Efficacy and Tolerability of ClO₂-Generating Gloves

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The transmission of pathogenic microorganisms by the hands of workers continues to be a problem in the medical field and the food industry. Compliance with hand hygiene is often poor, and gloves may be contaminated after being donned and may transmit microorganisms. A novel, patented technology allows materials to be impregnated with microspheres that, when activated by light or moisture, generate ClO₂ at sustained rates to produce a disinfecting microatmosphere. Gloves that were seeded with bacteria and then exposed to light were able to reduce the numbers of Staphylococcus aureus, Escherichia coli, Salmonella serotype Typhi-murium, and Listeria monocytogenes by 1–3 logs within 20 min, both on the gloves and on the hands of wearers. The gloves look and feel like their standard counterparts and were well tolerated in the Draize test. This technology holds promise for reducing cross-contamination and the transmission of pathogens in the medical and food handling environments.

Hand washing and the wearing of gloves are crucial aspects of infection control in hospitals. Both measures are intended to reduce the transmission of microorganisms such as Staphylococcus aureus and gram-negative bacilli between patients and hospital staff. Hospital staff are instructed to wash their hands both before donning and after removing gloves and to remove gloves after each episode of patient contact [1–3]. Nevertheless, compliance with these measures is poor [4], even after intensive education [5]. Failure to comply is considered to be a major contributor to hospital-acquired infections [6]. The addition of alcohol-based hand rubs probably will improve the situation with respect to reliance on hand washing, because the rubs are easy to apply and some may have a persisting antibacterial effect. A recent study showed that an alcohol-based hand rub was superior to plain soap and water in reducing the counts of gram-negative bacilli or "any transient organism" [7]; however, it would be desirable to have additional means of reducing the transmission of microorganisms by the hands of health care workers, both because compliance with hand hygiene is often inadequate and because hand hygiene does not address the problem of organisms contaminating the outside of the glove.

Similar principles apply to the transmission of infectious microorganisms by the hands of food handlers. Bacteria may be picked up from a contaminated food source, another environmental source, a coworker, or the food handler’s own flora, and may contaminate food products. A simple means of reducing such transmission would be in the interests of the public and of food services.

ClO₂ is a water-soluble gas that has a broad spectrum of activity against bacteria, viruses, protozoa, and even bacterial spores. In low parts-per-million concentrations it is environmentally safe and well tolerated by humans. A patented technology allows for the slow generation and release of ClO₂ at sustained rates from microspheres incorporated into the glove materials upon activation by moisture [8, 9] or light [10]. The system can be incorporated into standard vinyl and polyethylene gloves during production and, when activated, produces a sustained antiinfective microenvironment near the surfaces of the gloves. The gloves are...
indistinguishable from standard gloves to the wearer. The purpose of this article is to describe the antifungal effects and dermal tolerability of these gloves.

METHODS

Antibacterial activity of vinyl gloves in vitro. Disposable, single-use vinyl gloves suitable for medical use and meeting American Society for Testing and Materials (ASTM) D5250-00e (Standard Specification for Poly [vinyl chloride] Gloves for Medical Application) requirements were produced by a major manufacturer of vinyl gloves under the direction of Bernard Technologies Asia Pacific (Republic of Singapore). Some of the gloves were prepared containing the ClO₂-emitting material. These experiments were conducted by R and F Laboratories (West Chicago, IL). The studies were designed by me and my colleagues at Bernard Technologies. The data were analyzed and the manuscript was written by the author.

Cultures of S. aureus (ATCC 6538), Listeria monocytogenes (ATCC 19114), Escherichia coli O157:H7 (ATCC 43894), and Salmonella serotype Typhimurium (ATCC 6994) were grown in brain-heart infusion broth (Difco) and incubated at 35°C for 24 h. The suspensions were centrifuged at 1450 g for 30 min at room temperature. The pellets were resuspended in 5 mL of sterile saline and stored at 4°C until use.

For the in vitro experiments with S. aureus and L. monocytogenes, the gloves were placed on a clean tray under a biological hood. A plastic culture dish containing a piece of filter paper moistened with 1 mL of sterile water was placed into each glove to simulate the moisture from perspiration. The fingers were removed using scissors. A sanitized perfume atomizer (Sally Beauty Supply) was filled with the bacterial suspension, and 3 sprays (0.16 mL) were applied to the outer surface of the palm of each glove, with the atomizer held at an angle 8–15 cm from the glove. The inoculum on the gloves was not allowed to dry.

