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RIR-MAPLE Deposition of Multifunctional Films Combining Biocidal and Fouling Release Properties †

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Multifunctional films with both antimicrobial activity and fouling-release ability based on a biocidal quaternary ammonium salt (QAS) and thermo-responsive poly(*N*-isopropylacrylamide) (PNIPAAm) were deposited on substrates using resonant infrared, matrix-assisted pulsed laser evaporation (RIR-MAPLE). The surface properties of these films were characterized by X-ray photoelectron spectroscopy (XPS), Fourier transform infrared (FTIR) spectroscopy, atomic force microscopy (AFM), and water contact angle measurements. The biocidal and release properties of the films were tested against *Escherichia coli* K12 and *Staphylococcus epidermidis*. At 37°C, the deposited film facilitated bacterial attachment and killed a majority of attached bacteria. Decrease of the temperature to 25°C promoted the hydration and at least partial dissolution of PNIPAAm, leading to bacterial detachment from the film. To enhance the retention of PNIPAAm on the substrate, a small amount of (3-aminopropyl) triethoxysilane (APTES) was incorporated as a stabilizer, resulting in a ternary film with biocidal activity and bacterial-release ability after several attach-kill-release cycles. The simplicity and universality of RIR-MAPLE to form films on a wide range of substrata make it a promising technique to deposit multifunctional films to actively mitigate bacterial biofouling.

1 Introduction

The attachment of bacterial cells to surfaces of synthetic materials often leads to colonization resulting in the formation of biofilms. The creation of these unwanted biofilms, known as biofouling, causes a variety of serious health problems, including failure of implanted and submerged materials and devices, as well as the spread of infection within public health and food production settings. [1-3] Developing methods to prevent biofouling on synthetic surfaces is, thus, of great interest.[4-6] One approach to management of biofouling is to develop surfaces that can exhibit three functions: (i) strong biocidal activity to kill the attached bacteria; (ii) fouling-release ability to release the dead bacteria and debris; and (iii) reusability for several attach-kill-release cycles. There are considerable reports on antimicrobial surfaces by immobilization biocides (such as polycations,[7] antimicrobial peptides,[8] enzymes,[9] and quaternary ammonium compounds[10]) and on fouling release surfaces by modification with stimuli-responsive polymers[11-12] or amphiphilic polymers.[13-14] However, integration of biocides with fouling-release materials in order to endow synthetic surfaces with multifunctionality has been rarely reported so far.[15-17] Recently, Yu *et al*. developed a model multifunctional surface exhibiting the ability to control the attachment, killing and release of bacteria in response to temperature changes.[17] The multifunctional surface comprises a bacterial release control component, nanopatterned poly(*N*-isopropylacrylamide) (PNIPAAm) brushes prepared by the combination of interferometric lithography (IL) and surface initiated polymerization (SIP),[18] and a biocidal component, quaternary ammonium salt (QAS), which is immobilized into polymer-free regions between brushes. PNIPAAm is a well-studied thermally responsive polymer that displays a sharp, reversible solubility phase transition at a lower critical solution temperature (LCST) of ~32°C in aqueous solution.[19] Above the LCST, the nanopatterned PNIPAAm brushes collapse, exposing QAS that can then kill attached bacteria. Rinsing the surface with water at temperatures below the LCST induces swelling of the PNIPAAm brushes to release the dead bacteria, thereby restoring the surface for reuse. This strategy is general and has been applied to other biocides, such as antimicrobial enzymes.[16]

While nanopatterned PNIPAAm/QAS hybrid surfaces can effectively achieve both biocidal and fouling-release functionalities, the preparation of these surfaces involves multiple steps (i.e., IL patterning of monolayer of initiator, SIP to graft PNIPAAm, and backfilling with QAS) and may not be suitable for other non-silica based substrates, which may limit its broad application. It is thus desirable to find a simple, universal technique to deposit such multifunctional films on a wide variety of substrates. Resonant infrared, matrix-assisted pulsed laser evaporation (RIR-MAPLE) is a promising deposition technique for this purpose, in which an organic thin film is gently deposited onto substrate surfaces by infrared laser ablation of a host

emulsion matrix.[20-21] The detailed growth mechanism of film deposited by emulsion based RIR-MAPLE was introduced previously.[21] Importantly, the laser energy (Er:YAG, 2.9 μm) is resonant with OH groups in the emulsion matrix and is not absorbed by the material targeted for deposition.[22] As a result, the deposited organic material retains its structural and functional integrity. In addition, the entire deposition process is one-step and can be applied to most organic materials without OH groups, as well as any solid substrate. Previously, RIR-MAPLE has been successfully used to deposit films of common polymers such as polystyrene and poly(methyl methacrylate),[23] conjugated polymers such as poly(3-hexylthiophene-2,5-diyl) and poly(phenylenevinylene),[22] and colloidal CdSe quantum dots[24]. In addition, we recently deposited an antimicrobial oligo (*p*-phenylene-ethynylene) (OPE) film using RIR-MAPLE and demonstrated its UV light-induced biocidal activity.[25] Importantly, we found enhanced bacterial attachment and biocidal efficiency of the films deposited by RIR-MAPLE compared with those prepared by spin-coating and drop-casting methods, which can be attributed to nanoscale surface topography of the thin film. In addition, compared with other common deposition techniques, such as spin-coating and drop-casting, RIR-MAPLE provides an easy way to deposit multi-component films with nanoscale domain sizes of the constituent materials, regardless of the solubility characteristics of each component.[23] Such versatility is advantageous for the design and preparation of multifunctional, antifouling surfaces, especially for functional components that are not soluble in a single solution (such as hydrophobic molecules with hydrophilic molecules).

