

Effect of alginate impression disinfection with sodium hypochlorite and castor oil on *Candida albicans* counts and dimensional stability of the study model

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ABSTRACT

Alginate is the most common used impression material because it is easy to use, affordable, and well accepted by patients. When impression is performed, alginate would be in contacting with the surface of the oral cavity and Saliva. It caused the microflora, like *Candida albicans* would adhere to the surface so that it can cause cross-infection. Cross-infection prevention could be done by disinfecting the impression material before filling with gypsum. The main thing of choosing a disinfectant is that the disinfectant material could eliminate microorganisms and affect the dimensional stability of impression material. This study aimed to determine the effect of alginate impression disinfection with sodium hypochlorite and castor oil (*Ricinus communis oil*) on the *C. albicans* counts and the dimensional stability of the study model. The sample of *C. albicans* was counted by colony counter and the dimensional stability tested by digital caliper. This study showed that alginate impression disinfection with castor oil had almost the same effectiveness as sodium hypochlorite in reducing the *C. albicans* counts, but the dimension was changed even though the value of the dimensional changes is still within the tolerance limit.

Keywords: alginate, sodium hypochlorite, castor oil, *Candida albicans*, dimensional stability

INTRODUCTION

Dental Impression is the stage of making the negative imprint of the oral cavity tissue obtained from the impression material using an impression tray or custom impression tray into the oral cavity until the impression material is setting, then the impression was made into a study model or working model. Alginate is the most common used material because it is easy to use, affordable, and well accepted by patients.² One study found that 67% of the materials dentists sent to the dental laboratory were contaminated with *Streptococci*, *Staphylococci*, *P. aeruginosa*, *methicillin-resistant S. aureus* (MRSA), and *Candida* spp. *Candida albicans* are normal microflora in the oral cavity, which is 45% in neonates, 45-65% in healthy children and 39-45% in healthy adults, 50-65% in removable denture wearers, 90% in chemotherapy patients, and 95% in HIV patients. *C. albicans* could be pathogenic and causes opportunistic infection if there were predisposing factors such as low salivary pH and low salivary flow, tooth loss, poor oral hygiene, weakened immune system and systemic diseases. *C. albicans* count in the oral cavity will increase if there is one of these predisposing factors. This allowed cross-infection from patients, tools, and impression materials to dentists and dental technicians in the dental laboratory, thus preventing infection transmission and reducing cross-contamination before the impressions are sent to the laboratory, infection

control must be carried out by disinfecting the impression before filling it with dental stones.³⁻⁷

There are two types of impression disinfection, such as immersion and spraying. Saber claimed that impression disinfection by immersion showed the same antimicrobial activity with the spraying technique.⁸ Alginate impression should not be soaked for too long in disinfectant liquid because the impression quality would decrease due to water absorption. The sprayed alginate impression had smaller dimensional changes compared to the immersion because less liquid was absorbed. The dimensional stability of the alginate impression is a critical aspect to the success of making accurate impression. Therefore, the spraying was the most effective technique to reduce imbibition that could affect the dimensional stability of the impression. Before disinfecting, the impression material was rinsed under running water to clean the debris and saliva attached. Thereafter, the impression was sprayed with 3 mL of disinfectant solution for 30 seconds at a distance of ± 5 cm and the impression was inserted into a sterile plastic clip bag for 10 minutes. According to the American National Standards Institute/American Dental Association (ANSI/ADA) specification No. 18 changes in dimensions that occur in impression material less than 0,5%.⁹

Chemical disinfections were recommended by ADA guidelines because they are virusids, bactericids, and sporocids. Disinfecting materials that

were often used to disinfect impression material were divided into two types, namely non-traditional and traditional chemicals.¹⁰ Sodium hypochlorite is a commonly used, has prices that are not too expensive and show good bactericidal and fungicide properties compared to other chemical disinfectants.¹¹ Sodium hypochlorite also has disadvantages, such as the compound irritating mucous membranes, unpleasant odor, and a corrosive effect on metals. Therefore, it was necessary to search alternative natural materials with non-toxic disinfectant properties to prevent cross-infection.¹² One of the natural products that is commonly being studied in medicine is castor oil. It is relatively safe, biocompatible in periapical tissue, antimicrobial, bactericides, fungicides, and anti-inflammatory.¹³

This study was aimed to analyze the effect of alginate impression disinfection with 0.5% sodium hypochlorite and 10% castor oil on *C.albicans* count and dimensional stability of the study model.

