

# Antimicrobial Efficiency of Hypochlorous Acid Incorporation and its Effect on Surface Properties of Irreversible Hydrocolloid Materials

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## Abstract

**Objective:** Conventional approaches for disinfection, including spraying and immersion, resulted in only surface disinfection of impressions. As a result, self-disinfecting impression materials incorporated with antimicrobial compounds require more extensive studies. The incorporation of a disinfectant into irreversible hydrocolloid impression materials could eliminate the need for the disinfection step by conventional approaches, including spraying and immersion which only result in surface disinfection of impressions. The study was aimed to investigate the effect of incorporation of hypochlorous acid in irreversible hydrocolloid materials on antimicrobial efficiency, detail reproduction, and dimensional stability.

**Materials and Methods:** Hypochlorous acid (HOCl) was used in two concentrations, 100 ppm and 200 ppm, and mixed with alginate powder to compare with the control group (distilled water mixed with alginate). *Candida albicans*, *Staphylococcus aureus*, and *Pseudomonas aeruginosa* were chosen for assessing the antimicrobial activity with the disk diffusion test. In addition, the dimensional stability and reproduction of details were tested.

**Results:** The results revealed that both HOCl concentrations of 100 ppm and 200 ppm imposed significant antimicrobial activity against all three tested microorganisms. There was no significant difference regarding reproduction of details, but the addition of the antimicrobial had a significant adverse effect on the alginate's dimensional stability.

**Conclusion:** It may be concluded that the incorporation of HOCl into irreversible hydrocolloid impression material resulted in an impression with antimicrobial activity. In addition, there was no

effect on the impression materials ability to reproduce surface details, but the antimicrobial addition may affect its dimensional stability.

**Keywords:** antimicrobial efficiency, hypochlorous acid, incorporation, irreversible hydrocolloid, self disinfecting

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## Introduction

Controlling biologically associated risks in medical services and dental care clinic environments is one of the crucial measures necessary for preventing cross contamination among health workers and patients. The dentist requires an efficient, affordable, readily available, harmless, and practical disinfecting agent for controlling infectious diseases

[1,2]. Prosthetic appliances and their construction steps are a source of contamination, starting from the impression-making procedure, as they are easily polluted by the saliva and/or blood of the patient. As a result, strict measures must be implemented in dental offices and labs to minimize the risk of cross infection [3,4].

Several methods have been used for disinfecting dental impressions. Some of which are ultraviolet C (UVC) radiation, gaseous ozone, or using chemical disinfectant like 2% glutaraldehyde, 0.5% sodium hypochlorite, or propolis [5-9]. It is recommended that a hospital-grade disinfecting agent be used to disinfect dental impressions. There is shared agreement that further research is necessary to

achieve the best chemical disinfection composition, concentration, and ideal exposure duration. Also, there is always a need to evaluate the interaction between impression materials and disinfecting solutions [10,11].

Irreversible hydrocolloid impression materials are associated with high bacterial contamination because of their hydrophilicity [12,13]. Extensive studies have been done on various disinfecting agents, techniques, exposure durations, and antimicrobial chemicals. Most studies advised the use of sodium hypochlorite or glutaraldehyde as disinfectants, although they had adverse effects on the dimensional stability and detail reproduction [14]. Also, it is recommended that sodium hypochlorite at 0.5% or iodophors could be used for irreversible hydrocolloids (alginate) impression disinfection by spraying, as prolonged immersion can lead to damage and deterioration [2]. However, there is still a need for a leading global disinfectant. Hypochlorous acid (HOCl) is one of the most efficient disinfectants against germs while being completely harmless, non-toxic, and natural [15]. HOCl has a wide range of uses, such as in the

treatment of wounds, dentistry, and as an antibacterial and virucidal agent [16,17]. HOCl was shown to have a wide range of antimicrobial activity for the suppression of several gram positive and gram-negative pathogens. At the same time, it had no detrimental side effects such as mucosal irritation or discoloration of the extrinsic tooth surface and restoration [18,19]. In addition, *C. albicans*, *S. aureus*, and *P. aeruginosa* were effectively reduced in count with hypochlorous acid spray disinfection technique [20].

