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Electronic Supplementary Information (ESI)

A Self-Cleaning Surface Based on UV-Activatable, AgCl Micropumps for Bacterial Killing and Removal

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1. Supporting Videos

Videos were recorded through a 40x objective at 10 frames per second by a Basler ACE camera fitted on an Olympus IX73 microscope.

Video S1. Negatively-charged sulphonated polystyrene particles were pushed away from an immobilized AgCl microparticle and off the surface upon its UV-activation;

Video S2. Positively-charged melamine formaldehyde particles moved towards immobilized AgCl microparticles during UV-activation.

Video S3. Removal of attached *S. aureus* by micropumping.

Video S4. Removal of attached *P. aeruginosa* by micropumping.

2. Supporting Figures

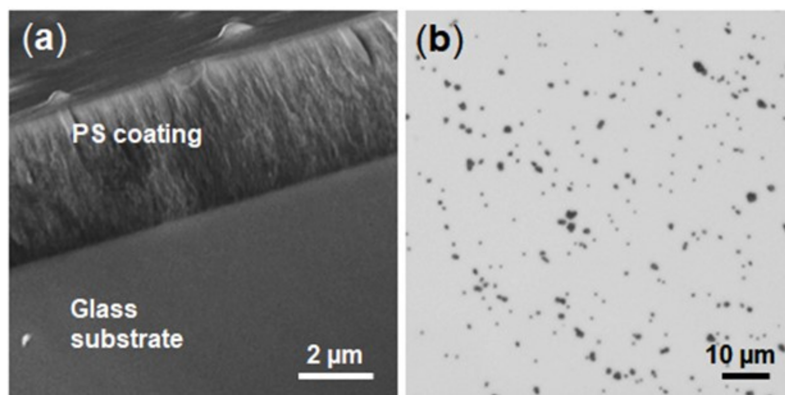

Fig. S1 (a) SEM micrograph of a cross-sectional view of a PS coating on a glass substrate with a thickness of about 5 μm. **(b)** A representative optical micrograph of AgCl microparticles distributed over a PS coating.

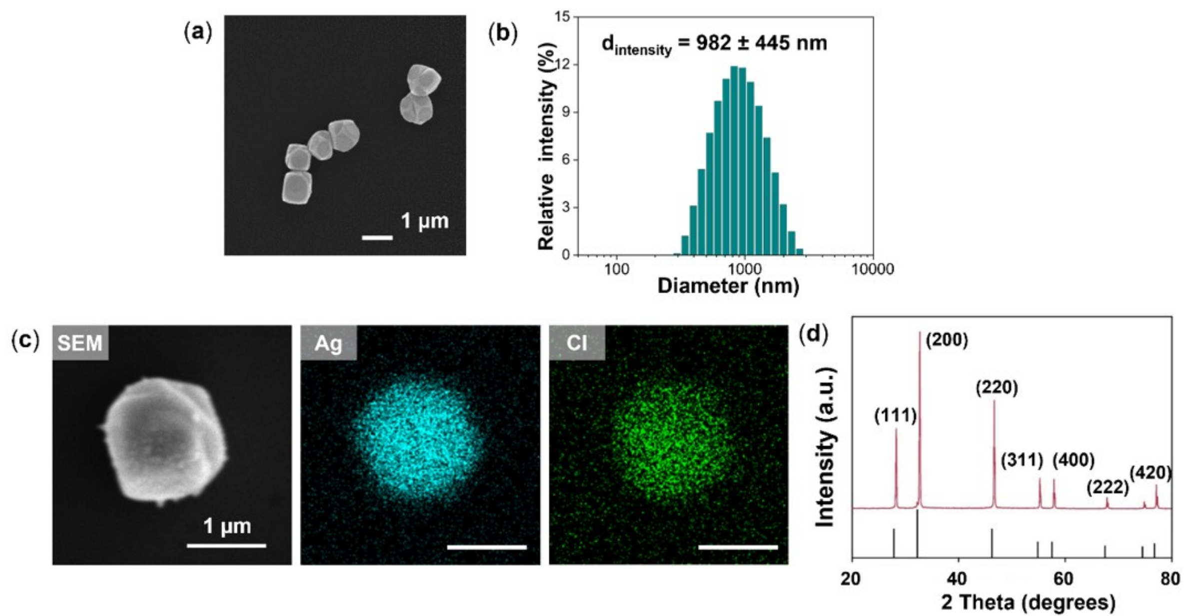

Fig. S2 (a) Low magnification SEM micrograph of truncated AgCl microparticles. **(b)** Diameter distribution of AgCl microparticles determined by dynamic light scattering. **(c)** High magnification SEM micrograph and corresponding EDX images of a AgCl microparticle. **(d)** X-ray diffraction pattern of AgCl microparticles, indicating different crystallographic planes.

