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ORIGINAL ARTICLE

Bactericidal and sporicidal performance of a polymer-encapsulated chlorine dioxide-coated surface

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Abstract

Aims: To investigate the physical characteristics and the bactericidal and sporicidal potential of a polymer-encapsulated ClO_2 coating.
Methods and Results: An antimicrobial coating based on polymer-encapsulated ClO_2 was developed. A low viscosity, water/oil/water double emulsion coating was formulated for easy on-site application. *Escherichia coli*, *Pseudomonas aeruginosa*, *Bacillus subtilis* and *Staphylococcus aureus* were applied onto the coating to study the bactericidal capabilities of the coating. The bactericidal performance of the coating increased when the contact time with the tested bacteria increased. Over 99% of the *E. coli*, *Ps. aeruginosa*, *B. subtilis* were killed with a contact time of 30 min. Although endospores of *B. subtilis* are more resistant, about 75% of the spores were killed after 72 h on the coating. Moreover, a sustained release of gaseous ClO_2 was achieved to maintain about 90% removal of *B. subtilis* with a 10-min contact time during a 28-day study period. The coating also exhibits antiadhesive properties against bacteria.
Conclusions: A polymer-encapsulated ClO_2 coating with sustained release of ClO_2 and promising bactericidal and sporicidal features was tested for 28 days.
Significance and Impact of the Study: This study provides a new direction for developing polymer-encapsulated ClO_2 coatings that possess persistent bactericidal and sporicidal properties.

Introduction

Micro-organisms are ubiquitous in our environment. Although many are harmless and even beneficial, some are well-known pathogens while others can elicit allergic responses in humans. Casual contact with surfaces and objects contaminated with infectious droplets (i.e. fomites) is an established route for transmission of many diseases (Pruss *et al.* 1999). Maintaining clean and pathogen-free surfaces is necessary in controlling the spread of infectious diseases. Metal surfaces containing silver, copper and brass possess intrinsic germicidal properties that can kill many pathogenic micro-organisms upon contact. They are commonly known as 'contact-killing' surfaces (Fang 1997; Block 2001). Similarly, chromium and nickel in stainless steel and zinc in galvanized iron are known to inhibit microbial growth (Fang 1997). Nanosilvers (Fu *et al.* 2006; Yu *et al.* 2007), photocatalytic TiO_2 (Sunada *et al.* 2003; Liu *et al.* 2007) and surface-tethered bactericides (e.g. quarternary ammonium compounds, phosphonium salts) (Isquith *et al.* 1972; Cen *et al.* 2003; Popa *et al.* 2003) are among the new generation of contact-killing, antimicrobial surface coatings developed in recent years. Nonmetals such as ceramics and enamels whose surfaces are physically nonadhesive and resist biofilm formation are also extensively used in sanitary settings. Microbes adhere poorly to ultra-hydrophobic surfaces and bacteria-repelling poly(ethylene glycol) (Desai *et al.* 1992). However, the antimicrobial properties of these materials diminish rapidly when the surface is fouled by dirt and contaminants, thus requiring frequent cleaning to maintain their effectiveness.

It is possible to store biocides (e.g. phenols, halogens) (Chung *et al.* 2003) and metals (e.g. silver ions) (Klueh *et al.* 2000) in bulk materials and coatings for slow and constant release into the environment to provide sustained 'release-killing' until the biocide content is exhausted. For example, a chlorine dioxide (ClO_2) biocide is generated from the reaction between stored sodium chlorite salt and acids released from a polymer matrix by either hydrolysis or photolysis reactions (Callerame 1989; Wellinghoff 1997). The latest developments in new antimicrobial coatings are oriented towards multiple mechanistic approaches for surface disinfection. Cohen's group (Li *et al.* 2006) employed layer-by-layer, self-assembly methods to produce antibacterial coatings capable of both release-killing and contact-killing based on stored silver salts and surface-grafted quarternary ammonium. Ho *et al.* (2004) used a polymer film to immobilize nanosilver to achieve both contact- and release-killing effects, while a grafted layer of polyethylene glycol repelled the adhesion of bacteria.

Chlorine dioxide is a fast-acting biocide that is effective against a broad spectrum of micro-organisms including, bacteria, fungi, spores, mold and viruses (Huang *et al.* 1997; Young and Setlow 2003; Sy *et al.* 2005; Simonet and Gantzer 2006). Released as a gas, the antimicrobial action of ClO_2 is less affected by dirt and surface fouling. The gaseous ClO_2 was originally used for large-scale disinfection by bottling companies and as a replacement for the more toxic chlorine in drinking water treatment. The successful stabilization of ClO_2 in liquid form has resulted in broad applications, including the use of a ClO_2 fumigant to disinfect the air ducts in the US Capitol following the Anthrax episode in 2001 (Gordon and Rosenblatt 2005). ClO_2 is safe compared with other oxidizing disinfectants (e.g. chlorine and ozone) and is approved by US FDA for disinfection of poultry and beef products, fruits and vegetables.