In this study, a multifunctional film containing both biocidal QAS and the fouling-release polymer PNIPAAm was simply deposited on substrates using RIR-MAPLE. The surface properties of these films were characterized by X-ray photoelectron spectroscopy (XPS), Fourier transform infrared (FTIR) spectroscopy, atomic force microscopy (AFM), and water contact angle measurements in order to demonstrate that both materials are present on the surface. Two model bacterial strains, *Escherichia coli* K12 (Gram-negative) and *Staphylococcus epidermidis* (Gram-positive), were used to test the biocidal and bacterial release performance. We show that the deposited PNIPAAm/QAS hybrid films killed large numbers of attached bacteria when the temperature is above the LCST, and that the dead bacteria are released upon subsequent exposure to water below the LCST due to the dissolution of the PNIPAAm component. To enhance the retention of PNIPAAm on the substrate, we incorporated a very small amount of (3-aminopropyl) triethoxysilane (APTES) as a stabilizer in the hybrid film, and the resultant ternary film maintains both biocidal activity and bacterial-release ability for several attach-kill-release cycles.

2 Experimental section

2.1 Materials

Poly (*N*-isopropylacrylamide) (PNIPAAm, M_W=19,000-30,000), dimethyloctadecyl [3-(trimethoxysilyl)propyl] ammonium chloride (QAS, 42 wt.% in methanol), (3-aminopropyl) triethoxysilane (APTES, ≥99%), and methanol (MeOH, ≥99.8%) were purchased from Sigma-Aldrich (St. Louis, MO). Silicon wafers and cover slips (size=25 x 50 mm, thickness=0.13 mm) were purchased from University Wafer and VWR, respectively. Before use, all the silicon wafers and cover slips were cleaned with "Piranha" solution (7:3(v/v) 98%H_2SO_4:30%H_2O_2; ***caution: piranha solution reacts violently with organic materials and should be handled carefully!***) to remove the organic residue, rinsed with an abundance of de-ionized (DI) water and dried under a dry nitrogen stream.

2.2 Bacterial strains

Escherichia coli K12 (ATCC 29425) and *Staphylococcus epidermidis* (ATCC 14990) were received as lyophilate from the American Type Culture Collection (Bethesda, MD) and stored as frozen stock aliquots in Difco nutrient broth (NB) +20% glycerol at -80°C. Experimental stock cultures were maintained on NB slants and were stored at 4°C for up to 2 weeks. A single colony from the slants was incubated in 50 mL of NB and grown overnight with shaking at 37°C. After growth, the bacterial culture was centrifuged at a relative centrifugal force of 11,952 × g for 10 min at 4°C. The pellet was then suspended in 0.85% NaCl (for *E. coli*) or phosphate buffered saline (PBS) (for *S.epidermidis*). This washing procedure was repeated twice.[17] The final concentrations of *E. coli* and *S. epidermidis* were ~1×10^8 cells/mL and ~3×10^7 cells/mL, respectively, as measured using a hemocytometer (C-chip CYTO Corp, Sunnyvale, CA) and phase contrast microscopy (Axioimager, Carl Zeiss Microimaging, Inc., Jena) through a 40X objective.

2.3 Preparation of multifunctional films

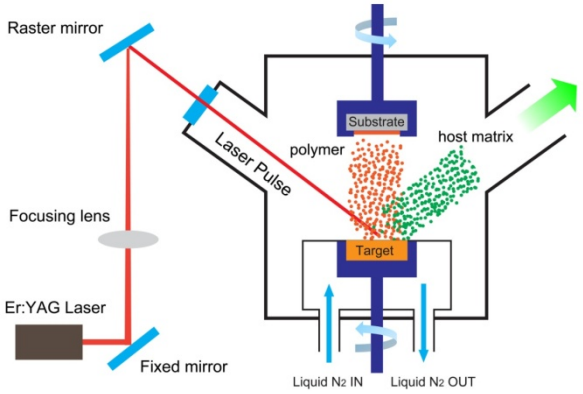
Target preparation for RIR-MAPLE

The target solutions for PNIPAAm and QAS comprised a mixture of MeOH and DI water at a ratio 3:7. Either PNIPAAm or QAS was dispersed in the mixture at the concentration of 5 mg/mL and a 5-min sonication was typically applied to ensure a good and uniform target solution. The mixtures were then injected into the chilled target holder (-190°C, cooled by liquid nitrogen) and were frozen into a solid ice instantly (less than 5 seconds). For PNIPAAm/QAS hybrid films, the target preparation was similar except that a split target was used for sequential deposition.[26] The split target method was adopted in order to avoid possible phase separation between PNIPAAm and QAS when they are co-dissolved into the MeOH/water mixture used as the target solution. For PNIPAAm/QAS/APTES ternary film, the APTES and PNIPAAm were blended in the mixture of methanol and water (3:7), at a ratio of 1:4 (total concentration is still 5mg/mL). This mixture comprised part of the partitioned target, while QAS alone (5 mg/mL) comprised the remaining target. In all, the ratio of APTES/PNIPAAm/QAS across the entire target was 1:4:5.

