Original Article

A Self-Disinfecting Irreversible Hydrocolloid Impression Material Mixed with Chlorhexidine Solution

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ABSTRACT

Objective: To examine the antibacterial effect and several physical properties of an irreversible hydrocolloid impression material mixed with chlorhexidine solution.

Materials and Methods: The experimental irreversible hydrocolloid specimens were prepared and allocated into four groups ($Group_{0.1\ g/L}$, $Group_{0.2\ g/L}$, $Group_{0.5\ g/L}$, $Group_{1.0\ g/L}$) according to the concentrations of chlorhexidine solution used as the mixing liquid. Specimens mixed with distilled water served as a control. The antibacterial effect, three-dimensional accuracy, flowability, and setting time were tested. Statistical analysis was performed using a one-way analysis of variance and a Tukey test, which was used for multiple comparisons ($\alpha = .05$).

Results: Zones of growth inhibition were observed around the test specimens, but not around the control specimens, and there were significant intergroup differences in the diameters of the inhibition zones. In the accuracy test, no significant differences ($P > .05$) were detected among all the measurements for all groups, and the accuracy was clinically acceptable. Also, no significant differences in the flowability ($P = .987$) and setting time ($P = .103$) were detected.

Conclusion: Chlorhexidine self-disinfecting irreversible hydrocolloid impression material can exhibit varying degrees of antibacterial activity without influencing the three-dimensional accuracy, flowability, and setting time.

KEY WORDS: Impression; Disinfection; Accuracy; Setting time

INTRODUCTION

Dental practitioners, patients, and laboratory personnel are subject to notable risks with respect to infectious diseases, which can be spread by saliva or blood from contaminated impression material, particularly irreversible hydrocolloid impression material.[1–3] Guidelines have been established by the American Dental Association (ADA) to limit cross-contamination during dental clinical and laboratory procedures such as impression disinfection.[4]

On the basis of these guidelines, researchers have proposed many methods of disinfection for irreversible hydrocolloid impression material. Among them, spray and immersion are the two most widely used techniques in clinical practice. However, these conventional strategies present several disadvantages. Although disinfection by immersion or spraying could be effective in reducing the chances of cross-infection, compliance by dental offices/clinics has been uneven.[5] Surveys indicate that a range of 37.5% to 90% of impressions are routinely disinfected,[6] and until now, many impressions have been sent to laboratories without having gone through any disinfection process.[7–9] The reasons for this include the following: (1) disinfection involves an overt effort or action; (2) spraying or immersing impression material with disinfectants may cause a loss of surface detail and dimensional accuracy of the impression[10–14]; (3) most of the disinfectants used for spray and immersion techniques are irritants and, therefore, inhalation of the disinfectant vapors may present health risks to the dental team; and (4) toxic disinfectants may also result in the corrosion of metal trays or abnormal dislodgement of the impression from the tray.[15]

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The difficulties associated with disinfecting irreversible hydrocolloid impression material have resulted in the development of self-disinfecting irreversible hydrocolloid impression materials that are preimpregnated with disinfectants such as didecyldimethyl ammonium chloride. Follow-up studies[5,16,17] have shown that this technique reduced the overall quantity of bacteria on the impression material, demonstrated greater dimensional stability than spray and immersion techniques, and saved disinfection time.

However, for most of the self-disinfecting irreversible hydrocolloid impression materials, disinfectants are impregnated into the powder of impression material and few attempts have been made to add disinfectants into the mixing liquid. Therefore, in this study various concentrations of chlorhexidine acetate solution[18] were used to mix the irreversible hydrocolloid impression material powder, and the antibacterial effect, three-dimensional accuracy, flowability, and setting time of this chlorhexidine self-disinfecting impression material were then evaluated.

The purpose of the study was to determine the following: (1) whether in vitro antibacterial activity against eight representative pathogenic microbes could be obtained after chlorhexidine was used to mix the irreversible hydrocolloid impression material; (2) the effects of chlorhexidine solution on the three-dimensional accuracy, flowability, and setting time of the irreversible hydrocolloid impression material; and (3) the concentration of chlorhexidine recommended for producing the self-disinfecting impression material in clinical conditions.