The aim of this work was to develop a polymer micro-encapsulating liquid ClO_2 coating that could provide long-term surface disinfection through the sustained release of gaseous ClO_2 from encapsulated liquid ClO_2. This study describes: (i) the physical characteristics of the polymer-encapsulated ClO_2 coating, (ii) the bactericidal performance of the coating against Gram-positive and Gram-negative bacteria, (iii) the sporicidal performance of the coating against the endospores of the *B. subtilis* and (iv) the potential antiadhesive property of the coating against bacteria.

Materials and methods

Preparation and coating of polymer-encapsulated ClO_2

The basic rules for preparing stable water-in-oil-in-water (w/o/w) emulsions described by Ficheux *et al.* (1998) were followed. The stabilized ClO_2 aqueous solution (United Laboratories Inc., IL, USA) was encapsulated by a water-in-oil-in-water (w/o/w) emulsion method (Pays *et al.* 2002), in which 25 ml of 1% (v/v) ClO_2 was suspended in lemon oil (Dreamworld, Guangzhou, China) and 25 ml of 5% (w/v) Pluronic P123 surfactant solution ($EO_{20}PO_{70}EO_{20,}$ MW 5750 g mol^{-1}, with a critical micelle concentration of 0·03 wt% at 25°C, BASF) was added by gentle stirring. The resulting emulsion was then added to an aqueous suspension of Pluronic F127 ($EO_{106}PO_{70}EO_{106}$, MW 12600 g mol^{-1}, critical micelle concentration 0·7 wt% at 25°C, BASF) obtained by dissolving 2·5 g of F127 in 50 ml of deionized water. This formulation had a storage capacity of 18% (w/w) or 180 mg ClO_2 per gram of solid. The emulsion was stored at 4°C before use, and as the antimicrobial coating was not immediately apparent to the eye, lemon oil was added to provide an olfactory cue.

Glass was chosen as the carrier substrate for the study. Rigorous screening was carried out because different components of the manufactured glass (e.g. ZnO and TiO_2) might have intrinsic bactericidal properties that could interfere with the measurements. Glass manufactured by Sail Brand, China was selected for its inertness and availability. The glass was cut into $2{\cdot}5 \times 2{\cdot}5$ cm^2 pieces and prepared according to the Association of Official Analytical Chemists (AOAC) guideline (2005a). Any defective glass was eliminated and the remaining substrates were rinsed once with distilled water and thrice with ethanol (99·9%; Merck) followed by another rinse with excess amounts of distilled water. The glasses were then sterilized in an autoclave (Hirayama, HA-300P) at 121°C for 20 min. Next, 60 μl of the formulation was coated on the cleaned glass to give a coating of about 1 mg ClO_2 cm^{-2} loading. The uniformity of the coating was ensured by the good wettability of the P123/F127 surfactant polymers used in the formulation. Cleaned glass pieces without any coating (uncoated) served as the control.

Characterization of coated glass samples

The coated glass samples were examined under a JEOL 6300 scanning electron microscope (JEOL Ltd, Tokyo, Japan) at an acceleration voltage of 10–15 kV. The samples were mounted on aluminum stubs using a conducting carbon tape and sputter-coated with a thin layer of gold (*c.* 10 nm) to prevent sample charging. The scanning electron micrographs provided information about the uniformity of the coating and the morphology of the deposited polymer microcapsules. The composition of the coating surface was analysed by time-of-flight secondary ion mass spectrometry (ToF-SIMS) (Physical Electronics PHI 7200). A finely focused ion beam (10^{12} ions per

cm^2) was scanned across the sample and the ionized molecular fragments from the surface were collected and analysed by the spectrometer.

Quantification of the ClO_2 released from the coating

In assessing the amount of ClO_2 released from the encapsulated coatings, a batch of the prepared coatings was exposed in ambient room conditions (23 ± 2°C, 70% R.H.) for a period of 28 days. Five coatings were collected randomly immediately after they were prepared (day 0), and at 12 h, 1 day, 2, 3, 7, 14 and 28 days after the coating preparation to quantify the amount of ClO_2 that remained in the coatings by a titration method. The sampled coatings were sonicated in 20 ml deionized distilled water to dissolve the coatings. An excess amount of 0·1 mol l^{-1} potassium iodide (KI, BDH) was added and iodometric titration was carried out in an acidic medium. The free iodine (I_2), the product of the oxidation of KI by ClO_2, was titrated with 0·1 mol l^{-1} sodium thiosulfate ($Na_2S_2O_3$, RDH) using starch as the indicator. The titration results were summarized as follows:

$$ClO_2^- + 4H^+ + 4I^- \rightarrow 2I_2 + 2H_2O + Cl^-$$

$$I_2 + 2Na_2S_2O_3 \rightarrow 2NaI + NaS_4O_6$$

The amount of ClO_2 that remained in the coating was calculated with a molar ratio of ½ of the free I_2. The amount of ClO_2 released from the coating on the day it was sampled was then calculated from the difference between the residual amount of ClO_2 in the coating and that of the freshly prepared coating (day 0).