The contaminated gloves were placed in individual trays under four 40-W fluorescent lamps 43 cm from the gloves to activate the ClO₂-generating system. At intervals of up to 15 min after the time of inoculation, impregnated and control gloves were subjected to microbial enumeration using sterile technique.

For bacterial enumeration, each glove was placed in a 120-mL jar containing 20 mL of sterile DeyEnglye (D/E) neutralizing broth (Difco). This broth was chosen to counteract the antimicrobial effects of the ClO₂. The jar was shaken vigorously for 20 s to release microorganisms from the surface of the gloves. Serial dilutions of the broth were made with 0.1% sterile peptone water. Trypticase soy agar (Difco) pour plates were made from these dilutions and incubated at 35°C for 24–48 h. Broth samples were plated on duplicate plates, and the geometric mean of the 2 values was used to calculate the number of bacteria on each glove.

To evaluate the consistency of results between lots of vinyl gloves, 3 separate lots of gloves were studied using S. aureus and the same protocol as above, except the experimental intervals were up to 120 min.

Antibacterial activity of polyethylene gloves on hands of volunteers. To simulate more closely the activity of the gloves in a situation of intended use, 2 kinds of experiments were done with 3 volunteers: in one, bacteria were applied directly to the outer surface of gloves on the hands of volunteers; in the other, bacteria were applied to the volunteers’ hands, which were then covered by the gloves. Prior written informed consent was obtained from each participant.

Disposable single-use polyethylene gloves, which were made with standard components and impregnated or not with the ClO₂-generating product, were produced by a major manufacturer of polyethylene gloves under the same direction as above. E. coli O157:H7 (ATCC 43894), E. coli (ATCC 25922), S. Typhimurium (ATCC 6994), L. monocytogenes (ATCC 19114), and S. aureus (ATCC 6538) were used in these experiments. Suspensions were prepared in a manner similar to that described above, except that pellets of bacteria were suspended in 10 mL of sterile saline or, in the case of L. monocytogenes, 2 mL of sterile saline.

For the first set of experiments, in which bacteria were applied to the outside of the gloves, 3 volunteers washed their hands with Ivory soap (Procter & Gamble) and then twice with 70% ethanol followed each time by air drying. A nonsterile latex glove was placed on each hand to minimize the transfer of bacteria to the polyethylene glove. The right hand was then covered by a ClO₂-emitting polyethylene glove, and the left hand was covered by a control glove. The 2 types of gloves had a different appearance (translucent vs. opaque white), but the volunteers did not know which glove contained the patented technology.

A pair of cotton swabs was dipped into the washed suspension of E. coli O157:H7 or S. Typhimurium, and excess fluid was drained on the inside of the tube. The swabs were rubbed on the surface of one palm area and, after being moistened again, were rubbed on the back area of the glove. The inoculated gloves on the hands were allowed to air-dry for 2 min. Both hands of each volunteer were used. The volunteers placed their hands between fluorescent lights so that both the palm and back of the gloves were illuminated simultaneously. At predetermined intervals, the gloves were partially removed and the fingertips (not inoculated) were cut off with sterile scissors in order to prevent entrapment of broth within the glove. The remainder of each glove was aseptically removed and placed in a stomacher bag (Seward Stomacher Lab System) containing 100 mL of D/E neutralizing broth. The contents of the bag were mixed and the bacterial content of the bag was enumerated.

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were agitated for 2 min at high speed. Bacteria in the broth were enumerated as described above.

For the second set of experiments, 3 volunteers again washed their hands with Ivory soap and ethanol as described above. The palms and backs of the hands of each volunteer were inoculated with *E. coli* or *S. aureus* by means of a sterile cotton swab moistened with the bacterial suspension. The inocula on the hands were allowed to air-dry for 2 min. A ClO₂-generating glove was placed on one hand and a control glove on the other of each volunteer. The gloved hands then were placed between fluorescent lights, as described above, for periods of up to 45 min.

The gloves were then removed carefully, and the hands were placed in a stomacher bag containing 100 mL of D/E neutralizing broth; a rubber band was applied over the wrist to prevent spillage of the broth. The hand in the bag was massaged for 2 min. Bacteria in the broth were enumerated as in the experiments with *S. aureus* and *L. monocytogenes*. A new set of gloves was used for each time interval.

**Statistical analysis.** Bacterial counts on gloves or hands exposed to standard gloves or ClO₂-generating gloves were compared by the Wilcoxon signed rank test using SPSS Base, version 10.0 (SPSS).

