

Microbiological evaluation of dental stone casts after immersion in sodium hypochlorite and peracetic acid

Avaliação microbiológica de gesso odontológico após imersão em hipoclorito de sódio e ácido peracético

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ABSTRACT

Objective: the purpose of this study was to evaluate the efficacy of disinfection of type III dental stone by immersion in 1% sodium hypochlorite and 0.25% peracetic acid at different periods of time (1, 5 and 10 min). **Material and Methods:** silicon dies were previously infected with strains of *Bacillus subtilis* for 15 min. Then, type III gypsum stone (Herodent, Vigodent COLTÈNE SA, Rio de Janeiro, Brazil) was inserted into the cavities to obtain contaminated specimens. A sterile silicone die was used to obtain uncontaminated specimens. The specimens were separated into positive and negative control groups, and further divided into the following groups: blocks immersed in sterile physiologic solution for 1, 5 or 10 min; blocks immersed in 1% sodium hypochlorite for 1, 5 or 10 min; and blocks immersed in 0.25% peracetic acid for 1, 5 or 10 min. All the groups were double-plated and incubated at 37°C for 24 h. **Results:** the results were expressed in colony forming units (CFU/ml) and the data were submitted to the Kruskal-Wallis test followed by Dunn's test. The results showed that immersion in 1% sodium hypochlorite and 0.25% peracetic acid resulted in complete disinfection of the test specimens at all test periods ($p < 0.01$), whereas immersion in saline did not provide effective disinfection. **Conclusion:** it can be concluded that both 1% sodium hypochlorite and 0.25% peracetic acid provided effective disinfection in dental stone specimens immersed in the solutions described above, at different periods of time.

KEYWORDS

Peracetic acid; Disinfection; Sodium hypochlorite; Calcium sulfate.

RESUMO

Objetivo: o objetivo deste estudo foi avaliar a eficácia da desinfecção do gesso odontológico tipo III com uso de hipoclorito de sódio 1% e ácido peracético 0,25% em diferentes tempos (1, 5 e 10 min). **Material e Métodos:** matrizes de silicone foram previamente contaminadas com solução contendo *Bacillus subtilis* por 15 min. Gesso odontológico tipo III (Herodent, Vigodent COLTÈNE SA, Rio de Janeiro, Brasil) foi inserido nas matrizes, obtendo-se espécimes contaminados. Uma matriz estéril foi utilizada para obter corpos de prova livres de contaminação. Além do controle positivo e negativo, os espécimes foram divididos nos seguintes grupos: blocos imersos em solução salina por 1, 5 ou 10 min; blocos imersos em hipoclorito de sódio 1% por 1, 5 ou 10 min; blocos imersos em ácido peracético 0,25% por 1, 5 ou 10 min. Foi realizado plaqueamento de todos os grupos e incubados por 24 horas a 37°C. **Resultados:** os resultados foram expressos por meio das contagens das unidades formadoras de colônias (UFC/ml) e os dados foram submetidos aos testes de Kruskal-Wallis seguido pelo de Dunn. Os resultados mostraram que a imersão em hipoclorito de sódio 1% e em ácido peracético 0,25% resultou em completa desinfecção dos corpos de prova em todos os tempos testados ($p < 0,01$), enquanto que a imersão em solução salina não proporcionou efetiva desinfecção em nenhum dos tempos testados. **Conclusão:** pode-se concluir que houve eficácia do hipoclorito de sódio 1% e do ácido peracético 0,25% ao imergir o gesso odontológico tipo III em qualquer dos tempos de avaliação.

KEYWORDS

Acido peracético; Desinfecção; Hipoclorito de sódio; Sulfato de cálcio.

INTRODUCTION

Dental materials that come into contact with oral cavity fluids, such as the materials used for dental impressions, casts and prostheses, are contaminated by microorganisms present in saliva and blood [1,2]. Thus, it is important to adopt procedures to disinfect the materials that are manipulated by the dental staff or that are sent to a dental laboratory, to prevent cross-contamination, which may extend to dentists, the dental office staff, dental technician and patients [3-6].

To this end, there are several recommendations and protocols to prevent cross-contamination [7,8]. Despite the well-documented methods of disinfecting impressions using sodium hypochlorite and glutaraldehyde [9-11], some studies detect the presence of lingering microorganisms in the materials sent to dental laboratories [2,4,12]. Regarding dental stone casts, the American Dental Association (ADA) recommends disinfection with sodium hypochlorite or iodophor by spray or immersion [3]. Some authors have investigated the use of chlorhexidine and glutaraldehyde, added during the cast die stone setting time, as another disinfection method [13]. Recently, studies have demonstrated the efficacy of disinfection of dental stone casts using microwave technology [5,6].