Bactericidal and sporicidal properties of polymer-encapsulated ClO_2 coating

Preparation of bacteria cells

Stock *Bacillus subtilis* (Carolina 15-4921A), *Escherichia coli K12* (Carolina 15-5065A), *Pseudomonas aeruginosa* (Carolina 15-5250A) and *Staphlococcus aureus* cells (Department of Biology, Hong Kong University of Science and Technology) were kept on tryptone soya agar (TSA, Oxoid) plates (Difco) and stored at 4°C. The bacteria were activated by subculturing a loopful of inoculum in 10 ml of Oxoid nutrient broth in a culture tube and gently shaking the tube in an incubator (Gallenkamp) at 37 ± 0·1°C and 80 rev min^{-1} for 18 h. The viable cell concentration was determined by the plate counting technique on TSA plates after serial dilution. The nutrient broth and TSA plates were sterilized in an autoclave at 121°C for 20 min.

Preparation of B. subtilis *endospores*

The endospores of *B. subtilis* (Carolina 15-4921A) were prepared with some modification from the reported procedure (Riesenman and Nicholson 2000). The bacteria were cultivated on TSA plates at 37 ± 0·1°C for 3 days to obtain a high spore yield. One or two bacterial colonies were harvested from the plates and transferred to a 15-ml centrifuge tube containing 5 ml sterilized deionized water. The suspension was mixed well with a vortex. The spores were purified by centrifugation and water washing. Two millilitres of the suspension was transferred to an Eppendorf tube. The suspension was centrifuged at 10 000 ***g*** for 20 min at 4°C. The supernatant was decanted and 1 ml of cold sterilized deionized water (4°C) was added and the sample was resuspended at 4°C. An aliquot of the suspension was examined under a phase contrast microscope. The centrifugation and washing steps were repeated until more than 99% free spores were obtained as indicated by phase contrast microscopy. The purified spores were suspended in a phosphate-buffered saline (pH 7·4) solution and stored in the dark at 4°C for no more than 7 days. The concentration of the viable spores in the suspension was determined by the plate counting technique on TSA plates following serial dilution of an aliquot of the suspension.

Testing the bactericidal properties of the polymer-encapsulated ClO_2 coating

The tests followed the standard operating procedure of AOAC International (2005b,c) and were conducted in a sterilized biological safety cabinet (NuAire, Nu-425-400E) under ambient conditions (23 ± 2°C, 70% r.h.). To investigate the bactericidal performance of the coatings, a suspension of 100 μl of 10^7 cell ml^{-1} bacteria was spread evenly on the coated and uncoated (control) glass pieces for a specific contact time, which was 10, 20, 30 and 60 min, respectively. At the end of each designated contact time, the amount of viable bacteria that remained on the coating or control surface was enumerated. The tested glass pieces were firstly immersed into a culture tube containing 20 ml neutralizer for 30 min to stabilize and wash off the still surviving bacteria from the surface. The sterile neutralizer solution was freshly prepared by adding 1% (v/v) 0·1 mol l^{-1} $Na_2S_2O_3$ 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 autoclaving at 121°C for 20 min. The glass pieces were then drip-dried and transferred to a second culture tube containing 20 ml of sterile nutrient broth (Nutrient broth No. 2, Oxoid) for 10 min to collect the residual bacteria, if any, on the surface after bathing in the neutralizer solution. A 100 μl aliquot of the neutralizer solution and nutrient broth collecting the

residual bacteria were spread onto separate TSA plates for viable culturing. The plates were incubated at 37 ± 0·1°C for 24 h. The viable bacteria were enumerated from the number of colonies formed and the concentration was calculated by normalizing with the volume (100 μl) applied. Three runs with five coating samples in each run were carried out for each contact time.

Persistency study

To investigate the persistency in the bactericidal performance of the coating, a batch of the coatings was prepared and left under ambient room conditions for a period of 28 days. Five coatings were randomly selected after 10 and 30 min and on day 1, 2, 3, 7, 14 and 28 after the coating preparation for the bactericidal test on *B. subtilis* with a standard contact time of 10 min.

