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A Multilevel Antimicrobial Coating Based on Polymer-Encapsulated ClO_2

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A multilevel antimicrobial coating with "release-killing", "contact-killing" and "anti-adhesion" properties was prepared from polymer-encapsulated chlorine dioxide (ClO_2), water-in-oil-in-water (w/o/w) double emulsion. A slow sustained release of gaseous ClO_2 at a rate sufficient to inhibit bacterial growth (~1300 μg of $ClO_2 \cdot g^{-1} \cdot day^{-1}$) was demonstrated for a prolonged period of time (i.e., 28 days). Touch and infectious droplets triggered an increased release of the biocides at the sites of contamination, resulting in rapid disinfection. Zinc chloride (i.e., 30 ppm) was added to provide "contact-killing" properties, while bacterial adhesion was prevented by the Pluronic polymer used to encapsulate ClO_2. The new antimicrobial coating is effective against Gram positive and Gram negative bacteria, including *Bacillus subtilis*, *Staphylococcus aureus*, and *Escherichia coli*. A greater than 5 log (i.e., ≥ 99.999%) reduction of viable bacteria was obtained at a short contact time of 10 min.

1. Introduction

The World Health Organization (WHO) reported that one of the most common routes for transmission of infectious diseases is by indirect contact with surfaces contaminated with infectious droplets produced by the patient's coughing, sneezing, and talking.[1,2] The study carried out by Bellamy et al.[3] found amylase from saliva in close to 29% of the exposed surfaces in domestic households, and a subsequent study by the same group[4] showed that up to 15% of the toilet samples and 12% of the living room samples from domestic homes were contaminated with fecal material. Reynolds and co-workers [5] extended the study to public places. They detected the presence of hemoglobin (blood marker) in 3%, amylase (saliva marker) in 15%, urea (urine marker) in 6%, and protein (general hygiene marker) in 26% of the 1061 samples taken from the daycare centers, shopping malls, offices, airports, movie theaters, restaurants, and gymnasia. They also found that an alarming 30% of the surfaces that tested positive for biochemical markers were also contaminated with fecal coliforms.

Many microbes, including viruses, can survive for days on surfaces.[2] Influenza virus can remain viable for 24−48 h, while parainfluenza and severe acute respiratory syndrome (SARS) viruses are known to survive for hours and days on most surfaces.[6−8] Hand contact with contaminated surfaces (i.e., fomites) and subsequent transfer of microbes to the mucosal membranes of the mouth, nose, and eyes is the cause of many reported gastroenteritis outbreaks (i.e., norovirus) and rhinovirus infections. Other pathogens known to transmit through fomites include cold virus, rotavirus, *Pseudomonas aeruginosa* and methicillin-resistant *Staphylococcus aureus* (MRSA).[2] Regular cleaning and disinfection are therefore important for breaking the chain of infection, and the use of antimicrobial surface coating provides an additional safeguard against disease transmission. Antimicrobial surfaces and coatings based on nanosilvers,[9−11] photocatalytic TiO_2,[12−16] and surface-tethered bactericides (e.g., quarternary ammonium compounds (QACs), phosphonium salts)[17−22] exhibit "contact-killing" properties and are proven effective against many microorganisms. The nano-TiO_2 also self-cleans and is active against other airborne pollutants.[23−30] Ultrahydrophobic

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coating and bacteria-repelling poly(ethylene glycol) protect surfaces from microbes by preventing their adhesion.[31] Another strategy is to store antibiotics,[32] biocides (e.g., phenols, halogens),[33] and metals (e.g., silver ions)[34] in bulk materials and coatings for a slow gradual release to provide a sustained "release-killing".

New antimicrobial coatings using more than one approach were reported to perform better. Cohen's and Tiller's groups [35,36] employed bactericidal silver to create effective antibacterial coatings. Cohen's group[35] employed a layer-by-layer, self-assembly method to produce a two-level antibacterial coating with both "release-killing" and "contact-killing" capabilities based on stored silver salt and surface-grafted quarternary ammonium, while Ho et al.[36] used a polymer film to immobilize nanosilver to achieve both "contact-killing" and "release-killing" effects while a grafted layer of poly(ethylene glycol) repels the adhesion of bacteria. This work investigates the performance of polymer-encapsulated chlorine dioxide (ClO_2) with zinc chloride ($ZnCl_2$) as a general surface, antimicrobial coating with "release-killing", "contact-killing", and "anti-adhesion" properties with the aim of providing a long-term surface disinfectant that has the ability to control the release of the ClO_2 in response to surface contamination by touch and infectious droplets.

ClO_2 is approved for treatments of drinking water, poultry and beef products, fruits, and vegetables.[37] Today, there is increasing use of chlorine dioxide as an active ingredient for the topical treatments of human immunodeficiency virus (HIV) lesions, eczema and acne, and in personal care products including toothpaste and deodorant.[38,39] Patent literature[40,41] has also described the generation of ClO_2 gas from sodium chlorite salts stored within a polymer matrix for gradual biocide release for disinfection. ClO_2 is a very reactive but selective biocide that attacks the electron-rich centers in organic molecules[42,43] and disinfects by oxidation of the cell membrane[44] and by denaturation and inhibition of protein synthesis.[45] It damages the inner membrane of spores, preventing proper germination,[46] and reacts with the viral envelope, causing severe damage and inactivation.[47] ClO_2 is fast acting and effective against a broad spectrum of bacteria, spores, viruses, and protozoae over a wide pH range (pH 2−8)[46−50] including the bioterror weapon *Bacillus anthracis* (anthrax) cells and spores,[46,51] the highly infectious SARS-associated coronavirus (SARS-CoV),[52] and the emerging pandemic influenza A virus (H1N1).[47]

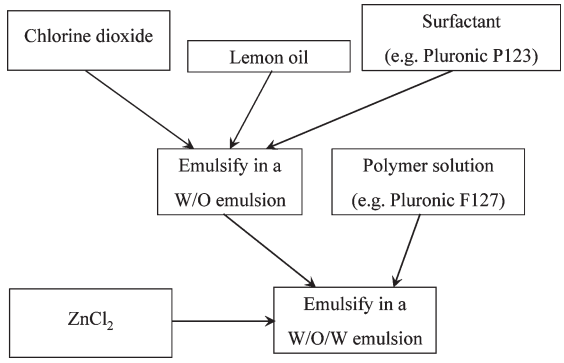

Figure 1. Preparation scheme for polymer-encapsulated ClO_2 w/o/w emulsion.