RIR-MAPLE system

The primary components of the RIR-MAPLE system are an infrared laser, an optical system, a rotating frozen target, a rotating substrate, and a pumping system, as depicted in **Scheme 1**, which also describes an ideal process for RIR-MAPLE deposition. Typically, the target solution is first frozen into a

solid target using liquid nitrogen. Then the infrared radiation coming from the laser is directed by the optical system and swept evenly across the rotating solid target. Ideally, only host matrix molecules with vibrational modes resonant with the laser are pumped away upon evaporation, while polymers are gently deposited onto a rotating substrate. It is important to note that, small amounts of target solvent may be deposited onto the substrate along with the polymer films. In our system, an Er: YAG laser at wavelength 2.9 μm is used to target hydroxyl bonds in the target solution.



Scheme 1 Schematic depiction of RIR-MAPLE system and process: an incident laser pulse enters a vacuum chamber through an optical window. The laser ablates a solid frozen target consisting of a guest material (polymer, QAS) in the hydroxyl rich solvent host matrix. The polymer has a high sticking coefficient and is deposited onto the substrate, whereas the frozen host matrix is evaporated by the laser energy and pumped away.

2.4 Surface analysis

X-ray photoelectron spectroscopy (XPS)

The elemental composition of surfaces was determined with a Kratos Analytical Axis Ultra X-ray photoelectron spectrometer (XPS) equipped with a monochromatic Al Kα source. High-resolution scans were acquired at a pass energy of 20 eV and a resolution of 0.1 eV. Survey scans were acquired with a pass energy of 160 eV and a resolution of 1.0 eV. All XPS data were analyzed using CASA XPS software. All binding energies were referenced to the main hydrocarbon peak designated as 285.0 eV. Peak resolution was performed using a linear peak base and symmetric 30/70 Gaussian–Lorentzian component peaks.

Fourier transform infrared spectroscopy (FTIR)

FTIR spectra were obtained using a Thermo Nicolet 8700 spectrometer equipped with a TE cooled DLaTGS detector. All spectra were taken at ambient temperature and under dry conditions with a nominal spectral resolution of 2 cm^{-1} in absorbance mode. FTIR samples were made by depositing PNIPAAm, QAS, and PNIPAAm/QAS on semi-insulating silicon wafers (IR window material) by RIR-MAPLE. To ensure the decent signal intensity (enough thickness of the film), a higher concentration (10 mg/mL) and longer deposition time (6 h) are adopted for the deposition of FTIR samples using RIR-MAPLE. The background from the silicon wafer was subtracted to obtain the final FTIR spectra.

Atomic force microscopy (AFM)

Tapping-mode topographical and phase measurements of deposited functional films in air were obtained with a Digital Instruments multimode atomic force microscope (AFM) with a Nanoscope IIIa controller. The corresponding section analysis was performed using the Nanoscope Analysis software.

Contact angle goniometry

Static contact angles were measured in air using a Rame-Hart model 100-00 contact angle goniometer at 25°C. Contact angle values reported are the average of six replicates.

Ellipsometry

The thickness of deposited multifunctional films was measured with an M-88 spectroscopic ellipsometer (J. A. Woollam Co., Inc.). The thickness values reported are the average of three replicates.

2.5 Attachment and detachment of bacteria

Attachment and detachment of bacteria on the sample surfaces were assessed using *E. coli* suspension (1×10^8 cells/mL in 0.85% NaCl) and *S. epidermidis* suspension (3×10^7 cells/mL in PBS).[11] Briefly, prior to introduction of the sample surfaces, the cell suspensions were pre-equilibrated at 37°C in glass Petri dishes. The sample surfaces were placed on the bottom of a glass Petri dish, test surface up, and incubated in these suspensions at 37°C for 2 h unstirred.[11-12,16-17] They were then rinsed gently with ultrapure water pre-equilibrated at 37°C to remove loosely attached cells and salts and dried under a low-pressure stream of dry nitrogen. For bacterial detachment, the sample surfaces were washed under shear (estimated shear rate = 0.04 Pa) with 60 mL of 0.85% NaCl (for *E. coli*) or PBS (for *S. epidermidis*) at 25°C delivered from a syringe, rinsed in ultrapure water, and dried. The attached bacteria were examined using a phase contrast optical microscope (Axioimager, Carl Zeiss Microimaging, Inc., Jena) through a 40X objective, and images of 15 randomly chosen fields of view were captured. For each sample, three replicates were performed, and the density of adherent bacteria was analyzed by ImageJ (National Institutes of Health) to obtain the average and standard deviation.

2.6 Live/Dead assays.

A standard live/dead staining assay was performed using the BacLight kit (Invitrogen, Grand Island, NY) to examine the biocidal activity of sample surfaces. Upon completion of the experimental treatments described above, the sample surfaces were immersed into a staining solution containing 1:1 mixture of SYTO 9 (3.34 mM) and propidium iodide (20 mM).[27] After incubation at 37°C for 15 min in the dark, the surfaces were rinsed with ultrapure water at 37°C and examined by fluorescence microscopy (Axioimager, Carl Zeiss Microimaging, Inc., USA) through a 40X air objective, and images of 15 randomly chosen fields of view were captured. For each sample, three replicates were performed and the relative number of live (green) *vs.* dead (red) bacteria was analyzed by ImageJ (National Institutes of Health) to obtain the average and standard deviation.

