A self-cleaning surface based on UV-activatable, AgCl micropumps for bacterial killing and removal†

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We report a self-cleaning, bacterial killing surface by immobilization of AgCl microparticles on a surface, acting as chemical micropumps. The surface shows a high bacterial killing efficacy of attached bacteria and exhibits sustainable removal of bacteria as a result of UV-activatable micropumping originating from the photocatalytic reaction of AgCl microparticles. Our work provides an advance in the sustainable use of bacterial contact-killing surfaces stricto sensu through removal of dead bacteria and debris that may shield contact-killing sites.

Bacterial attachment to surfaces and their growth into a biofilm is troublesome in many public health and industrial processes. For example, bacterial attachment to biomedical implants and devices, surfaces involved in food processing or water treatment may lead to health problems including death. In the marine industry, biocorrosion-induced corrosion of ship hulls or oil-rigs yields safety threats and high maintenance costs. Attached bacteria in a biofilm-mode of growth can be several hundred times more resistant to antimicrobials than bacteria in a planktonic-mode and are hard to eradicate. In addition, development of antimicrobial resistance is accelerating at an alarming rate.

A considerable amount of work has focused on contact-killing coatings that kill bacteria upon attachment such as quaternary-ammonium coated surfaces, but dead bacteria often remain attached to the surface shielding the contact-killing sites for newly arriving bacteria. Accordingly, contact-killing surfaces should not only kill the attached bacteria, but also remove them. Smart, antibacterial coatings currently combine (contact-)killing and anti-adhesive properties and are based on switchable polymer surfaces that change from a killing to anti-adhesive mode upon an external stimulus (pH, temperature or light). However, the changes brought about by these external stimuli do not remove dead bacteria and often additional cleaning is required to maintain the killing properties. Micropumps immobilized on a surface might offer a possibility for additional self-cleaning, induced by an external stimulus.

Micropumps convert chemical energy into a fluid flow that can be used to detach attached bacteria and guide them away from a surface. Photo-responsive micropumps convert chemical energy into mechanical energy upon UV-irradiation. Micropumps have been applied in different areas, including drug delivery, chemical sensing, and the assembly and crystallization of colloidal particles. AgCl microparticles have recently been described to act as UV-activatable micropumps. Moreover, silver ions are known to kill a range of different bacterial strains and species. Here, we verify the hypothesis that an AgCl microparticle-based coating exhibits both bacterial killing and removal of dead bacteria upon UV-irradiation (Scheme 1). AgCl microparticle-based coatings were prepared, after which bacterial killing and micropumping were evaluated by immobilizing AgCl microparticles onto a polystyrene surface, towards a Gram-positive Staphylococcus aureus and a Gram-negative Pseudomonas aeruginosa strain.

AgCl microparticle coated surfaces were fabricated by a two-step method, including polystyrene coating of a glass surface and subsequent immobilization of AgCl microparticles (Scheme 1). The thickness of the spin-coated polystyrene film was about 5 μm, as determined using scanning electron microscopy (Fig. S1a, ESI†). Polystyrene coating increased the water contact angle on the glass surface from 7 to 102 degrees (Fig. 1a), due to the hydrophobic nature of polystyrene. Subsequently, truncated AgCl microparticles (Fig. S2a, ESI†)
with a diameter of around 982 nm (Fig. S2b, ESI†) were immobilized on the polystyrene coating by heating (Fig. 1b). Using SEM (Fig. 1b) and optical micrographs (Fig. S1b, ESI†), it was found that approximately $5 \times 10^6$ cm$^{-2}$ AgCl microparticles were immobilized on the PS coating. EDX (Fig. S2c, ESI†) and XRD (Fig. S2d, ESI†) confirmed the composition and crystalline nature of the microparticles. SEM micrographs of transverse cross-sections showed that the AgCl microparticles were partially embedded into the polystyrene coating (Fig. 1c). Immobilization of the AgCl microparticles decreased the water contact angle of the polystyrene film from 102 to 86 degrees (Fig. 1a), as a result of the hydrophilicity of the AgCl microparticle. EDX and XRD (Fig. S2d, ESI†) confirmed the composition and crystalline nature of the microparticles. SEM micrographs of transverse cross-sections showed that the AgCl microparticles were partially embedded into the polystyrene coating (Fig. 1c). Immobilization of the AgCl microparticles decreased the water contact angle of the polystyrene film from 102 to 86 degrees (Fig. 1a), as a result of the hydrophilicity of the AgCl microparticle.

In order to evaluate bacterial killing, *S. aureus* or *P. aeruginosa* were attached by sedimentation to the polystyrene surfaces with and without immobilized AgCl microparticles. Similar numbers of bacteria ($0.9 - 1.7 \times 10^7$ per cm$^2$) were found to attach to both surfaces (Fig. S3, ESI†). For polystyrene surfaces without immobilized AgCl microparticles, UV-irradiation only killed maximally 10% of bacteria (Fig. 2a). In contrast, for polystyrene surfaces with immobilized AgCl microparticles, even in the absence of UV irradiation, 86% of *S. aureus* and 89% of *P. aeruginosa* were killed upon attachment due to the release of Ag$^+$ ions (Fig. 2b). Then upon UV-irradiation, the killing of attached bacteria further increased to 99.9% due to photo-initiated generation of reactive oxygen species (ROS, Fig. 2c). Note that the Ag$^+$ ion concentration decreased slightly due to formation of non-ionic Ag resulting from the photo-catalytic reaction of AgCl. Moreover, killing of attached bacteria on AgCl microparticle coatings was accompanied by severe damage to the bacteria and disintegration of the cell wall (Fig. 2d).