The effectiveness of disinfection on impression materials is widely overlooked in dental practices. Since none of the available impression materials were specifically developed for microbial decontamination, disinfecting solutions may have an undesirable effect on the dimensional stability of impressions and the fabrication of the gypsum model. The disinfection procedure for impression materials was evaluated by various studies to assess the surface characteristics and dimensional stability [21,22]. Dental impressions were better disinfected with the immersion than the spray disinfection technique [23]. Some limitations

of the immersion and spray disinfection procedures have been identified, including dimensional changes and inadequate disinfection, respectively. Incorporation of disinfectants into irreversible hydrocolloid impression materials has been developed to address the shortcomings of spray and immersion disinfection procedures [24]. Therefore, this study was aimed to evaluate the effect of incorporating HOCl at two concentration of 100 and 200 ppm on some properties of irreversible hydrocolloid materials in addition to its antimicrobial efficiency.

## Methods

This work was approved by the research ethics committee of the College of Dentistry, University of Baghdad (ref. number 660, project number 660222 at 13.9.2022). The participants gave a written informed consent for the study. Specimens were prepared from an irreversible hydrocolloid impression material (Alginate, Zhermack, Tropicalgin, Italy) and according to the requirements for each test. The powder/liquid ratio for mixing the impression material was as recommended by the manufacturer. However, for the test groups, instead of distilled water (DW), HOCl was added at

concentrations of 100 and 200 ppm.

### Specimens' Grouping

The total number of specimens included in this study were 75 specimens. They were subdivided according to the test into 45 specimens for the antimicrobial test and 30 specimens for reproduction of details test and dimensional stability test. The following represent the three main specimen groups for each test:

DW (control group): alginate mixed with distilled water (no additive).

HOCl 100: alginate mixed with 100 ppm of HOCl.

HOCl 200: alginate mixed with 200 ppm of HOCl.

### Hypochlorous acid disinfectant solution preparation

The hypochlorous acid disinfection solutions were produced on-site with the use of the Eco One system (EcoloxTech, West Palm Beach, FL., USA) by adding one teaspoon of white vinegar (5%) and 2 g of non-iodized salt (kosher salt) to one liter of water. The manufacturer's instruction were followed for producing the two HOCl concentrations of 100 ppm and 200 ppm [4].

### Specimen preparation for antimicrobial test

Forty-five alginate specimens with 1 mm thickness and 12 mm in diameter were prepared and distributed evenly for the three groups. These specimens were fabricated from alginate powder mixed with sterile distilled water, 100 ppm of HOCl, or 200 ppm of HOCl for 45 seconds using hand mixing in a rubber bowl and with a spatula corresponding to the instructions of the manufacturer. The end of a 12 mm internal diameter disposable plastic hypodermic syringe was cut off and filled with the alginate mix. The set material was pushed out of the syringe by 1 mm thickness and sliced at the end of the syringe with a number 11 surgical blade [5].

### Antimicrobial activity test

*Staphylococcus aureus*, *Pseudomonas aeruginosa*, and *Candida albicans* were used in this study for testing the antimicrobial activity test. *Staphylococcus aureus* was used as a Gram-positive bacteria, *Pseudomonas aeruginosa* was chosen as a Gram-negative bacteria, and *Candida albicans* for Fungi [25]. Isolation of the microorganisms was from patients at the teaching hospital of Baghdad College of Dentistry in

accordance with the ethical approval of the ethical committee and a written informed consent obtained from the patients. The Vitek 2 was used for identification of the three microorganisms [26-28]. The microorganisms were cultivated and incubated for 24 hours in a Mueller-Hinton broth.

The antimicrobial activity for each group was tested separately with the disk diffusion test against the different microorganisms; *Candida albicans*, *Staphylococcus aureus*, and *Pseudomonas aeruginosa*. Inoculum suspensions for each of the above-mentioned microorganisms was prepared with a turbidity to the 0.5 McFarland standard, which corresponds to approximately  $1.5 \times 10^8$  colony forming unit (CFU)/ml. Three specimens were positioned evenly spaced apart in each petri dish. Then, inoculum suspension for each microorganism ( $1.5 \times 10^8$  CFU/ml) was seeded in 15 ml of Mueller-Hinton broth and poured over the specimens and according to each group. The petri dishes were kept for 60 minutes at room temperature to allow the diffusion of antimicrobial agent. Then, they were incubated aerobically at 37°C for 24 hours. The inhibition zones, after incubation, became evident around the specimens. The antibiotic zone scale was used to

measure the inhibition zones for evaluation of the antimicrobial effect [29].

