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The effect of disinfection protocols on dimensional accuracy of irreversible hydrocolloids and *Candida albicans* colonisation

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Abstract

Irreversible hydrocolloid is a widely used impression material in dental practices. However, improper disinfectant protocols may affect the dimensional accuracy of the cast and increase susceptibility towards cross-infection. This study investigated the effect of disinfectants and disinfection protocols on the dimensional accuracy of alginate impression and *Candida albicans* biofilm formation. For methodology, an alginate impression was developed using an acrylic maxillary edentulous master cast with 3 reference points. 60 3cm round alginate beads were synthesised and treated with 2% Aseptoprint, 1% sodium hypochlorite (NaOCl) or 3% MD 520 solutions for 2 min, 1 h, 6 h, and 24 h. For the antibiofilm assay, beads were inoculated with *C. albicans* (ATCC MYA 4901) for 24 h, and Colony-Forming Units (CFUs) were counted using a haemocytometer, then analysed via two-way Analysis of Variance (ANOVA). Dimensional accuracy was assessed by treating the developed alginate impressions in 2% Aseptoprint, 1% NaOCl, and 3% MD 520, respectively, for 2 min, 1 h, 6 h, and 24 h. Three linear measurements were obtained and compared against the master cast and analysed statistically using Friedman Tests. The results showed that a significant reduction of CFUs was recorded after disinfection with 3% MD 520 and 2% Aseptoprint ($P < 0.05$). Dimension of alginate impressions was significantly changed after 6 and 24 h of disinfection time. In conclusion, the use of 3% MD 520 and 2% Aseptoprint with 1 hour immersion time is recommended for effective alginate impression disinfection with minimal change in dimensional accuracy.

Keywords: *Candida albicans, dimensional accuracy, infection control, irreversible hydrocolloid*

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Introduction

Infection control is paramount in every dental protocol, such that healthcare professionals are compelled to practice standard infection control in clinical settings to avoid the risk of cross-infection between dental practitioners, patients, and other dental auxiliaries. The principal transmission route to dental technicians is via improperly disinfected dental impressions in the clinic. According to Arzmi *et al*. (2015), dental impressions taken using any type of materials will be contaminated with the patient's saliva and blood that contain various interkingdom microorganisms. Infectious diseases, such as Hepatitis B, have a higher risk of cross-contamination since the hepatitis B virus surface antigen is commonly detected in the saliva of infected individuals (Kamimura *et al.*, 2021). Additionally, a recent study reported that approximately 67% of materials delivered to dental laboratories are exposed to diverse infectious microorganisms (Jain & Nur, 2018). Given that the spread of infectious diseases can be prevented through proper infection control protocols, disinfection of dental impressions is considered a top priority, which can be attained through the spray or immersion technique.

In dental clinics, impression-making is a common procedure that records hard and soft tissue details for diagnostic investigation, prostheses construction, orthodontics study models, and other purposes. An excellent impression should reproduce accurate details, exhibit good tear strength, and is unaltered when removed from the mouth. Impression materials can be classified into two types: elastic and non-elastic. In addition, the elastic type of impression can be further divided into aqueous and non-aqueous. The irreversible hydrocolloid (alginate) is an example of aqueous elastic impression material and has been broadly used as an impression material in dental practices due to its low cost, hydrophilic, ease of handling, and pleasant taste and odour. Nevertheless, alginates exhibit weak dimensional stability and are more vulnerable to alterations during the disinfection process compared to elastomeric materials (Al-Harby & Ibrahim, 2011). A recent study by Joana *et al.* (2013) concluded that irreversible hydrocolloid is a potential vehicle for microbial transmission and carries two to three times more microbes than elastomeric impressions. Besides, alginates' porous nature and high water content require special considerations in the disinfection protocol (Doddamani, 2011), which imparts additional labour, cost, and inconvenience for its practical application.