Sodium hypochlorite is a powerful disinfectant agent against bacteria and viruses, it has advantages, such as a broad antimicrobial spectrum, ease of use, water solubility, rapid bactericidal action, relative non-toxicity in certain concentrations, non-flammability, colorless aspect and low cost [14]. Sodium hypochlorite can be used to disinfect instruments contaminated by human immunodeficiency virus and hepatitis B virus [15]. Moreover, it has been found to be suitable to disinfect impressions and dental stone casts [4-6,9,10,16-18]. However,

there are few studies showing the effectiveness of this material to disinfect dental stone casts, especially in regard to disinfection period [4-6].

Peracetic acid is considered a high level disinfectant by the Food and Drug Association, and is widely used as an alternative to glutaraldehyde, because of its broad spectrum activity, even in the presence of organic matter, and because it dissociates into non-toxic products [19,20]. This product is considered a more potent biocide than hydrogen peroxide at low concentrations [15]. The efficacy of decontamination of acrylic resins after immersion in 0.2% peracetic acid for 5 min [21]. Was observed disinfection of chemically activated resin by immersing it in 0.25% peracetic acid for 1 min, or else in a diluted concentration of 0.025% for 10 min [22], and confirmed the efficacy of 2% peracetic acid for a quick disinfection (1-2.5 min) of contaminated gutta-percha. No studies were found relating the use of this product to dental stone cast disinfection [23].

Considering that a dental stone cast is a source of cross-contamination [6,24,25,26], especially when the methods of disinfecting an impression are carried out improperly or are nonexistent, it is understandable that these casts must be disinfected properly. Thus, this study was designed to evaluate the effectiveness of disinfecting type III dental stone by immersing it in 1% sodium hypochlorite or 0.25% peracetic acid at different periods of time.

MATERIALS AND METHODS

Three silicone matrices (SPLAB Comércio de Produtos Laboratoriais Ltda., São Paulo, SP, Brazil), each containing 21 cavities (dimensions of 5 mm high x 14 mm wide x 4 mm deep per cavity) were used to prepare the specimens. The matrices were sterilized with ethylene oxide (ACECIL Central de Esterilização Com. Ind. Ltda., Campinas, SP, Brazil) prior to contamination.

Bacillus subtilis was used because it is considered a non-pathogenic microorganism and

is more resistant than some viruses [18]. Strains of *Bacillus subtilis* (ATCC 19659) were activated by culturing in brain heart infusion (BHI) agar and incubated at 37°C for 24 h. Single colonies taken from the plates were transferred into 5ml of sterile saline solution and homogenized in a vibration device (AP-56, Phoenix Lufarco, Araraquara, SP, Brazil). This procedure was repeated until a 200ml solution was prepared with Factor 5 of the McFarland Scale, about 15×10^8 organisms / ml (NEFELOBAC - Nephelometric McFarland scale).

Afterwards, the silicone matrices were contaminated by immersing them in a 600ml autoclavable beaker containing the contaminated solution with the strains of *Bacillus subtilis* described above, for 15 min.

The type III dental stone (Herodent, Vigodent COLTÈNE SA, Rio de Janeiro, Brazil) was prepared according to the manufacturer's instructions. It was inserted into the silicone matrix cavities in small portions, so that it would not overflow, using the vibrating apparatus (VH Gold line, Essence Dental, Araraquara, SP, Brazil) to facilitate product flow and prevent bubble formation. After 60 min, the specimens were removed from the matrix and stored in a sterile glass beaker. The process was repeated for each of the three matrices used. A total of 57 contaminated specimens were prepared using this type III dental stone.

A sterile matrix was used for the preparation of specimens without microorganism contamination to obtain the negative control group (NC). The dental stone was handled in the same manner as described above, using all sterile materials, and 60 min were allowed to elapse for the material to set. The gypsum blocks were removed individually from the matrix and placed in a sterile glass beaker, thus yielding three specimens free from contamination.

Of the 57 contaminated specimens, three were used as the positive control (PC) to highlight the presence of the contamination. The 54 contaminated samples were divided into nine experimental groups ($n = 6$), according

to the disinfectant solution used (sterile saline solution, 1% sodium hypochlorite - Asfer Indústria Química Ltda - Sao Caetano do Sul, SP, Brazil; and 0.25% peracetic acid - THECH Desinfecção Ltda. - Cotia, SP, Brazil) and the immersion time (1, 5 and 10 min).