MATERIALS AND METHODS

In this study, the irreversible hydrocolloid impression material powder (Heraplast NF, Heraeus Kulzer Dental Ltd, Shanghai, China) was mixed with chlorhexidine acetate solution (Chlorhexidine acetate CP 2000, Jiutai Pharmaceutical Co, Jinzhou, China) of various concentrations. The antibacterial effect, three-dimensional accuracy, flowability, and setting time of this self-disinfecting impression material were determined.

Preparation of Test and Control Specimens

Specimens made from irreversible hydrocolloid impression material were prepared in accordance with the specific requirements in different tests. The specimens were divided into five groups in each test: specimens mixed with 0.1 g/L chlorhexidine solution ($Group_{0.1\ g/L}$), specimens mixed with 0.2 g/L chlorhexidine solution ($Group_{0.2\ g/L}$), specimens mixed with 0.5 g/L chlorhexidine solution ($Group_{0.5\ g/L}$), specimens mixed with 1.0 g/L chlorhexidine solution ($Group_{1.0\ g/L}$), and specimens mixed with distilled water (control group). The group allocations were consistent for all tests.

Measurement of Antibacterial Effect

The agar well technique was used to assess the antibacterial activity of the specimens.[14] First, the irreversible hydrocolloid impression material was mixed according to the powder/liquid ratio (10 g/23 mL) recommended by the manufacturer. Immediately after mixing, the material was placed in a mold and kept under slight pressure (2 kg) for 1 minute. Then impression disks, 10 mm in diameter by 1 mm in thickness, were prepared. Mean weight of the disks was 0.1014 ± 0.003273 g. After that, wells of the same size as the impression disk were cut into nutrient agar plates (Nutrient Agar, Difco 213000, BD, Franklin Lakes, NJ) previously inoculated with the appropriate microorganisms under sterile conditions. On each agar plate, five wells were cut and specimen was selected from each of the four test groups ($Group_{0.1\ g/L}$, $Group_{0.2\ g/L}$, $Group_{0.5\ g/L}$, $Group_{1.0\ g/L)}$ and put into four of the agar wells, respectively. The control specimen was placed in the fifth or center well of each plate. Three independent assays were performed for each microorganism ($n = 3$). Finally, all plates were incubated in the appropriate aerobic and anaerobic environment for 24 to 48 hours at 37°C.

After incubation, the clear zones or inhibitory areas around the specimens were measured with an intelligent analyzer of bacteria inhibiting ring (ZY-300IV, Xianqu Weifeng Co, Beijing, China) to evaluate the antibacterial effect. Three plates for each microorganism were put into this machine at one time, the plates were scanned by a charge-coupled device scanner, and the digital images were transferred to the computer. Finally, the inhibition zones on the plates were automatically measured by the accompanying software. The following microorganisms were used: *Streptococcus mutans* ATCC (American Type Culture Collection, The Global Bioresource Center) 25175, *Actinomyces viscosus* ATCC 19246, *Porphyromonas gingivalis* ATCC 33277, *Lactobacillus acidophilus* ATCC 4356, *Staphylococcus aureus* ATCC 12600, *Staphylococcus epidermidis* ATCC 14990, *Escherichia coli* ATCC 35328, and *Pseudomonas aeruginosa* ATCC 25314. A one-way analysis of variance (ANOVA) test ($\alpha = .05$) was used to determine if a significant difference existed between groups, and a Tukey test was conducted for multiple comparisons.

Measurement of Three-dimensional Accuracy

The three-dimensional accuracy was evaluated with an indirect technique that consisted of four steps. First, a stainless steel master die, designed by Tjan[19] was

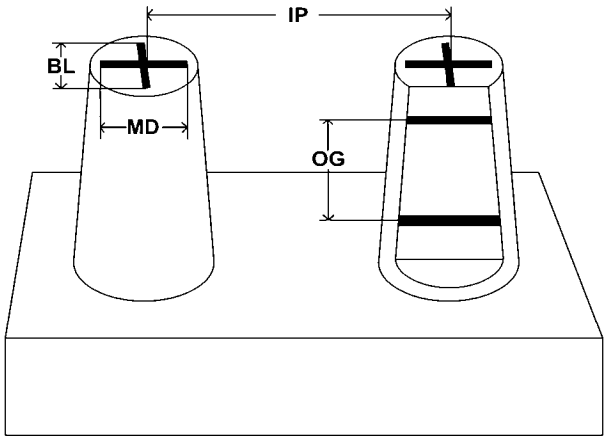

Figure 1. Schematic diagram of stainless steel master die used to simulate abutments of fixed partial denture. Reference lines are inscribed for measurement. IP indicates interpreparation; MD, mesiodistal; BL, buccolingual; and OG, occlusogingival.

