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RESEARCH ARTICLE

THE DISINFECTION METHODS OF ALGINATE IMPRESSIONS

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ABSTRACT

Background: Dentists, dental materials, and dental laboratories are exposed to different types of pathogenic microorganisms. Impression materials, impression trays, and poured stone cast have been said to be the main source of cross infection between patients and dentists. The aim of the present study consisted in evaluating the effectiveness of water washing and sodium hypochlorite disinfection in reducing the microbial load of alginate after mouth contact. ***Materials and Methods:*** In this in vitro experimental study twenty students voluntarily participated. The inclusion criteria were age between 21 and 24 years, and inexistence of systemic and salivary gland pathologies. For each participant, one impression was taken in alginate from the mandibular arch. These samples were submitted to water wash and sodium hypochlorite disinfection and to subsequent microbiological analysis. Statistical analysis included the analysis of variance for multiple comparisons (one-way ANOVA) followed by Student's t-test. ***Results*:** After mouth contact, alginate microbial count increased from 1.72 ± 2.92 to 2.81 × 103 ± 5.36 × 102 CFU/mm2. It was seen that after water wash the microbial count decreased to 48.5% while after sodium hypochlorite disinfection the microbial count decreased to 99.99%. ***Conclusion*:** This study revealed that alginate impression can be effectively disinfected by sodium hypochlorite. Tap water rinsing reduces microbial load but does not eliminate the cross-infection potential of alginate impressions.

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INTRODUCTION

Dentists, dental materials, and dental laboratories are exposed to different types of pathogenic microorganisms. Impression materials, impression trays, and poured stone cast have been said to be the main source of cross infection between patients and dentists (Orsi and Andrade, 2004). The control of cross-infection is an imperative issue when dealing with dental impression materials in Dentistry. Dental impressions are inevitably in contact with saliva, plaque, and blood, all of which containing potential pathogenic microorganisms. Therefore, dental care providers as well as dental assistants, staff and laboratory technicians are possible targets of contamination (Kotsiomiti *et al*., 2008). New researches have shown that 67% of materials sent to dental laboratories are infected by various microorganisms. The most frequently identified microorganisms are Streptococcus species, Staphylococcus species, Escherichia coli species, Actinomyces species, Antitratus species, Pseudomonas species, Enterobacter species, Klebsiella pneumonia, and Candidaspecies (Pang and Millar, 2006). Taking this into account, we should make an effort to eliminate most of these microorganisms and reduce the rate of infection transmission in dental laboratories. For that reason, the American Dental Association (ADA), Centers for Disease Control and Prevention (CDC) as well as the Australian Dental Association published guidelines for disinfection of dental impressions (ADA, 1996; ADA, 2012). However, the majority of professionals who work in hospitals, private clinics, dental schools and prosthetic laboratories do not follow the published recommendations. In dentistry there are several impression materials that have as main features: accuracy, elastic recovery, dimensional stability, flow, flexibility, workability, hydrophilicity, a long shelf-life, patient comfort and economics (Donovan and Chee, 2004). Of all materials used for impressions, hydrocolloids and elastomers are the most important in this field. The hydrocolloids are subdivided in reversible and irreversible. Alginate is an example of irreversible hydrocolloid and is the most commonly used material in Dentistry since it is easy to manipulate, does not imply specialized equipment and is low-priced (Rubel, 2007). As irreversible hydrocolloids are composed of 80% of water they are subject to the phenomena of imbibition (absorption of water) and syneresis (evaporation of water) (Nassar *et al*., 2011). The selection of a disinfectant depends on the impression material chosen, given that it should be efficient and should not alter the material's properties.[10]In some studies, it has been declared that washing the impression materials with tap water only removes 40% of bacteria; however, some studies reported that it has the capacity to reduce 90%, microorganisms (Al-Jabrah *et al*., 2007).