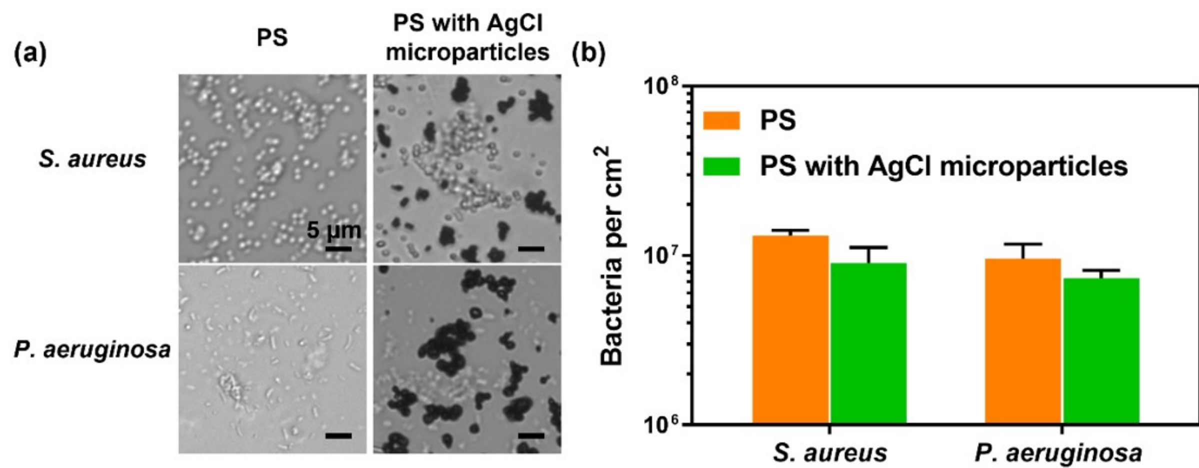

Fig. S3 (a) Optical micrographs of bacteria attached to PS with and without immobilized AgCl microparticles. AgCl microparticles appear black bacteria are white. **(b)** Number of attached bacteria, as enumerated using optical microscopy (example micrographs in panel (a)). Data represent means ± standard deviations over three experiments with separately prepared materials and bacterial cultures.

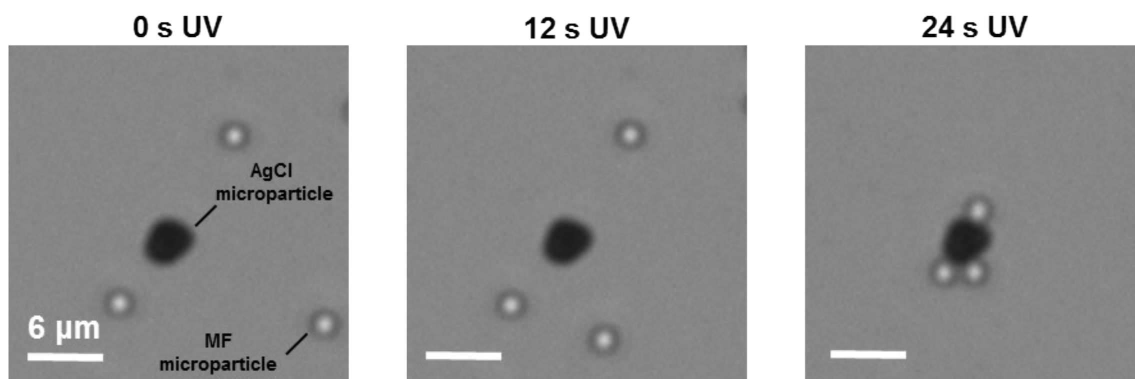

Fig. S4 Optical micrographs showing the effects of UV-activatable AgCl microparticles on attached, positively-charged melamine formaldehyde (MF) particles at different points in time after initiating UV-activated micropumping (32 mW/cm^2, 365 nm).