machined. It consisted of two stainless posts on a stainless steel base simulating a three-unit fixed partial denture. Lines were inscribed on this master die to provide references for the measurement (Figure 1). Second, a custom tray of this steel die was fabricated. A wax spacer was positioned over the entire master die, resembling an inverted loaf, to provide uniform spacing (2 mm) and consistent seating against the die base for the acrylic resin trays. Uniformly spaced perforations were placed in the trays with a round bur (3 mm in diameter) to retain the irreversible hydrocolloid without an adhesive. Impressions of this master die were poured in die stone (die-stone, type IV; Heraeus Kulzer Dental Ltd). Impressions of this steel die were taken with the custom tray, and stone casts were recovered. The impressions were allowed to set for 6 minutes at room temperature before they were poured in die stone. The die stone was hand-mixed to wet the powder, then mechanically spatulated with an automatic vacuum mixing and stirring instrument (JG-5812; Jing-Gong Medical Equipment Co, Tianjing, China) for 15 seconds. A water/powder ratio of 22 mL water to 100 g powder was used for each mix.

The stone casts were allowed to set for 2 hours before separation and were dried at room temperature for at least 24 hours before being measured. Finally, measurements of four dimensions were recorded for the recovered stone casts to indirectly assess the three-dimensional accuracy. The dimensions measured included interpreparation (IP), mesiodistal (MD), buccolingual (BL), and occlusogingival (OG). Four test groups and one control group were tested with 10 replications of each group in a total of 50 trials for each dimension ($n = 10$). Measurements of the metal master die and stone casts were recorded using an electronic digital caliper (electronic digital calipers HY-097, 0.01 mm; Huayi Co, Hangzhou, China). Differences between the mean dimensions of the stone casts and the steel master die were expressed as percentage of deviation. One-way ANOVA test was used to analyze the results ($\alpha = .05$).

Measurement of Flowability

Flowability was measured by comparing the diameter of the impression disks. These were fabricated by injecting 0.5 mL impression material onto a glass slab ($15 \times 15 \times 2$ mm) using a disposable syringe within 60 seconds of mixing. Another glass slab was then placed on top of the impression material, and a standard weight of 1.5 kg was placed on the upper plate. Five seconds later, the weight was removed and the diameters of the impression disks were measured using the intelligent analyzer of bacteria-inhibiting ring. Means and standard deviations were recorded to indirectly assess the flowability. Three specimens were included in each group ($n = 3$). A one-way ANOVA test was performed to detect the presence of group differences ($\alpha = .05$). All procedures were performed in accordance with American National Standards Institute (ANSI)/ADA specification no. 18 for irreversible hydrocolloid impression material.[20]

Measurement of Setting Time

Setting time was tested according to the method introduced by Lemon et al.[21] The impression material was mixed for 60 seconds and syringed on the surface of a flat glass slab. Sixty seconds after mixing, the flat end of a polished poly (methyl methacrylate) rod measuring 6 mm in diameter and 10 cm in length was placed in contact with the exposed surface of the material and then immediately withdrawn. This procedure was repeated at 3-second intervals in the early stages of setting and at 1-second intervals at the later stages until the impression material no longer adhered to the end of the rod. Setting time was established as beginning at the start of the mix and ending at the point at which the impression material no longer adhered to the end of the rod. Also, there were three replicate specimens in each group ($n = 3$).

Statistics

The results were reported and submitted to one-way ANOVA test ($\alpha = .05$). All the data were evaluated by ANSI/ADA specification no. 18. One-way ANOVA analyses and the Tukey test were performed using a statistical analysis program (SPSS 12.0, SPSS Inc, Chicago, Ill) and the significance level was .05.