This study reports the preparation of a multilevel antimicrobial coating based on "release-killing" polymer-encapsulated ClO_2 containing "contact-killing" zinc and "anti-adhesion" polymers. The bactericidal activity of the coating was tested against *Bacillus subtilis*, *S. aureus*, and *Escherichia coli* bacteria. The observed correlation between the elevated malondialdehyde (MDA) level and cell death, and the antiadhesive property of the antimicrobial coating are also discussed.

2. Experimental Section

2.1. Preparation of Multilevel Antimicrobial Formulation. The stabilized ClO_2 aqueous solution was purchased from United Laboratories, Ltd. and encapsulated in active triblock copolymers of polyoxyethylene−polyoxypropylene (i.e., Pluronic P123 and F127) by a water-in-oil-in-water (w/o/w) emulsion method according to the procedure shown in Figure 1 [53]. The Pluronic P123 ($EO_{20}PO_{70}EO_{20}$, MW 5750 g/mol) and Pluronic F127 ($EO_{106}PO_{70}EO_{106}$, MW 12 600 g/mol) were supplied free of charge by BASF Corp. The lemon oil purchased from Dreamworld was used as the oil phase for the w/o emulsion and to provide an olfactory cue to an otherwise colorless and odorless formulation. The lemon oil consisted of 10% (v/v) essential oil from natural extract dissolved in paraffin solvent and had a low evaporation rate of 0.1 based on BuAc = 1 (Note: H_2O = 0.3). Twenty-five milliliters of 5% (v/v) ClO_2 was suspended in the lemon oil and 25 mL of 5% (w/v) Pluronic P123 surfactant solution was added with stirring (i.e., 400 rpm). The resulting emulsion was then added to an aqueous suspension of Pluronic F127 (2.5 g dissolved in 50 mL deionized water) at a stirring rate of 200 rpm to give a 1:1:2 w/o/w double emulsions. A 0.25 mL portion of 50 mM $ZnCl_2$ (99%, Aldrich) was added, and the final emulsion was stored at 4 °C before use. This formulation gave a storage capacity of 18% (w/w) or 180 mg of ClO_2 per gram of solid as determined by the iodometric titration using 0.1 M sodium thiosulfate ($Na_2S_2O_3$, RDH) and starch indicator. The encapsulated ClO_2 was examined by an optical microscope (Olympus BH2-MJLT) and an ultraviolet−visible (UV−vis) spectrophotometer (Ultrospec 4300 pro). The spectra were taken between 200 to 1100 nm at a resolution of 0.5 nm to test for the presence of other oxychlorine species such as chlorite and hydrochlorite.

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2.2. Characterization of Multilevel Antimicrobial Coating. The double emulsion was deposited on substrates to produce a surface coating. The deposited double emulsion was examined by an optical microscope and JEOL 6300 scanning electron microscope (SEM) at an acceleration voltage of 10−15 kV. The composition of the sample was analyzed by X-ray photoelectron spectroscopy (XPS, Physical Electronics PHI 5600) equipped with a monochromatic Al X-ray source, and a chemical map of the surface was obtained by time-of-flight secondary ion mass spectroscopy (ToF-SIMS, Physical Electronics PHI 7200). The ClO_2 content of the surface coating was measured at different times and temperatures to monitor the release of ClO_2. The coated sample was sonicated in 20 mL of deionized distilled water to dissolve the coating. An excess amount of potassium iodide (KI, BDH) was added, and iodometric titration was carried out in an acidic medium. The free iodine (I_2) was titrated by 0.1 M sodium thiosulfate ($Na_2S_2O_3$, RDH) with starch indicator.

2.3. Bactericidal Property of the Multilevel Antimicrobial Coating. A set of standard operating procedures (SOPs) from the Association of Official Analytical Chemists (AOAC International) and approved by the U.S. Environmental Protection Agency (EPA)[54] was adopted for this study with some modifications. These include AOAC standards for carrier screening (MB-03-03), bacteria enumeration (MB-04-02), and antibacterial efficacy test (MB-06-02).[55−57]

Substrate Screening (*55*). Glass was chosen as the carrier substrate for the study, and a rigorous screening was carried out as the different components of the manufactured glass (e.g., ZnO and TiO_2) may possess intrinsic bactericidal properties that could interfere with the measurements. Glass manufactured by Sail Brand, China (Table 1) was selected for its inertness and availability. The glasses were cut into 2.5×2.5 cm^2 pieces and prepared according to AOAC guidelines. The defective glasses were eliminated, and the remaining substrates were rinsed once with distilled water and three times with ethanol (99.9%, Merck) followed by an excess amount of distilled water, before being sterilized in an autoclave (Hirayama, HA-300P) at 121 °C for 20 min.

Bacteria Cell Preparation and Enumeration (*56*). The *B. subtilis* (15-4921A) and *E. coli K12* (15-5065A) were purchased from Carolina Biological Supply Co., while the *S. aureus* was provided free by the Department of Biology, HKUST. The stock bacteria were kept on a tryptone soya agar (TSA) plate (Oxoid) and stored at 4 °C. The bacteria cells were activated by subculturing a loopful of inoculum in 10 mL of Oxoid nutrient broth in a culture tube and gently shaken in an incubator (Gallenkamp) at 37 ± 0.1 °C and 80 rpm for 18 h. The viable cell concentration was determined by plate counting technique on TSA plates after a serial dilution. The nutrient broth and TSA plates were sterilized by an autoclave at 121 °C for 15 min.

Bactericidal Activity Tests. The bactericidal property of the chlorine dioxide gas released from the coated glass plate was investigated for *S. aureus* cells. Sterile TSA plates were evenly inoculated with a loopful (ca. 100 μL) of *S. aureus* inoculum from the broth culture (ca. $10^6 \cdot cm^{-3}$). Glasses coated with 1 $mg \cdot cm^{-2}$ of encapsulated ClO_2 were placed at fixed distances of 0.6, 3, and 10 mm from the surface of the TSA plate using sterilized U-shaped paper frames of fixed thicknesses. The TSA plate was incubated upside down overnight at 37 ± 0.1 °C, and the agar beneath the coated glasses was examined for bacterial growth.