Testing the sporicidal properties of the polymer-encapsulated ClO_2 coating

Endospores of *B. subtilis* (Carolina 15-4921A) were used as a model to study the sporicidal capability of the coating. The testing procedures were similar to those for vegetative cells according to the standard operating procedures of AOAC International (2003) to test sporicidal activity. In view of the resistant nature of the spores, longer contact times of the spores on the coatings were allowed from 0·5 to 2, 8, 24, 48 and 72 h. The initial spore concentration was 10^5 spore ml^{-1}. The viability of the spores at the end of each contact time was enumerated by the plate-spreading method.

Testing the antiadhesive properties of the polymer-encapsulant matrix

The adhesion of *E. coli K12* (Carolina 15-5065A) and *B. subtilis* (Carolina 15-4921A) on clean glass and glass coated with placebo encapsulants (polymer encapsulating sterilized distilled water) was examined. The coated glass was prepared by the same procedure described above, but the ClO_2 solution was replaced with sterilized, distilled water to eliminate possible bactericidal effects. To intensify the possible adhesion effect, a 10-fold higher bacterial cell concentration was used. Hence, 200 μl of the cell suspension with 10^8 cell ml^{-1} *E. coli* and *B. subtilis* was uniformly spread on the coated and uncoated glass surfaces, and incubated at 37°C for 4 h without shaking to allow the bacteria cells to settle on the surface. The samples were then washed gently with sterile distilled water to remove any nonadherent bacteria (Veyries *et al.* 2000). Gram staining was performed on the coated and uncoated glass pieces and images taken by an optical microscope (magnification of 1000×) were recorded to reveal the qualitative degree of bacterial adhesion on the coated and uncoated surfaces.

Results

Polymer-encapsulated ClO_2 coating

The water-in-oil-in-water double emulsion approach that encapsulates ClO_2 in the triblock copolymers Pluronic P123 and F127 results in a uniform, transparent and tactilely smooth coating as shown in Fig. 1a. The coating on the glass was allowed to dry under ambient conditions (i.e., 23 ± 2°C, 70% r.h.) for 24 h. Syneresis or liquid bleeding from the polymer was not observed. A scanning electron micrograph of the coating was taken at 100x magnification and it is shown in Fig. 1b. It is apparent that the film displays uniform features that are similar to those of deposited microcapsules. The ToF-SIMS mapping (Fig. 1c) detected only organic molecular fragments originating from the polymer on the surface of the coating and none of the nonvolatile salts found in encapsulated ClO_2 solution, suggesting that the encapsulation of ClO_2 in the polymer matrix was successful.

Bactericidal and sporicidal potential of the polymer-encapsulated coating

The bactericidal potential of the polymer-encapsulated ClO_2 coating is summarized in Fig. 2. Different bacteria have different susceptibilities to the coating, yet, in general, the reduction profiles increase with the duration of the contact time of the tested bacteria on the coating. With a 10-min contact time, about 0·5-log, 0·6-log, 0·7-log and >2-log reduction of *Staph. aureus*, *Ps. aerugenos*, *B. subtilis*, and *E. coli* were recorded, respectively. When the contact time was increased to 30 min, the reduction further increased to 0·8-log, 3-log, 2·7-log and over 5-log reduction, correspondingly. Over 99·99% reduction of *Ps. Aerugenosa* and *E. coli*, *B. subtilis* and 90% reduction of *Staph. aureus* were achieved when the contact time increased to 60 min.

The sporicidal potential of the coating on the endospores of *B. subtilis* is summarized in Fig. 3. The plain glass surface (control) showed no sporicidal effect; on the contrary, the endospores on the glass started to germinate after 72 h as reflected in the increase in the viable count. However, with the polymer encapsulated ClO_2 coating, there is a persistent reduction in the viability of the endospores, from 0·1-log reduction at 2 h to about 0·7-log reduction (75%) at 72 h.

Release of encapsulated ClO_2 from the polymer matrix

The persistence of antibacterial activities is shown in the sustained release of gaseous ClO_2 from the coated glass

