JKAU: Med. Sci., **Vol. 21** No. 3, pp: 3-15 (2014 A.D. / 1435 A.H.)
DOI: 10.4197/Med. 21-3.1

The Effect of Disinfecting Irreversible Hydrocolloid Impressions on Bacterial Count

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Abstract. This study aims to investigate the efficacy of the different concentrations of sodium hypochlorite on the count of bacteria found in irreversible hydrocolloid impressions. Forty-six mandibular irreversible hydrocolloid impressions were collected at King Abdulaziz University. They were submersed in sterilized containers with sterile distilled water. Different concentrations of sodium hypochlorite solutions were used as a disinfectant with a submersion time of one minute. All samples investigated exhibited bacterial growth. The geometric mean of bacterial count was significantly different according to the patients' gender. However, the difference was significant for sodium hypochlorite concentrations depending on gender and type of bacteria. The highest geometric mean of bacterial count was for male, while the lowest was for female. There was significant difference between types of bacteria with higher count of gram positive cocci. Sodium hypochlorite at 1% concentration and one minute immersion time has been shown to be an effective disinfectant to eliminate bacteria efficiently from irreversible hydrocolloid impressions.

Keywords: Impression material, Alginate, Bacteria, Gender, Disinfection.

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Accepted for publication: 22 May 2014. Received: 10 February 2014.

Introduction

Although, the oral environment harbors a large number of microorganisms in the saliva[1], the majority of organisms pose no significant risk to dental personnel[2]. Nevertheless, a number of them cause infectious diseases that may be incurable, such as Hepatitis B and human immunodeficiency viruses[3]. The risk of transmitting pathogenic micro-organisms from patients to health–care workers *via* contaminated impressions, appliances, and prosthetic materials has been considered a topic of importance[4,5]. Kugel *et al.*[6] surveyed dental laboratories and found only 44% knew that the impressions they received had been disinfected.

To avoid cross contamination, impressions should be disinfected immediately after their removal from the mouth[7-10]. Disinfection eliminates virtually all recognized pathogenic micro-organisms, but not necessarily all microbial forms such as spores. This may be accomplished by using a chemical disinfectant able to kill vegetative forms of pathogenic organisms, including influenza, enteroviruses, and tubercle bacillus[5]. Rinsing of the impression is necessary to remove blood, saliva, and debris that may prevent exposure of the impression surface to disinfectant[8,11-13]. However, it is not sufficient to simply rinse the impressions with water without adopting a further disinfection procedure, washing impression materials with water alone remove only 40% to 90% of bacteria[14]. It should be regarded as merely a gross decontamination, since it does not eliminate the cross-infection potential of alginate[15].

No universally accepted method appeared sufficient to fulfill the disinfection requirements without affecting the precision of impressions[16] and may partly explain the relatively limited use of chemicals for disinfection of impressions. The American Dental Association recommends a 10-minute immersion in a 1:10 dilution of sodium hypochlorite to disinfect irreversible hydrocolloid impressions[10]. Sodium hypochlorite is inexpensive, convenient to use, and one of the most commonly used disinfecting solutions[14]. Correia-Sousa *et al.*[15] recommended that disinfection with sodium hypochlorite was the most efficient, reducing alginate adhered

microorganism. Moreover, Jennings and Samaranayak[17] reported reduced colony forming units (cfu) of Pseudomonas aeruginosa and Candida albicans when contaminated impression materials were disinfected with a 0.0125% solution of sodium hypochlorite for 30 min. Also, Streptococcus sobrinus showed no growth after a 10-min immersion in 0.5% sodium hypochlorite.

Standardized procedures for susceptibility testing are available for a wide range of organisms, and in general, the guidelines provided by the National Committee for Clinical Laboratory Standards[18] are the most widely used. Several methods can be used to determine bacterial concentrations. These include direct counts, plate counts, filtration, and turbidimetric measurements. Bacterial concentrations can also be estimated with McFarland standards. Plate count requires that a measured volume of material be added to agar either by the pour plate or spread plate technique. If the original sample is highly contaminated, dilutions are prepared and plated[18].