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Table 1. Chemical Composition of the Glass Substrates

	composition (atom %)	
compounds	glass 1 (Sail Brand)	glass 2 (Corning)
SiO_2	74.0	70.0
Al_2O_3	1.5	4.3
Na_2O	12.4	6.6
K_2O	0.2	7.7
CaO	8.0	nil
MgO	4.0	nil
ZnO	nil	6.4
TiO_2	nil	4.7

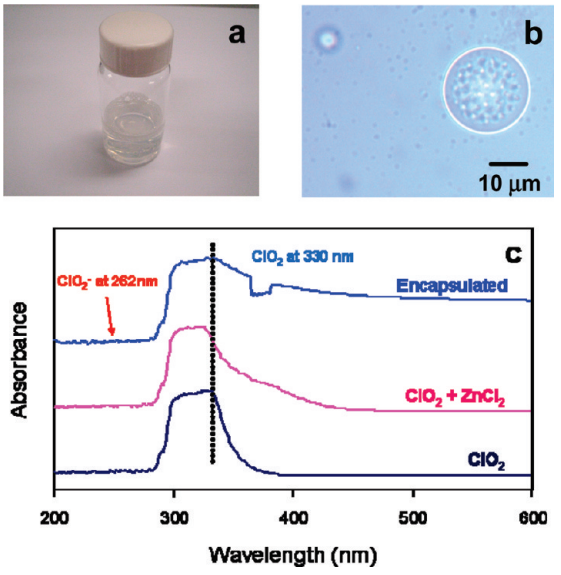

Figure 2. (a) Polymer-encapsulated ClO_2 w/o/w emulsion after a month in storage and the (b) optical microscope picture and (c) UV−vis spectrum of the prepared double emulsion.

Table 2. Surface Elemental Composition of the Coated and Uncoated Glass and Stainless Steel

	glass				stainless steel		
elements	uncoated	coated	wiped[a]	elements	uncoated	coated	wiped[a]
Si(2p)	20.7	0.5	4.8	Fe(2p)	24.6	1.2	0.6
Al(2p)	0.6	0.0	0.0	Cr(2p)	6.6	0.7	0.7
Mg(1s)	5.1	0.0	0.0	Ni(LMM)	1.9	0.0	0.0
O(1s)	54.0	29.0	39.6	O(1s)	47.2	34.6	34.6
Na(1s)	6,9	0.5	5.9	Na(1s)	0.0	10.5	9.2
C(1s)	12.7	69.5	48.6	C(1s)	19.4	49.2	54.3

[a] Wiped 30 times in repeating motion with a cotton cloth with a 20 N force normal to the surface.

Surface contamination often originates from infectious droplets, and a bactericidal test was carried out to simulate this situation. One hundred microliters of $10^6 \cdot cm^{-3}$ bacteria cell suspension was placed in contact with both coated and uncoated glass substrates at ambient conditions (23 ± 2 °C, 70% relative humidity (RH)) in a sterilized biological safety cabinet (NuAire, Nu-425-400E). Three sets of runs of five samples each were carried out at the fixed contact times of 1, 5, 10, 30, and 60 min. The samples were immersed in a primary subculture tube containing 20 mL neutralizer for 30 min to stabilize the surviving bacteria cells. The sample was drip-dried and transferred to a second subculture tube containing 20 mL of sterile nutrient broth (Nutrient broth no. 2, Oxoid) for 10 min. One hundred microliter aliquots from the neutralizer and nutrient broth were separately cultured on the TSA plates. Additional cultures were made for *E. coli* and *S. aureus* on the MacConkey and Mannitol salt agar

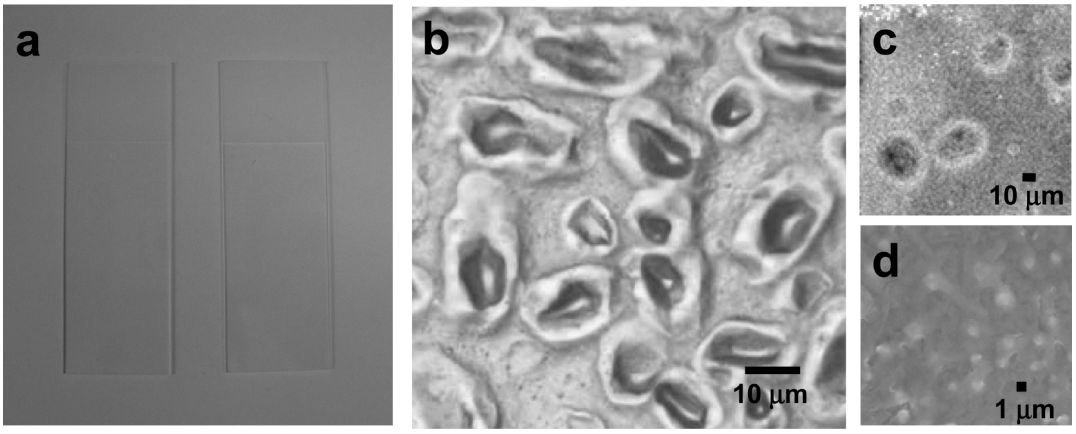

Figure 3. (a) Images of glass slide with (left) and without (right) a coating of the polymer-encapsulated ClO_2, (b) optical and (c,d) SEM pictures of the coated glass, showing a uniform surface coating (b) consisting of globules (c) containing 0.5−1 μm-sized emulsion clusters (d).

plates, respectively. The numbers of viable bacteria were counted after incubating the plates for 24 h at 37 ± 0.1 °C. The sterile neutralizer solution was freshly prepared by adding 1% (v/v) 0.1 M $Na_2S_2O_3$ to 600 mL of 0.85% (w/v) normal saline (NaCl, RDH) solution containing 0.1% (v/v) (final concentration) of polyoxyethylenesorbitan monooleate (Tween 80) followed by placement in an autoclave at 121 °C for 20 min.

Measurement of Cell Membrane Damage. The level of MDA produced by the peroxidation of membrane lipid is considered to be indicative of the oxidative stress and cell membrane damage. The MDA was measured by thiobarbituric acid assay.[58] One hundred microliters of $10^7 \cdot cm^{-3}$ *B. subtilis* cell suspension was deposited on the coated glass substrate and allowed 10 min contact. The bacteria were recovered and mixed with 5% trichloroacetic acid (99.0%, Sigma-Aldrich) in an eppendorf tube, before adding 0.6% 2-thiobarbituric acid (98%, Sigma). The solution was heated to 95 °C for 15 min, cooled to room temperature, and centrifuged (Eppendorf 5415C) at 10000 rpm for 10 min. The optical density of the supernatant was recorded between 534 and 600 nm by a spectrophotometer (ICN Biomedicals, 156812), and the MDA concentration was calculated against calibration standards.