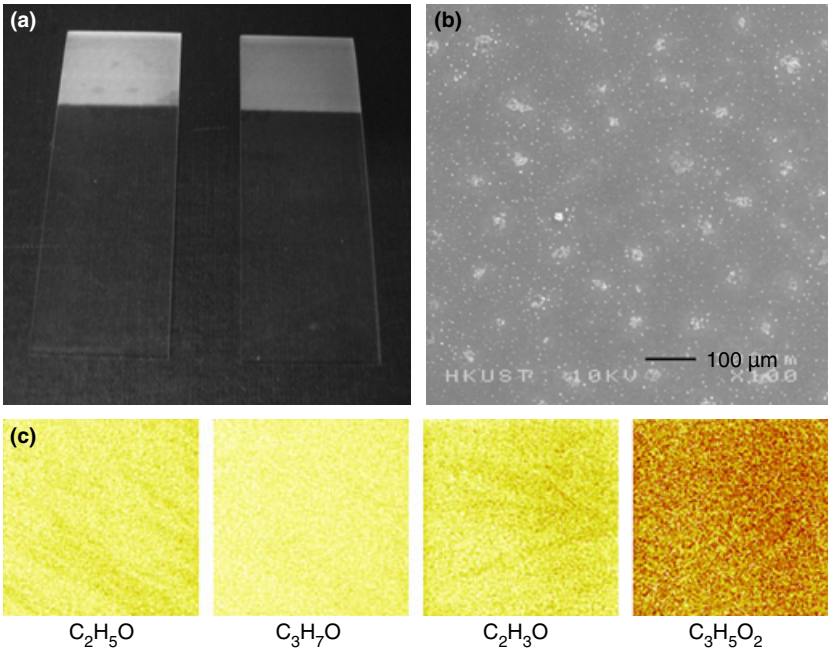

Figure 1 (a) Images of glass slides with (left) and without (right) the antimicrobial coating, (b) a scanning electron micrograph and (c) ToF-SIMS maps of the glass coated with encapsulated ClO_2.

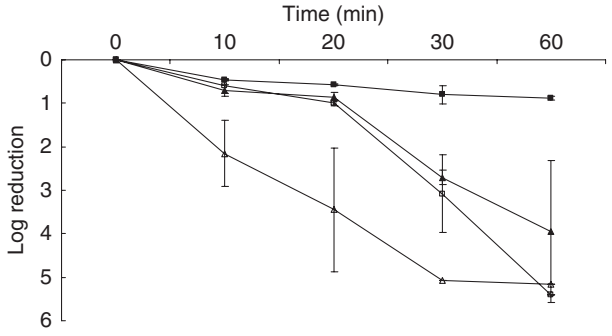

Figure 2 The reduction profile of the tested bacteria with respect to the contact time on the polymer coating with encapsulated ClO_2 (1 mg cm^{-2}). Each data point represents the mean of triplicate sets of five samples with the standard error bar. (—△—, *E. Coli*; —□—, *PS. aeruginosa*; —■—, *staph. aureus*; —▲—, *B. Subtilis*).

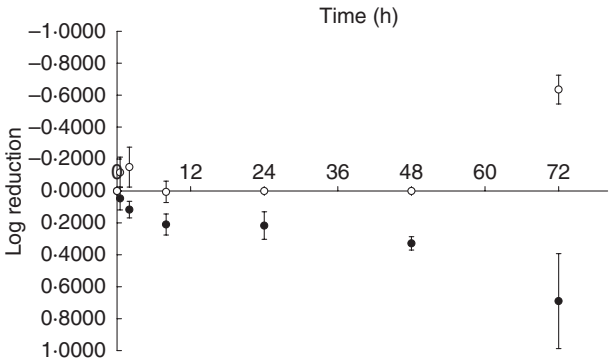

Figure 3 The reduction profile of the tested endospores of *Bacillus subtilis* with respect to the contact time on the polymer coating with encapsulated ClO_2 (1 mg cm^{-2}) (solid circles) compared with those on glass without the coating (open circles). Each data point represents the mean of triplicate sets of five samples with the standard error bar.

surface and its sustained bactericidal potential (Fig. 4). Over the study period of 28 days (4 weeks), about 20% of the stored ClO_2, equivalent to *c.* 1300 μg $ClO_2.g^{-1}$-day^{-1}, were released (Fig. 4a). This amount is sufficient to reduce microbial growth. A parallel bactericidal test on *B. subtilis* showed that a sustained 0·8-log reduction was achieved after a 10-min contact time over the 28 day study period (Fig. 4b). This is equivalent to the disinfecting power of a 300 ppm ClO_2 solution. By extrapolating the rate of release from the 28-day study, we estimated that the stored ClO_2 in the reported preparation would last for at least 3 months with sustained bactericidal potential.