2.7 Scanning electron microscopy.

To observe the morphology of attached bacteria, the sample surfaces were rinsed gently in ultrapure water to remove the unattached cells, fixed by 2.5% glutaraldehyde solution for 2 h, dehydrated in a series of ethanol solutions (30-100%), and air-dried.[28] Before characterization, the samples were sputter coated with a 5 nm layer of gold. The surfaces were then examined using an FEI XL30 scanning electron microscope (SEM) at an accelerating voltage of 7 kV.

3 Results and discussion

3.1 Preparation and characterization of films deposited by RIR-MAPLE

Thin films formed from QAS, PNIPAAm, and PNIPAAm/QAS were deposited onto glass coverslips and silicon wafers using RIR-MAPLE. Under identical deposition times (3 h) and concentrations of target solution (5 mg/mL), all three types of film showed similar film thickness of ≈110 nm (as measured by ellipsometry, the film thickness can be controlled by the deposition time, 3-h deposition time was chosen here to yield a thick film that is contiguous), but the surface wettability of the films was significantly different. The measured water contact angle on the QAS films was 92.5±3.4°, which is similar to the value of QAS layers formed by self-assembly from solution as reported previously.[17] In contrast, the PNIPAAm films deposited by RIR-MAPLE were hydrophilic with a contact angle of 20.7±0.9°, which is much lower compared to PNIPAAm films prepared by surface initiated polymerization or drop-casting methods.[28] Considering the absence of differences in chemical composition (see below), we hypothesize that differences in the surface roughness lead to the difference in wettability (detailed eluidation can be found in **ESI, Figure S1**). The PNIPAAm/QAS film showed an intermediate contact angle of 57.8±2.8°, suggesting that both components are on the surface.

The surface chemistry of deposited films was examined by FTIR and XPS. As shown in **Figure 1a**, characteristic IR absorbance peaks of QAS (1191 cm^{-1} (OCH_3), 916 cm^{-1} (Si-OH), and 825 cm^{-1} (Si-O-C))[29] and PNIPAAm (N-H at 3309cm^{-1}, C=O at 1648 cm^{-1}, C-N at 1535 cm^{-1} and the doublet for $–HC(CH_3)_2$ at 1386 cm^{-1} and 1365 cm^{-1}) [30] were observed on the corresponding films. These characteristic peaks were observed also on the PNIPAAm/QAS film, confirming the existence of both components on the hybrid film. It was also found that the strength of the IR peaks near 2850-3000 cm^{-1} (C-H) substantially reduced for the pure PNIPAAm film (bottom) compared with the hybrid film (middle) and the pure QAS film (top). The observed differences for this peak range are inherent to the materials and unrelated to the deposition technique, that is, PNIPAAm has weaker C-H stretching peaks than pure QAS. We also compared the FTIR spectra of the corresponding films deposited by drop-casting with those prepared by RIR-MAPLE. The positions of characteristic FTIR absorption peaks were identical, suggesting that no significant chemical degradation occurred during RIR-MAPLE process (**ESI, Figure S2**). In addition, XPS was performed to further determine the chemical composition of the surface of deposited films (**Table 1**). The C/N ratio for the QAS film and PNIPAAm film were 27.7 and 6.3, respectively, consistent with their chemical structures (see inset in **Figure 1a**). Analysis of the N1s spectral region showed that these two films each contain one unique peak that can be used to identify the material. For QAS, a single peak was observed at 402 eV, and is attributed to nitrogen in the cationic quaternary ammonium species. For PNIPAAm, a single peak was observed at 399 eV, and is attributed to nitrogen in the amide group. As with the FTIR results, these two peaks were both found on PNIPAAm/QAS hybrid films.

Table 1 Chemical composition of film surfaces (Data shows the mean ± the standard error (n=4))

Surface	C(%)	N(%)	O(%)	Si(%)	Cl (%)	C/N
QAS	83.1±0.6	3.0±0.1	8.7±0.5	3.1±0.2	2.1±0.1	27.7±0.7
PNIPAAm	75.7±0.1	11.9±0.1	12.4±0.1	N.D.	N.D.	6.3±0.2
PNIPAAm/QAS	80.3±0.3	5.5±0.2	9.9±0.2	2.4±0.1	1.8±0.1	14.5±0.8

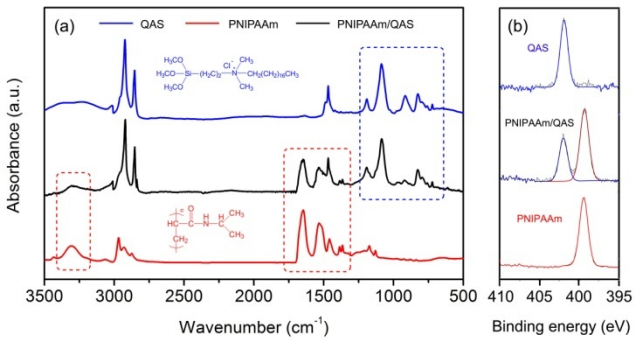


Figure 1 (a) Normalized FTIR spectra and (b) high-resolution XPS N1s spectra of QAS, PNIPAAm, and PNIPAAm/QAS hybrid films deposited by RIR-MAPLE. The chemical structures of QAS and PNIPAAm are shown as insets in (a). Red boxes represent PNIPAAm specific peaks and blue box represents QAS specific peaks.