While many publications have examined the validity of various disinfectants on impression materials[19-29], no mention has been made in the literature regarding the microorganism counts on the impressions. The purpose of this study was to investigate the count of the bacteria on irreversible hydrocolloid impressions after their submersion in different concentrations of sodium hypochlorite for 1 minute. The null hypothesis was that the use of sodium hypochlorite disinfectant would have no influence on the bacteria count in irreversible hydrocolloid impressions.

Materials and Methods

Irreversible hydrocolloid primary impressions (Kromopan, Lascod, Italy) of 46 candidates were collected. 23 males and 23 females (20-57 years old) were randomly selected from patients seeking prosthodontic treatment at the Faculty of Dentistry, King Abdulaziz University, Saudi Arabia. A detailed medical and dental history was obtained from each patient. Patients medically compromised or have received antibiotic treatment in the last 3 months were excluded from the study. The selection criterion was

that most of the natural teeth in the patient's mandibular arch were present (not less than 12 teeth). However, age matching was not possible. The Research Ethics Committee at King Abdulaziz University approved a protocol describing the specimen collection for this investigation.

Prior to dental treatment, mandibular impressions were made using irreversible hydrocolloid impression material. Material was mixed with sterilized water and prepared according to the manufacturer's recommendations at an ambient temperature of 20˚C ± 1˚C. Alginate adhesive (Hold; Water Pik, Fort Collins, CO USA) was placed on the sterile metal trays prior to making the impression. Each tray was filled with the impression material, and immediately overturned into the patient's mouth. A gradual, constantly increasing pressure was applied until the excess impression material was expelled to ensure contact between the impression material and the teeth. Impressions were removed with a snapping motion two min after the recommended time period to ensure complete setting and rinsed under running water for 30 seconds. Impressions were submersed immediately in sterilized plastic containers with 100 mL sterile distilled water for one minute to form a bacteria suspension. Each plastic container was numbered, so the evaluator was blinded to its content. Specimens were transported to the microbiology laboratory within two hrs.

To facilitate the bacteria count and prevent colonies from growing together and forming a solid layer, bacteria suspensions were shaken thoroughly in a mixer for 60 seconds (Vortex, Marconi, São Paulo, Brazil). Serial 10-fold dilutions were made from 1^{-1} to 1^{-4} with a series of tubes before plating. A clean, sterile, dry pipet was used to remove 0.1 mL of the bacterial suspension and blow it into the dilution fluid. Suspensions were filtered through separate 47 mm membrane filters (Micro-Funnel, Gelman Sciences, Ann Arbor, MI USA), and the filters were removed aseptically and inoculated on a sterile R2A agar plate (R2A Agar, Difc, Becton Dickinson Microbiology Systems, Sparks, MD USA). The plates were incubated aerobically at 37˚C for 72 hrs. This medium supports not only anaerobic bacteria, and spores but also the aerobic bacteria.

Bacterial colonies were counted and dilution factors applied to obtain the total number of colony-forming units (cfu/mL). An aseptic technique was followed by using gloves, protective clothing, masks, and eye protection during all culture procedures.

Using this protocol, one control and three experimental groups (n = 46) were established to ultimately determine the antimicrobial efficacy of sodium hypochlorite (Sainsbury's bleach; Sainsbury, London, UK) disinfection on irreversible hydrocolloid. Serial concentrations of sodium hypochlorite (Fox Packing Co., St. Paul, MN USA., batch number 62207-6) solutions: 0.5, 1, and 2 were used as a disinfectant with a submersion time of 1 minute. The data were analyzed statistically using SPSS Version 10 (SPSS Inc., Chicago IL USA). Repeated measures ANOVA were used, and as Mauchly's sphericity test indicated violation of sphericity assumption, therefore, degrees of freedom of within subjects' effects were corrected using the Greenhouse-Geisser Epsilon.

Results

The geometric mean of bacterial count at different sodium hypochlorite concentrations is shown in Table 1. Repeated measures ANOVA, demonstrated significant variation by concentration of sodium hypochlorite with sharp drop in geometric mean bacterial count with 0.5% concentrations from (1082) to (66).

Table 2 showed that the effect of concentration was significantly modified by both gender ($p < 0.001$) and type of bacteria ($p < 0.000$). The drop in geometric mean of bacterial count was faster among males than females, and with gram positive cocci and gram negative bacilli, rather than gram positive bacilli.