A mould must be dimensionally stable and has highly detailed reproduction for the restorative treatment to be clinically successful according to Doddamani, 2011. Previously, Guiraldo *et al.* (2014) discovered that the final alginate structure is extremely sensitive to the volume of water in the fibrillar structure. Consequently, the dimensional stability of an irreversible hydrocolloid mould is highly vulnerable to changes in the water content. Irreversible hydrocolloids may undergo mould expansion by imbibition (water sorption) or mould shrinkage due to syneresis (water loss), which compromises the detailed reproduction and dimensional stability of the mould. Fahimeh *et al.* (2011) deduced that the use of the immersion technique is bound by a time limit as the material is susceptible to wet conditions.

An ideal disinfectant should possess a dual functionality, which includes serving as an effective antimicrobial agent and simultaneously preserving the dimensional accuracy and surface features of the impression material as well as the resultant gypsum cast without any side effects (Walker *et al.*, 2010 and Amin *et al.,* 2009). Practically, it is rarely possible to disinfect the impressions within the recommended disinfection time due to common setbacks in dental clinics, such as the long queue of patients. Generally, dental impressions are soaked in the disinfectant longer than the recommended immersion time. However, this practice could induce changes in the dimensional accuracy of the impression and

potentially affect the fit of future prostheses or appliances (Kotsiomiti *et al.*, 2008).

To date, numerous disinfectants are available and are utilised according to the manufacturer's instructions. Sodium hypochlorite (NaOCl), glutaraldehyde, iodophor, and phenol are among the frequently used disinfecting agents, particularly for dental impressions in dentistry. However, the suitability of certain disinfectants has been disputed due to the potential effect of the disinfectant and the extent of distortion or degradation on the impressions (Rentzia *et al.,* 2011). Thus, selecting compatible disinfection and proper protocol is essential to minimise the risk of microbial infections, simultaneously preserve the integrity and dimension of the impressions, and ensure a fitting prosthesis and accurate model construction study.

Apparently, various studies have explored the effect of disinfectants on the dimensional accuracy of alginate impressions. Nevertheless, the antimicrobial activity and performance of three types of the most common disinfectants, including Aseptoprint, NaOCl, and MD 520, have not been reported. Thus, this two-stage study was performed to investigate the effect of several disinfectants and treatment protocols on the dimensional accuracy of irreversible hydrocolloid and biofilm formation of *Candida albicans*.

Materials and Methods

Preparation of disinfectants

Three types of commonly used disinfectant solutions in clinical settings were selected for this study, namely: Aseptoprint (OCC Switzerland), sodium hypochlorite (NaOCl), and MD 520 (Durr Dental). The disinfectants were prepared as follows: 10 mL of Aseptoprint was mixed with 500 mL of water to achieve 2% Aseptoprint; 1 mL of Clorox was mixed with 100 mL of water to yield 1% NaOCl; 30 mL of MD 520 was mixed with 970 mL of water to produce 3% MD 520. The study design is an in vitro study.

Preparation of impression

An acrylic maxillary edentulous master cast was constructed with reference points A, B, and C. The reference points were made by small pointed acrylic prominences at the approximate position of the incisive papilla (A) and left and right maxillary tuberosity (B and C), as shown in Figure 1.

The alginate impression was developed using the Hydrocolor 5 alginate powder (Zhermack SpA, Italy) on a spaced and perforated special tray (Vertex, Netherlands). The alginate powder-to-water ratio was measured using respective measuring scoops according to the manufacturer's instructions. An alginate mixer (Motion F1, Taiwan) was used to blend the mixture for 10 sec to form a homogenous alginate mixture. The impressions were then immediately cast with Gypsum Type 3 (Sheralpin, Germany) mixed in a Vacuum Mixer (Motova 100, Bremen, Germany) and allowed to set at room temperature. The vibrator (Denstar 510, Korea) was used to eliminate small air bubbles on the cast surface during the pouring procedure. Finger pressure was used to fix the special tray loaded with alginate to the master cast. For standardisation, one operator repeated the same steps to all 12 impressions. The impressions were then left to set for 5 min. The experimental casts were allowed to sit at room temperature for an hour before being separated from the impressions.