2.4. Antiadhesion Property of the Multilevel Antimicrobial Coating. The adhesion of *E. coli K12* (Carolina 15-5065A) on clean glass and glass coated with placebo encapsulants was examined. The placebo emulsion was prepared using the same procedure described in Figure 1, but replacing the biocides with distilled water to eliminate possible bactericidal effect. Two hundred microliters of $10^8 \cdot cm^{-3}$ *E. coli* suspensions were uniformly spread on the coated and uncoated glass surface and incubated at 37 °C for 4 h without shaking. The samples were washed gently with sterile distilled water to remove nonadherent bacteria.[59] Gram staining[60] was performed on the samples, and images observed under the optical microscope (magnification of 100×) were recorded to quantify the degree of bacteria adhesion on the coated and uncoated surfaces.

3. Results and Discussion

3.1. Multilevel Antimicrobial Formulation. The ClO_2 biocide was encapsulated in a w/o/w double emulsion following the basic procedure outlined by Ficheux et al.[61] The ClO_2 solution was first encapsulated by P123 with a hydrophilic−lipophilic balance (HLB) of 8 in lemon oil to create a stable w/o emulsion. The final w/o/w double emulsion was prepared by using F127 with an HLB of 22. The material selection and formulation are important and must take into account the reactivity of ClO_2. The traditional surfactants commonly used in emulsion were readily oxidized by ClO_2, resulting in instability and phase separation (Figure S1a, Supporting Information), and ClO_2 can denature oils and fragrances resulting in discoloration and altered scent (Figure S1b). The lemon oil resisted ClO_2 oxidation and was used as the oil phase in the w/o/w double emulsion. The Pluronic family of polyoxyethylene−polyoxypropylene triblock copolymers is safe and widely studied for use in medicine and cosmetics.[62] These polymers are also known to react to different environmental cues such as pH, temperature, and moisture, and are responsible for several new drug delivery systems. Zinc was added as a metabolic poison[63] and to provide a "contact-killing" property. Although silver and copper ions are better bactericides, silver reacts to form insoluble salts in the presence of halides (e.g., ClO_2) while copper has a strong color.

The double emulsion prepared by this procedure is clear and transparent, as shown in Figure 2a, and is stable for at least 12 months in 4 °C storage. Tiny emulsion capsules measuring 10−20 μm in diameter were observed under the optical microscope (Figure 2b), and it is possible to see the smaller micrometer-sized w/o emulsion within the capsules. The ClO_2 content can be adjusted from 0 (i.e., placebo) to 750 mg of ClO_2 per gram coating, but samples containing higher than 250 mg of ClO_2 per gram coating left behind a white residue from salts contained in the commercial formulation. A 180 mg of ClO_2 per gram coating formulation was prepared for this work. The formulation contained a trace concentration of zinc (i.e., 30 ppm) added as $ZnCl_2$. The UV−vis spectroscopy of the ClO_2 solution in Figure 2c shows a broad peak at 330 nm belonging to ClO_2. No signals were detected from the oxychlorine species (i.e., ClO_2^- at 260 nm and ClO^- at 290 nm). The spectra in Figure 2c show that the addition of $ZnCl_2$ and its encapsulation in a w/o/w double emulsion did not appear to affect the ClO_2.

3.2. Multilevel Antimicrobial Coating. The prepared formulation has a low viscosity and is easily coated on surfaces. The Pluronic polymers act as a surfactant and the double emulsion

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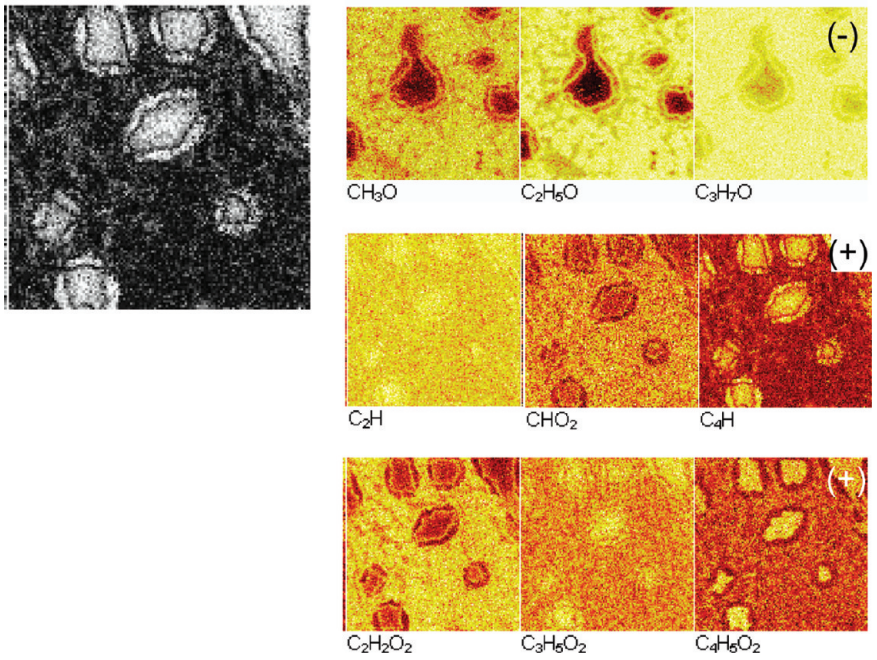

Figure 4. A total ion image ($200 \times 200\ \mu m^2$) of the glass coated with polymer-encapsulated ClO_2 (left) and the ToF-SIMS ($200 \times 200\ \mu m^2$) maps of the surface reconstructed from negative and positively charged molecular fragments (right).