Preparation of the specimens for the reproduction of details test and dimensional stability test

The surface detail reproduction, and dimensional stability of irreversible hydrocolloid impression materials were evaluated with the use of a ruled test block according to ISO specification number 1563:1990 for alginate impression material [30]. The alginate impression material was hand mixed according to the manufacturer's instructions, applied, and maintained against the ruled test block for a minimum of 3 minutes before removal. This ruled test block had three lines ( $50 \pm 8 \mu\text{m}$ ,  $20 \pm 4 \mu\text{m}$  and  $75 \pm 8 \mu\text{m}$  wide) inscribed on the superior surface with crosslines X and X', as seen in Figure 1. A ring mold surrounded the test block around the border and acted as a mold for the alginate impression material and was used for preparation of thirty specimens, 10 specimens for each test group.

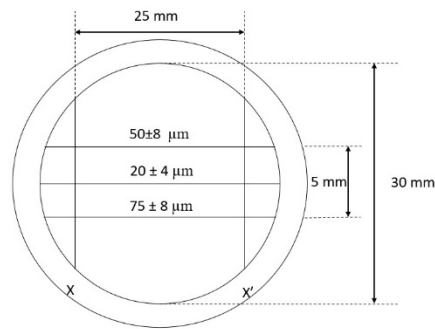


Figure 1. Diagram showing the dimensions of the ruled test block used for dimensional stability and reproduction of details tests.

### Dimensional Stability

Measurement A represented the measurement of the distance between the crossline X to X' on the ruled test block while the same measurement on the alginate impression specimens was recorded as measurement B. The measurements were made at the edges of the cross lines [30]. Measurements were made for each alginate impression specimen in every group and the following formula was used for calculating the dimensional change [31]:

$$\text{Dimensional change\% (percentage)} = (A - B) / A \times 100$$

### Reproduction of Details

The alginate impression specimens were inspected visually with low-angle illumination without magnification immediately after separation from the ring mold and test block. The reproduction of

details for the intended line ( $50 \pm 8 \mu\text{m}$ ) reproduced in the alginate impression specimen was considered satisfactory if continuous for the full 25 mm between the crosslines, according to ISO specification number 1563:1990 [30].

The data for all tests were statistically analyzed with GraphPad Prism version 10.2.0 (392)

### Results

All the tested groups showed inhibition zones for all tested microorganisms with an increase in the diameter of the zone with the higher concentration of HOCl (Figure 2). *Candida albicans* was the most sensitive to HOCl. In contrast, the control group (DW) did not show any inhibition zone for any of the tested microorganisms.

There was a significant difference between the groups for each of the different microorganisms when tested for antimicrobial activity (Figures 3-5). Further post hoc test with Tukey's HSD showed significant differences between the control group and each of the tested groups with exception for *Staphylococcus aureus* microorganism which was not significant ( $P = 0.1591$ ) between

the HOCl 100 and HOCl 200 test groups.

Dimensional stability of alginate was affected by the addition of HOCl. Statistical analysis for the dimensional stability test with one way ANOVA and the post hoc test (Tukey's HSD) showed significant differences  $P < 0.0001$  between the control group and each of the tested groups (HOCl 100 and HOCl 200). It was not significant

( $P=0.6784$ ) between the HOCl 100 and HOCl 200 test group as shown in Figure 6.

The results of details reproduction test presented a well-defined, sharp detail and continuous line for the full 25 mm between cross lines for all specimens in control group and tested groups (100 and 200 ppm HOCl). The means and standard deviations can be seen in

Figure 7 for the values of details reproduction for all groups.