Preparation of alginate beads

Rubber cups were used as the mould to synthesise 60 round alginate beads with the size of 3 cm in diameter each. The beads were then wrapped with wet gauze and sealed in plastic bags to prevent water from evaporating the beads. Then, the beads were treated with 2% Aseptoprint, 1% NaOCl or 3% MD 520 solutions at different immersion times of 2 min, 1 h, 6 h, and 24 h. The beads were soaked in distilled water at each immersion time to serve as the control. Once the disinfection is complete, the beads were stored at 4 °C.

Growth of microorganism

Candida albicans (ATCC MYA 4901) were provided by the Cluster of Cancer Research Initiative IIUM (COCRII) for this study. The yeast was revived from glycerol stock in a sterile nutrient broth (NB) and incubated at 37 °C aerobically for 24 h.

Antibiofilm assay

Approximately 5 mL of *C. albicans* suspension was inserted into new sterile containers using a pipette. A single alginate bead was then transferred into the yeast suspension and incubated at 37 °C for 24 h. Note that the bead was fully submerged in the yeast suspension to ensure full contact between the bead and the yeast. Following the incubation, the bead was removed from the suspension and rinsed with 1 mL of phosphate buffer saline (PBS) before being transferred into a new sterile container containing sterile PBS. The containers were agitated for 60 sec to remove the biofilm from the bead. Next, approximately 100 µL of each sample was pipetted and inserted onto a haemocytometer (Neubauer Assistant, Germany) to quantify the Colony-Forming Unit (CFU) of the viable *C. albicans* cell under a light microscope (Olympus, Japan). The assay was conducted in triplicates.

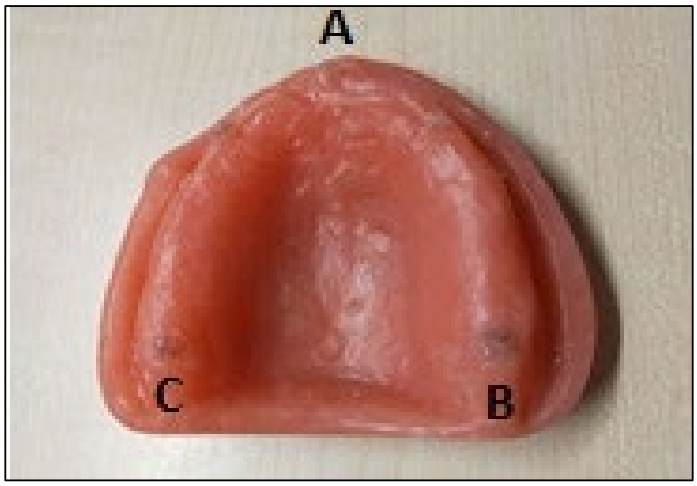

Figure 1. Maxillary edentulous master cast and the position of reference points.

Dimensional accuracy

The developed impressions were divided into three main groups. The first group of four impressions were immersed in 2% Aseptoprint, while the second group of four impressions were immersed in 1% NaOCl. The third group of four impressions were immersed in 3% MD 520. All three groups were immersed in the respective disinfectant for 2 min, 1 h, 6 h, and 24 h. Following the immersion process, all impressions were wrapped with damp gauze and sealed in a plastic bag during transportation to the dental laboratory to prevent water evaporation.

For the dimensional accuracy analysis, three readings were measured for each linear measurement (A-B, A-C, B-C) of each experimental cast, including the master cast as the control. A digital calliper (Mitutoyo 0–150 mm; Japan) with an accuracy of $\pm$0.01 mm was used for the measurement. The collected data were analysed.

Statistical analysis

The statistical analysis was conducted using SPSS Statistic software version 27. The two-way ANOVA was applied to assess the anti-biofilm activity by determining the significant difference between the disinfection protocols. A *P*-value of 0.05 was considered significant. In addition, the dimensional accuracy was evaluated using the Friedman Test at a significance value of 0.05 based on the setting in the SPSS software. The test was

performed to investigate the differences in the dimension between the master cast and the three groups of different disinfectant solutions with different immersion times.