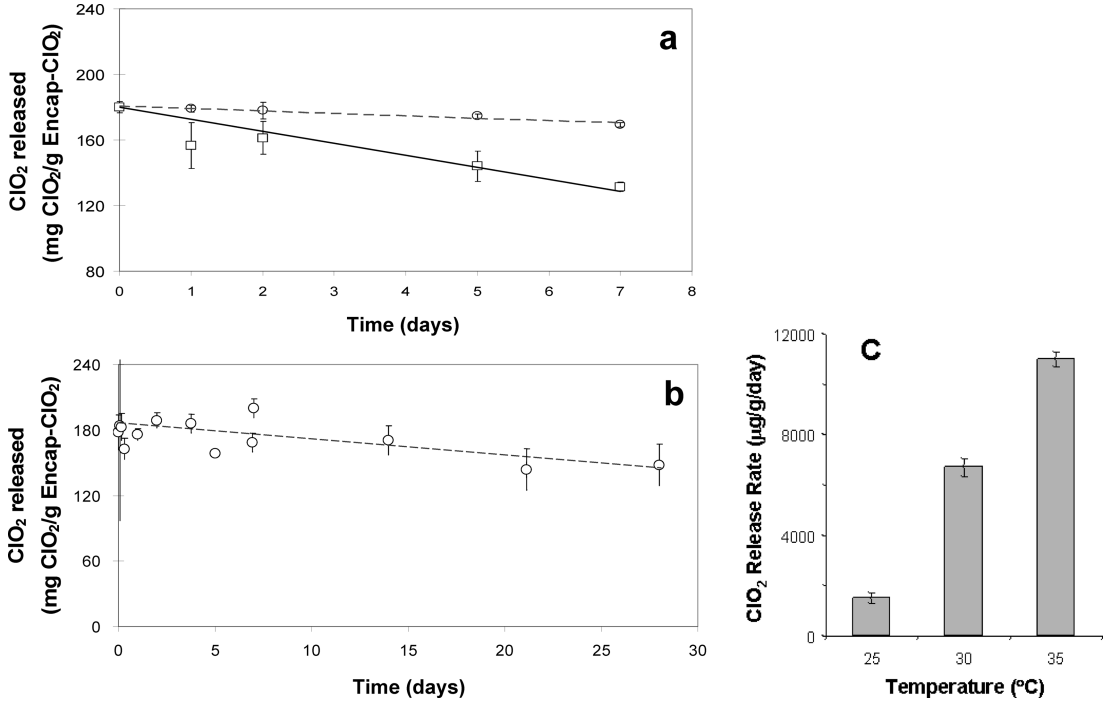

Figure 5. (a) The amount of ClO_2 remaining in the coating during the 7 day release experiment at 25° (○) and 35 °C (□) in a constant temperature oven with a relative air humidity of 60−80%. (b) 28 day release experiment at ambient temperature (20−26 °C) and conditions (RH = 60−90%). (c) Average release rate of ClO_2 gas from the coated glasses obtained over a period of 7 days. Please note that data points were obtained from triplicate runs of five samples each, and the lines in panels a and b were drawn to guide the eyes.

readily wets glass, metal, ceramic, and wood surfaces, depositing a thin uniform layer that resists normal wear (Table 2). The samples were wiped 30 times with a clean cotton cloth at 20 N force normal to the surface to simulate wear. The double emulsion coating was durable and remained intact even after the vigorous wiping (Table 2), but could be washed off with detergent water. The deposited coating forms a transparent and tactilely smooth coating, as shown in Figure 3a for glass. The scent from the lemon oil served as an olfactory cue as the coating is not immediately apparent to the eyes. Syneresis, which is a common problem for gels and hydrogels, was not observed.

The coated glass was examined under an optical microscope, and the coating displays uniform features that are reminiscent of the deposited microcapsules (Figure 3b). A closer examination at a higher magnification revealed the globular shapes of the deposited microcapsules (Figure 3c) and the smaller 0.5−1 μm capsules they contained (Figure 3d). The discrepancy in size between the optical microscope and SEM images could be due

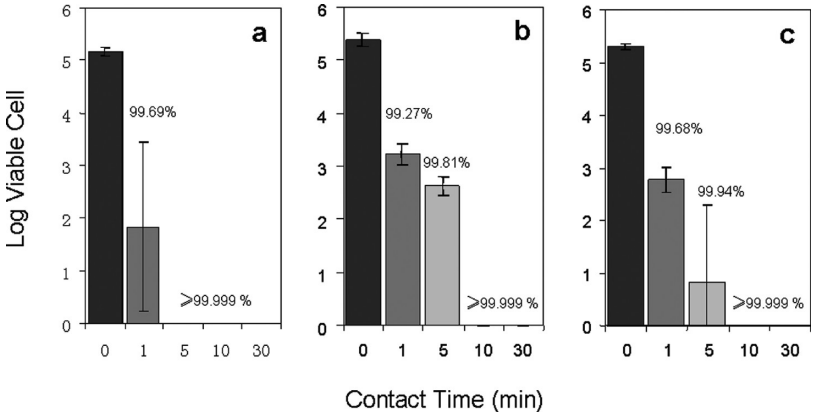

Figure 6. Surviving (a) *B. subtilis*, (b) *S. aureus*, and (b) *E. coli* bacteria cells after 1, 5, 10, and 30 min contact with a glass coated with 1 mg/cm^2 multilevel antimicrobial coating. Please note the error bars represent the standard deviation from five samples.

to desiccation at high vacuum conditions of the latter sample. A ToF-SIMS analysis of the sample was done, and the surface chemical maps are displayed in Figure 4. The maps were generated from the molecular fragments produced by the interactions between the ion beam and the sample. The figure shows that the interior and interface of the capsules are chemically different, as was expected for a double emulsion with a P123 w/o emulsion encapsulated within F127.

The release of gaseous ClO_2 from encapsulated ClO_2 coating on glasses was monitored for 7 days at room and body temperatures. The coated glasses were placed in constant temperature ovens at 60−80% RH, and the ClO_2 content of the coating was measured at fixed time intervals by titration. Figure 5a shows that approximately 1500 μg of gaseous ClO_2 was released per gram of coating material each day at 298 K. The addition of 30 ppm $ZnCl_2$ did not affect the release of the encapsulated ClO_2, and a comparable ClO_2 release rate of ca. 1600 $\mu m \cdot g^{-1} \cdot day^{-1}$ was obtained over a 7 day period. Under ambient temperature and conditions, the biocide is transported from the internal phase to the exterior via diffusion. Studies showed that molecules can migrate through the oil phase without affecting the double emulsion stability [64].

The use of polymer (i.e., P123) instead of the usual hydrophobic surfactant eliminated the faster transport route via inverted micelles in the oil phase [64]. This enabled a slow, sustained release of ClO_2. Figure 5b shows that approximately 20% of the stored ClO_2 were released during a twenty-eight days study at a rate of ~1300 μg $ClO_2.g^{-1}day^{-1}$. The concentration of ClO_2 gas on the coating surface was about 80 ppm_v, which rapidly dissipated to a low, undetectable value away from the surface. The elevated ClO_2 concentration at the surface ensured high effectiveness against most microorganisms, yet the overall chlorine dioxide concentration in the air are maintained low compared to the permissible exposure level of 0.1 ppm_v set by the US OSHA for long-term exposure in the workplace [65].