METHODS

The research sample for calculating *C.albicans* counts used alginate impression obtained from the impression to the master model, while the sample for measuring dimensional stability in the study was a study model made by the type III plaster obtained from the results of pouring the maxillary master model. The model was prepared with a round bur on the mesioincisal of right central incisor, cusp of mesiopalatal of left first molar, and cusp of mesiopalatal of right first molar which was used as antero-posterior (AP) line and cross arch (CA) line.

Preparation of alginate samples

In the manufacturing of alginate impression samples, the master model was first disinfected by spraying 70% alcohol to avoid contamination with other microorganisms. Alginate impression material with a ratio of powder and liquid according to the manufacturer's instructions was put into a rubber bowl and stirred with a spatula until homogeneous. Alginate impression material that has been homogeneous is inserted into the appropriate tray and then impressed on the master model. After setting, then the tray is removed from the master model.

Procedure for making castor oil 10%

Prepare a 10 mL of castor oil solution and dissolve it into 100 mL of aquadest by adding 0,5 mL of Tween 80 solvent. The solution is stirred until homogeneous, then filtered with paper filter.

Candida albicans counts

The samples were divided into 3 groups; they

are A1 (aquadest), A2 (0.5% sodium hypochlorite, and A3 (10% castor oil). The impressions were stored in a plastic bag for 10 minutes, swabbed using a cotton swab and transferred to a test tube containing phosphate-buffered saline. The test tubes were vibrated using a vortex for 30 seconds so that *Candida* attached to the cotton swab could be released. Using a micropipette, 100 µL of phosphate buffer saline containing the *C.albicans* was transferred to a petri dish. Pour the thawed Sabouraud dextrose agar into a petri dish and then gently shake it over a flat surface and leave it until hardens. The petri dish was put into the incubator for 24 hours at 37°C. *C.albicans* counts was carried out after 24 hours being removed from the incubator, using a colony counter.

Dimensional stability (DS) measurement

In measuring dimensional stability, the contaminated alginate impression was rinsed with running water for 15 seconds. The samples were divided into 3 groups, namely group B1 (aquadest), group B2 (sodium hypochlorite 0,5%), group B3 (castor oil 10%). The alginate impressions were sprayed with 3 mL of disinfectant solution for 30 seconds at a distance 5 cm. Each impression was inserted into a sterile plastic clip bag for 10 minutes. The alginate impression was filled using a type-III dental stone on top of the vibrator and waited until the cast setting for about 45-60 minutes and then removed. The measurements were carried out by measuring the distance from the mesioincisal right central incisor to the mesiopalatal cusp of the maxillary left first molar (AP line) and measuring the distance between the mesial cusp of the palatal right first molar to the mesial cusp of the palatal left of the left first molar maxillary (CA line) using a digital caliper. The percentage of the dimensional changing obtains by using the formula

$$DS = \frac{\text{Master model} - \text{gypsum model}}{\text{Master model}} \times 100\%$$

RESULT

After the univariate analysis, *C.albicans* counts in alginate impression after disinfection was known group A1, the smallest value was 18 CFU/mL, the largest was 43 CFU/mL, and the mean value was 27.70±8.02. In group B1 the smallest value was 0 CFU/mL, the largest value was 1 CFU/mL, and the mean value was 0.20±0.42. In group C1 the smallest value was 0 CFU/mL, the largest was 3 CFU/mL, and the mean value was 1.00±0.81 (Table 1).

