Electronic Supplementary Information (ESI)

Colloidal antibiotic mimics: selective capture and killing of microorganisms by shape-anisotropic colloids

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1. Supporting Figures

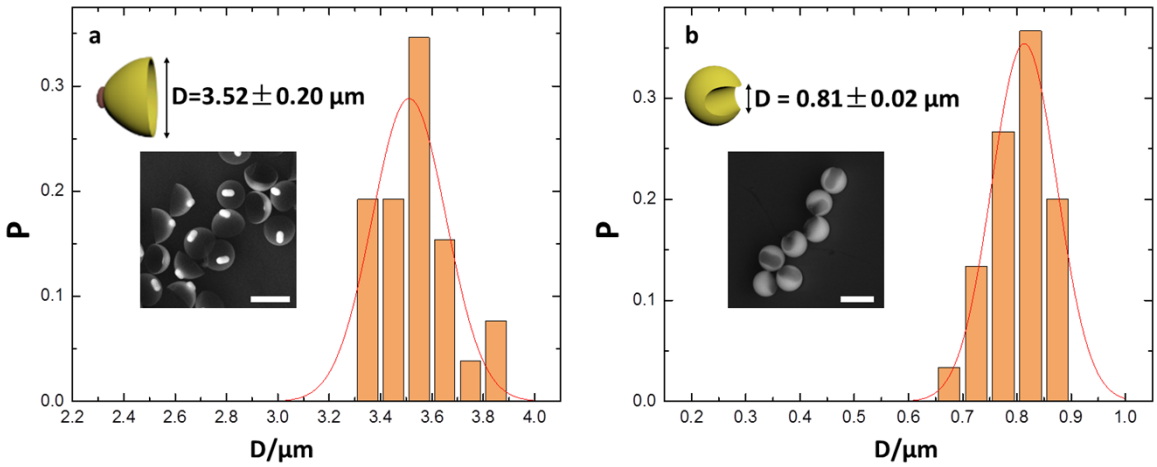

Figure S1. Size distributions of the bowl and rod cavities. The size is obtained by measurements of ~ 50 particles using ImageJ (NIH). The insets are the corresponding SEM images of the colloids. Scale bars: 5 μm (a) and 1 μm (b).

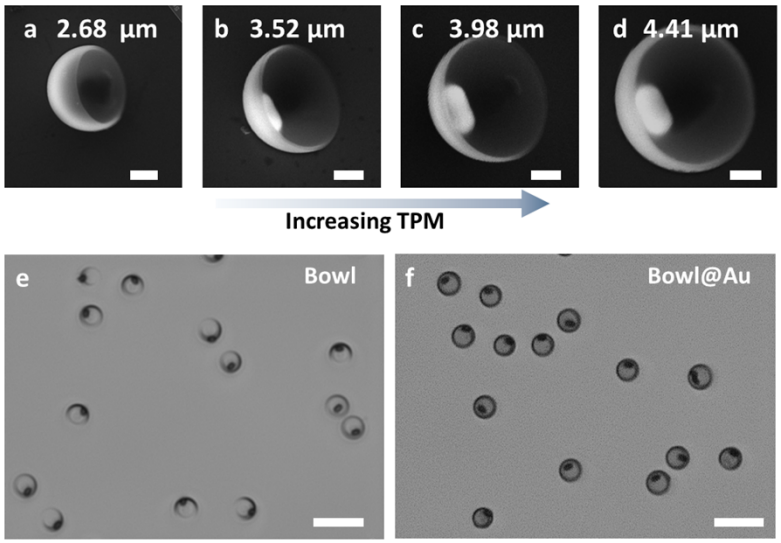

Figure S2. Bowl-shaped colloidal antibiotic mimics with tunable sizes. (a-d) SEM images of colloidal bowls. Colloidal bowls with a series of different sizes can be synthesized by changing the amount of TPM: 2.0 mL (a), 2.5 mL (b), 3.0 mL (c), and 4.0 mL (d). The sizes of the mimics are indicated. Scale bars: 1 µm. It is noted that the colloidal bowls in (b) match the shape of the yeast cells used in this work. (e, f) Bright-field optical microscope images of colloidal bowls before (e) and after (f) coated with Au nanoparticles. Scale bars: 10 µm.

