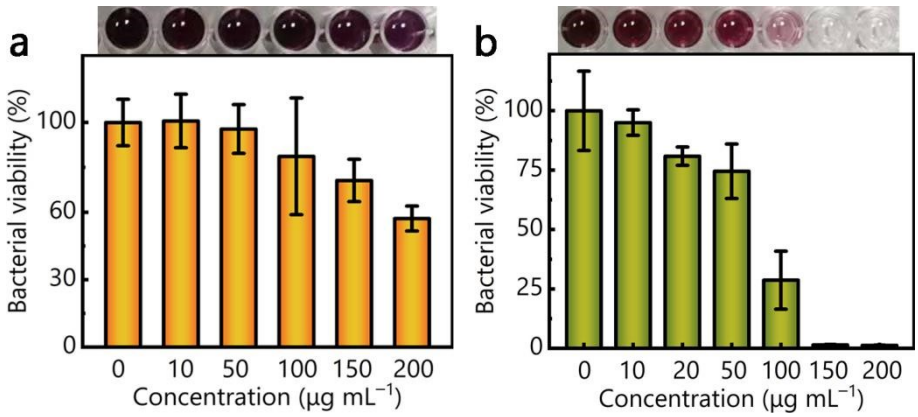

Fig. S4. (a) Upper panel: Photograph of the mature *S. aureus* biofilms treated with different concentrations of TS4 (0−200 μg mL^{-1}). Before photographing, the biofilms were stained by MTT. Lower panel: Corresponding statistical results of the MTT assay. (b) Upper panel: Photographs of the biofilms formed by the 10% LB media containing *S. aureus* cells and different concentrations of TS4 (0−200 μg mL^{-1}). Before photographing, the biofilms were stained by MTT. Lower panel: Corresponding statistical results of the MTT assay.

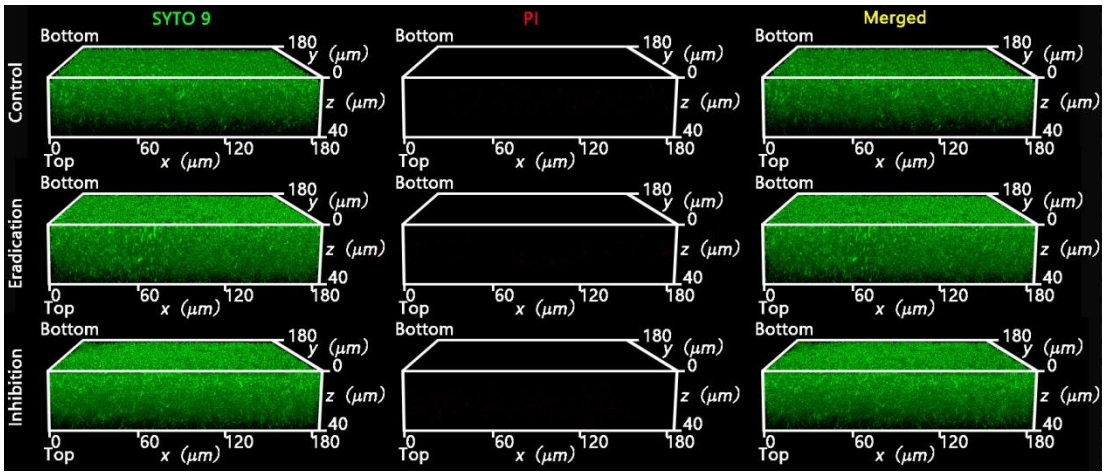

Fig. S5. Three-dimensionally reconstructed confocal fluorescence images of the *E. coli* biofilms that were left untreated (control) and treated with TS4 (100 μg mL^{-1}, 24 h, eradication), and the biofilms formed by the 10% LB medium containing *E. coli* cells and TS4 (100 μg mL^{-1}, inhibition). Before imaging, the biofilms were stained by the Live/Dead BacLight Bacterial Viability Kit.