The experimental groups were divided as described in Table 1.

Table 1 - Experimental groups

| Group | Quantity (n) | Immersion Solution | Immersion Time |
|-------|--------------|-------------------------|----------------|
| PC | 3 | - | - |
| NC | 3 | - | - |
| SS1 | 6 | Sterile Saline Solution | 1 min |
| SS5 | 6 | Sterile Saline Solution | 5 min |
| SS10 | 6 | Sterile Saline Solution | 10 min |
| SH1 | 6 | 1% Sodium Hypochlorite | 1 min |
| SH5 | 6 | 1% Sodium Hypochlorite | 5 min |
| SH10 | 6 | 1% Sodium Hypochlorite | 10 min |
| PA1 | 6 | 0.25% Peracetic Acid | 1 min |
| PA5 | 6 | 0.25% Peracetic Acid | 5 min |
| PA10 | 6 | 0.25% Peracetic Acid | 10 min |

In preparing the negative control group, non-contaminated gypsum blocks were immersed in 5 ml of sterile saline solution, followed by homogenization in a vibration device for 30 s. Aliquots of 100µL were plated in duplicate on BHI agar using a micropipette tip. The sample collected was spread across the board with a previously flamed Drigalski loop. The same process was used to prepare the positive control group, except that the blocks were contaminated with *Bacillus subtilis*.

As for preparation of the SS, SH and PA groups, contaminated gypsum blocks were

immersed in sterile test tubes containing 5ml of sterile saline solution and 1% sodium hypochlorite or 0.25% peracetic acid, and allowed to rest for 1, 5 or 10 min. During the time intervals, the tubes were kept upright in a test tube stand and underwent no movement. After these periods of time, the gypsum blocks were transferred individually to other individual test tubes containing 5ml of sterile saline solution. Each test tube was homogenized for 30 s, and 100 μ L of each sample was collected in the same manner as that described above.

Next, the seeded Petri dishes were incubated at 37°C for 24 h in a microbiological oven (Odontobrás, Ribeirão Preto, SP, Brazil). The Petri dishes were removed from the microbiological oven to count the colony forming units / ml (CFU / ml) and calculate the percentage of inhibition of the bacterial seedlings, achieved by disinfectants. The results indicated the number of CFU / ml when there was growth, and zero (0) when there was not any.

The statistical analysis was descriptive, characterizing the sample by mean and standard deviation. The SS, HS and PA groups were evaluated by Kolmogorov-Smirnov as the normality of the data. Because all the groups were not normal, the Kruskal-Wallis test was performed for comparative purposes. When a statistically significant difference was found, Dunn's post hoc test was performed to assess the difference among the groups.

The results were considered statistically significant at $p < 0.05$. The software used for the statistical analysis was SPSS Statistics version 20.0 (SPSS Inc., Chicago, IL, USA).

RESULTS

All samples of the positive control group produced large amounts of CFU/ml, whereas none of the samples of the negative control group produced any CFU/ml.

The results showed that there was partial disinfection of the specimens immersed in sterile saline solution. The specimens immersed in 1% sodium hypochlorite and 0.25% peracetic acid showed complete disinfection, at all test periods.

Table 2 shows the comparison of the experimental group results.

Table 2 - Mean and standard deviation of CFU / ml for groups immersed in different solutions at 1, 5 and 10 min

| Time | Positive Control | SS | SH | PA |
|--------|---------------------------------|---------------------------------|------------------------|------------------------|
| 1 min | 159.33 \pm 11.35 ^A | 32.50 \pm 16.82 ^B | 0 \pm 0 ^C | 0 \pm 0 ^C |
| 5 min | 159.33 \pm 11.35 ^A | 26.33 \pm 20.887 ^B | 0 \pm 0 ^C | 0 \pm 0 ^C |
| 10 min | 159.33 \pm 11.35 ^A | 32.00 \pm 13.957 ^B | 0 \pm 0 ^C | 0 \pm 0 ^C |

Different letters in rows mean a statistically significant difference ($p < 0.01$) by the Kruskal-Wallis test

DISCUSSION

The ADA7 reports that microorganisms present in saliva or blood from patients may cause infections, leading to illnesses such as the common cold, pneumonia, tuberculosis, herpes, hepatitis B and HIV. Furthermore, microorganisms with different degrees of pathogenicity can be identified in materials sent to laboratories, including *Enterobacter cloacae*, *Escherichia coli*, *Klebsiella oxytoca* and *Pseudomonas aeruginosa*, which, under the right circumstances, such as tears or cuts in the skin, could result in infection [2].