Considering between subject variations, Table 3 demonstrated a significant difference between males and females with higher geometric mean among males than females at each concentration. There was significant difference between types of bacteria with higher count of gram positive cocci (2314) and gram negative bacilli (1019), than gram positive bacilli (723).

Table 1. Geometric mean of bacterial counts at different concentrations of disinfectant (cfu/10^3).

Bacteria	Conc.	Females		Males		Total	
		N	Geometric Mean	N	Geometric Mean	N	Geometric Mean
G+ve Cocci	0.0%	6	719	7	6299	13	2314
	0.5%	6	275	7	1372	13	654
	1%	6	0	7	66	13	9
	2%	6	0	7	0	13	0
G+ve Bacilli	0.0%	11	335	12	1463	23	723
	0.5%	11	2	12	27	23	8
	1%	11	0	12	11	23	3
	2%	11	0	12	1	23	1
G-ve Bacilli	0.0%	6	512	4	2855	10	1019
	0.5%	6	177	4	1034	10	359
	1%	6	0	4	26	10	3
	2%	6	0	4	0	10	0
Total	0.0%	23	457	23	2563	46	1082
	0.5%	23	25	23	170	46	66
	1%	23	0	23	23	46	4
	2%	23	0	23	1	46	0

Table 2. Repeated measures ANOVA tests of within-subjects effects.

Source of variation	Sum of Squares	Df	Mean Square	F-value	P
Conc.	222.70	2.44	91.08	170.12	.000*
Conc.* Gender	8.90	2.44	3.64	6.80	.001*
Conc.* Bacteria	25.00	4.89	5.11	9.55	.000*
Conc.* Gender* Bacteria	1.83	4.89	.37	.70	.622
Error (Conc.)	52.36	97.80	.54		

*$P < 0.05$ (Significant)
Df: Degree of freedom, P: Probability

Table 3. Repeated measures ANOVA tests of between-subjects effects.

Source	Sum of Squares	Df.	Mean Square	F-value	P
Intercept	358.17	1	358.17	182.12	.000*
Gender	25.23	1	25.23	12.83	.001*
Bacteria	15.26	2	7.63	3.88	.029*
Gender* Bacteria	.10	2	.05	.03	.974
Error	78.67	40	1.97		

*$P < 0.05$ (Significant)
Df: Degree of freedom, P: Probability

Figure 1 shows the effect of different concentration on the bacterial count in male and female participants. The highest geometric mean bacterial count (2563) was for male patients, while the geometric mean bacterial count for female patients was (457). However, Figure 2 shows the effect of different concentration of sodium hypochlorite on the different type of bacterial.

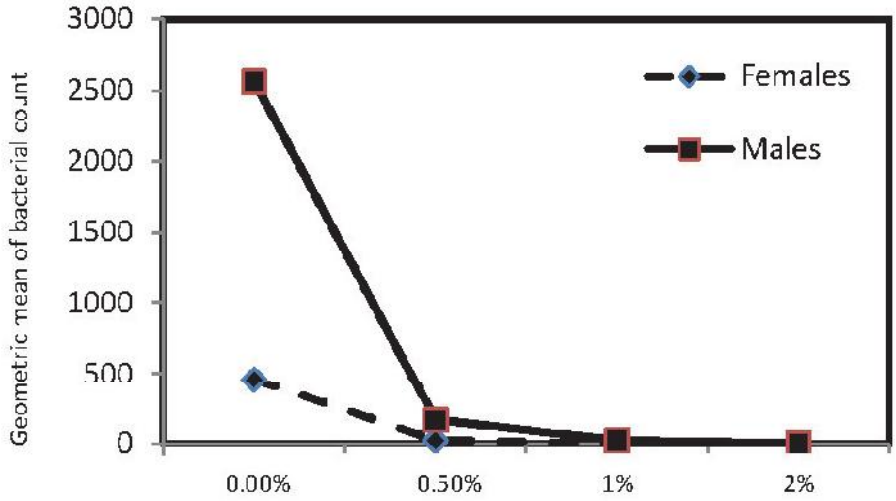

Fig. 1. Effect of different concentration on the bacterial count in males and females.