The dimensional stability value of the study model in group A2 viewed from the AP line the smallest value was 0.084% and the largest was 0.225%,

Table 1 *C.albican* counts in alginate impression after disinfection with sodium 0.5% hypochlorite and 10% castor oil

Sample	<i>C.albicans</i> counts (CFU/mL)		
	Aquadest (Group A1)	Sodium Hypochlorite (Group B1)	Castor oil (Group C1)
1	19	1**	1*
2	25	0*	3**
3	35	1	1
4	27	0	0
5	18*	0	0
6	22	0	1
7	24	0	1
8	36	0	1
9	28	0	1
10	43**	0	1
$\bar{X} \pm SD$	27.70 ± 8.02	0.20 ± 0.42	1.00 ± 0.81

Table 2 The dimensional stability value of the study model after disinfection with sodium hypochlorite 0,5% and castor oil 10% viewed from AP dan CA line

Sample	The dimensional stability value of the study model (%)					
	Aquadest (Group A2)		Sodium Hypochlorite (Group B2)		Castro oil (Group C2)	
	AP	CA	AP	CA	AP	CA
1	0.084*	0.115	0.169*	0.231	0.112*	0.202*
2	0.140	0.086	0.309	0.289	0.169	0.231
3	0.112	0.057*	0.252	0.202*	0.281	0.289
4	0.169	0.115	0.366	0.376	0.366	0.202
5	0.197	0.144	0.479**	0.550**	0.309	0.347
6	0.112	0.086	0.422	0.434	0.422	0.492**
7	0.225**	0.057	0.281	0.463	0.253	0.405
8	0.197	0.115	0.338	0.347	0.281	0.463
9	0.140	0.202**	0.450	0.289	0.450**	0.318
10	0.169	0.173	0.253	0.318	0.338	0.347
$\bar{X} \pm SD$	0.154 ± 0.044	0.115 ± 0.047	0.332 ± 0.098	0.349 ± 0.108	0.298 ± 0.104	0.329 ± 0.102

and the mean value was 0.154%±0.044, viewed from the CA line the smallest value was 0.057% and the largest was 0.202% with the mean value was 0.115%±0.047. In group B2 viewed from the AP line the smallest value was 0.169% and the largest was 0.479% with the mean value was 0.332% ±0.098; viewed from the CA line the smallest value was 0.202% and the largest was 0.550% with the mean value was 0.349%±0.108. In group C2, viewed from the AP line the smallest value was 0.112% and the largest was 0.450% with the mean value was 0.298%±0.104; viewed from the CA line the smallest value was 0.202% and the largest was 0.492% with the mean values was 0.329%±0.102 (Table 2).

The normality test was carried out on *C.albicans* counts using Shapiro-Wilk test and an abnormal distribution was known; so to determine the effect of disinfection of alginate impression with group A1, group B1, group C1 on *C.albicans* counts was tested statistically with the Kruskal-Wallis test. That found a significance level 0.0001 (p <0.05). It indicates that there was an effect of disinfection of alginate impression disinfection with group A1, group B1, group C1 on *C.albicans* counts (Table 3).

Table 3 Effect of alginate impression disinfection with 0.5% sodium hypochlorite and 10% castor oil on *C.albicans* counts

Group	N	<i>C.albicans</i> Counts	p
		$\bar{X} \pm SD$	
A1	10	27.70±8.02	0.0001*
B1	10	0.20±0.42	
C1	10	1.00±0.81	

The result of normality test of dimensional stability value using the Shapiro-Wilk test was an abnormal distribution so that to determine the effect of alginate impression disinfection with group A2, group B2, group C2 on study models dimensional stability, the study models were tested statistically with the one-way Anova test. The results of the statistical test from the AP line obtained a significant level of p = 0,0001 (p <0,05) and from the CA line obtained a significant level of p = 0.0001 (p <0.05). It indicates that there was an effect of disinfection of alginate impression with group A2, group B2, group C2 on dimensional stability value (Table 4).