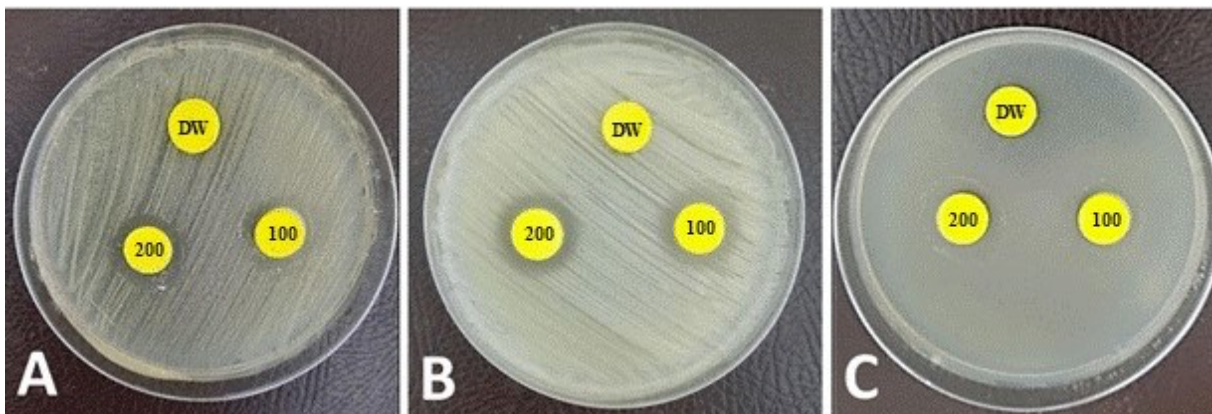


Figure 2. Inhibition zone around test group specimens for A) *C. albicans*, B) *S. aureus*, C) *P. aeruginosa* in which DW represent control group, 100 represent HOCl 100, and 200 represent HOCl 200 test group.

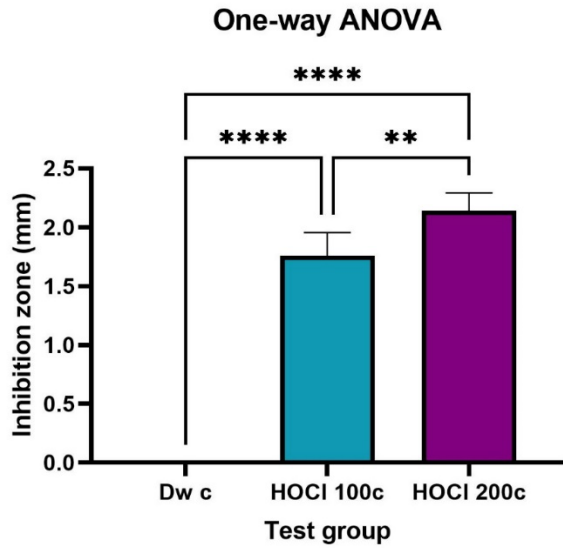


Figure 3. Means and standard deviations of inhibition zones (in mm), One way ANOVA and Tukey's HSD statistical analysis of disk diffusion test for *Candida albicans*. (\*\*\*\*) represented  $P < 0.0001$  and (\*\*) represent  $P = 0.0032$ .

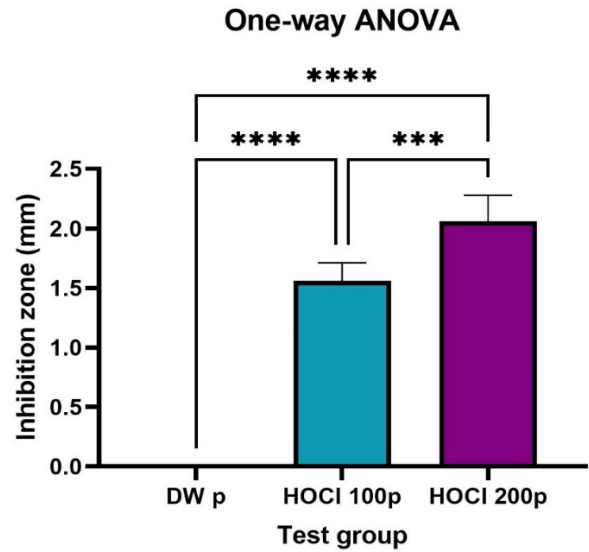


Figure 5. Means and standard deviations of inhibition zones (in mm), One way ANOVA and Tukey's HSD statistical analysis of disk diffusion test for *Pseudomonas aeruginosa*. (\*\*\*\*) represented  $P < 0.0001$  and (\*\*\*) represented  $P = 0.0007$ .