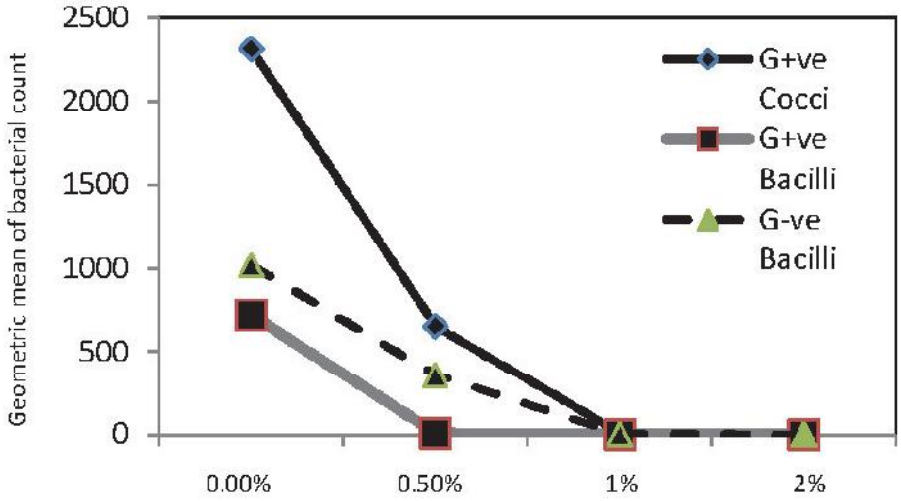

Fig. 2. Effect of different concentrations on bacterial count by type of bacteria.

Discussion

In this study, bacteria identified from the impressions were gram positive cocci and bacilli, and gram negative bacilli. The gram positive cocci included *Streptococcus mutans, Staphylococcus auricularis, Staphylococcus hominis, Staphylococcus warneri,* and *Staphylococcus chromogenes*. The gram positive bacilli were *Coryne bacterium* and *Lactobacillous,* whereas, gram negative bacilli were *E. coli* and *Enterobacter*. It is already known that prosthodontic patients are generally at high risk of transmitting and acquiring infectious diseases[3]. The prevalence of these diseases and their potentially harmful effects mandate adherence to infection control procedures in the dental office and laboratory[8-10].

The results of this research do not support the null hypothesis, since disinfecting with sodium hypochlorite appeared most effective in reducing the micro-organism count on irreversible hydrocolloid impressions. Some methodological considerations are warranted, as the present study may have underestimated the contamination level on the impressions. All impressions were studied immediately during the process of making the impressions to reduce the number of bacteria. Plate count is among the most frequently used methods of counting bacteria, is quite accurate in determining the number of living bacteria in a fluid, and can measure low concentrations of cells. When a suspension contains bacteria in higher concentrations, serial dilutions are prepared and plated[18].

Guidelines for Infection Control in Dental Health-Care Settings were developed by Center for Disease Control in collaboration with a working group of infection control experts in 2003. The document contains a review of the scientific evidence regarding dental infection, control issues as well as consensus, evidence-based recommendations[9]. American Dental Association (ADA) Infection Control Guidelines recommend disinfecting irreversible hydrocolloid impressions by immersion[5], and this study further defines the parameters for using sodium hypochlorite by increasing the concentration and shortening the application time. The antimicrobial effect of sodium hypochlorite is produced when it is added to water and

hypochlorous acid (HOCl) is formed. Hypochlorous acid (HOCl) contains active chlorine, a strong oxidizing agent, which exerts its antibacterial effect by the irreversible oxidation of –SH groups of essential enzymes, disrupting the metabolic functions of the bacterial cell. Moreover, chlorine may combine with cytoblasmic components to form N-chloro compounds, toxic complexes which destroy the microorganism.