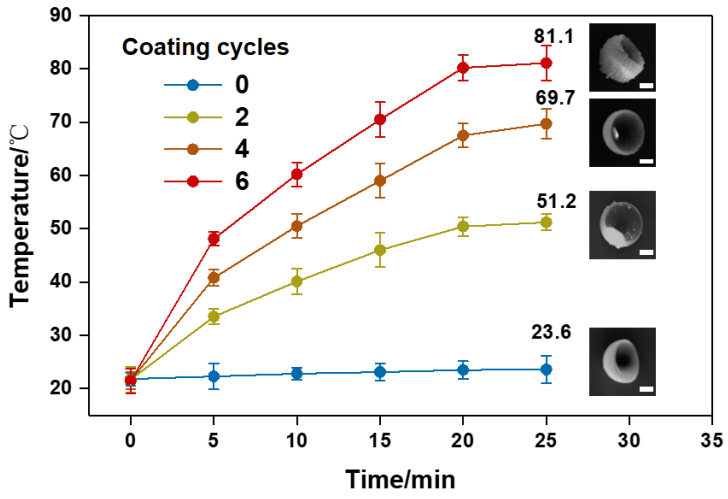

Figure S3. The temperature of the bowl-shaped colloidal antibiotic mimics after different Au coating cycles under NIR irradiation. The photothermal effect of the colloidal bowls is enhanced with increasing coating cycles. The colloidal bowls coated for 4 cycles were selected for the photothermal killing of the microorganisms. The insets show the SEM images of the corresponding colloids. Scales bars: 1 µm.

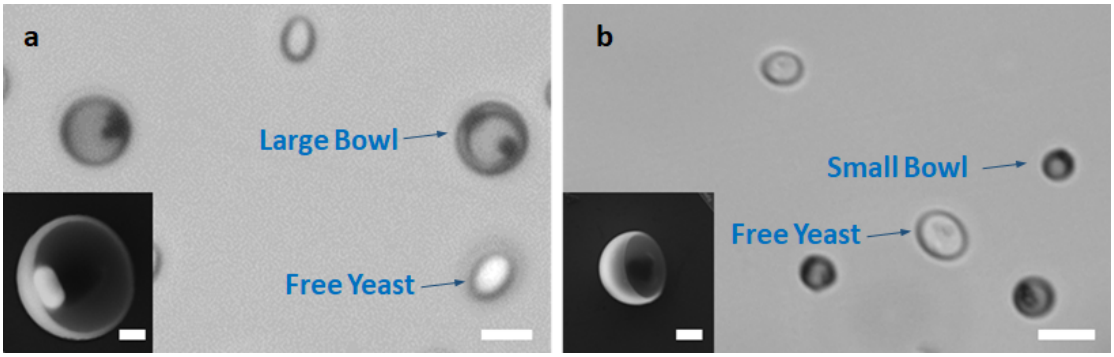

Figure S4. Optical microscopy images of yeast cells cultured with bowls with sizes of 4.41 µm (a) and 2.68 µm (b) for 12h, showing no capture of the yeast cells by the bowls. Scale bars: 5 µm. Insets: the corresponding SEM images of the large and small bowls. Scale bars: 1 µm.