3.2 Biocidal activity

The biocidal activity and bacterial release ability of the PNIPAAm/QAS films were assessed using two model bacteria, the Gram-negative bacterium, *E. coli,* and the Gram-positive bacterium, *S. epidermidis*.[16-17] QAS and PNIPAAm films were used as controls. All the films were incubated in an *E. coli* suspension (1×10^8 cells/mL) or a *S. epidermidis* (3×10^7 cells/mL) at 37°C for 2 h, and the viability of attached bacteria was determined by a standard live/dead staining assay using fluorescence microscopy (**Figure 2a-f**). Among these three types of films, QAS films showed the highest levels of attachment of bacteria, regardless of the bacterial strain. Importantly, more than 90% of the attached bacteria were noted to be dead, as they were stained by propidium iodide (red fluorescence), which cannot permeate and stain viable cells with intact membranes. It has been proposed that QAS moieties immobilized on a surface can attract bacteria by electrostatic and/or hydrophobic interactions, and then degrade the cell membrane and destabilize the intracellular matrix of a bacterium through a contact mechanism.[10, 31-32] In contrast, PNIPAAm films showed lower

levels of bacterial attachment and no obvious biocidal activity as the majority of attached bacteria were only stained upon exposure to the cell-permeating dye, Syto 9 (green fluorescence). The hybrid PNIPAAm/QAS hybrid films showed intermediate levels of bacterial attachment as compared to the two control films, but comparable killing efficiency (defined as the ratio of the amount of dead bacteria to total bacteria) to that of QAS surface (see **Figure 2g,h**).

The killing efficiency of PNIPAAm/QAS hybrid films is ~80% against *E. coli* and ~85% against *S. epidermidis*, which is higher than our previously reported nanopatterned PNIPAAm/QAS surfaces.[17] The enhanced biocidal activity may be related to the increased surface roughness (the root mean squared (RMS) roughness according to AFM analysis is 21.2 nm and 6.5 nm for PNIPAAm/QAS film and nanopatterned PNIPAAm/QAS surface, respectively).[25] We also compared the killing efficacy of the films under different incubation temperature (37°C *vs.* 25°C, **ESI, Figure S3**). For the control films (QAS or PNIPAAm), no significant difference in killing efficiency was observed with incubation temperature. In contrast, the PNIPAAm/QAS hybrid films exhibited more effective biocidal activity at 37°C than at 25°C (79.7±6.1% *vs.* 51.3±3.3% for *E. coli* and 86.7±6.0% *vs.* 18.8±4.5% for *S. epidermidis*). We attribute this temperature-dependent killing ability to the change of solubility of PNIPAAm chains in the film. At temperatures below the LCST (e.g., 25°C), PNIPAAm chains hydrate, thus increasing their excluded volume and may still remain associated with the surface (e.g., because of chain entanglement) to block the contact of bacteria with QAS on the surface, resulting in the decrease of biocidal activity.