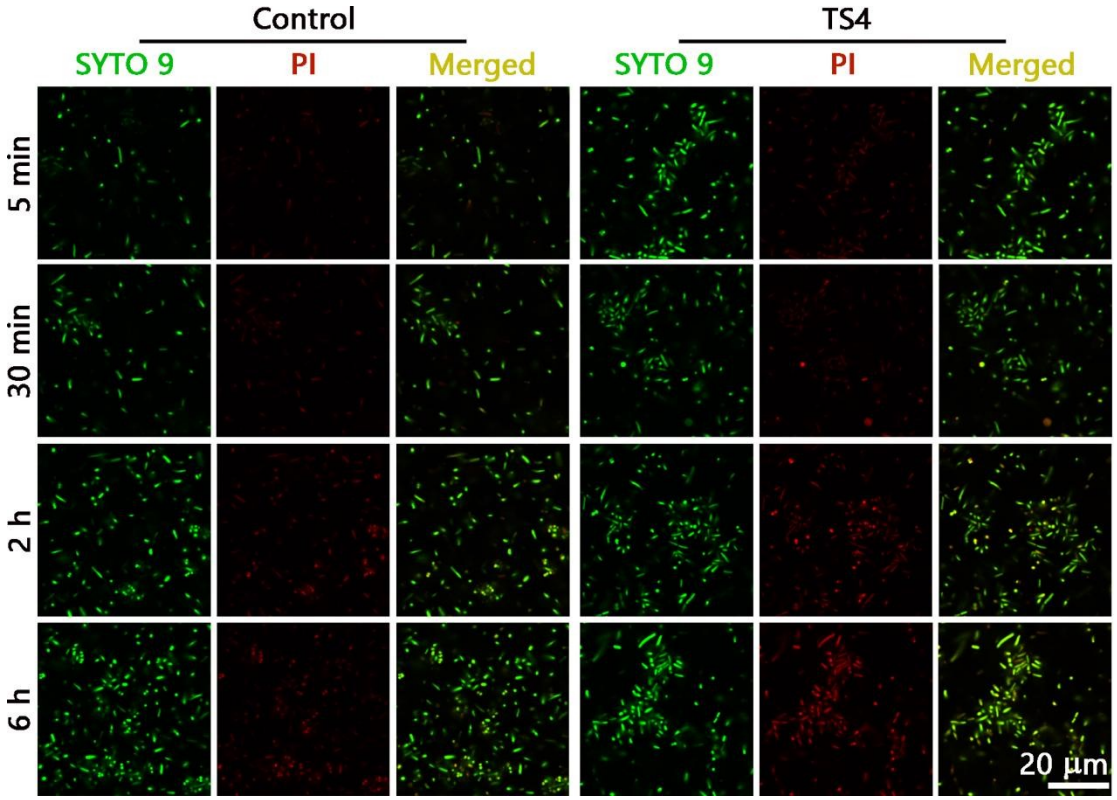

Fig. S6. Confocal fluorescence images of *E. coli* cells that were left untreated (control)

and treated with 100 μg mL^{-1} TS4 at 37 °C for different time periods. Before imaging, the bacteria were stained by the Live/Dead BacLight Bacterial Viability Kit.

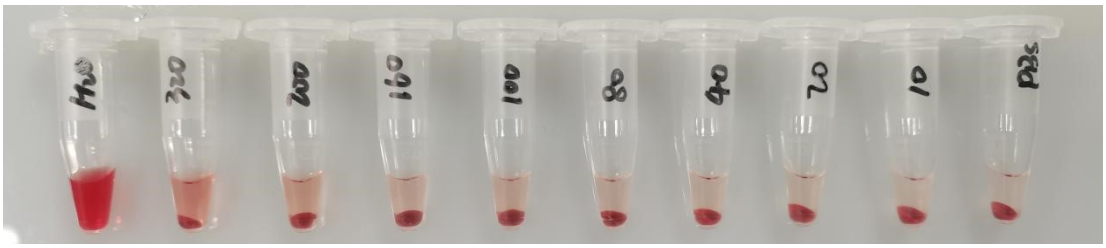

Fig. S7. Photograph of the RBC dispersions treated with different concentrations of TS4 (320, 200, 160, 100, 80, 40, 20, and 10 μg mL^{-1}). RBCs treated with water and PBS were set as the positive control and negative control, respectively. The samples were pictured after centrifugation at 2000 rpm for 5 min.