Result

Antibiofilm activity

Table 1 shows the mean count of the viable *C. albicans* from all impressions that were immersed in the respective disinfectants. From the table, the lowest CFU count across all immersion times was recorded by the 3% MD 520 compared to other disinfectants. However, the 3% MD 520 and 2% Aseptoprint showed a significant decrease in the CFU count compared to the control group at 2 min and 1 h immersion times ($P < 0.05$). In addition, the 6 h immersion time was insignificant for all disinfectants ($P > 0.05$). In terms of the immersion time, it was found that a prolonged immersion time leads to a lower *C. albicans* count.

A linear graph of CFU count at different immersion times is illustrated in Figure 2. The graph depicts the disinfection efficacy of each disinfectant with the lowest mean of *C. albicans* count obtained using 3% MD 520 solution, followed by 2% Aseptoprint and 1% NaOCl.

Table 1. Means and standard deviations of CFU of C. albicans counted from alginate beads immersed in different disinfectants and immersion time. The study was conducted in triplicate (N = 3).

	CFU x 10^4 (mL)			
Time	**Control**	**2% Aseptoprint**	**1% NaOCl**	**3% MD 520**
2 min	3.17 (0.14)	1.90 (0.97)[a]	2.50 (0.50)	1.33 (1.50)[a]
1 h	2.83 (0.14)	1.70 (0.34)[a]	1.30 (1.20)	0.67 (0.58)[a]
6 h	1.25 (0.43)	1.30 (0.34)	1.90 (0.51)	0.67 (0.58)
24 h	1.22 (0.19)	1.10 (1.20)	1.30 (0.58)	0.00 (0.00)[a]

[a]Significant difference (P < 0.05)

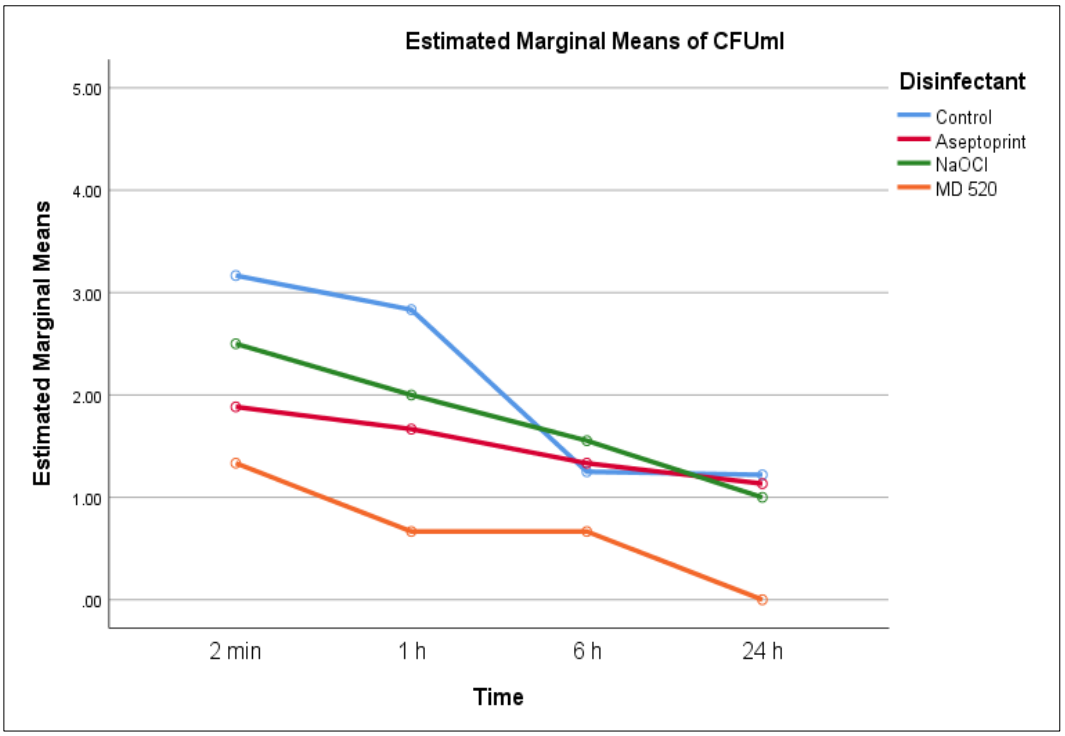

Figure 2. C. albicans count (CFU) in alginate beads immersed in different disinfectants and at different immersion times.