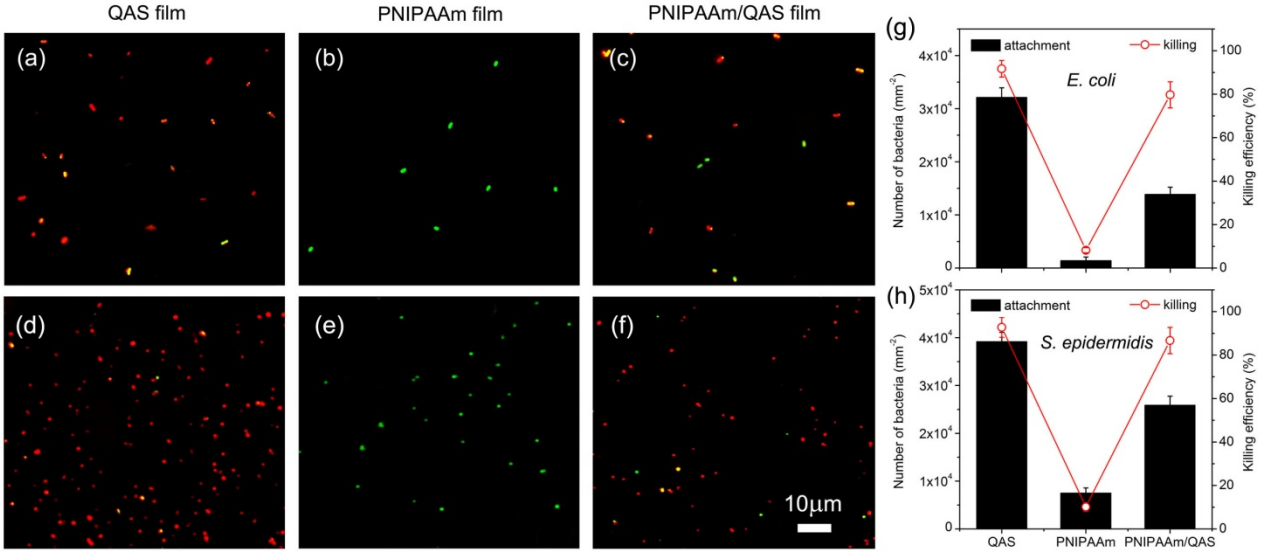


Figure 2. Dual channel (green/red) fluorescence micrographs of attached bacteria exposed to live / dead stains (see text for details) on (a)(d) QAS film, (b)(e) PNIPAAm film and (c)(f) PNIPAAm/QAS hybrid film after incubation in suspensions of (a-c) *E. coli* or (d-f) *S. epidermidis* at 37°C for 2 h. Green staining indicates live bacteria, and red or yellow staining indicates dead bacteria. The corresponding attachment and killing efficiency (defined as the ratio of dead bacteria and total bacteria) are summarized in (g) and (h). Data shows the mean ± the standard error (n=3).

The morphology of attached bacteria was observed using SEM. The attached bacteria on the PNIPAAm control film were intact, indicating that the cells were normal and healthy prior to fixation, but those on the QAS control film and the PNIPAAm/QAS hybrid film exhibited significant damage to the outer membrane and the cellular integrity was lost, regardless of the bacterial species (see **Figure 3**).

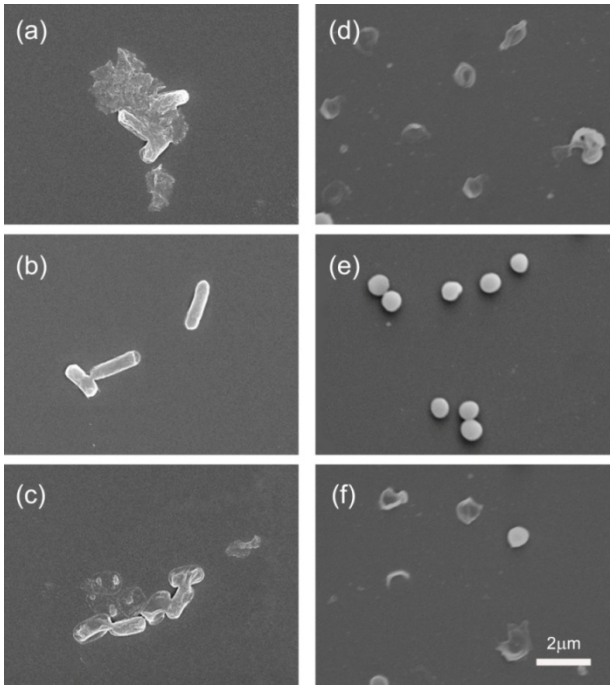


Figure 3. SEM images of attached bacteria on a QAS film (a)(d), a PNIPAAm film (b)(e) and a PNIPAAm/QAS hybrid film (c)(f) after incubation in suspensions of *E. coli* (a-c) or *S. epidermidis* (d-f) at 37°C for 2 h.

3.3 Attachment and release of bacteria

The ideal biocidal films should not only kill the attached bacteria, but also release the dead bacteria and related debris. To test the bacterial release capability, the different films were first incubated in suspension of bacteria at 37°C for 2 h, and then rinsed with either 0.85% NaCl aqueous solution (for *E. coli*) or PBS (for *S. epidermidis*) at 25°C. **Figure 4** summarizes the number of bacteria attached initially and remaining after rinsing the films. The QAS films did not show significant release of bacteria, probably due to the strong electrostatic and/or hydrophobic interactions between the QAS and bacteria.[33] In contrast, more than 80% of attached *E. coli* and 90% of attached *S. epidermidis* were removed from PNIPAAm films after rinsing with cold buffer solution. It should be noted that the mechanism of "fouling-release" ability of PNIPAAm films deposited by RIR-MAPLE is likely different from that of PNIPAAm grafted surfaces prepared by SIP.[34] For PNIPAAm grafted surfaces, the ends of PNIPAAm chains are strongly anchored on the substrates by covalent bonds. Lowering the temperature results in the changes of degree of hydration and conformation of the PNIPAAm chains, leading to the change in the grafted surfaces from a bacteria-attractive state to a bacteria-repellent state.[34-35] In contrast, in the RIR-MAPLE process used here for PNIPAAm, there should be no chemical reaction and thus no covalent bonds between PNIPAAm films and underlying substrates. Therefore, although PNIPAAm films are stable and insoluble in an aqueous medium when they are in a dehydrated form at temperature above LCST (e.g. 37°C), decease of the temperature below LCST (e.g. 25°C) will promote the hydration and potential dissolution of PNIPAAm layer, such that the dissolved PNIPAAm may be easily washed away during rinsing process. This dissolution may lead to the loss of anchorage points of bacteria and bacterial detachment. The instability of PNIPAAm films prepared by RIR-MAPLE in cold aqueous solution was confirmed by ellipsometry, XPS and AFM (**ESI, Figures S4 and S5**), suggesting that these films will not likely be suitable for multiple bacterial attachment and release cycles. PNIPAAm/QAS hybrid films exhibited the bacterial release ability due to the existence of PNIPAAm. Significant release of bacteria was found on these films (65.7±5.4 for *E. coli* and for 60.7±6.8% for *S. epidermidis*, $p<0.01$), although the release ratio is a little lower than observed for PNIPAAm films. As controls, these surfaces were rinsed with the same buffer at 37°C in a similar manner (data not shown) and they showed less than 20% bacterial detachment, consistent with our previous work with PNIPAAm modified surfaces.[17]Furthermore, we found both killing efficiency and release ratio of the PNIPAAm/QAS films were independent of the film thickness (from ~10.3 nm to ~103.4 nm, **ESI**, **Figure S6**). Considering the surface wettability is also similar for these films (**ESI, Figure S7**), we assume that the topmost layer of PNIPAAm/QAS films is homogeneous and independent of thickness.