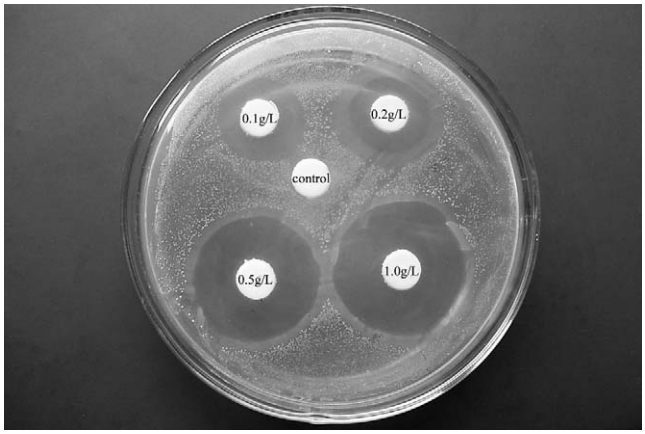

Figure 2. Well-defined zones of inhibited growth of *Streptococcus mutans* around impression disks after incubation. Concentrations of chlorhexidine solution used to fabricate impression disks were labeled, and the central disk served as control.

RESULTS

Well-defined zones of inhibited growth became apparent after this incubation period and allowed for consistent measuring of inhibitory fields (Figure 2). Mean diameters of inhibited zones for each microorganism are presented in Table 1. The results demonstrated that zones of growth inhibition around the specimens were observed on all plates. On the plates that were inoculated with *P aeruginosa*, inhibition zones were observed only around the specimens of $Group_{1.0\ g/L}$, whereas on the plates inoculated with the other seven bacteria, growth inhibition was detected around all the test specimens ($Group_{0.1\ g/L}$, $Group_{0.2\ g/L}$, $Group_{0.5\ g/L}$, $Group_{1.0\ g/L}$). No zones of inhibited growth were observed around the control wells on all agar plates. One-way analysis of variance and the Tukey test revealed that the inhibition zones tested became significantly larger ($P < .001$) for each microorganism when the concentrations of chlorhexidine solution were raised from 0.1g/L to 1.0 g/L.

The means and standard deviations of the dimensional changes measured at four dimensions are presented in Table 2. No significant differences ($P > .05$) were identified between groups for all dimensions (IP, MD, OG, and BL). The discrepancies between the master die and stone casts in the BL and OG dimensions were positive for each group, which indicated that the stone casts were larger in these dimensions than the metal master die. However, the discrepancies in the IP and MD dimensions were negative, which indicated that the stone casts were smaller in these dimensions.

In the flowability test, the mean diameters of the impression specimens for the four test groups were 32.0, 32.4, 32.0, and 32.2 mm, respectively; the mean diameter for the control group was 32.3 mm (Table 3). One-way analysis of variance ($\alpha = .05$) revealed no significant differences between groups ($P = .987$). The average setting time for the control group was 140 seconds, and setting time for the four test groups was between 140 and 150 seconds (Table 4). Statistical analysis of the data by one-way analysis of variance ($\alpha = .05$) indicated no significant differences in the setting time between groups ($P = .103$). The flowability and setting time of all specimens satisfied all the requirements of ANSI/ADA specification no. 18.

Table 1. Mean diameter of inhibition zone (mm) and standard deviation for each bacterial species and group

Species	0.1 g/L	0.2 g/L	0.5 g/L	1.0 g/L	Control
Streptococcus mutans	20.3 ± 0.9	27.2 ± 0.2	33.0 ± 0.7	37.2 ± 0.8	0
Actinomyces viscosus	12.9 ± 0.1	19.3 ± 0.2	27.0 ± 0.2	30.1 ± 0.8	0
Porphyromonas gingivalis	18.9 ± 0.6	20.7 ± 0.1	27.3 ± 0.5	28.9 ± 0.5	0
Lactobacillus acidophilus	12.5 ± 0.1	19.3 ± 0.6	28.1 ± 0.5	31.1 ± 1.2	0
Staphylococcus aureus	12.0 ± 0.2	14.5 ± 0.5	18.5 ± 0.5	21.4 ± 0.9	0
Staphylococcus epidermidis	10.7 ± 0.1	14.3 ± 0.4	16.3 ± 0.3	19.4 ± 0.7	0
Escherichia coli	11.4 ± 0.3	13.5 ± 0.4	16.0 ± 0.5	18.1 ± 0.4	0
Pseudomonas aeruginosa	0	0	0	15.2 ± 0.5	0

Table 2. Mean ± standard deviation and percent of dimensional change between master die and stone casts (mm) for each group (n =10)[a]

