Factors affecting the stability of sodium hypochlorite solutions used to disinfect dental impressions

Donald E. Gerhardt* / Henry N. Williams**

Increased concern over the transmission of acquired immunodeficiency syndrome, hepatitis B, herpes, and other diseases has prompted research into the disinfection of dental impressions. Among the factors to be considered when dental impressions are disinfected is the stability of the disinfectant solutions during storage and use. This study is concerned with the effect on disinfectant solutions of repeated immersion of alginate dental impressions taken in metal trays. The effects of the impression materials, metal trays, and dilution were evaluated, and the impact of light, heat, and storage were also addressed. The findings indicated that in the test solutions, although considerable chlorine was consumed during the disinfection procedures, bactericidal activity was maintained, while in the control solution both chlorine content and bactericidal activity were remarkably stable. (Quintessence Int, 1991:22:587-591.)

Introduction

Increased concern over the transmission of infectious diseases in the dental office, especially hepatitis B, acquired immunodeficiency syndrome, and herpes, has prompted the dental profession to investigate all possible routes of infection. One area of interest is the transmission of diseases to dental and laboratory personnel via dental impressions and the stone casts made from these impressions. The effectiveness of this mode of transmission has been shown by Leung and Schonfeld,1 and the matter of disinfecting dental impressions is now being addressed by the profession.

Three important factors must be considered when dental impressions are disinfected: (1) how are the impression material and resultant cast affected, (2) how stable are the disinfectant solutions, and (3) how effective are the disinfection procedures? The first consideration has been addressed by several studies that assessed the accuracy of stone casts made from alginate dental impressions disinfected in sodium hypochlorite (NaOCl) solutions.2-7 All studies concluded that the dimensional changes after disinfection are either insignificant or at least acceptable for most clinical applications. Assuming that immersing alginate impressions in NaOCl disinfecting solutions is clinically acceptable, the next question is how are these solutions affected by the disinfecting procedures, and how long are they able to maintain their effectiveness (ie, how stable are the solutions)? Various internal and external factors that act on a disinfectant solution may alter the effectiveness of the disinfectant and influence the stability of the solution.

The literature suggests that chlorine solutions are inherently unstable and if used for disinfecting should be made fresh daily.8 However, other reports indicate that chlorine solutions are stable over long periods of time under varying conditions of storage and use.9-11 Since studies have not addressed the stability of chlorine solutions as used in a clinical environment, many questions remain to be answered about the proper use of chlorine disinfectants. For example, can they be used indefinitely, or must they be discarded after a specific period of time?

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Assuming that chlorine disinfectants do lose their effectiveness during a period of clinical use, what is the critical factor: time, conditions of storage, or use? The answer to this question is extremely important and could strongly influence disinfecting procedures. This study evaluates the effects of time, storage, and use on the stability of chlorine solutions prepared to disinfect dental impressions.

**Method and materials**

Clorox bleach (The Clorox Co), diluted 1:10 in chlorine-demand-free water, was used to prepare experimental and control solutions of sodium hypochlorite. This dilution has been used in previous studies involving both surface and dental impression disinfection.²⁻¹² Prior to testing, and after each set of 16 impressions was immersed in the experimental solution, determinations were made of the bactericidal activity and the amount of both total and free chlorine.

The experimental protocol consisted of making impressions of dentoform models using irreversible hydrocolloid (alginate) impression material (Jeltrate, type 1, LD Caulk Co). The impressions were made in chrome-plated brass trays with soldered rims, washed in running tap water, and then “disinfected” by immersion for 10 minutes in a 1-L volume of the 0.6% solution of NaOCl. A second liter of the solution was kept as a control. Both solutions were stored at room temperature in opaque, covered containers except for the testing periods, when both solutions were uncovered. A total of 80 impressions, 16 per day, were made and immersed over a 5-day period.