3. Experimental Details

3.1 Materials. Silver nitrate ($AgNO_3$) was purchased from Sinopharm Chemical Reagent Co., Ltd, China. Styrene, 2,2'-azobis(2-methylpropionitrile) (AIBN), ethylene glycol and polyvinylpyrrolidone (PVP, Mw 58000), sodium chloride (NaCl) were purchased from Aladdin, China. Sulfuric acid (H_2SO_4) was purchased from Chinasun Specialty Products Co., Ltd., China. Ethanol (100%) was purchased from Shanghai Lingfeng Chemical Reagent Co., Ltd., China. Tryptic Soy Broth (TSB) was purchased from Dalian Meilun Biotechnology Co., Ltd., China. Melamine formaldehyde particles were purchased from Micropheres-Nanopheres, USA. All chemicals were used as received without further purification. Deionized water was produced by a Milli-Q Ultrapure Water System (Millipore Sigma) and used for preparation of all aqueous solutions.

3.2 Synthesis and Characterization of AgCl Microparticles. AgCl microparticles were synthesized as described before.[1] $AgNO_3$ (425 mg) and PVP (411 mg) were dissolved in 50 mL ethylene glycol under magnetic stirring in a 100 mL three-neck flask wrapped in a tin foil to avoid light. NaCl solution (876 mg in 5 mL deionized water) was dropped into the above mixture at a rate of one droplet per 2 s, using a peristaltic pump (BQ50-1J-A, Longer Precision Pump Co., Ltd.， China). Next, the mixed solution was heated to 150 °C in an oil bath (DF-101S, Gongyi Yuhua Instrument Co., Ltd., China) for 15 min and the resulting precipitate containing AgCl microparticles was centrifuged and washed with deionized water three times to remove residual reagents. Particles were then dried overnight at 60 °C and stored in dry condition for further experiments.
The morphology and corresponding Energy Dispersive X-ray (EDX) of AgCl microparticles were observed using Scanning Electron Microscopy (Regulus 8230, Hitachi, Japan), while X-ray diffraction (XRD, Bruker D2 PHASER, Germany) was employed for structural characterization. First, dried microparticles were placed on the sample holder and gently pressed to create a flat and tight layer. Then, samples were placed in the measuring chamber and XRD patterns were collected at room temperature at a step rate of 0.2^o/s, using monochromatic Cu Kα radiation (λ =1.54 Å).

Zeta potentials were measured in deionized water using a Malvern ZetaSizer ZS2000 (UK).

3.3 Synthesis of Sulfonated Polystyrene Particles. Polystyrene particles were prepared by dispersion polymerization.[2] Briefly, PVP (0.01 g), styrene (6 g) and AIBN (0.18 g) were dissolved in ethanol (25 mL) in a four-necked round-bottom flask. Under vigorous stirring and N_2 flushing, polymerization was initiated at 72 °C, continuing for 12 h. The resulting polystyrene particles were cleaned by triple centrifugation (3850 x g), washing, and resuspended in deionized water. The suspension was dried in a vacuum oven at 60 °C for 48 h to yield dry powder. In order to sulfonate the particles, the dry powder (50 mg) was resuspended in 20 mL sulfuric acid (18.4 mol·L^{-1}) under magnetically stirring for 4 h at 40 °C.

After dilution with 140 mL deionized water, the suspension was washed three times with ethanol followed by triple washing in water, and finally dried in vacuum at 60 °C for 48 h. Zeta potentials were measured as described above.

3.4 Coating Polystyrene Surfaces with AgCl Microparticles and Characterization. First, a thin polystyrene film was spin coated on a clean glass slide according to a previously reported method.[3] The final thickness of the PS film amounted approximately 5 µm, as determined using Scanning Electron Microscopy (SEM, see Figure S1a). For immobilization of AgCl microparticles, 50 µL AgCl microparticle suspension (1 mg/mL in ethanol) was drop-cast on the polystyrene film and then the sample was placed in an oven at 120 ^{o}C to soften the PS film and allow the AgCl microparticles to be partially embedded into the film. After 5 min, the sample was cooled down to fix the microparticle. Loose, non-immobilized AgCl microparticles were removed by washing with deionized water. Finally, samples were cut into small pieces (0.6 × 0.6 cm) for further use.