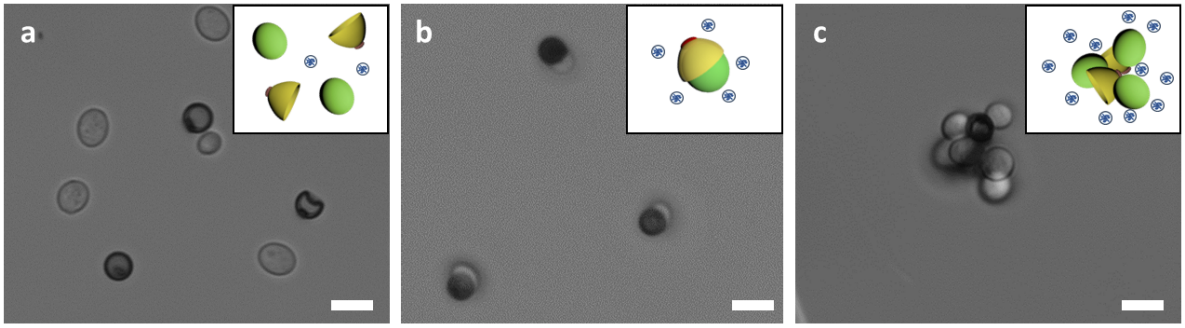

Figure S5. Bright-field micrographs of the yeast cells cultured with the colloidal mimics for 12h at different depletant concentrations: 0.2 g/L (a), 1.2 g/L (b), and 10 g/L (c). Scale bars: 5 µm.

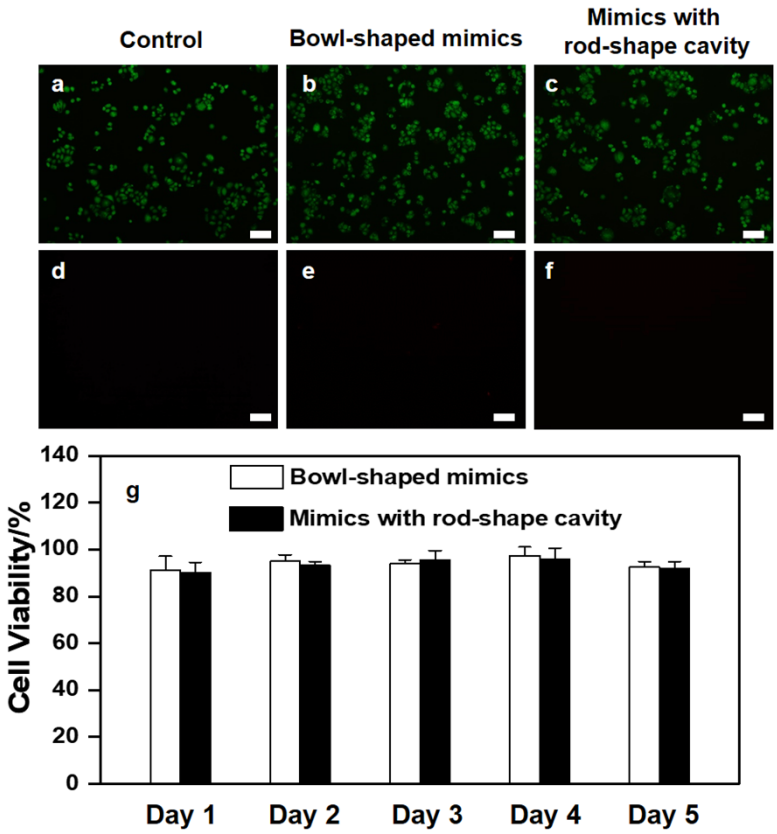

Figure S6. The biocompatibility of the two types of colloidal antibiotic mimics. (a-f) Representative fluorescence micrographs of L929 cells stained with dye after cultivation with the colloidal mimics. The green fluorescence indicates the living cells (a-c) and the red fluorescence indicates the dead cells (d-f). The red fluorescence signal is absent in d-f, which demonstrates the excellent biocompatibility of the colloidal mimics. Scale bars: 200 μm. (g) Quantification of the viability of L929 cells that were cultivated with the colloidal mimics for different days.