Dimensional accuracy

Table 2 lists the mean and standard deviation of the linear measurements of the constructed alginate impression casts that were disinfected using 2% Aseptoprint, 1% NaOCl, and 3% MD 520 at different immersion times. Generally, the dimensions of all disinfectants recorded an insignificant difference at 2 min and an hour of immersion time. However, a significant difference ($P < 0.05$) was observed in the dimensions when immersed in 1% NaOCl and 3% MD 520 for 6 h. Additionally, the measured dimension of all three disinfectants exhibited a significant difference when immersed in the disinfectants for 24 h ($P < 0.05$).

Figure 3 illustrates demonstrates the increasing patterns for all linear measurements (A-B, A-C, B-C). The results imply that the dimensions of the experimental casts increased following the immersion in all three disinfectants for 2 min, 1 h, 6 h, and 24 h compared to the constant dimension of the master cast.

Table 2. Mean and standard deviation of all linear measurements on the master cast and experimental casts constructed from alginate impressions immersed in different disinfectants and immersion times.

Disinfectant	Time	Mean distance (standard deviation) in mm
Master cast	-	48.94 (0.77)
2% Aseptoprint	2 min	49.17 (0.67)
	1 h	49.26 (0.26)
	6 h	49.37 (0.60)
	24 h	49.47 (0.59)[a]
1% NaOCl	2 min	49.14 (0.67)
	1 h	49.29 (0.61)
	6 h	49.37 (0.59)[a]
	24 h	49.45 (0.62)[a]
3% MD 520	2 min	49.15 (0.67)
	1 h	49.22 (0.65)
	6 h	49.40 (0.57)[a]
	24 h	49.46 (0.58)[a]

[a]Significant difference (P < 0.05)

Discussion

Disinfection procedure is a routine clinical practice that essentially protects dental personnel against exposure to infectious microbial diseases, including viruses; hepatitis B, hepatitis C, herpes, Human Immunodeficiency Virus (HIV), and Mycobacterium tuberculosis, when directly handling the impressions, according to Hemalatha & Ganapathy, 2018. Previously, it was reported that microorganisms were more persistent in irreversible hydrocolloid impressions, which makes it more challenging during the disinfection process (Vatansever, 2013). Additionally, a recent study by Sukhija *et al.* (2010) revealed that impressions of dentulous patients convey twice the amount of microbial load compared to that of edentulous patients. Thus, proper disinfection protocol is vital to ensure effective antimicrobial activity and to prevent cross-infection among dental practitioners. Concurrently, it is crucial to determine the optimal immersion time so that the physical properties of the irreversible hydrocolloid impressions are maintained since the slightest change in the dimensional accuracy of the constructed cast may affect the fit of future prostheses or appliances.