Antiadhesive property of the polymer coating

Members of the Pluronic family have been reported to exhibit antiadhesive properties against micro-organisms such as *Ps. aeruginosa, Staph. aureus and Staph. epidermidis* and are used as detergents in contact lens cleaning solutions (Portole's *et al.* 1994). Water-containing emulsion microcapsules were prepared using the same procedure, with the ClO_2 solution replaced with sterilized, distilled water. This 'placebo' emulsion was coated on glass pieces and aliquots of *E. coli* and *B. subtilis* were added. It can be seen from the microscope images

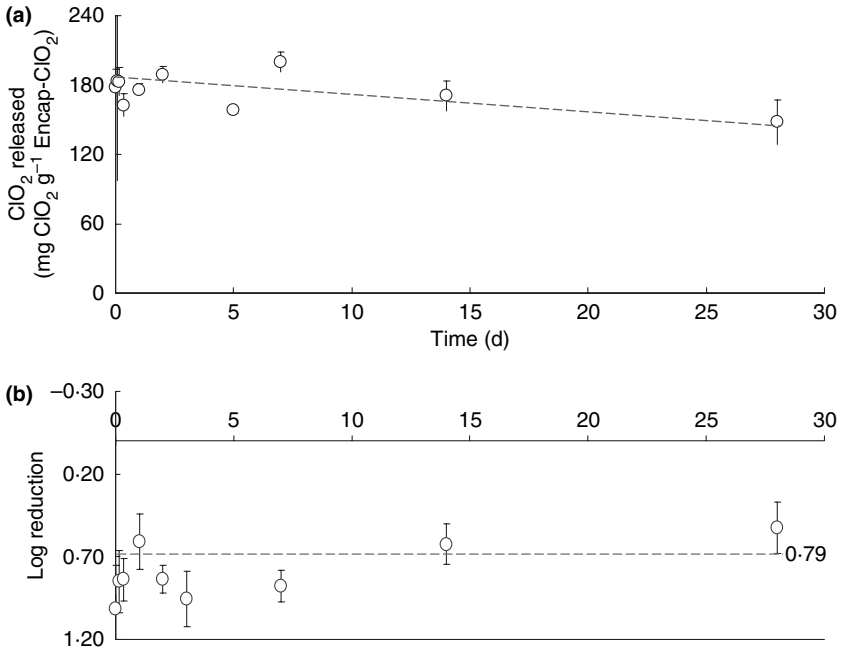

Figure 4 (a) The persistence of ClO_2 released from the coating with encapsulated ClO_2 (1 mg cm^{-2}) as reflected from the amount of ClO_2 remaining on the coating over time, (b) the sustained bactericidal performance on *Bacillus subtilis* in log reduction during the course of the study. The bactericidal performance employed the 10-min contact testing protocol as described in the text. Each data point represents the mean of triplicate sets of five samples with the standard error bar.

(Fig. 5a,c) that the amount of *E. coli* and *B. subtilis* on the coated glass was significantly less (i.e. <10%) compared with that on the uncoated glass (Fig. 5b,d). This indicates that the polymers used for encapsulation prevented the adhesion of *E. coli* and *B. subtilis* on the glass surface.

Discussion

The World Health Organization has reported that the most common route for transmission of infectious diseases is by indirect contact with surfaces contaminated by infectious droplets produced by an infected person's coughing, sneezing or talking (Pruss *et al.* 1999). Bellamy *et al.* (1998) found amylase from saliva on close to 29% of exposed surfaces in domestic households. Human expiratory droplets and hence any inherent pathogens or embedded bacteria can settle on surfaces for days. Fomites, inanimate surfaces capable carrying germs, had been associated with contact transmission of viral, bacterial and fungal pathogens. Regular surface cleaning and disinfection are therefore important and recommended for breaking the chain of transmission. Chemical disinfectants are commonly employed for maintaining the cleanliness of surfaces.

The oxidizing biocide, ClO_2, is a common disinfectant. It is safe (McDonnel and Russell 1999) and effective against a broad spectrum of bacteria, spores and viruses over a wide pH range from 2 to 8 (Huang *et al.* 1997; Young and Setlow 2003; Sy *et al.* 2005; Simonet and Gantzer 2006). The disinfection ability of ClO_2 has also been reported for *B. anthrax* cells and spores (Canter *et al.* 2005), the severe acute respiratory syndrome-associated coronavirus (Wang *et al.* 2005) and the influenza A virus (H1N1) (Ogata and Shibata 2005). ClO_2 is a very reactive free radical molecule. Owing to its unique one-electron transfer reaction mechanism, it is also a highly selective oxidant (Gordon and Rosenblatt 2005). It attacks electron-rich centres in organic molecules (Gordon and Rosenblatt 2005) and kills micro-organisms through oxidizing their cell membranes (Berg *et al.* 1986) and denaturing their proteins (Ogata 2007). It breaks the inner membrane of spores preventing their proper germination (Young and Setlow 2003) and reacts with the viral envelope to cause irreparable damage and inactivation (Ogata and Shibata 2005). ClO_2 solution can be applied or sprayed directly onto the target surface for action. The disinfection ability, however, diminishes with time in terms of minutes as the ClO_2 vaporizes. In enabling a longer lasting disinfection performance, ClO_2 gas was generated from sodium chlorite salts stored in a polymer matrix through a reaction with an acid (Callerame 1989; Wellinghoff 1997), and the acid was either stored with the salt or formed as byproduct of polymer decomposition. This process is inevitably slow as the generation of ClO_2 gas depends on the release or production of acid and its subsequent reaction with sodium chlorite.