Figure 5a shows the chlorine dioxide was released at 11 mg. $g^{-1}day^{-1}$ at near body temperature of 308 K or eight times higher than at room temperature, therefore touch could trigger a faster release of the biocide (Figure 5c & Figure S2). Close to the body temperature, the P123 film between the internal water phase and the oil interface become unstable and merges with the F127 film encapsulating the w/o emulsion droplets resulting in "coalescence" [61]

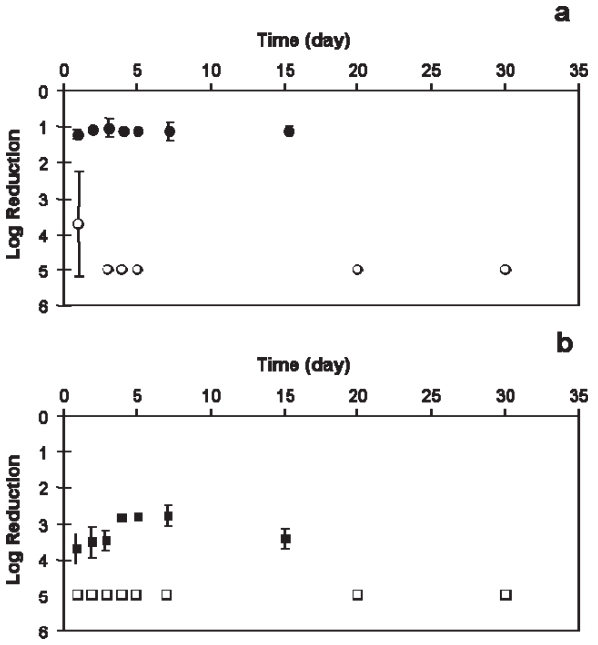

Figure 7. Number of (a) *S. aureus* and (b) *E. coli* bacteria cells killed on contact with glass coated with 1 mg/cm^2 multilevel antimicrobial coating (open symbols) and polymer-encapsulated ClO_2 without 30 ppm $ZnCl_2$ (filled symbols) at different days after coating (30 min contact time, neutral pH). Please note the each datum was obtained from at least five samples, and some of the data points were repeated more than once.

and a rapid release of the ClO_2. Sputum and infectious droplets rehydrate the coating and destabilize the outer F127 polymer membrane triggering a rapid release of ClO_2. The ClO_2 release is sustained by a mismatch between the osmotic pressures of the internal and external water phases. Thus, unlike other "release-killing" antimicrobial systems using ClO_2 [40,41], the new coating can actively self-disinfects the areas contaminated by touch and infectious droplets.

3.3. Bactericidal Properties of Multilevel Antimicrobial Coating. The multilevel antimicrobial coating was tested for two Gram positive (i.e., *B. subtilis* and *S. aureus*) and one Gram negative (*E. coli*) bacteria. Figure 6 plots the viable bacteria cell after 1, 5, 10, and 30 min of contact with glass surfaces coated with 1 mg$\cdot$cm^{-2} multilevel antimicrobial coating. The results show that the coating has excellent bactericidal properties, and a 5 log reduction in viable bacteria (i.e., 99.999% kill) was obtained at a

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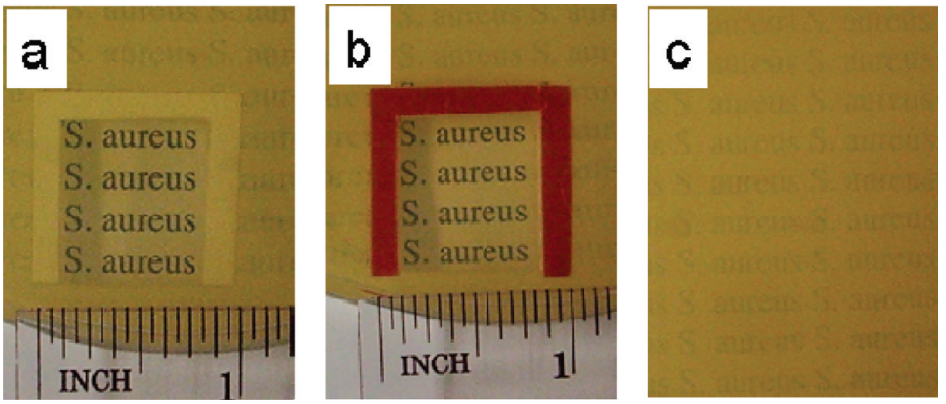

Figure 8. Photographs of *S. aureus* growth on TSA plate with glasses coated with polymer-encapsulated ClO_2 (i.e., 1 mg/cm^2) placed at (a) 0.6, (b) 3, and (c) 10 mm from the surface of the TSA plate. Please note the transparent area is free of bacterial growth.

contact time of 10 min or less. A better than 5 log reduction in viable *B. subtilis* was obtained after 5 min of contact with the coated glass surface, as shown in Figure 6a. Even at a short contact time of 1 min, close to 3 log reduction in viable bacteria was observed, but the measurement error tends to be large, as time is needed to neutralize the biocides. *Staphylococci*, the other Gram positive bacteria tested in this study, appeared to be less susceptible than *B. subtilis* (cf. Figure 6b). This could be explained by the presence of carotenoid pigments in *S. aureus* that are antioxidative in nature, providing the bacteria with some degree of protection from oxidizing biocides such as ClO_2. [66,67] Nevertheless, a 5 log reduction in viable *S. aureus* was obtained for longer than 10 min contact with the coated glasses. Figure 6c shows that the multilevel antimicrobial coating was also effective against the Gram negative *E. coli* bacteria, and a 10 min contact with the coated glasses is sufficient to decrease the viable *E. coli* by 5 log.

In contrast, the clean glass and glasses coated with the individual component of the formulation displayed modest bactericidal activity. The glasses coated with $ZnCl_2$, ClO_2, and polymer gave at most 0.8, 0.6, and 0.2 log reduction in viable bacteria after 30 min of contact, while the clean glass exhibited 0.15 ± 0.1 log reduction. Zinc itself is a modest bactericide, but is often reported to exhibit synergistic effect when used in combination with other biocides (e.g., Cu and Ag) resulting in an enhanced bactericidal activity [68]. Although the ClO_2 can rapidly disinfect surfaces, it evaporates after drying, leaving only residual oxychloride salts. This could explain the low bactericidal activity (i.e., 0.6 log reduction) of ClO_2-treated glasses after drying. Polymer encapsulation enabled ClO_2 to persist longer on surfaces by controlling its release (Figure 5).