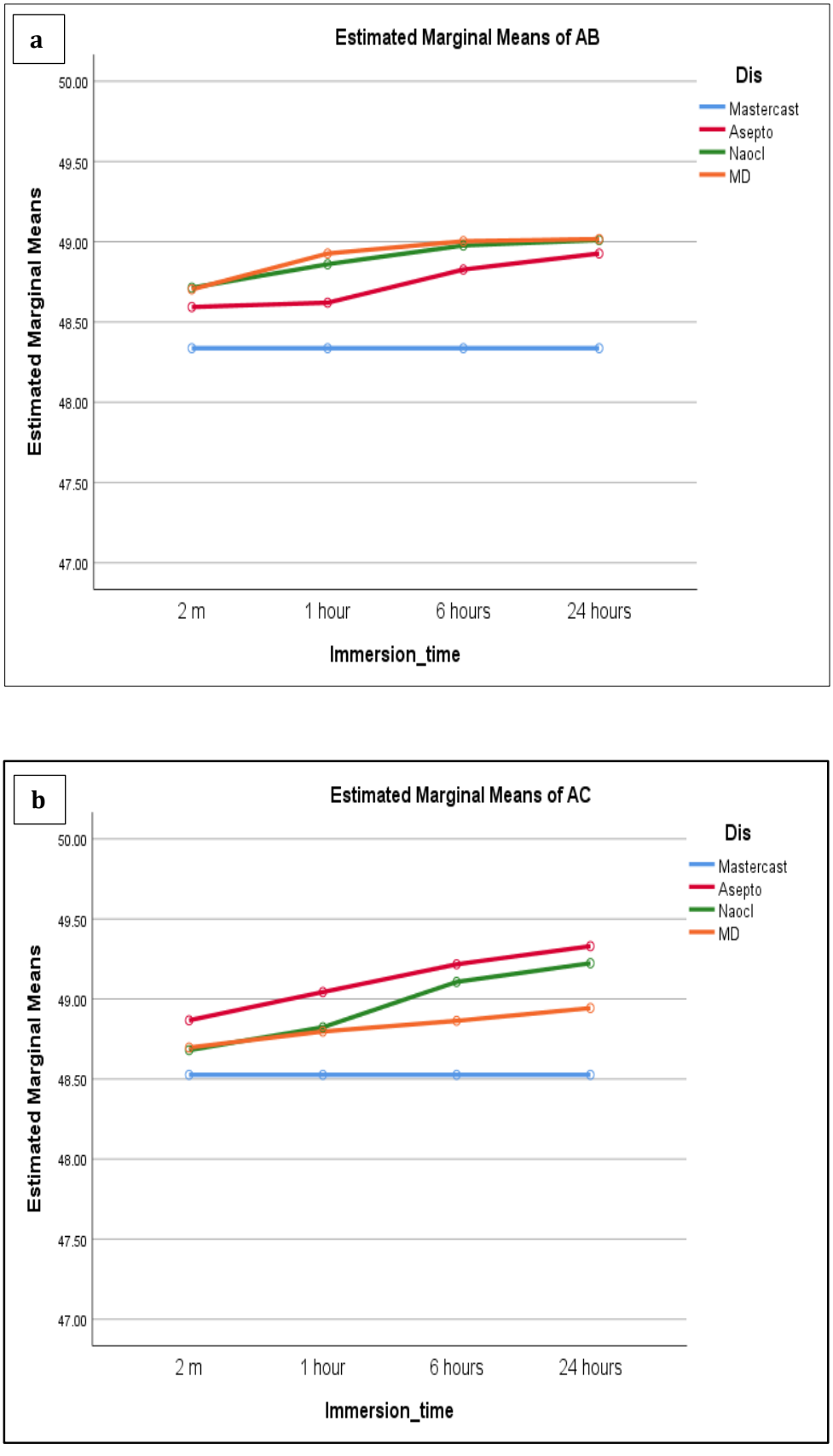

Figure 3. Means of dimensions for linear measurements A-B (a), A-C (b) and B-C(c) on casts produced from impressions immersed in different disinfectants and different immersion times.