**Dermal tolerance of glove materials.** The dermal tolerability of the gloves was assessed using a standard test [11] done with rabbits, in accordance with the US Food and Drug Administration (FDA) Title 21 of the US Code of Federal Regulations, Part 58. The gloves were produced using standard techniques for the production of disposable gloves as described above. Corresponding, commercially available, disposable single-use vinyl and polyethylene gloves without the technology were purchased as controls. Covance Laboratories (Vienna, VA) conducted these studies. The studies were approved by the company’s Animal Care and Use Committee.

Each type of glove, both test and control, was evaluated under occlusive dressings on abraded and intact skin of 6 rabbits. A 2.5 × 2.5-cm piece of glove was applied to 2 test areas (abraded and nonabraded) on each rabbit. Each area of application was covered with a 5 × 5-cm gauze patch secured with paper tape, covered with Saran Wrap (S. C. Johnson & Sons), and secured with Elastoplast tape (Elastoplast). The animals were collared for the 24-h test period. The patches were then removed; the area was rinsed with tap water and gently tamped dry. Dermal irritation was evaluated at 24, 48 and 72 h after removal of the glove samples. Animals tolerated the test without apparent discomfort.

**RESULTS**

**Antibacterial activity of vinyl gloves in vitro.** Table 1 summarizes the results of in vitro experiments that used *S. aureus* and *L. monocytogenes* sprayed onto the surface of standard and ClO₂-generating vinyl gloves. Each value represents the geometric mean log₁₀ colony-forming units calculated from duplicate platings of bacteria obtained from a single glove. For both *S. aureus* and *L. monocytogenes*, the number of bacteria on control gloves decreased by <1 log over the experimental period. By contrast, on the treated gloves, the numbers for both species decreased by ≥2 logs within 2 min, compared with the corresponding counts on control gloves. There was a continued decrease in the number of bacteria over the next 15 min. The difference in values between control and treated gloves was highly significant (*P* = .005) for both species of bacteria by the Wilcoxon signed rank test.

<table>
<thead>
<tr>
<th>Time after inoculation, min</th>
<th><em>S. aureus</em> ATCC 6538</th>
<th><em>L. monocytogenes</em> ATCC 19114</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control gloves (n = 2)</td>
<td>ClO₂-generating gloves (n = 2)</td>
<td>Mean reduction</td>
</tr>
<tr>
<td>0</td>
<td>8.71, 8.45</td>
<td>Not tested</td>
</tr>
<tr>
<td>1</td>
<td>8.66, 8.47</td>
<td>5.99, 5.98</td>
</tr>
<tr>
<td>2</td>
<td>8.41, 8.19</td>
<td>4.93, 4.82</td>
</tr>
<tr>
<td>5</td>
<td>8.36, 8.44</td>
<td>4.42, 4.33</td>
</tr>
<tr>
<td>10</td>
<td>8.28, 8.29</td>
<td>4.44, 4.52</td>
</tr>
<tr>
<td>15</td>
<td>8.28, 8.34</td>
<td>4.40, 4.28</td>
</tr>
</tbody>
</table>

**NOTE.** Each value represents 1 glove evaluated in duplicate plates; the geometric mean was calculated from these values. The difference in values between control and treated gloves at intervals of 1–15 min was highly significant (*P* = .005) for both species of bacteria, as determined by the Wilcoxon signed rank test. The mean number of bacteria on uninoculated gloves (“background number”) was 365 cfu/mL for impregnated gloves and 972 cfu/mL for control gloves (data not shown).
Figure 1 shows the results of the experiment in which 3 lots of vinyl gloves were inoculated with *S. aureus*. For each of the lots, there was a decrease in bacterial numbers of $>3$ logs within 15 min and a slow continued decrease thereafter. The results were similar for all 3 lots.

**Antibacterial activity of polyethylene gloves worn by volunteers.** The first part of this experiment examined the effects of the gloves incorporating microspheres on bacteria on the outside of the glove (table 2). This study differed from the one above in that polyethylene gloves—rather than vinyl gloves—were used, and the gloves were inoculated while on the hands of volunteers to represent more closely conditions of actual use. Also, gram-negative rather than gram-positive species were studied. Within 5 min after application, there was a 1.54-log decrease in the counts of *E. coli* O157:H7 and a 0.54-log decrease in the counts of *S. Typhimurium*, compared with the corresponding numbers for control gloves. By 20 min, the reductions were 3.98 and 1.14 logs, respectively. The difference in values between control and treated gloves was highly significant ($P = .008$) for both species of bacteria, as revealed by the Wilcoxon signed rank test.