Several studies relate to the efficacy of different concentrations of sodium hypochlorite. McNiell *et al.*[24] reported that both the polio virus and *Streptococcus sanguinis* on irreversible hydrocolloid could be deactivated by a 7.5 min immersion in 0.1% hypochlorite solution. Schwartz *et al.*[25] also reported 4-log 10 reductions in bacteria on irreversible hydrocolloid impressions when the contaminated materials were immersed in 0.5% sodium hypochlorite solution for 10 min. The results of the current study showed all of the examined impression samples were contaminated with bacteria, but most often at a low level. However, the same percentage of bacterial count reduction with less immersion time was demonstrated with 1% sodium hypochlorite. Westerholm *et al.*[26] found a more than 4-log 10 reduction of *Mycobacterium phlei* on irreversible hydrocolloid impressions after only 1 min exposure to full strength (5.25%) sodium hypochlorite spray. Although, Best *et al.*[19] reported only 3.2-log 10 reduction of *M. tuberculosis* when 1% sodium hypochlorite was used for 1 min. Bacterial types may explain the variations between researchers.

No universally accepted method, however, fulfills disinfection requirements without the risk of affecting the precision of the impressions. Haralur *et al.*[27] reported the least amount of bacterial colonies was found on impression treated with sodium hypochlorite with 0.18 on impression and 0.82 colonies on dental cast, while group treated with iodophor had the mean bacterial colonies 56.82, 53.09 for impression and dental cast, respectively.

The current study is in agreement with other studies[26-28] reported that sodium hypochlorite effectively disinfects alginate impression material. In Badrian *et al.*[28] study, it was claimed that 0.525%

hypochlorite sodium could efficiently prevent microorganisms' growth and disinfect the impression materials. It eradicated (98.84%) of *Staphylococcus aureus*, and (99.54%) of *Pseudomonas aeruginosa* in 10 min. However, Westerholm *et al.*[26] stated that sodium hypochlorite can absolutely (99.99%) prevent the growth of *Staphylococcus aureus.* Ghahramanloo *et al.*[29] found that sodium hypochlorite 0.525% could disinfect samples effectively (96.6%) in 10 min.

Future research is required to compare the current results to those from other patients having dental treatment. The present study was limited, although, microorganisms were counted. Further work should identify the type of bacteria. Moreover, only one type of irreversible hydrocolloid impression material was tested; different brands of irreversible hydrocolloid may retain different counts of bacteria.

Conclusions

Within the limitations of this study, the following conclusions were drawn:

1. The bacteria counts on irreversible hydrocolloid impressions were reduced with the minimum concentration of sodium hypochlorite solution.
2. The disinfection of irreversible hydrocolloid impressions in a 1% solution of sodium hypochlorite for 1 minute was as effective as the ADA's protocol of using a 0.5% sodium hypochlorite solution for 10 min.

Acknowledgments

The author would like to express her sincere appreciations to Professor Mohamed Ayad for his help and assistance. In addition, she would like to thank Dr. Mohamed Kurban at Microbiology Laboratory, King Abdulaziz University for his assistance.

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تأثير تعقيم طبعات الهيدروكلويد غير المرتدة على عدد البكتيريا

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المستخلص. الهدف دراسة عدد البكتيريا الموجودة في طبعات الهيدروكلويد غير المرتدة بعد غمسه في تركيزات مختلفة من محلول هيبوكلوريت الصوديوم. تم جمع ٤٦ طبعة للفك السفلي في جامعة الملك عبد العزيز، ثم غمرت الطبعات في حاويات معقمة فيها ماء مقطر، ثم استخدمت تركيزات مختلفة من محلول هيبوكلوريت الصوديوم كمطهر لمدة دقيقة واحدة. أظهرت جميع العينات نمو البكتيريا. كانت هناك فروق ذات دلالة إحصائية بالنسبة للمتوسط الهندسي لعدد البكتيريا وفقاً لنوع المرضى، كما كان الفرق كبيراً لتركيزات هيبوكلورايت الصوديوم اعتماداً على نوع الجنس ونوع البكتيريا. كان أعلى متوسط هندسي لعدد البكتيريا للذكور في حين كان الأدنى للإناث. كان هناك اختلافاً كبيراً بين أنواع البكتيريا حيث كان العدد الأكبر لبكتيريا جرام المكورات الإيجابية. أثبتت هذه الدراسة أن غمر طبعات الهيدروكلويد غير المرتدة في هيبوكلوريت الصوديوم بتركيز ١٪ لمدة دقيقة واحدة يعتبر مطهر فعال للقضاء على البكتيريا.