2. Materials and Methods

2.1 Materials

All the reagents were analytical grade and used without further purification. 3-methacryloxypropyltrimethoxysilane (TPM), polyvinylpyrrolidone (PVP, Mw = 58000 g/mol), Azobisisobutyronitrile (AIBN), and ammonia ($NH_3 \cdot H_2O$) were obtained from Sigma-Aldrich (Shanghai) Trading Co., Ltd. LIVE/DEAD bacterial staining kit was obtained from Invitrogen. *Escherichia coli* (*E. coli* ATCC 700926) was obtained from Biofeng. *Saccharomyces cerevisiae* (*S. cerevisiae* ATCC 204508) was obtained from Biobw. Cell counting kit-8 (CCK-8) was purchased from Beyotime (Shanghai, China). Mouse fibroblast cells (L929) were purchased from the cell bank of the Type Culture Collection Committee of the Chinese Academy of Sciences.

2.2 Synthesis of bowl-shaped colloidal antibiotic mimics

The bowl-shaped colloidal antibiotic mimics were prepared based on our previous work.[1] Specifically, 10 mg Fe_2O_3 ellipsoids were added into 5 ml PVP solution (10 mg/ml) and stirred for 4 hours. 2.5 mL of the 3-methacryloxypropyltrimethoxysilane (TPM) monomer was hydrolyzed in 50 ml of deionized water until a clear solution was formed. Then, 15 ml of the above-hydrolyzed solution and 2 mg of pretreated Fe_2O_3 ellipsoids were added into a flask. Deionized water was added to this flask to keep the total volume at 49 ml. The growth of TPM on Fe_2O_3 ellipsoids was started by injecting 1 ml ammonia into this mixture solution. Under vigorous magnetic stirring for 30 minutes, TPM oil droplets on the ellipsoids were bent by the shear flow and became bowl shape. Finally, 12 mg AIBN was added to this solution to initiate the polymerization of TPM oil droplets at 80°C for 6 h. The prepared colloids were washed three times with ethanol and water and dried. The size of the colloidal bowls can be controllably adjusted by changing the amount of TPM (Figure S1).

2.3 Synthesis of colloidal antibiotic mimics with rod-shaped cavity

We prepared the colloidal antibiotic mimics with one rod-shaped cavity by etching the Fe_2O_3@pTPM single-patch colloids.[2] In a typical experiment, 20 mg of Fe_2O_3 particles was dissolved in 30 mL of deionized water and sonicated for 15 min. Then, 100 μL of ammonia was added,

followed by the addition of 300 μL of TPM monomer, and the mixture was kept under stirring for 1 h. Finally, 10 mg AIBN was added to this solution to initiate the polymerization of TPM oil droplets at 80°C for 6 h to obtain the Fe_2O_3@pTPM single-patch colloids. The colloidal antibiotic mimics with one rod-shaped cavity are obtained by etching Fe_2O_3 seeds with 0.1M hydrochloric acid solution.

2.4 Photothermal modification

We use gold nanoparticles (AuNPs) to modify the prepared colloidal antibiotic mimics.[3] For a typical experiment, 150 μL of $HAuCl_4$ (2 wt%) was dispersed in 30 mL of deionized water, stirred, and heated to boiling. Then 900 μL of sodium citrate (1 wt%) was added to obtain AuNPs dispersion solution. Subsequently, 25 mL of the AuNPs dispersion solution was mixed with 25 mL of a 10 mg/mL poly(allylamine hydrochloride) (PAH) solution and stirred for 10 minutes under ultrasonication. Next, 20 mg of bowl-shaped colloids were added to the AuNPs/PAH solution and incubated with slow stirring for 20 minutes to obtain coated Bowl@Au particles through electrostatic adsorption.