Dead bacteria, killed by Ag$^+$ ions, remained on the surface without UV-irradiation (Fig. 3a and b, left images). On the contrary, the number of dead bacteria was significantly less under UV-irradiation, which activated micropumping by AgCl microparticles and enhanced bacterial removal (Fig. 3a and b, right images). Quantitative statistics show that after self-cleaning by micropumping, the number of residual dead bacteria on the surface was reduced by 95% compared to that without micropumping (Fig. 3c).

In order to investigate the sustainability of bacterial killing and removal by micropumping, consecutive cycles of bacterial attachment and UV-activated micropumping were performed. In the absence of UV-activated micropumping, bacteria remained on the surface between cycles, and the killing efficacy of both *S. aureus* and *P. aeruginosa* decreased upon multiple cycles (Fig. 4a and b). In the presence of UV-activated micropumping, high killing efficacies were maintained for both strains for at least three cycles, indicative of the self-cleaning ability of AgCl microparticle coated surfaces (Fig. 4a and b). The coated surfaces exhibited not only sustainable bacterial killing in multiple, consecutive cycles of bacterial attachment, killing and removal by micro-pumping, but also in multiple cycles of use after external cleaning (Fig. 4c and d).
In order to explore the mechanism of AgCl micropumping and self-cleaning, we visualized micropumping and removal of micron-sized sulfonated polystyrene particles (SPS) carrying a negative zeta potential of $-49 \pm 1$ mV, *i.e.* similarly negative as the *S. aureus* and *P. aeruginosa* strains used ($-41 \pm 1$ and $-35 \pm 1$ mV, respectively). Using real-time, optical-microscopy, negatively-charged SPS can be seen to be pushed away from an immobilized AgCl microparticle and off the surface upon its UV-activation (Video S1, ESI†). This leaves a clean area around an immobilized AgCl micropump with a diameter of 20 to 30 µm (Fig. 5a). The area cleaned is far larger than the range of the electrostatic double-layer forces between the AgCl microparticles (zeta potential $-36 \pm 1$ mV) and SPS particles, indicating the development of an electric field around the microparticles (Fig. 5b) due to the faster diffusion of $\text{H}^+$ compared to larger $\text{Cl}^-$ ions, which generated upon UV-activated decomposition of AgCl according to the following equation:

$$4\text{AgCl} + 2\text{H}_2\text{O} \xrightarrow{\text{hv}} 4\text{Ag} + 4\text{H}^+ + 4\text{Cl}^- + \text{O}_2$$

Negatively-charged SPS particles will be pushed away from the micropumps, while being UV-activated. This mechanism is confirmed by the opposite behavior of positively-charged, micron-sized melamine formaldehyde particles (zeta potential $39 \pm 1$ mV), that moved towards immobilized AgCl microparticles during UV-activation (Video S2, ESI†) and attached to them through electrostatic double-layer attraction without detaching from the surface (Fig. S4, ESI†). Bacterial removal by micropumping (Videos S3 and S4, ESI†) occurs similarly as observed for negatively-charged SPS. These observations and proposed mechanism demonstrate that the possibilities of micropumping based on UV-activated AgCl microparticles are confined to negatively-charged bacteria, which the great majority of bacterial strains and species are, and accordingly will function best in low ionic strength applications.

In summary, we have described a self-cleaning surface based on UV-activatable, AgCl micropumps for bacterial killing and removal of dead bacteria and bacterial debris. Killing in the absence of UV-activation is achieved through the release of Ag$^+$ ions and is enhanced by ROS-generation during UV-activation.
Moreover, UV-activation results in micropumping to clean the surface from dead bacteria and bacterial debris.

Prevention of growth of attached bacteria is a key issue in many different applications, because once in an adhering biofilm, eradication is highly difficult. In this sense, it must be noted that although our AgCl microparticle coated surfaces kill attached bacteria, they do not constitute a bacterial contact-killing surface *stricto sensu*. Contact-killing surfaces *stricto sensu* have been described as possessing a high potential as antibacterial surfaces, but shielding of the contact-killing sites has so far impeded wide-spread use. In the biomedical field for antibacterial surfaces, but shielding of the contact-killing sites has so far impeded wide-spread use. In the biomedical field for antibacterial surfaces, but shielding of the contact-killing sites has so far impeded wide-spread use. In the biomedical field for antibacterial surfaces, but shielding of the contact-killing sites has so far impeded wide-spread use. In the biomedical field for antibacterial surfaces, but shielding of the contact-killing sites has so far impeded wide-spread use. In the biomedical field for antibacterial surfaces, but shielding of the contact-killing sites has so far impeded wide-spread use.

We envision many applications for such self-cleaning surfaces. Moreover, AgCl microparticles can also be immobilized on contact-killing surfaces *stricto sensu*, solving the problem of active-site blockage by dead bacteria by their removal through micropumping.

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Conflicts of interest

H.J.B. is also a director of a consulting company, SASA BV (GN Schutterlaan 4, 9797 PC Thesinge, The Netherlands). The authors declare no potential conflicts of interest with respect to authorship and/or publication of this article.

Notes and references


5 J. A. Callow and M. E. Callow, *Nat. Commun.*, 2011, 2, 244.