The polymer-encapsulated ClO_2 coating system described in this study has promising antibacterial potential. A stabilized ClO_2 solution was microencapsulated in the active triblock copolymers Pluronic P123 and F127 to form a stable water-in-oil-in-water (w/o/w) double

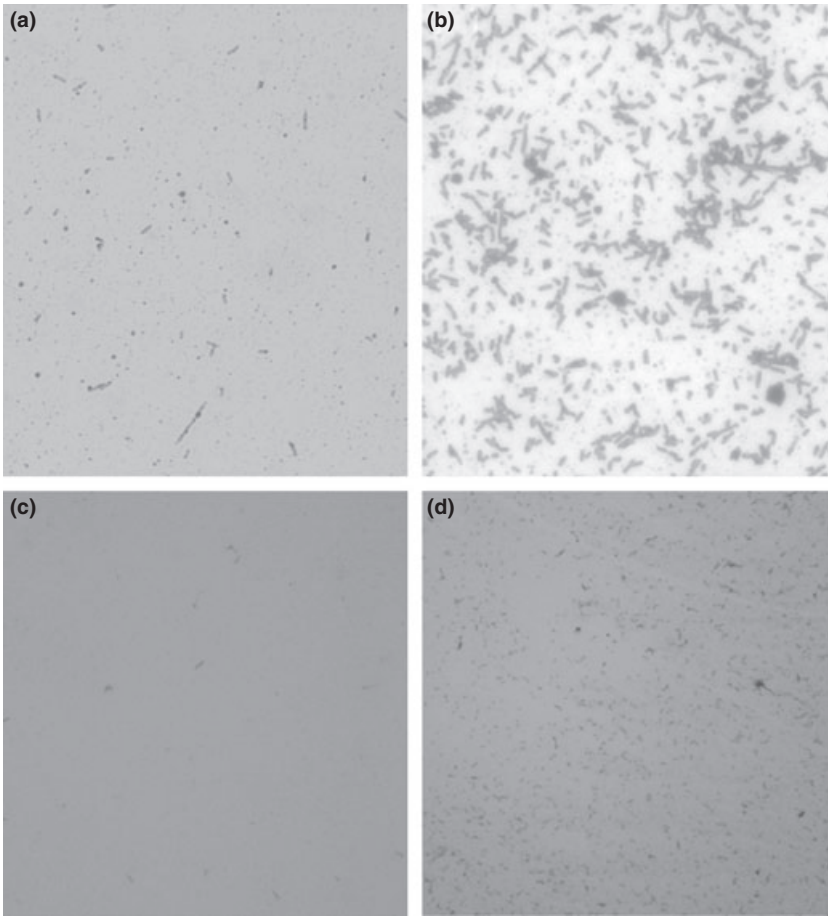

Figure 5 Optical images of pinkish stained *Escherichia coli* cells on (a) glass coated with encapsulated sterile water (i.e., placebo) and (b) uncoated glass, and bluish stained *Bacillus subtilis* cells on (c) glass coated with encapsulated sterile water (i.e. placebo) and (d) uncoated glass at a magnification of 1000×.

emulsion (Fig. 1). This process does not require any acid activation to generate the ClO_2 biocide, which eliminates the need for acid storage and subsequent reactions. The Pluronic family of triblock copolymers was selected because it is safe and widely used in medicines and cosmetics (Thies 1990; Morishita *et al.* 2001). Also, their responsiveness to different environmental cues such as temperature, pH and moisture has been amply demonstrated for controlled drug delivery (Tarcha 1991; Sosnik and Cohn 2004). The emulsion was readily sprayable and with the Pluronic polymers acting as a surfactant. The emulsion droplets deposited uniformly on surfaces as shown by the SEM picture in Fig. 1b. The chemical composition of the surface indicated that the ClO_2 was completely encapsulated in the polymer matrix instead of residing on the surface layer (Fig. 1c).

The experimental results in Figs 2 and 4 indicated that ClO_2 remained encapsulated after surface coating. A slow but sustained release of ClO_2 at about 1300 μg ClO_2 g^{-1} day^{-1} from a coating area of 1 mg cm^{-2} loaded with 180 mg ClO_2 per gram of polymer was reported in this study. Assuming that there is a constant rate of ClO_2 release from the polymer, the coating prepared in this study can optimally last for 3 months. The P123 polymer does not favour a fast transport route via inverted micelles of encapsulates in the oil phase (Matsumoto and Kang 1989; Sela *et al.* 1995). Studies showed that molecules can migrate through the oil phase without affecting the double emulsion stability (Bibette *et al.* 1999). The ClO_2 encapsulated in this study is likely transported from the interior of the polymer to the peripheral surfaces via diffusion, resulting in a sustained release of ClO_2 over the 28-day study period.