Figure 7 compares the bactericidal property of glasses coated with multilevel antimicrobial coating and polymer-encapsulated ClO_2 for 28 days. The figure plots the log reduction in viable *S. aureus* (Figure 7a) and *E. coli* bacteria (Figure 7b) after 30 min of contact with the coated glasses. The glasses coated with the multilevel antimicrobial coating maintain a 5 log reduction in viable bacteria over 28 days. This demonstrates that the new antimicrobial coating can provide an effective and long-term surface disinfection. The glasses coated with polymer-encapsulated ClO_2 but without the 30 ppm $ZnCl_2$ are less potent and display 1.1 ± 0.1 log reduction in viable *S. aureus* (Figure 7a) and

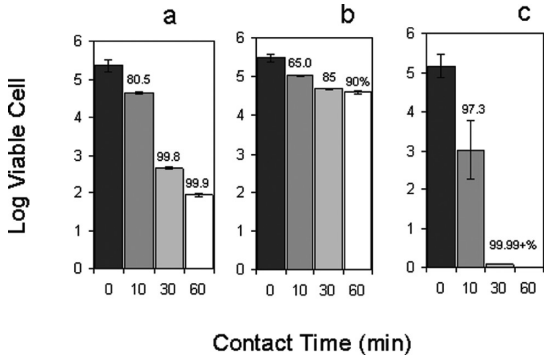

Figure 9. Surviving (a) *B. subtilis*, (b) *S. aureus*, and (c) *E. coli* bacteria cells after 10, 30, and 60 min contact with a glass coated with 1 mg/cm^2 polymer-encapsulated ClO_2. Please note that the error bars represent the standard deviation from five samples.

3.3 ± 0.8 log reduction in viable *E. coli* (Figure 7b). The results indicate a substantial enhancement by adding trace amount of the modest bactericide, $ZnCl_2$, to the formulation. It is believed that the inactivation mechanisms of the two biocides (i.e., zinc being a metabolic poison and "contact-killing" biocide, while ClO_2 is an oxidizing and "release-killing" biocide) complemented each other, resulting in a synergistic improvement. There is also a strong possibility that zinc oxide nanoparticles are present from the reactions between ClO_2 and zinc chloride; unfortunately, the zinc concentration in the formulation is too low to detect and allow a proper characterization (cf. surface XPS data in Table 2).

3.4. Bactericidal Properties of Polymer-Encapsulated ClO_2 Coating. The "release-killing" property of the polymer-encapsulated ClO_2 coating was investigated. Figure 5 shows that a sustained release of ClO_2 can be obtained from substrates coated with the polymer-encapsulated ClO_2 at ambient and near-body temperatures. The bactericidal property of the ClO_2 gas released from the coating was examined by placing the coated glasses 0.6, 3.0, and 10.0 mm from the TSA plate inoculated with *S. aureus*. The bactericidal activity observed in Figure 8 is consistent with the mass measurement data. It is apparent from Figure 8a that the 80 ppm_v ClO_2 gas concentration near the coated surface is sufficient to prevent bacterial growth on the agar placed 0.6 mm away from the coated glass. Bacterial growth was also not observed on the agar immediately above the coated glass placed at a distance of 3 mm from the surface (Figure 8b), but at 10 mm distance, the ClO_2 concentration has decreased sufficiently that no bactericidal activity was observed (Figure 8c).

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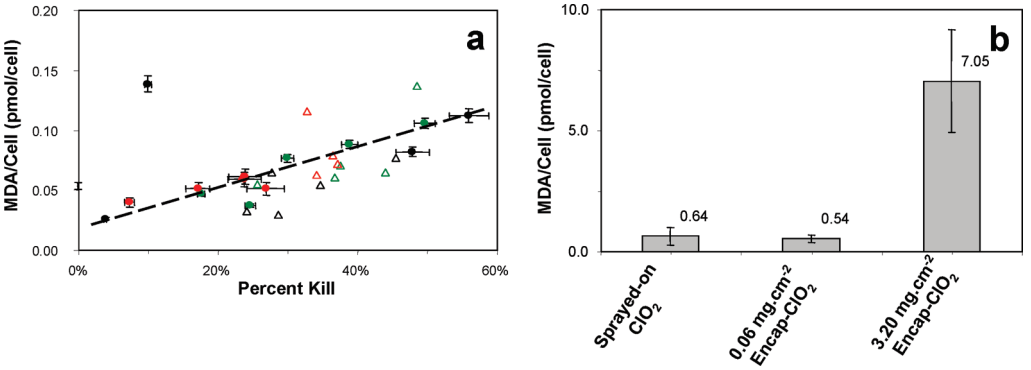

Figure 10. Plots of (a) MDA level in viable *B. subtilis* cells after contact with the polymer-encapsulated ClO_2 coating (filled symbols) and sprayed on ClO_2 (open symbols) for different lengths of time, and (b) MDA level for the different bactericidal systems that yield the same 98% kill (20 min contact with sprayed on 300 ppm ClO_2; 30 min contact with 0.06 mg·cm^{-2} polymer-encapsulated ClO_2 coating; 10 min contact with 3.20 mg·cm^{-2} polymer-encapsulated ClO_2 coating). The error bars represent the standard deviation from five samples, and please note that the lines were drawn to guide the eyes.

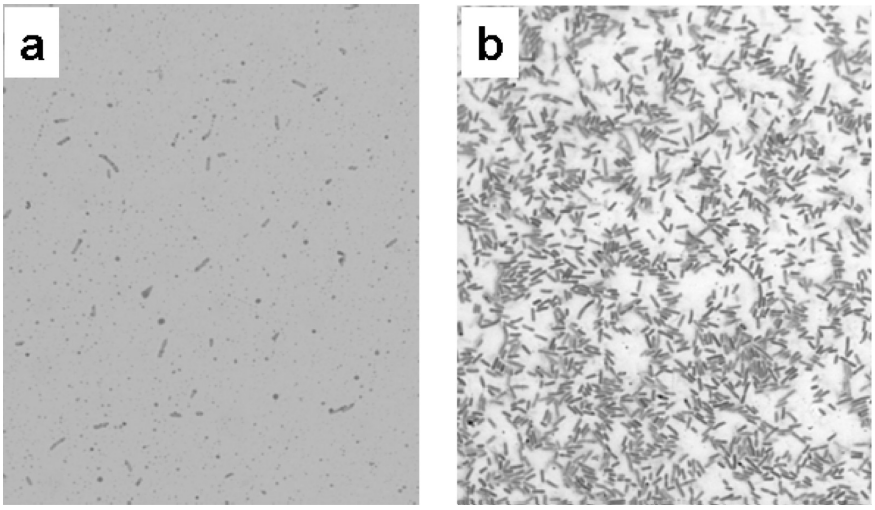

Figure 11. (a) Optical images of adherent *E. coli* cells on (a) glass coated with encapsulated sterile water (i.e., placebo) and (b) uncoated glass at a magnification of 1000×.