Chlorine determinations were made using the DPD (n,n diethyl-p-phenylenediamine) colorimetric method (Spectrokit Reagent Systems, Milton Roy Co) prior to the start of testing and each day after 16 impressions had been immersed. Measurements of absorbance were obtained in a Spectronic 20 photometer (Bausch & Lomb, Inc). Absorbance readings obtained from the photometer were converted to milligrams per liter of chlorine using conversion tables supplied with the chlorine testing kit. The percentage of chlorine and the parts per million of chlorine in each of the solutions were derived from these figures. Freshly diluted solutions used in this study contained 0.60% total chlorine (6,000 ppm) and 0.45% free chlorine (4,500 ppm).

The bactericidal activity of the disinfectant was evaluated each day, after 16 impressions had been immersed. A sterile, blank, round disk, measuring 10 mm in diameter, was held with forceps and momentarily immersed in the disinfectant solution. The disk was removed and drained by pressing it to the sides of the vessel containing the disinfectant and placed on the surface of a trypticase soy agar plate that had just been inoculated with a culture of *Staphylococcus aureus*, *Pseudomonas aeruginosa*, or *Bacillus subtilis*. The plates were incubated for 24 hours and examined for zones of inhibition surrounding the disinfectant disk. The diameter of any observed zone was measured. This procedure was repeated on both the test and experimental solutions daily for the duration of the study.

**Results**

Both total chlorine and free chlorine levels in the experimental solution fell steadily throughout the test period (Fig 1). Total chlorine of 6,000 ppm in the fresh solution dropped to 2,000 ppm after 80 impressions had been immersed. The free chlorine component decreased from 4,500 ppm to 1,500 ppm, and the combined chlorine from 1,500 to 500 ppm. The control solution remained constant at 6,000 ppm total chlorine and 4,500 ppm free chlorine.

The results of the bactericidal evaluation showed the sodium hypochlorite disinfectant produced zones of inhibition against the three bacterial species each day over the duration of the study. On the first day, immediately following preparation and prior to use, the zones of inhibition measured approximately 40 mm on the *S aureus* plates, 35 mm on the *P aeruginosa* plates, and 30 mm on the *B subtilis* plates. The zone sizes remained virtually unchanged during the course of the study, revealing that there was little change in the bactericidal activity of the solutions (Table 1).

**Discussion**

The results indicated that, within the parameters of this study, hypochlorite solutions lost chlorine with use, but not over time or under conditions of storage. However, the loss of chlorine did not correlate with a loss of bactericidal activity of the disinfectant solution.

Once hypochlorite solutions are prepared, their stability is affected by organic contaminants, heavy metal ions, dilution, time, light, and temperature. The major factor contributing to the loss of chlorine in this study was most likely the alginate impression material. Alginate contains large amounts of sodium, potassium,
and calcium, all of which react readily with chlorine and could be expected to reduce the amount of chlorine available for disinfecting. Organic material in the impression material consumes chlorine and reduces its capacity for bactericidal activity. The alginate impression material itself, an organic compound, may have been responsible for consuming large amounts of the available chlorine.

The second factor responsible for the loss of chlorine was the reaction between the hypochlorite solution and the metal trays. Because of its electronic configuration, chlorine has a tendency to acquire extra electrons. Chlorine in water reacts quickly with inorganic reducing substances, such as copper, nickel, and cobalt, which are powerful catalysts of decomposition, changing the solution from chlorine to inorganic chloride ions. The chlorine thus reduced to chloride is lost as a disinfectant. The reactivity with metals in this study was evident from the appearance of the metal impression trays after a 10-minute immersion in the hypochlorite solution (Fig 2). The primary point of attack was the solder used to attach the rims. This reaction between chlorine and metal undoubtedly consumed considerable amounts of free chlorine.

A third reason for the loss of chlorine is simply the dilution of the test solution with water. In this study, as is common in clinical practice, the impressions were rinsed in running water and merely shaken to remove excess water. Water on the impression introduced into the solution and hypochlorite removed during the retrieval of 80 impression trays may have been a significant factor in the overall loss of chlorine.