DISCUSSION

C.albicans counts in groups A1, B1, C1 had different variations. The difference in *C.albicans*

Table 4 Effect of alginate impression disinfection with 0.5% sodium hypochlorite and 10% castor oil on dimensional stability value of the study model

Group	Line	The dimensional stability value (%)		
		n	$\bar{X} \pm SD$	p
A2	AP	10	0.154±0.044	0.0001*
B2		10	0.332±0.098	
C2		10	0.298±0.104	
A2	CA	10	0.115±0.047	0.0001*
B2		10	0.349±0.108	
C2		10	0.329±0.102	

counts were caused by effectiveness differences of disinfection between aquadest, 0.5% sodium hypochlorite, and 10% castor oil. Group A1 showed the highest *C.albicans* counts compared to other groups because the aquadest does not contain any disinfectants, so *C.albicans* was still attached to the alginate impression. It showed that washing the alginate impression under running water could only reduce the number of microorganisms attached to the impression by 40-90%.¹⁴ Therefore, the ADA said impression materials been washed under running water should be disinfected before pouring to prevent cross-infection to operators and dental laboratories.¹² Group B1 showed the least amount of *C.albicans* where the chlorine content in the released sodium hypochlorite adhered to the cell cytoplasm which could destroy important *C.albicans* proteins.¹⁵

The results of this study are in accordance with Ahirwar's research which shows that the amount of microorganisms in alginate impression is less after disinfection with sodium hypochlorite compared to aquadest.¹⁶ Group C1 also showed a lower amount of *C.albicans* than group A1 where the sodium ricinoleate component in castor oil correlated with a decrease in acid production, thereby inhibiting the formation of biofilms.^{17,18} The results of this study are in accordance with the research of Hanoem; disinfection of alginate impression with 50% neem oil showed a smaller amount of microorganism colonies compared to aquadest.¹⁹ The results of this study are in accordance with Basofi's research that disinfection of alginate impression with galangal rhizome decoction showed fewer bacterial colonies than aquadest.²⁰ The results of this study are also in accordance with the Trivedi research that disinfection of alginate impression with aloe vera showed a lower amount of *C.albicans* compared to aquadest.²¹

Values of DS were varied in each sample in one group; they might be due to the simple measuring tools used such as digital calipers which allow scratches on the distance between lines to be

measured in the study model so it can cause inaccurate measurements by the operator. In addition, the compressed stress was not matched by the strain when releasing the spoon from the master model which was not fast enough, so that the stress received would be greater than the strain, this could cause permanent deformation.²² Another possibility that could affect dimensional stability was air humidity. Accordance Arini, et al alginate impression stored in plastic would expand due to high humidity.²³ Based on ADA specification No.18, the dimensional stability value of the study model in the three groups (A2, B2, and C2) was still within tolerable limits (<0.5%). Group A2, the mean value of dimensional stability was smaller than the other groups because in the control group, the alginate impression was only sprayed with aquadest, so that the dimensional stability did not change too much. Group B2 showed an average value of dimensional stability that is greater than group C2 where sodium hypochlorite when reacted with water would decompose slowly, which would release chlorine, oxygen and sodium hydroxide causing an oxidation process. Oxygen was a strong oxidizing agent that could cause pressure fluctuations in the solution. During the disinfection process, the sodium hypochlorite solution was in contact with the alginate impression material, the imbibition nature of the alginate which absorbed water and the pressure on the absorbed solution causes the alginate impression to expand and the dimensional stability of the study model can change.²⁴

Group C2 shows a smaller mean value of dimensional stability than group B2, this might be due to the presence of phenol in castor oil which when in contact with alginate impression material would cause an esterification reaction that produces esters, such as the reaction of ester formation by bonding with carboxylic acids which form an ester contained in the chemical structure of the alginate impression material. The esterification reaction would produce esters and H₂O, while alginate had properties that were easy to imbibe causing the increase of the disinfectant solution absorption and affecting the dimensional stability of the study model.^{9,24,25}