Group	MD (10.40)[b]		BL (10.32)[b]		IP (41.12)[b]		OG (14.74)[b]	
Control	10.36 ± 0.06	−0.38%	10.34 ± 0.04	0.19%	40.90 ± 0.04	−0.54%	14.76 ± 0.06	0.14%
0.1 g/L	10.36 ± 0.06	−0.38%	10.34 ± 0.04	0.19%	40.90 ± 0.06	−0.54%	14.77 ± 0.07	0.20%
0.2 g/L	10.37 ± 0.05	−0.29%	10.35 ± 0.03	0.29%	40.92 ± 0.04	−0.49%	14.78 ± 0.08	0.27%
0.5 g/L	10.38 ± 0.05	−0.19%	10.36 ± 0.05	0.39%	40.95 ± 0.08	−0.41%	14.81 ± 0.05	0.47%
1.0 g/L	10.39 ± 0.05	−0.00%	10.37 ± 0.03	0.48%	40.95 ± 0.04	−0.41%	14.82 ± 0.06	0.54%

[a] MD, mesiodistal; BL, buccolingual; IP, interpreparation; OG, occlusogingival. Numbers set in boldface represent percent of dimensional change between master die and stone casts.

[b] Measurements of metal master die in four dimensions.

Table 3. Data for flowability test (mm)[a]

Group	$\overline{D}_1$	$\overline{D}_2$	$\overline{D}_3$	Mean	SD
Control	34.1	30.1	32.7	32.3*	2.03
0.1 g/L	32.7	31.3	31.9	32.0*	0.70
0.2 g/L	31.6	33.3	32.2	32.4*	0.86
0.5 g/L	32.3	30.6	32.9	32.0*	1.19
1.0 g/L	32.9	31.7	31.9	32.2*	0.64

[a] $\overline{D}_1$, $\overline{D}_2$, $\overline{D}_3$, mean of flowability values for three specimens in each group. SD indicates standard deviation.
* $P = .987 > .05$, 1-way analysis of variance test ($\alpha = .05$).

Table 4. Test values for each of three setting times (seconds)[a]

Group	t_1	t_2	t_3	Mean	SD
Control	140	144	145	144*	2.5
0.1 g/L	144	145	146	144*	2.1
0.2 g/L	150	143	147	147*	3.5
0.5 g/L	149	144	150	148*	3.2
1.0 g/L	140	142	144	142*	2.0

[a] t_1, t_2, t_3, indicates mean setting time for three specimens in each group. SD indicates standard deviation.
* $P = .103 > .05$, 1-way analysis of variance test ($\alpha = .05$).

DISCUSSION

Greater efficiency and effectiveness could be achieved through the use of disinfectant-supplemented irreversible hydrocolloid impression materials compared with the other disinfection techniques. Flanagan et al[5] tested the antibacterial effects of two alginates with no added disinfectant and three others supplemented with chlorhexidine or quaternary ammonium compounds. The results revealed that the quaternary-ammonium-containing alginates were completely effective against all five test microorganisms. The alginate with chlorhexidine killed all the gram-negative bacilli and the majority (95% to 99%) of the gram-positive cocci and yeast. However, those alginates without supplements had no antimicrobial effects. The study of Cserna et al[16] confirmed that the irreversible hydrocolloids with chlorhexidine and quaternary ammonium were effective in reducing surface growth of the bacteria studied; so did the studies of Tobias et al[14] and Rice et al.[17] In our study, we have come to a similar result that chlorhexidine-containing irreversible hydrocolloid impression material possessed surface antibacterial effects on all the eight tested microbial species, and alginates without supplements had no antimicrobial effects.

Self-disinfecting irreversible hydrocolloid impression materials have another specific advantage. As is known, oral microorganisms can easily become incorporated into setting impression materials. Immersing or spraying rinsed impressions can only provide a surface disinfection effect. However, the self-disinfecting impression would be disinfected throughout the material and not just on the surface as would normally occur. Evidence shows that microorganisms are present within the material as the material takes up oral fluids and microbes while setting. Flanagan et al[5] verified this statement. Therefore, disinfectant-implemented alginates could eliminate most of the test microbes that were incorporated into the set impression materials and, in most cases, no viable cells could be recovered even when the specimens were processed immediately after setting.