Ultraviolet light and temperature probably had little effect on these results. Both solutions, test and control, were stored in opaque plastic containers with covers so that light would not be a decomposing factor. All solutions were stored at room temperature (approximately 20°C) for the duration of the study.

### Table 1 Diameters of the zones of inhibition (cm)

<table>
<thead>
<tr>
<th>No. of impressions</th>
<th>Pretest</th>
<th>16</th>
<th>32</th>
<th>48</th>
<th>64</th>
<th>80</th>
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</thead>
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<tr>
<td><strong>S aureus</strong></td>
<td></td>
<td>4.0</td>
<td>4.0</td>
<td>4.0</td>
<td>4.0</td>
<td>3.0</td>
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<tr>
<td>Experimental</td>
<td>4.0</td>
<td>3.0</td>
<td>4.0</td>
<td>4.0</td>
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<td>3.5</td>
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<tr>
<td>Control</td>
<td>4.0</td>
<td>3.0</td>
<td>4.0</td>
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<tr>
<td><strong>P aeruginosa</strong></td>
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<td>3.0</td>
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<td>Experimental</td>
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<td>Control</td>
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<tr>
<td><strong>B subtilis</strong></td>
<td></td>
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<td>2.5</td>
<td>3.0</td>
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<td>Experimental</td>
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Fig 2 Corrosion precipitate (arrows) resulting from the immersion of metal trays in the NaOCl disinfecting solution.

.. approximately 24 °C) over the span of the study. No loss of chlorine was found in the control solution over the period of 1 week. Although, under the experimental conditions used, light and temperature were observed to have minimal effect on the results, these factors have the potential to affect prepared solutions and are therefore variables that must be considered in any clinical situation.

Chlorines are selective in their attacks on various types of organic material, and not all organic material slows the germicidal effectiveness of hypochlorite solutions. However, other organic material, such as microbes and blood, will consume chlorine until the demand is satisfied. Consequently, hypochlorite solutions tend to lose effectiveness rapidly in the presence of blood and saliva. This will have a substantial impact on the disinfection of dental impressions should they be heavily covered with blood or saliva when placed into the solutions. The effect would be especially critical in solutions with lower levels of chlorine, which could rapidly lose their effectiveness unless the chlorine dosage is adjusted to overcome the demand.

Both the test and control solutions retained their biocidal activity throughout the study. This reflects the fact that chlorine solutions are effective even at low concentrations of free chlorine. Dychdala has reported that a chlorine solution at pH 7.2 and 25 °C and containing on average 0.8 ppm chlorine requires only 30 seconds to accomplish a 100% biocidal result against \textit{S. aureus}. The solutions used in this study initially contained free chlorine concentrations of 4,500 ppm and at the conclusion of testing still contained 1,500 ppm of free chlorine. This concentration was more than sufficient to maintain a biocidal effectiveness against the three strains of organisms used.

Conclusions

Three factors affecting the stability of chlorine solutions, time, conditions of storage, and use, were evaluated to ascertain whether chlorine solutions must be changed daily or can be retained for extended use. The results indicated that chlorine disinfecting solutions of sufficient concentration can be retained for periods up to 1 week and still maintain their effectiveness.

The critical factor of those tested was use. The way in which a chlorine disinfecting solution is used will determine how frequently it must be replaced or replenished. In the present study, three elements, alginate impression material, metal trays, and dilution, were responsible for reducing the total available chlorine in the disinfectant solution by two thirds. Time and conditions of storage were of secondary importance. Properly stored, and unused, a dilute chlorine solution was observed to remain stable over a 1-week period.

After 5 days, during which time 80 impressions were immersed in the chlorine solution, the residual unreacted chlorine was sufficient to maintain bactericidal activity against selected organisms.

References


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