According to the Guidelines previously mentioned, the products recommended for the disinfection of impression materials are chlorhexidine, sodium hypochlorite, glutaraldehyde and iodine agents. Sodium hypochlorite is the elected disinfecting solution for alginate (Kohn *et al*., 2003). In addition, sodium hypochlorite is recommended by the Environmental Protection Agency (EPA) and is considered to be a good surface disinfectant, non-irritating and efficient against wide-spectrum microorganisms; however, it has an unpleasant odor and a relevant chemical instability. There are two disinfection techniques for impression materials: immersion and spraying. Disinfection by immersion allows the solution to contact with all surfaces of the impression (Blair and Wassell, 1996). Spraying has a lower probability of distortion than the other technique, but it may not reach all surfaces (Al-Jabrah *et al*., 2007). Yet, the antimicrobial activity of both techniques is considered similar. Alginate impressions should not be immersed in the disinfectant solution for more than a few seconds because it could compromise the quality of the impression given its propensity for absorbing water (Johnson *et al*., 1998).

Before disinfection, a pre-wash of the material with running water is also recommended to remove all debris, blood and saliva. Rueggeberg *et al*. in 1992, found that spraying disinfectants on alginate does not cause dimensional distortion in poured stone casts compared to casts from water-rinsed controls. It was shown that immersion method cause dimensional distortion in both anterior and posterior segments. Both spraying and immersing decrease surface details to the same extent. The antimicrobial effect of spraying and immersing methods was almost equal while mere water rinsing showed no significant disinfection effect (Rueggeberg *et al*., 1992).

Since none of the mentioned disinfection protocols have been accepted as a standard gold for disinfecting dental materials and the presence of hazardous microorganism on dental impression can impose detrimental effects, the present study was designed to investigate the efficiency of water wash and sodium hypochlorite disinfection of alginate impression. With that purpose we aimed to: (Orsi and Andrade, 2004) evaluate the microbial load of alginate without mouth contact; (Kotsiomiti *et al*., 2008) evaluate the number of microorganisms transferred to the alginate after the dental impression; (Powell *et al*., 1990) evaluate the reduction of microbial load after water pre-wash and (Pang and Millar, 2006) evaluate the disinfecting efficiency of sodium hypochlorite.

MATERIALS AND METHODS

The present randomized experimental study was carried out with the cooperation of Department of Conservative dentistry & endodontics, Rajarajeswari dental college, Bangalore aiming at evaluating the disinfection effect of water and 0.5% sodium hypochlorite (Chloran, Tehran, Iran). Twenty students, 13 men and 7 women were invited to voluntarily participate in the present study. Inclusion criteria included age between 21 and 24 years, absence of systemic or salivary gland pathologies as well as participants with DMFT (decayed, missing and filled teeth) index ≤ 5 (after a clinical examination). The medical and dental histories as well as oral hygiene habits of each subject were obtained by interview in order to characterize the population. For each participant, one alginate impression (Orthodontic Alginate impression Material Orthoprint, Rovigo, Italy) was performed at the mandibular arch. Informed consent forms, approved by ethical committee, were signed and obtained from each participant. After opening, the alginate was sealed in a sterile bottle and stored in a dry and clean environment. After hand disinfection, the alginate was handmixed to a homogenous consistency for 30 s using sterile water. An impression was made using artificial sterilized teeth (Frasaco) in order to evaluate the microbial load of alginate previous to mouth contact. Simultaneously, a universal, sterile, non-perforated impression tray was loaded with the same alginate impression material and transferred to the mouth. After 2minutes the impression was separated from the mouth. The selected impression was dissected into three parts under aseptic conditions.

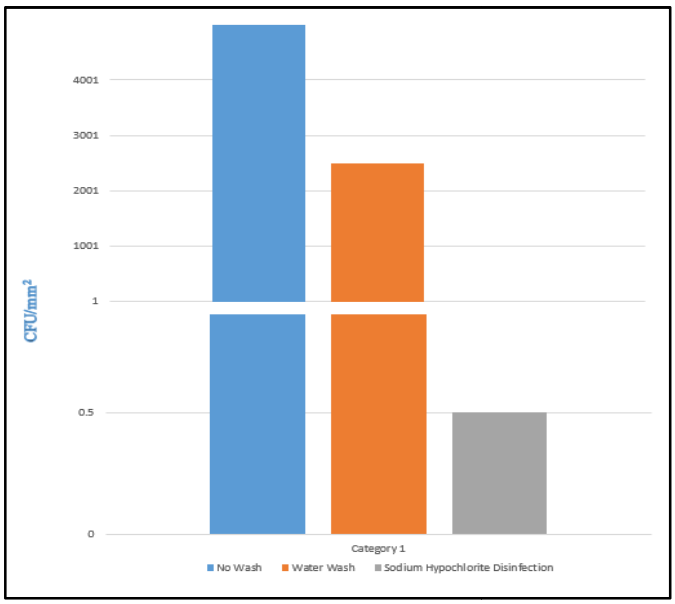
Each part of impression was submitted to one of the following treatments

- Group I - Samples were left untreated, without any disinfection methodology.
- Group II - Samples were washed with running tap water for 15 s.
- Group III - Samples were disinfected by immersion in 0.5% of sodium hypochlorite (Hipoclorito, Lisboa, Portugal) for 15 s followed by placing the sample in a gauze embedded with the same disinfectant for 10 more minutes.