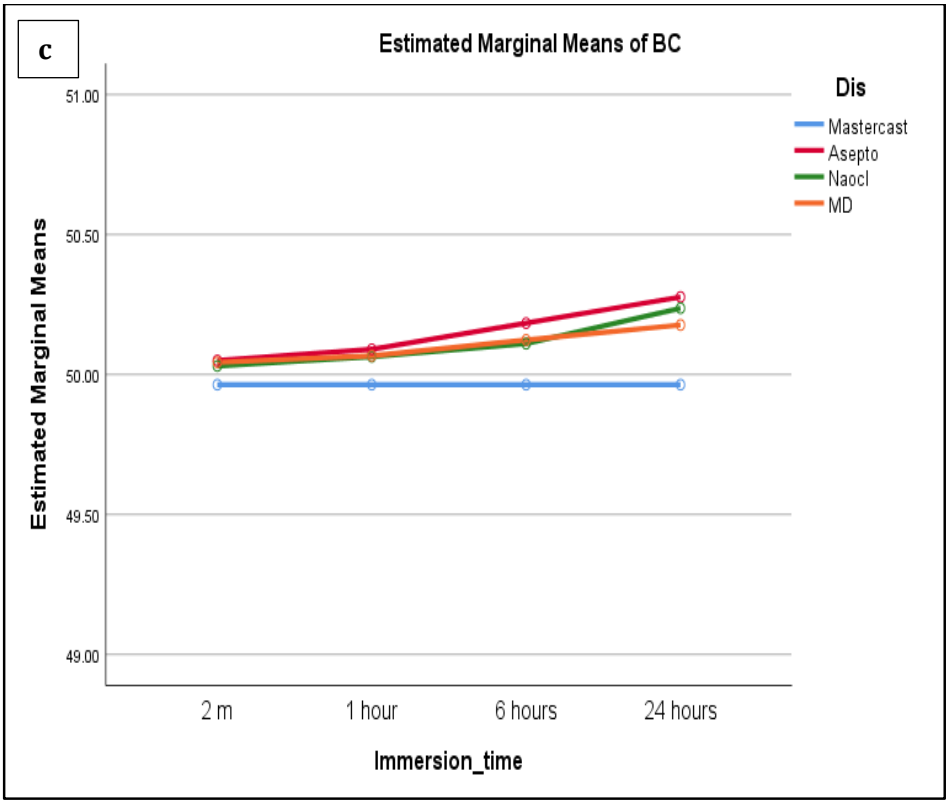

Figure 3 (continued).

C. albicans was employed in this study since it is one of the most prevalent oral microflora species. In addition, numerous studies including Dasgupta *et al.,* 2013 and Samra & Bhide (2010) have reported the presence of yeast on alginate impressions. According to Ferreira *et al*. (2017), *C. albicans* is the main causative microorganism for denture-related infections, which induces inflammation and erythematous condition of the oral mucosa or denture stomatitis, especially the upper dentures. Moreover, Badrian *et al.* (2012) stated that Candida is a common opportunistic fungal organism that causes oral candidiasis in immunocompromised patients. Among all fungi, Candida species are the most persistent colonisers in the oral cavity and have adapted to reside as a commensal (Patel, 2022), indicating the importance of effective disinfection of all impression materials prior to sending them to the laboratory.

For comparative purposes, the control group was not treated with any disinfectants but was only immersed in distilled water. As expected it was found that the fungal colony was still present after being immersed in distilled water. This is consistent with the study by Taylor *et al.* (2002), who reported that using distilled water could reduce the microbial count up to 50–90%, but not 100%. In another study, Joana *et al.* (2013) recorded that the microbial load of alginate impression decreased significantly by 48.5% after being washed with distilled water. Nevertheless, an effective disinfection procedure is still needed due to the significant presence of other microorganisms that remain on the dental impressions.

This study applied the immersion disinfection technique over the spray method. This was based on the report by Fahimeh *et al.* (2011) that considered the spraying technique's effectiveness inferior to the immersion technique due to less contact time between the impression and the disinfectant. Samra and Bhide (2010) also stated that the immersion method is the most reliable disinfection method because the surface of the impressions is fully

submerged and in direct contact with the disinfectant. Additionally, chemical disinfectants can be divided into three categories. High-level disinfectants, such as glutaraldehyde, can deactivate spores, while intermediate-level disinfectants, which include formaldehyde, chlorine-based compounds, iodophors, and alcohols phenolic compounds, may not deactivate spores but can eliminate other microorganisms. In contrast, low-level disinfectants, such as quaternary ammonium compounds and simple phenols, are unqualified to function as disinfecting agents to treat contaminated dental impressions.

For this study, the performance of two types of high-level disinfectants comprising NaOCl (a derivative of chlorine compound) and MD 520 (glutaraldehyde-based) and one intermediate-level disinfectant, namely Aseptoprint (alcohol-based) was evaluated. Based on the results, the lowest Candida count was obtained from impressions immersed in 3% MD 520 solution in 24 h, indicating the highest antibiofilm activity, followed by 2% Aseptoprint and 1% NaOCl. Hence, 1% NaOCl showed the least efficiency for the removal of *C. albicans.*