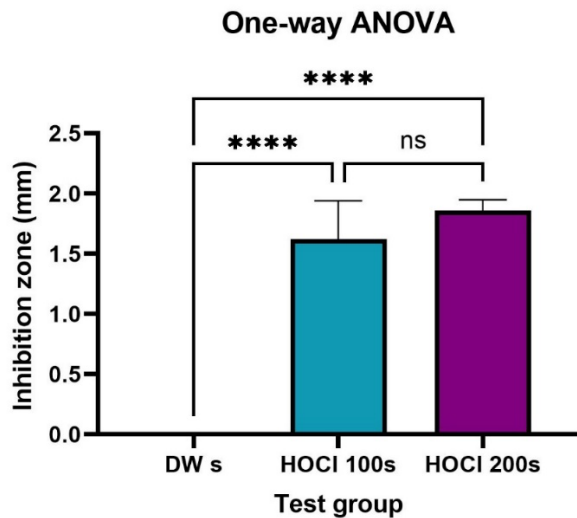


Figure 4. Means and standard deviations of inhibition zones (in mm), One way ANOVA and Tukey's HSD statistical analysis of disk diffusion test for *Staphylococcus aureus*. (\*\*\*\*) represented  $P < 0.0001$  and (ns) represent  $P \text{ value} = 0.1591$ .

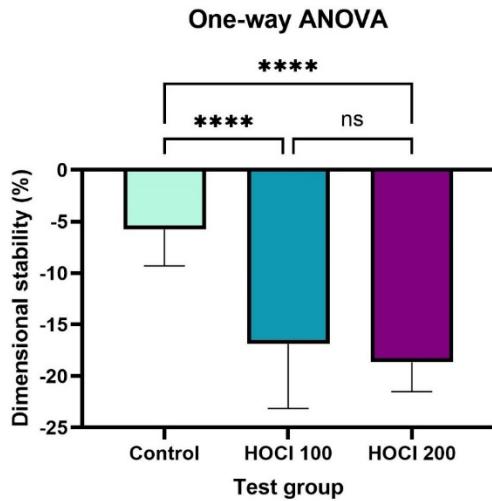


Figure 6. Means of dimensional stability in (%) and standard deviations, One way ANOVA and Tukey's HSD statistical analysis of dimensional stability test. (\*\*\*\*) represented  $P < 0.0001$  and (ns) represented  $P = 0.6784$ .

## Discussion

Disinfection is necessary for the different steps in prosthodontic treatment to prevent cross contamination starting from diagnosis to delivery of the prosthesis. This includes different impression materials, dental casts, occlusal rims, and dentures. They may be disinfected by different methods like microwave irradiation, immersion, or spraying with a disinfectant such as SOLO and sodium hypochlorite, or incorporating chemicals into gypsum materials like povidone iodine, glutaraldehyde, or sodium hypochlorite. These methods of disinfection and chemicals may

have some influence on the properties of the materials being disinfected [4,32-38].

Disinfection of irreversible hydrocolloid impression material was generally conducted by spraying or immersing the impression in disinfectant solutions. However, oral microbes are easily integrated into the impression materials during the setting step, so spraying or soaking the impression provides merely surface decontamination. As a result, there is a need for the development of self-disinfecting impression materials [39,40].

An ideal disinfecting agent should be harmless to the surface of the impressions, noncorrosive, efficient in a variety of forms, and reasonably priced [20]. HOCl

meets all those requirements in addition to that it exhibits broad-spectrum antibacterial, antiviral,

## Mean and SD

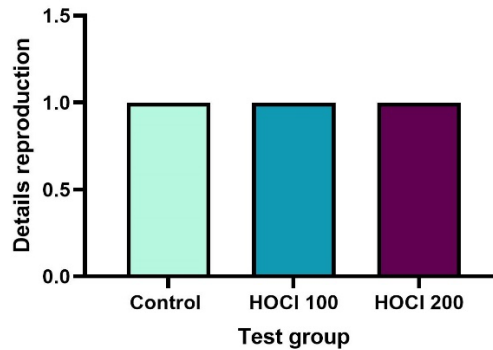


Figure 7. Means and standard deviations of details reproduction test.

and antifungal properties [41,42]. In this study HOCl was incorporated into irreversible hydrocolloid impression material to evaluate its antimicrobial efficacy and its effect on the impression material's dimensional stability and reproduction of details.