The coating also demonstrates consistent bactericidal potential. The ClO_2 released from the coating killed over 90% of the *E. coli*, *Ps. aerugenosa*, *B. subtilis*, and *Staph. aureus* and about 10% of *B. subtilis* endospores with a contact time of 30 min. The amount of *B. subtilis* killed was comparable with that killed after exposure to 300 ppm ClO_2 solution. The relative susceptibility of *B. subtilis* spores and the range of bacteria killed followed with the trend reported by Russell (2003). Gram-negative *E. coli* was most susceptible and endospores of *B. subtilis* were the least susceptible (Figs 2 and 3). *Staphylococcus*

aureus was the least susceptible to ClO_2 among the tested vegetative species. *Staphylococcus aureus* also exhibited similar low susceptibility to a hypochlorite (i.e. bleach) solution (data not shown). This may be related to the presence of carotenoid pigments in *Staph. aureus* that are antioxidative and provide the bacteria with some degree of protection from oxidizing biocides (Clauditz *et al.* 2006).

Despite the different susceptibilities of the bacteria to polymer-encapsulated ClO_2, the bactericidal and sporicidal performance generally increased as the contact time was prolonged. This trend favours the use of the encapsulated coating as an antimicrobial surface. Under ambient conditions, droplets or fomites from whatever source will be deposited onto surfaces and remain on the site until they are transferred to other surfaces or objects after contact. This means that there is ample time for the ClO_2 to act on the species resting on the coating. We speculate that the bactericidal or sporicidal activity is caused by multiple actions of the coating. Primarily, both dissolved and gaseous ClO_2 is released through diffusion from the ClO_2 encapsulated in the polymer matrix. In addition, the coating is rehydrated by droplets or fomites upon deposition. This rehydration destabilizes the outermost membrane film of the coating, resulting in its rupture and rapid flooding of the contact area with the encapsulated ClO_2. The release is sustained by the difference in the osmotic pressures between the exterior and interior water phases of the polymer formulation. The rehydration thus initiates the sporadic release of ClO_2 in addition to the usual release-killing mechanism employed by antimicrobial systems using ClO_2 (Callerame 1989; Wellinghoff 1997). This sporadic release potential gives the coating the advantage of self-disinfection of areas contaminated by droplets or fomites.

Formation of biofilms is detrimental to the coating surface. Phenotypic tolerance to oxidizing biocide can arise from biofilm formation via consumption of the biocide by the organic constituents of the biofilm (Chen and Stewart 1996), or by a 'population-based' resistance strategy (Cochran *et al.* 2000). No biofilm was observed in this reported polymeric matrix coating. The active biocide, ClO_2, used in the formulation prevents indiscriminate reactions and rapid consumption of ClO_2 by the biofilm because of rapid diffusion (Hosni *et al.* 2006) and greater reaction selectivity (Gordon and Rosenblatt 2005). The absence of a biofilm may also be due to the antiadhesive properties of the P123 and P127 polymers that prevent the adhesion both of the Gram-positive and Gram-negative bacteria (e.g. *B. subtilis and E. coli*) on its surface (Fig. 5). The chance of seeding a biofilm on the coating is also reduced. The antiadhesive property of the polymers is probably related to the anchorage of the hydrophobic core of the triblock polymers on the material surface, while its hydrophilic chains form sterically stabilized barriers against adhesion in the periphery (Bridgett *et al.* 1992). The antiadhesive property of the coating surface reduces the chance of the coating degeneration as a result of biofilm formation and allows the coating surface to be a self-maintaining bactericidal system.

In conclusion, a prototype of an antimicrobial coating was prepared with polymer-encapsulated ClO_2 through the double w/o/w emulsion system for general surface application. The coating is easily sprayable because of its low viscosity. A sustained release of gaseous ClO_2 at a maintenance level was demonstrated for a prolonged period (i.e. 28 days). A surface concentration of about 300 ppm ClO_2 was obtained to prevent the bacteria or endospores from attaching on the coating. Contamination by droplets or fomites triggers an increased release of biocide in the affected area, resulting in rapid disinfection. The polymer-encapsulated ClO_2 coating also exhibited promising bactericidal potential against a spectrum of bacterial species and sporicidal potential against *Bacillus* endospores over a contact time of 60 min.

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