Therefore, it is recommended that used molds should be disinfected after an impression is made [9-11]. The disinfection process involves rinsing the impression to remove blood, saliva and other debris, followed by immersion in any disinfectant product compatible with the impression material, to remove microorganisms [7]. In this process, it is essential that the disinfection be made with some disinfectant agent, because it has been shown that simple rinsing with tap water reduces the amount of microorganisms, but does not promote efficient disinfection [3,9].

Dental stone casts obtained by a contaminated impression are considered a source of cross-contamination [24-26]. In the present study, it was found that the positive control group had a large number of microorganisms, in agreement with the authors cited above. Therefore, gypsum blocks can be considered vehicles of transmission of microorganisms, and appropriate disinfection measures should be adopted before they are handled by any professional.

The blocks that were simply rinsed in tap water (SS group) had statistically significant fewer organisms than the positive control, but disinfection was not complete. Bacterial colonies were found in all the test periods (1, 5 or 10 min), and there was no difference between leaving the blocks in the disinfectant solution for 1 or 10 min. This result was corroborated by Mitchell et al. [26].

Among the existing disinfection products, sodium hypochlorite has been studied in the dental field for some time in different situations, as a disinfectant solution for impressions and for gutta-percha [3,9,10,11,23]. According to these studies, the time required for efficient disinfection depends on the type of microorganism and the concentration of the product. Regarding dental stone cast disinfection [18], obtained complete disinfection by immersion in 5.525% sodium hypochlorite saturated with calcium sulfate dehydrate for 1 h, without damage to surface details. Goel et al. [4] obtained clinically acceptable disinfection after 10 min of immersion in 0.07% sodium hypochlorite with no significant dimensional change, and was achieved a high level of disinfection after immersion in 0.5% sodium hypochlorite for 10 min. The present study evaluated the complete disinfection of the bacteria tested by immersion in 1% sodium hypochlorite from the time of 1 min to 10 min. One min is a more feasible period of time to use clinically, considering that a sodium hypochlorite solution at a concentration of 1%

is easily found in dental materials stores, that a period of 1 min is considered a short period of time, and that this brevity decreases the possibility of damage occurring to the surface details. The study reported that immersing the dental stone cast in sodium hypochlorite (Clorox - The Clorox® Company, Oakland, CA, USA) for a 10 min interval produced the least amount of adverse physical property events compared with glutaraldehyde (Sporicidin - Contec® Inc., Spartanburg, SC, USA; Banicide - Pascal International, Inc., Bellevue, WA, USA; Glutarex - 3M, St. Paul, MN, USA; procide-30 - Cottrell, Ltda., USA; Metricide - Metre Research Corp., Orange, CA, USA) and with phenol compounds (Multicide, Biotrol International, Earth City, MO USA) [16]. In regard to dimensional stability, no statistically significant difference was found between the casts immersed in 0.07% sodium hypochlorite for 10 min, the models irradiated by microwave for 5 min, or the group in which no disinfection method was performed [4]. Further studies are necessary to evaluate the actual dimensional change in these immersion periods.

Peracetic acid has been studied in dentistry for disinfecting acrylic resins [21,22], gutta-percha [23] and impression materials [11]. The product was used in this study because of its good performance, as observed in the studies cited above, especially for its disinfecting effect and for the short time to achieve effectiveness. The results obtained in the studies by showed that peracetic acid became effective in a 1-min period [22,23]. These results differ from those obtained by other authors [21.], who immersed acrylic resins in the acid for 5 min, and immersed an optic bronchoscope in the acid for 10 min. It should be considered that all the studies differed in regard to the contact methods, the microorganisms used and the test time; these factors could account for this difference [19].

Although this study did not use organic matter (such as blood and saliva), it is important to note that some studies have shown that the

disinfectant effect of sodium hypochlorite is decreased in the presence of organic matter; however, peracetic acid retains constant properties [20,27].

The results of this study, within its limits, showed the efficacy of 1% sodium hypochlorite and 0.25% peracetic acid as disinfectants for type III dental stone. Thus, the immersion of dental stone casts in either of these two chemicals for at least 1 min is a viable alternative for controlling infections and reducing the risk of cross-contamination.

CONCLUSION

It can be concluded that both 1% sodium hypochlorite and 0.25% peracetic acid are effective in disinfecting type III dental stone in all of the time periods tested (1, 5 and 10 min).

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