Group B1 showed the greatest decrease in the amount of *C.albicans* caused by the disinfectant effect of sodium hypochlorite where the chlorine content of the released sodium hypochlorite adheres to the cell cytoplasm which could destroy important *C.albicans* proteins. The results of this study were in accordance with the research of Bustos, et al in their research explaining that 0.5% so-

dium hypochlorite could reduce the amount of bacteria including *C.albicans* in alginate impression.²⁶ The results of this study were also in accordance with research by Badrian, et al which showed a reduction in the amount of *C.albicans* in alginate impression after being sprayed using 0.525% sodium hypochlorite solution bacterial colonies on alginate impression after spraying with 0.5% sodium hypochlorite.²⁷ The results of this study are in accordance with Ahirwar's research that there was a reduction in the number bacterial colonies on alginate impression after spraying with 0.5% sodium hypochlorite.¹⁶

Group C1 showed a decrease in the amount of *C.albicans*. The main component of castor oil, such as sodium ricinoleate, correlates with a decrease in acid production which can inhibit the formation of biofilms. Castor oil had bactericidal and fungicidal effects because it contains substances such as alkaloids, saponins, tannins, terpenoids, steroids, glycosides, phenolics, and flavonoids.²⁸ According to Pisani, et al castor oil had a detergent action against microorganisms associated with cell wall damage, allowing cytoplasmic components to disappear and resulting in the cell death.¹⁷ The results of this study were in accordance with the research of Hanoem, et al in their research concluded that 50% neem oil spraying was effective in reducing the amount of microorganism colonies on alginate impression.¹⁹ The results of this study are also in accordance with the research of Basofi, et al that there are differences in the amount of bacterial colonies on alginate impression after immersed with galangal rhizome decoction.²⁰ According to Trivedi, et al spraying disinfection with aloe vera effectively reduced *C.albicans* counts in alginate impression.²¹

Disinfection of alginate impression with 0,5% sodium hypochlorite in group B2 showed an effect on the dimensional stability of the study model. This was because sodium hypochlorite when reacted with water would decompose slowly, which would release chlorine, oxygen, and sodium hydroxide causing an oxidation process. The oxygen was a

strong oxidizing agent that can cause pressure fluctuations in the solution. During the disinfection action, the sodium hypochlorite solution was in contact with the alginate impression material, the imbibition nature of the alginate which absorbs water, and the pressure on the adsorbed solution causes the alginate impression to expand and the dimensional stability of study model can change.²⁴ The results of this study were in accordance with previous research from Sari, et al spraying 0.5% sodium hypochlorite on alginate impression resulting in dimensional changes that were still within tolerance limits.²⁹ This study was also in accordance with the study of Amelia, et al. which showed there was a change in dimensional stability which was still within the tolerance limits of alginate impression after 0.5% sodium hypochlorite was sprayed.⁹

Disinfection of alginate impression with 10% castor oil in group C2 showed an effect on the dimensional stability of the study model. The results of this study were in accordance with previous study by Hasanah, et al concluded that disinfection of alginate impression by spraying 80% betel leaf solution resulted in insignificant changes in dimensional stability.³⁰ The results of this study are also in accordance with the study by Wirayuni, et al spraying noni extract on alginate impression produces dimensional changes that were still within tolerance limits.³¹ Betel leaf and noni had some of the same content as castor oil. The phenol content in castor oil if in contact with alginate impression material would occur an esterification reaction that produces esters and H₂O and the properties of alginate is easy to imbibition causing the increase of the disinfectant solution absorption so that the alginate impression changes the dimensions.^{24,25}

This study showed that alginate impression disinfection with castor oil had almost the same effectiveness as sodium hypochlorite in reducing *C.albicans* counts, but dimension was changed even though the value of the dimensional changes is still within the tolerance limit.

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