Chlorhexidine is a broad-spectrum disinfectant that is widely acknowledged as an extremely effective antiplaque and antigingivitis agent.[22] It has been studied mostly in mouth-rinse formulations and is safe and effective.[23] Irreversible hydrocolloid impression material will come in contact with the oral mucosa directly during setting, so we have selected the four test concentrations of chlorhexidine within the often used concentration range for mouthwash, which is between 0.1 and 2.0 g/L. Although mouth rinses containing 1.2 g/L chlorhexidine are ADA accepted[22] and available on a prescription basis for treating gingivitis, studies indicate that 1.2 g/L chlorhexidine is cytotoxic to human fibroblasts in vitro[24] and is able to induce primary DNA damage in leukocytes and oral mucosal cells.[25] In view of this, attempts have been made in this study to select a concentration that is lower than 1.2 g/L, but that can still achieve sufficient antibacterial activity. Therefore, 0.1, 0.2, 0.5, and 1.0 g/L were selected as the four test concentrations, with 1.0 g/L being the highest.

Further evidence includes the following. First, the disinfection time of chlorhexidine solution was significantly shortened when the concentration was raised from 0.1 to 1.0 g/L; however, the antibacterial activity did not increase accordingly when the concentration was higher than 1.0 g/L.[18] This statement was also verified in our preliminary trials in which the diameters of the inhibition zones did not increase when the concentration of chlorhexidine was higher than 1.0 g/L. Second, a literature review[26] indicated that the minimum inhibitory concentrations of chlorhexidine against most of the microorganisms tested in our study ranged from 0.00267 to 0.08 g/L, which were lower than the concentrations currently used in our research. Third, the irritation of high-concentration (higher than 1.0 g/L) chlorhexidine solution compromises patient comfort. Based on the results of this study, we suggest that a chlorhexidine solution of 1.0 g/L might prove useful as the mixing liquid to produce the self-disinfecting irreversible hydrocolloid impression material for clinical use.

Samaranayake et al[3] noted that the self-disinfecting impression material containing ammonium chloride showed a total kill of microorganisms immediately after impressions were made. Therefore, in the antibacterial

effect test, all specimens were prepared and put into the agar wells within 8 minutes from the start of mixing. Eight representative pathogenic microbes (four aerobes and four anaerobes) were used as indicators of antimicrobial activity, and the antimicrobial effect of chlorhexidine on most of the microorganisms tested has been shown previously.[27] *S mutans, L acidophilus,* and *A viscosus* are oral cariogenic bacteria[28] and *P gingivalis* is a known periopathogen.[29] *S aureus, S epidermidis, E coli,* and *P aeruginosa* are pathogenic bacteria that have been widely used by others[26,27,30] as indicators of the effectiveness of disinfection protocols.

The accuracy phase indicated that stone casts were smaller in the MD and IP dimensions than the metal die and larger in the BL and OG dimensions. Although there is no evident reason, it is speculated that it may be related to the tray design.[31] It was also examined in the results that maximal dimensional discrepancy was 0.54% for OG dimension and −0.54% for IP dimension, but all specimens appeared comparable with values reported by other studies[12,32] and were within clinically acceptable limits for accuracy.[13] In conclusion, the three-dimensional accuracy of the irreversible hydrocolloid was not influenced, even if chlorhexidine solution served as the mixing liquid.

The flowability and setting time are important working properties for irreversible hydrocolloid impression material, but they are also the properties that are liable to be influenced by variable factors, including water temperature, relative humidity, and mixing duration. For this reason, in the tests for flowability and setting time, the water temperature (20 ± 2°C), relative humidity (50 ± 5%), mixing duration (60 seconds), and powder/water ratio (10 g/23 mL) were kept constant throughout the procedures to reduce errors caused by these factors. The results showed that mixing irreversible hydrocolloid impression material with chlorhexidine solution would not affect the flowability and setting time.

It is important to recognize the limitations of in vitro antimicrobial susceptibility testing per se and the difficulty in correlating in vitro results with the in vivo activity. Therefore, further research is needed to substantiate this self-disinfecting impression material using other in vitro microbial assays, for example, whole plaque or *Candida* species or even an in vivo test to substantiate the present findings.

CONCLUSIONS

- Chlorhexidine self-disinfecting irreversible hydrocolloid impression material can exhibit varying degrees of antibacterial activity in vitro and its three-dimensional accuracy, flowability, and setting time will not be influenced.
- Based on the findings of this study, 1.0 g/L is the recommended concentration for chlorhexidine solution to produce the self-disinfecting impression material.

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