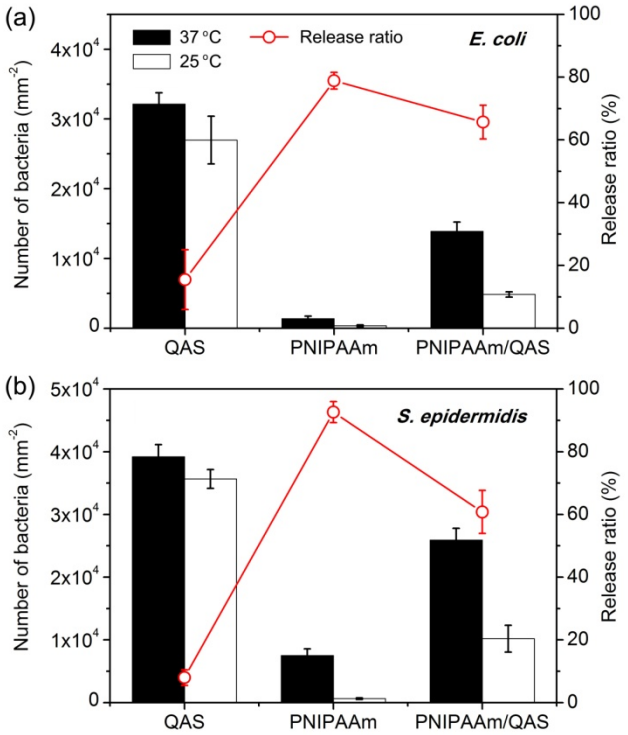


Figure 4. Attachment and detachment of (a) *E. coli* and (b) *S. epidermidis* on film surfaces. The surfaces were incubated in suspensions of *E. coli* or *S. epidermidis* at 37°C for 2 h and the average number of attached cells was determined. Then the surfaces were rinsed with 0.85% NaCl aqueous solution or PBS at 25°C and the remaining cells were counted. The bacterial release ratio is also shown. Error bars represent the standard deviation of the mean (n=3).

3.4. Recycle test

Reusability is often desirable for fouling-release surfaces. We thus examined PNIPAAm/QAS hybrid films after repeated cycles of attachment and release of *E. coli* (**Figure 5**). After two cycles

of attachment and release, the surface exhibited decreased killing efficiency from 79.7±6.1% to 50.7±6.5%, which may have resulted from accumulation of unreleased bacteria and debris. In addition, after one cycle of attachment and release, the bacterial attachment increased while the release ability was significantly depressed ($p<0.01$). We attribute these trends to the dissolution of PNIPAAm during rinsing with cold aqueous solution, leaving films comprising primarily of bacteria-attractive, biocidal QAS.

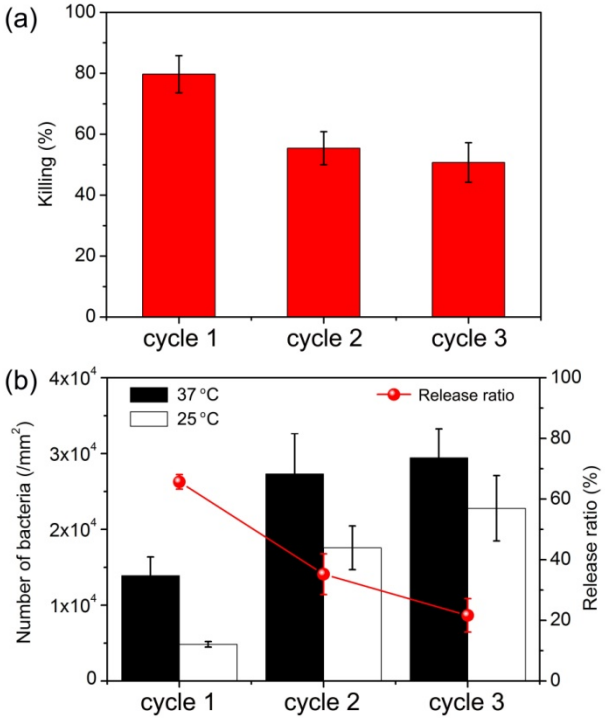


Figure 5. Comparison of (a) killing efficiency and (b) attachment and detachment of *E.coli* and corresponding bacterial release ratio of PNIPAAm/QAS hybrid films upon three cycles of repeated exposure to, and release of cells. Error bars represent the standard deviation of the mean (n=3).