2.5 Selective capture and photothermal killing of targeted microorganisms

Through preliminary theoretical calculation,[4] we determined the concentration of depletant (sodium carboxymethyl cellulose, $M_w \approx 700\,000$) to be 1.2 g/L. At this concentration, the overlap volume can induce an effective lock-key attraction resulting in a selective capture of microorganisms. In a typical experiment, 5 mg bowl-shaped mimics and 100 μL suspension of yeast (10^4 cells/mL) were gently cultured by rotating a petri dish for 10 h. Then, the medium was irradiated with near-infrared light with a wavelength of 808 nm and intensity of 0.75 W at a distance of 15 cm for 30 min, and the power density is about 1.5 W/cm^2. The experiment to capture and kill *E. coli* by the mimics with one rod-shaped cavity is similar.

2.6 The viability tests with microorganisms and counting methodology

The LIVE/DEAD BacLight Bacterial Viability Kits utilize mixtures of SYTO® 9 green-fluorescent nucleic acid stain and the red-fluorescent nucleic acid stain, propidium iodide.[5] These stains differ both in their spectral characteristics and in their ability to penetrate healthy bacterial cells. The

SYTO® 9 stain generally labels all bacteria in a population — those with intact membranes and those with damaged membranes. In contrast, propidium iodide penetrates only bacteria with damaged membranes. Thus, with an appropriate mixture of the SYTO® 9 and propidium iodide stains, bacteria with intact cell membranes stain fluorescent green, whereas bacteria with damaged membranes stain fluorescent red. This staining mechanism also applies to the structure of yeast cells.[5]

In a typical staining experiment, the dye was prepared by dispersing 1.5 μL green-fluorescent nucleic acid stain and 1.5 μL red-fluorescent nucleic acid stain in 1 mL of deionized water. A concentrated suspension of photothermally-treated microorganisms was prepared by centrifugation at 5000 rpm for 5 min and supernatant removal. Then 10 μL of the prepared dye was added to the concentrated microorganisms. After shaken slightly, and incubated in the dark for 10 min, the suspension was diluted with deionized water and the excess stain was washed off. The clear dead and living microorganisms were observed under the fluorescence microscope. The whole process is strictly protected from light.

To test the viability of microorganisms, we performed three groups of parallel experiments and selected 5 regions for statistics in each group of experiments. Then use ImageJ to count the number of dead and living microorganisms in all regions.

2.7 Biocompatibility

2.7.1 Cell culture

Mouse fibroblast cells (L929 cells) were cultured in an RPMI 1640 medium (ThermoFisher Scientific, Inc., Carlsbad, CA) supplemented with 10% fetal bovine serum (FBS, Gibco), 100 U/mL penicillin (Genview) and 100 μg/mL streptomycin (Solarbio). Cultural media were changed every two days. Cells were detached from the cell-culture flask by trypsinization, collected by centrifugation at 185 rad/s for 5 min, and re-suspended in a fresh cell culture medium before experiments.

2.7.2 CCK-8 assay

The L929 cells were cultured on 48-multiwell plates (2×10^4 cells/well) at 37°C for 12 h to allow

full spreading. The original cell medium was then replaced by the RPMI1640 containing anisotropic colloidal antimicrobials, and the cells were continually cultured at 37°C for 5 days. After washing the cells with PBS three times, 200 µL RPMI1640 and 20 µL CCK-8 dye was added, and the cells were further cultured at 37°C for 2 h. Next, 200 µL of the mixed solution was transferred to a 96-well plate. The absorbance of each solution at 450 nm was recorded by a microplate reader (Varioskan Flash, Thermo Scientific, USA). For each sample, six parallel replicates were performed. The relative cell viability (%) was evaluated by comparing the optical density value of each experimental group with a control group. Fluorescence images showed that particles have little effect on cell proliferation (Figure S6 a-f) and were confirmed by the statistical results (Figure S6g).

Reference

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2. S. Sacanna, M. Korpics, K. Rodriguez, L. Colon-Melendez, S. H. Kim, D. J. Pine and G. R. Yi, *Nat. Commun.*, 2013, **4**, 1688.
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5. M. Berney, F. Hammes, F. Bosshard, H. U. Weilenmann and T. Egli, *Appl. Environ. Microbiol.*, 2007, **73**, 3283-3290.