Similar findings were reported in past studies. For instance, Demajo *et al.* (2016) demonstrated the effective use of glutaraldehyde-based disinfectant (MD 520) and alcohol-based disinfectant on alginate impressions. The MD 520 eliminated all microbes compared to that of the alcohol-based disinfectant. This finding was supported by Aeran *et al.* (2015), who recorded 100% removal of microbial colonies via immersion in 2% glutaraldehyde. Another study by Ahsan *et al.* (2013) concluded that 2% glutaraldehyde exhibited a higher antimicrobial effect compared to 1% NaOCl. On the contrary, Samra *et al.* (2010) reported a contradicting result in which NaOCl immersion achieved a greater efficiency compared to that of glutaraldehyde, which might be due to the use of a higher concentration of NaOCl at 5.25%. This was in line with the American Dental Association (ADA) recommendation, which suggests NaOCl as an effective disinfectant at a 1:10 dilution with a 10-minute immersion duration to disinfect irreversible hydrocolloid impressions (ADA, 1996).

In terms of the immersion time, a longer immersion time yields a higher reduction of *C. albicans* cell count. However, Bustos *et al.* (2010) found that the immersion of impressions in 0.5% NaOCl or 2% glutaraldehyde for 5 min was sufficient to achieve effective disinfection. Besides, it is important to reduce immersion time to maintain the dimensional stability and surface integrity of the impression materials. This was demonstrated through the dimensional accuracy assessment, where the immersion in MD 520 and NaOCl for 6 h and 24 h, respectively, resulted in a discrepancy in the linear measurements compared to the master cast. However, immersion for 2 min and 1 h did not show any difference in the measured dimensions for all three disinfectants.

Previously, Taylor *et al.* (2002) stated that the immersion of alginate impressions for an hour or above substantially affected the dimensional accuracy of the materials. In another study, the effect on alginate after 60 min of immersion in 2% glutaraldehyde showed no significant different dimensional changes (Peutzfeldt & Asmussen, 1989). This finding contradicted a study by Ismail *et al.* (2017), who recorded a statistically significant difference in the dimensional accuracy of alginate impression after being disinfected with 1% NaOCl and 2% glutaraldehyde for 1 h. Meanwhile, Amalan *et al.* (2013) showed that NaOCl induced surface alterations and reduced the detailed reproduction of the irreversible hydrocolloids. However, these alterations were below the 1.5% acceptable clinical limit, as established by the ISO guideline (ADA, 1992).

In addition, Ulgey *et al.* (2020) studied the comparison of dimensional accuracy of alginate impression between 15- and 30-min immersion time and found that 15 min immersion time caused minimal distortion in all the distances of impression compared to that of 30 min. This indicates that the

dimensional accuracy correlates with immersion time and is consistent with the present study. The alginate impression experienced a higher water absorption with increasing immersion time, corresponding to the casts' increasing dimensions following the disinfection process. The structure of alginates is susceptible to expansion as a result of water absorption or shrinkage due to moisture loss via evaporation (Wadhwa *et al.*, 2013). The result is in agreement with the findings by Ghada *et al.* and Ismail *et al.*, where they recorded an increase in linear measurements after the immersion of impression in the disinfectants.

Furthermore, Joana *et al.* (2013) found that glutaraldehyde-based disinfectant was effectively applied to remove all microbial forms from the alginate impressions without altering the dimensional stability. This finding is similar to the result of the present study, where 3% of MD 520 achieved the best antibiofilm properties with the least dimensional change. Nevertheless, one of the limitations of the present study is the use of the digital calliper with +0.01 mm accuracy to measure the distance between the reference points. It is recommended that future studies should employ a more sophisticated measurement, such as a scanned digital image of the cast and software, to precisely measure the distances of the casts.

Conclusion

This study successfully demonstrated the use of 3% MD 520 with an immersion time of 1 h to achieve the highest reduction of fungal count and minimal dimensional distortion of the irreversible hydrocolloid impression. Additionally, the immersion of 2% Aseptoprint reduced fungal count more efficiently than 1% NaOCl. Although a longer immersion time was effective against high fungal count in the irreversible hydrocolloids, significant changes in dimensional accuracy were recorded with more than 6 h immersion times. Overall, 3% MD 520 is considered a promising disinfecting agent for dental impression disinfection and other dental-related treatment.

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