To enhance the retention of PNIPAAm in the PNIPAAm/QAS hybrid films, we introduced a common cross-linker, (3-aminopropyl) triehoxysilane (APTES), which has been shown to improve the retention of polymer thin films on a substrate surface due to the formation of a complex three-dimensional (3D) network, which lock the PNIPAAm chains to allow retention of otherwise soluble PNIPAAm chains on the substrate.[36] A small amount of APTES (see Experimental Section) was incorporated into the target solution and a ternary PNIPAAm/QAS/APTES film was deposited. The ternary film was then annealed at 180 °C for 24 h .This thermal annealing process will drive APTES condensation reactions to completion to extend the network and further enhance the retention of PNIPAAm. Results of ellipsometry and water contact angle indicated that there are no significant changes in surface wettability and film thickness of this hybrid film before and after incubation in cold water, suggesting the incorporation of APTES indeed increases the stability of the hybrid film (**ESI**, **Figure S5**). We tested the reusability of this improved hybrid film using repeated cycles of attachment and release. After two attach-kill-release cycles, only slight attenuation in the biocidal activity and bacterial release was observed, demonstrating that the film maintained its multifunctionality (**Figure 6**). In future studies, we will conduct a thorough investigation of a range of parameters (e.g. composition ratio of PNIPAAm and QAS) and binding methods (such as introduction of covalent bonding) to optimize the killing efficiency, release efficiency and reusability of the anti-fouling, hybrid surfaces.

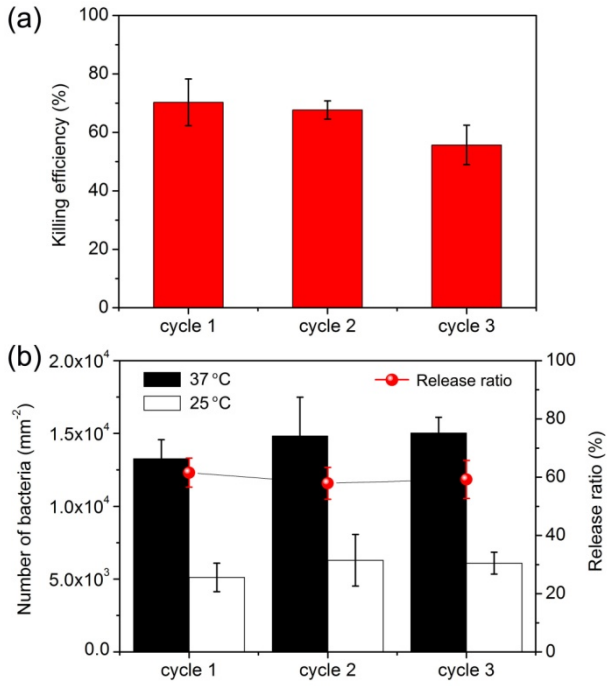


Figure 6. Comparison of (a) killing efficiency and (b) attachment and detachment of *E.coli* and corresponding bacterial release ratio of PNIPAAm/QAS/APTES ternary films upon three cycles of repeated exposure to, and release of cells. Error bars represent the standard deviation of the mean (n=3).

4 Conclusions

To realize a multi-functional film capable of both biocidal activity and release of resultant dead cells, we deposited hybrid films containing a biocidal agent (QAS) and a fouling-release polymer (PNIPAAm) by RIR-MAPLE. The resultant films were well characterized using XPS, FTIR, AFM, ellipsometry, and water contact angle measurement. The functionality of the films was tested against the Gram-negative bacterium, *E. coli*, and the Gram-positive bacterium, *S. epidermidis*. At 37°C (above the LCST of PNIPAAm), the film accumulated and killed large number of bacteria, which were released upon subsequent exposure to water below the LCST. To enhance the retention of PNIPAAm, we incorporated APTES to get ternary hybrid films, which maintained both biocidal activity and bacterial-release ability after several attach-kill-release cycles. Considering the simplicity of RIR-MAPLE and its applicability to form films on a wide range of substrata, this study demonstrates a new strategy to design multifunctional surfaces that can kill and release bacteria in a controllable manner, potentially enabling the design of new biomaterials for control of biofouling in a variety of contexts. The general strategy demonstrated here can also be extended to prepare other multi-functional films using two or more functional

polymers.

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Notes and references

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† Electronic Supplementary Information (ESI) available: surface morphology of PNIPAAm films prepared by different methods; confirmation of chemical integrity of films deposited by RIR-MAPLE; effect of incubation temperature on bacterial attachment and killing efficacy; stability of PNIPAAm containing films in aqueous solution; effect of film thickness on surface properties; and enhancement of stability by introduction of APTES. See DOI: 10.1039/b000000x/

‡ These authors contributed equally to this work.

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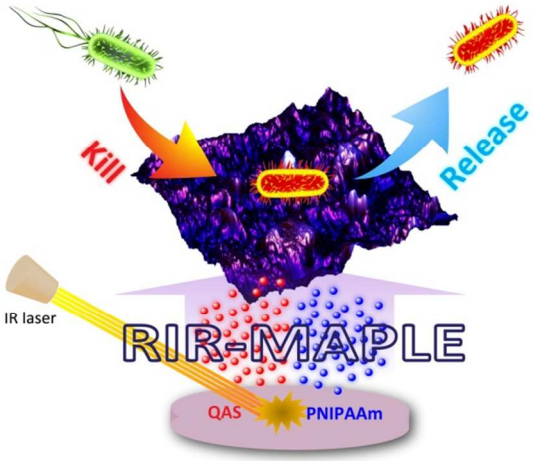

Multifunctional films combining switchable biocidal and fouling release properties were deposited on substrates using RIR-MAPLE.