Ag^+ ion release was quantified by immersing samples in deionized water (200 µL/0.36 cm^2) for 30, 60, 90 or 120 min. The concentration of released Ag^+ ion was subsequently measured using Atomic Absorption Spectrometry (AA240FS-GTA120, USA). For quantification of the generation of Reactive Oxygen Species (ROS) upon UV-irradiation, the above procedure was followed but with immersion of the samples in a 2′,7′-dichlorofluorescin solution (DCFH). DCFH is a ROS probe, obtained by mixing 10 µL DCFH-DA ((1 mM, Yeasen Biotechnology (Shanghai) Co., Ltd., China)) with 990 µL NaOH (10 mM) at room temperature for 30 min.[4] After 120 min, the samples were UV-irradiated (32 mW/cm^2) for 3 min at room temperature and fluorescence intensity measured using a spectrophotometer (Varioskan FLASH 3001, Thermo scientific, USA) at a wavelength of 525 nm.

3.5 Bacterial Strains. *S. aureus* ATCC 12600 and *P. aeruginosa* PAO1 were grown from frozen stock (7% DMSO, −80 °C) on TSB agar plates at 37 °C for 24 h. One bacterial colony of *S. aureus* or *P. aeruginosa* was transferred into 3 mL of TSB and incubated under shaking (190 rpm, KYC-100C, Shanghai Xinmiao Medical Treatment Apparatus Manufacturing Co., Ltd., China) at 37 °C. After 16 h, bacteria were harvested by centrifugation (5000g, 5 min, 10 °C), followed by washing twice in sterile phosphate buffered saline (PBS, 5 mM K_2HPO_4, 5 mM KH_2PO_4, 150 mM NaCl, pH 7.4). The bacterial suspension was sonicated (150 W, SB-80, Ningbo Scientz Biotechnology Bo., Ltd, China) three times 30 s in an ice-water bath. Bacterial zeta potentials were measured on freshly-cultured bacteria, as described above.

3.6 Contact-killing of Attached Bacteria on AgCl Microparticle Coated Surfaces. 200 µL of a bacterial suspension in sterile water (1 × 10^7 CFU/mL) was spread on a polystyrene surface and AgCl micropump-based surfaces and left at 37 ^{o}C for 2 h to allow bacterial sedimentation. After sedimentation, surfaces were gently washed twice with sterile water and then immersed in another 200 µL sterile water. Next, surfaces were UV-irradiated (Olympus U-RFL-T mercury lamp, 32 mW/cm^2, 365 nm) for 3 min with a distance of 1 cm

between the UV source and the surface. In this experiment, after UV-irradiation, bacteria remaining on the surfaces were detached by pipetting repeatedly and subsequently serially diluted for enumeration of the numbers of colony forming units (CFUs) using agar plating. Experiments on polystyrene surfaces without AgCl microparticles coating and in absence of UV-irradiation served as a control.

In addition, similar experiments were done in which the cycle of bacterial attachment and self-cleaning by UV-irradiation was repeated three consecutive times. There was no additional cleaning other that UV-activated self-cleaning in between cycles.

3.7 Efficacy of Self-Cleaning by UV-activated Micropumps. In order to evaluate self-cleaning by UV-activated micropumps, bacteria were attached to the surfaces and surfaces subjected to self-cleaning as described above. After UV-activated self-cleaning, remaining bacteria were stained with live/dead fluorescent stain (1:1 (v/v) mixture of Syto9 (3.34 mM) and propidium iodide (20 mM)) for 15 min at 37 ^{o}C in the dark for enumeration of green-fluorescent, live and red-fluorescent, dead (technically "cell-wall damaged") bacteria. After staining, surfaces were gently rinsed with sterile water and dried under a low-pressure flow of dry nitrogen. Bacteria attached to the surfaces were examined using an inverted fluorescence microscope (IX71, Olympus, Japan) with a 20 × objective, and images of 15 randomly chosen fields of view were taken. Experiments in which the surfaces were not UV-irradiated served as a control.

3.8 Statistical Analysis. Data were expressed as means ± standard deviations (SD). Three separately prepared samples were used in all experiments. Statistical analysis was performed using a two-tailed Student's t-test, accepting significance value of 0.05. Imaging data were analyzed using ImageJ (NIH Research Services Branch, USA).

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