The second part of the experiment involved the activity of the gloves on bacteria that had been inoculated directly onto the volunteers' hands (table 3). After as little as 5 min of glove wear, a modest drop in bacterial numbers was evident for both species. After 20 min, the numbers of both species decreased by $>1$ log, compared with the values for subjects wearing control gloves. The difference in values between control and treated gloves was highly significant ($P = .008$) for both species of bacteria, as revealed by the Wilcoxon signed rank test.

**Dermal tolerance of glove materials.** Exposure of the vinyl and polyethylene experimental glove materials and the corre-

Table 2. Effect of ClO$_2$-generating polyethylene gloves on numbers of *E. coli* O157:H7 and *Salmonella* serotype Typhimurium after inoculation onto the gloves during use.

<table>
<thead>
<tr>
<th>Time after inoculation, min</th>
<th>No. of bacteria on outside of gloves, log$_{10}$ cfu/mL</th>
<th>E. coli O157:H7 ATCC 43894</th>
<th>S. Typhimurium ATCC 6994</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Control gloves (n = 3)</td>
<td>ClO$_2$-generating gloves (n = 3)</td>
<td>Mean reduction</td>
</tr>
<tr>
<td>0</td>
<td>7.63, 7.77, 7.98</td>
<td>Not tested</td>
<td>...</td>
</tr>
<tr>
<td>5</td>
<td>7.90, 7.95, 8.03</td>
<td>6.89, 4.90, 7.46</td>
<td>1.54</td>
</tr>
<tr>
<td>20</td>
<td>7.16, 7.16, 7.54</td>
<td>3.02, 2.30, 4.59</td>
<td>3.98</td>
</tr>
<tr>
<td>45</td>
<td>7.15, 6.78, 6.81</td>
<td>2.15, 2.30, 2.15</td>
<td>4.71</td>
</tr>
</tbody>
</table>

**NOTE.** Each value represents 1 glove evaluated in duplicate plates; the geometric mean was calculated from these values. The difference in values between control and treated gloves at intervals of 5–45 min was highly significant ($P = .008$) for both species of bacteria, as determined by the Wilcoxon signed rank test. Baseline bacterial numbers of uninoculated gloves were $<10^2$ cfu/glove (data not shown).
sponding commercially available control gloves to intact skin resulted in no dermal irritation. The Dermal Irritation Index was calculated to be 0.0 at the intact sites. At the abraded sites, there was well-defined erythema noted at the 24-h observation that had resolved by the 72-h observation with all treated and control gloves. There also was no sign of corrosiveness at any of the intact or abraded sites. There was no distinguishable difference in dermal tolerance between the 2 types of gloves.

**DISCUSSION**

Single-use disposable gloves serve as a functional barrier to microorganisms by helping to control cross-contamination from an individual’s hands to another person or object and by protecting the wearer’s hands from contaminated sources. Disposable vinyl examination gloves are used widely in the health care and medical fields, where they are changed after each episode of human contact or procedure. They also are used in food handling, service, and processing applications, where the gloves usually are worn for periods of 1 h. Disposable polyethylene gloves are commonly used in food service applications and janitorial environments. The gloves used in these experiments meet the specifications for vinyl medical examination gloves defined in ASTM 5250-00e. They have a shelf life of at least 12 months, on the basis of experiments to date. Additional studies of their shelf life are in progress.

These experiments show that gloves incorporating the microspheres and exposed to light to activate the mechanism are able to reduce counts of *S. aureus*, *L. monocytogenes*, *E. coli*, and *S. Typhimurium* substantially and quickly on the surface of the gloves and on the hands of volunteers wearing the gloves. The effect of the gloves was highly significant (*P* < .008) for all comparisons by the Wilcoxon signed rank test. Strikingly, the counts of *S. aureus* were reduced within 1 min of wearing the gloves. We doubt that these sharp reductions in bacterial numbers are an artifact due to the carryover of ClO₂, because the effect of the ClO₂ would have been neutralized by the D/D neutralizing broth and by the simple effect of dilution. Overall, it appeared that the gram-positive species (*S. aureus* and *L. monocytogenes*) might have been more susceptible than the gram-negative species during the study period.

*S. aureus* is a common pathogen among hospitalized patients. This suggests that the ClO₂-generating vinyl gloves would have utility in medical settings. By contrast, *L. monocytogenes*, *E. coli* O157:H7, and *S. Typhimurium* are common foodborne pathogens, and the findings also give promise for the use of these gloves in the food industry.