The bactericidal activity of the encapsulated ClO_2 coated on glass was also tested for *B. subtilis*, *S. aureus*, and *E. coli* bacteria, and the results are summarized in Figure 9. The results show a consistently lower bactericidal activity compared to the multilevel antimicrobial coating based on the combination of polymer-encapsulated ClO_2 and $ZnCl_2$. Figure 9a plots the log reduction as a function of contact time after a 100 μm droplet containing 10^6·cm^{-3} *B. subtilis* cells was brought into contact with glasses coated with 1 mg·cm^{-2} encapsulated ClO_2. A 0.7 log reduction or 80% kill was obtained for the vegetative cell of *B. subtilis* after 10 min of contact with the coated glass substrate, and a 3 log reduction or 99.9% kill was obtained for 30 min contact. The control sample (i.e., placebo) shows a much smaller 0.15 log (28.6%) decrease in viable *B. subtilis* cells for a 30 min contact time. Figure 9b shows the antimicrobial coating also performed well against *S. aureus* with 65, 85, and 90% kill at contact times of 10, 30, and 60 min, respectively. The antimicrobial coating was also effective against the Gram negative bacteria *E. coli* (Figure 7c) with better than 95% kill for *E. coli* at a short contact time of 10 min, and a longer contact time of 30 min gave better than 99% kill.

3.5. Cell Membrane Peroxidation by Coating-Released ClO_2. The ClO_2 is a very reactive biocide that attacks multiple targets in the cell including the cell membrane. The *B. subtilis* cell exposed to ClO_2 displayed an elevated level of MDA from the peroxidation of the cell membrane. Figure 10a shows there is a strong correlation between the measured MDA concentration and the bactericidal activity of ClO_2. This is true for both encapsulated and sprayed-on ClO_2. This suggests that encapsulation at the very least does not alter the ability of ClO_2 to react with the membrane lipids and disrupt the cellular transport and, possibly, the cell integrity resulting in cell death.[44,69] It is important to note that the MDA level is indicative only of the damage to the cell membrane[58,70] and does not take into account other cellular damage caused by ClO_2, which is also known for its ability to denature proteins and oxidize biomolecules.[43,45] The correlation reported in Figure 10a fails at high cell death (>80%), as shown in Figure 10b. The figure plots the MDA level for three samples of comparable cell death of 98%. The MDA level and percent kill of *B. subtilis* from the sprayed-on and encapsulated ClO_2 are comparable (Figure 10b), indicating that the release of encapsulated ClO_2 is fast and its bactericidal activity comparable to free ClO_2. The very high MDA level from 3.2 mg·cm^{-2} coating did not translate to a higher kill. It is possible that most of the

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MDA produced in this sample was from the further peroxidation of the membrane materials from nonviable cells.

3.6. Antiadhesion Property of Encapsulated ClO_2 Coating. Surface fouling and biofilm growth can diminish the effectiveness of antimicrobial coatings. Phenotypic tolerance to oxidizing biocide can arise from biofilm growth as a result of biocide consumption by the organic constituents of the biofilm,[71] and by "population-based" resistance strategy.[72] Released as a gas, ClO_2 is less sensitive to dirt and surface fouling. It is also less susceptible to biofilm, because of its rapid diffusion and greater reaction selectivity.[42] The latter prevents the indiscriminate reaction and rapid consumption of ClO_2 by the biofilm. The Pluronic polymers used in the double emulsion are reported to be antiadhesive against microorganisms such as *P. aeruginosa*, *S. aureus*, and *S. epidermidis* and are used as detergents in contact lens cleaning solutions.[73]

Water-containing emulsion capsules (i.e., placebo) were prepared using the same procedure described in Figure 1, but replacing the biocides with distilled water. The emulsion was coated on glass, and aliquots of *E. coli* were added and incubated. It can be seen from the microscope picture (Figure 11a) that the number of *E. coli* on the coated glass is significantly less compared to that on uncoated glass (Figure 11b). This indicates that the polymers used for encapsulation prevent the adhesion of the bacteria. One possible explanation is that the hydrophobic core of the triblock polymer anchors to the material surface, while the hydrophilic chain forms a sterically stabilized barrier against adhesion.[74]

4. Concluding Remarks

A multilevel antimicrobial surface coating with "release-killing", "contact-killing", and "anti-adhesion" properties was prepared from polymer-encapsulated ClO_2, w/o/w double emulsion, and zinc chloride. A slow, sustained release of ClO_2 at a rate sufficient to inhibit bacterial growth ($\sim$1300 $\mu g\ ClO_2 \cdot g^{-1} \cdot day^{-1}$) was obtained at room temperature and conditions (i.e., 298 K, RH = 60−80%). Approximately 20% of the stored ClO_2 was released during a 28 day study, and the stored ClO_2 is expected to last for at least 3 months, providing long-term protection. The detergent actions of the Pluronic polymers also prevented the adhesion of bacteria on the surface. Touch and infectious droplets triggered an increased release of biocide at the sites of contamination through an increased transport rate and by destabilization of the emulsion capsule. This "smart response" led to rapid bactericidal activity against the Gram positive and Gram negative bacteria. The choice of "multiple-targets" biocides and the strategy of "biocide-flooding" by the "smart coating" served to prevent the emergence of biocide-resistant bacteria.

Besides the excellent bactericidal properties, a number of other considerations were taken into account during the formulation of the antimicrobial coating. The selected ingredients are safe, environmentally benign, and affordable. Designed for general surface coating, the low viscosity emulsion can be applied on-site with minimal skill and equipment to produce a transparent and tactilely smooth coating that is durable and long lasting, but could be washed off with detergent water if needed. Although not a substitute for a healthy hygiene practice, the new antimicrobial coating can provide an added measure of protection against the spread of diseases in high risk situations encountered in natural and manmade disasters, and during outbreaks of diseases in either human or animal populations.

Acknowledgment. The authors gratefully acknowledge the Institute for the Environment (IENV) and the Materials Characterization and Preparation Facility (MCPF) for the use of their equipment. We also thank BASF and Orkney Environmental Technology for supplying the Pluronic polymers and stabilized ClO_2 solution free of charge.

Supporting Information Available: Photos illustrating the destabilization and discoloration of poorly prepared double emulsions; infrared images of a handprint taken after 5 min of touching a glass plate coated with encapsulated ClO_2 and 5 min after lifting the hand from the glass; and a graph depicting the average ClO_2 release rate at various temperatures. This material is available free of charge via the Internet at http://pubs.acs.org.

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