The incorporation of HOCl in alginate in two concentrations of 100 ppm and 200 ppm resulted in anti-microbial properties of the alginate material against the different microorganisms used in this study. This could be explained by the microbicidal activity of HOCl against multiple gram-positive bacteria, gram-negative bacteria, and yeasts [43]. This microbicidal activity resulted from interaction of HOCl with different functional protein groups,

involving chlorination of amino groups and oxidation of thiol groups of cysteine residues, thioether groups of methionine residues, disulfide groups, tryptophan residues, and heme groups [44-46]. These interactions impair the chain of electron transport and cause a reduction in the amount of adenine nucleotides [44], which are the major contributors in energy storage and transfer. Others reported that HOCl specifically bonds to the unsaturated lipid layer which subsequently results in disrupting cellular integrity [18, 41]. The antimicrobial efficacy of HOCl in the specimens was more effective with the higher concentration. The HOCl 200 group was significantly more effective against all tested microorganisms than HOCl 100 group and this comes in agreement with Chen et al. [47].

In this study it was found that significant dimensional changes resulted from HOCl incorporation. This could be attributed to the presence of HOCl that may have dissociated in aqueous solution forming hydrogen ion  $H^+$  and the hypochlorite group  $OCl^-$ . The hypochlorite has high affinity to bond with sodium ions and calcium ions [41], which are present in alginate powder. This

resulted in formation of sodium hypochlorite and calcium hypochlorite which may have led to depletion of the water in the mix and affected the powder/water ratio. The water is important for dissociation of calcium sulfate dihydrate reactor present in the alginate powder. The importance of this reactor is to convert the alginate compound from soluble (sodium or potassium alginate) to insoluble (calcium alginate) [48,49]. So, this process may have affected the crosslinking and gelation of alginate, and this may have resulted in permanent deformation of alginate specimens and dimensional changes. The implication of these results is that such dimensional changes in the impressions may lead to formation of dental casts that are dimensionally different from the that of the oral cavity and this may have an unsatisfactory result. However, further investigations need to be done to evaluate the clinical significance of these changes by using different measuring procedures like pouring a simulated clinical cast and measuring the resulted dimensional accuracy [50], or measuring the trueness and precision of the impression using a laser scanner with three-dimensional superimposition software [51].

Different studies evaluated the effects of disinfectants on the dimensional stability of the alginate impression material with different outcomes. Most of them found that disinfection didn't affect the dimensional accuracy of the alginate. Ravishankar et al. [39] reported a non-significant difference in the dimensional stability after incorporation of different concentrations of chlorhexidine, povidone iodine, and benzydamine into alginate. Also, a study conducted by Benakatti et al. [52] showed no dimensional alterations in alginate after mixing with 0.12% and 0.2% chlorhexidine. Other investigations, however, showed dimensional alteration after incorporating disinfecting agents like Ismail et al. [48] who found that the addition of different percentages of PVP-iodine powder to alginate impression material resulted in dimensional changes.

There was no effect of incorporation of HOCl in two concentrations of 100 ppm and 200 ppm on the reproduction of details. There was no loss in the sharpness or continuity of the lines. This could be attributed to the fact that there wasn't any change observed in the flowability of the unset irreversible hydrocolloid when placed over the



mold which was considered to be the most important factor affecting details reproduction [39].

This study has some limitations, one of which was the need for a positive control for evaluating the efficacy of HOCl with a well-established disinfectant for the alginate impression material. The microorganisms used were limited to bacteria and fungi and no antiviral activities were tested. The other properties of the alginate material were not evaluated and need to be tested after the incorporation of HOCl including the working time, setting time, wettability, change in color, tear strength, and compatibility with the gypsum products. In addition, other antimicrobial tests could be used as the viable count method and evaluating the microbial adhesion and antibiofilm effect after HOCl incorporation. Also, the antiviral activity of the incorporation method could be evaluated against different orally transmitted viruses as herpes simplex virus 1 (HSV1) and corona virus (SARS-CoV-2).

## Conclusions

The incorporation of 100 ppm and 200 ppm HOCl into irreversible hydrocolloid impression material results in an impression material with antimicrobial activity against

*Candida albicans*, *Staphylococcus aureus* and *Pseudomonas aeruginosa*. This incorporation adversely affects the dimensional stability of the impression material. Therefore, this restricts the use of HOCl as a disinfecting agent for alginate impressions until further investigations could evaluate the clinical significance of this effect or overcomes such a drawback. The ability of the irreversible hydrocolloid impression material to reproduce details was not affected by the incorporation of HOCl into the impression material.

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