The ClO₂-generating system in the gloves used in these experiments is activated by light. The technology also can be triggered by moisture. It may be questioned whether the amount of ambient light in usual circumstances will be sufficient to activate the microspheres; however, the threshold of activation for the generation of ClO₂ is 1.88 lux (12.2 μW). Health care workers performing procedures usually work under lights that are of sufficient intensity to activate the gloves. Recently, a report was published of a light-activated antimicrobial coating with the potential for continuous disinfection of surfaces [12]. The antibacterial effect was not nearly as rapid or complete as that described in our experiments. The technology described in our studies offers immediate significant activity upon exposure to light, and the activity extends beyond the glove surface to the microenvironment. This latter advantage would be even more important in some other uses of the microsphere technology.

The gloves were well tolerated in these experiments, both by volunteers and by rabbits in the Draize tests. The 72-h duration of the Draize test comports with FDA guidelines for standard acute irritation and sensitivity tests. If the gloves are to be used in the medical or food industries, it will be necessary to monitor tolerance over longer periods.
Of interest, the ClO\textsubscript{2} acted not only upon bacteria on the outside of the glove but also, though perhaps to a lesser extent, on bacteria on the hand of the wearer. Thus, even if the hands of health care workers were contaminated with potential pathogens, as they frequently are [13], the number of organisms would presumably be reduced on the wearer’s hand, as well as on the outer surface of the glove. It is important to note that the density of organisms inoculated onto the gloves and hands of volunteers in these experiments exceeded by 5–6 logs the density that would be present in normal circumstances. For species other than coagulase-negative staphylococci, usual counts on the hands of health care workers are $<10^3$ cfu/mL [7, 13]. Thus, the findings in these studies may underestimate the beneficial impact of the technology.

Although these experiments dealt with only a few representative species of bacteria, the disinfectant, ClO\textsubscript{2}, is known to have an extremely broad spectrum of activity. For example, it is known to be highly active against viruses, including HIV, enteroviruses, herpesviruses, rotavirus, and polioviruses [14–20]. Among bacteria, it has demonstrated activity against *S. aureus*, *E. coli*, *Klebsiella pneumoniae*, *Salmonella* species, *Yersinia enterocolitica*, *Pseudomonas aeruginosa*, *Bacillus subtilis* spores, *Clostridium sporogenes* spores, and *Mycobacterium avium* [21–27]. It is active against fungal species, such as *Alternaria*, *Chaetomium*, and *Aspergillus* [24], and protozoa, including *Cryptosporidium* oocysts and *Giardia* cysts [23, 28–31].

The mechanism of antimicrobial action of ClO\textsubscript{2} is thought to lie in its potency as an oxidizing agent. Its virucidal activity is attributed to its ability to denature viral proteins and, possibly, nucleic acids [32, 33]. Its activity against bacteria may result from oxidative damage to the outer cell membrane, with loss of the transmembrane ionic gradient leading to the efflux of potassium [21]. The microbicidal activity of ClO\textsubscript{2} is proportional to the product of concentration and duration of exposure.

ClO\textsubscript{2} is generally recognized to be safe for humans, when used in accordance with regulatory guidelines. It is used in the disinfection of drinking water in communities in the United States (and is registered by the Environmental Protection Agency) and many other countries and is permitted by the FDA and the US Department of Agriculture for several uses during processing and packaging of fresh meats, seafood, and produce. It is also used for the disinfection of medical instruments and devices. Another attractive attribute is that ClO\textsubscript{2} neither induces nor selects for resistant organisms and is equally effective against pathogenic and nonpathogenic variants of the same species (e.g., generic *E. coli* and *E. coli* O157:H7). It is effective against both methicillin-susceptible and methicillin-resistant *S. aureus*. These properties, in addition to its noncarcinogenicity, good materials compatibility, lack of residues other than harmless inorganic reaction products, broad-spectrum microbicidal and sporicidal activity, ability to be generated on demand, and low sensitivity to pH, give ClO\textsubscript{2} the characteristics of an ideal disinfectant for many purposes.

In terms of its physicochemical properties, ClO\textsubscript{2} can be used as a gas or dissolved in water. The patented microspheres allow for the novel use of ClO\textsubscript{2} to produce an antiseptic microatmosphere. The microspheres can be “programmed” to release ClO\textsubscript{2} at various rates (e.g., over hours or days), depending on the intended use of the product. In contrast to “contact” biocides that require direct physical contact between the microbe and the surface in which the biocide is embedded, this technology produces a gas in the microenvironment so that direct contact of the microorganism with a surface is not necessary. The results described in this article are preliminary, but the technology described holds promise for use by health care workers to reduce nosocomial infections and by food service workers to reduce cross-contamination.

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**References**