Following the exposure to treatment regimes, the microbiological analysis was performed. For that purpose, each sample was placed in sterile tubes containing 3mL of 0.9% NaCl sterile solution and sterile glass beads. The tubes were then vortexed for 15 seconds to release the adhered microorganisms. Afterwards, the suspensions were serially diluted with 0.9% NaCl solution until 10^{-2}. The resulting samples were immediately plated in triplicate in Brain Heart Infusion (BHI) agar using the Miles and Misra method. The plates were incubated aerobically at 37 °C for 48h. The colonies were counted and expressed as colony-forming units per square millimeter (CFU/mm2). The statistical analysis was performed using Microsoft Excel. The categorical variables were described through relative frequencies (%) whereas continuous variables were described using mean ± standard deviation (SD). A level of 0.05 was considered significant (p). Statistical analysis was performed by Student's t-test for unpaired comparisons and one-way ANOVA followed by Student's t-test for paired comparisons.

RESULTS

As expected, alginate without mouth, water or disinfectant contact, presented very low microbial load, 1.72 ± 2.92 CFU/mm^2. After mouth contact, alginate microbial load increased significantly to $2.81 \times 10^3 \pm 5.36 \times 10^2$CFU/mm^2 (Student's t-test for unpaired comparisons, p = 0.0017). Afterwards, the alginate used in dental impression was washed with tap water and its microbial load decreased significantly by 48.5% (Fig. 1). Sodium hypochlorite disinfection of alginate decreased microbial count by 99.99% (Fig. 1). This reduction was statistically significant when compared to alginate with mouth contact and when compared to alginate with mouth contact followed by tap water wash (ANOVA, p = 0.00003).



DISCUSSION

Dentists practicing dentistry encounter potentially harmful microorganisms. Patients are the most important source of microorganisms. Studies indicate that the surface of impressions taken out of the mouth is polluted with bacteria (Jennings and Samaranayake, 1991). Egusa and colleagues in 2008 showed that alginate impressions taken from patient's mouths contain hazardous microorganisms like Staphy lococcus aureus, methicillin resistant Staphylococcus, Candida albicans, and Pseudomonas aeruginosa with rate of 55.6%, 25.9%, 25.9%, and 5.6%, respectively .The seareopportunistic pathogens that can be spread and transferred through the oral cavity (Egusa *et al*., 2008). The results obtained in this study demonstrate that alginate acts as a vehicle for microorganisms' transmission and that disinfection with sodium hypochlorite reduces the microbial load present in this dental material to residual levels. Water rinsing reduces alginate microbial load but does not disinfect efficiently the dental impression material, so, additional methods should be used. To evaluate the potential role of alginate in cross-infection in dental setting, some precautions were taken. Alginate was prepared with newly opened powder and blended with sterile water to avoid contamination of alginate with water-born microorganisms.

In addition, in order to understand the contribution of environmental contaminants and "alginate-born" microorganisms on total microbial load, samples of alginate without mouth contact were evaluated. Our results showed that extra-mouth contaminants represent only 0.06% of total microbial load of alginate after mouth contact. Two different methods of reducing the microbial load of alginate after mouth contact were evaluated: tap water wash and sodium hypochlorite disinfection. From the two methods employed, disinfection with sodium hypochlorite was the most efficient, reducing alginate adhered microorganism by 99.99%. In the present survey, 0.5% sodium hypochlorite agent which is common in housework was used. This disinfectant agent could effciently prevent microorganisms growth and disinfect the impression materials. Westerholm (1992) and Rueggeberg (Rueggeberg *et al*., 1992) and colleagues also showed that spraying sodium hypochlorite can effectively disinfect the impression materials. In a study done by Ghahramanloo and colleagues, using 0.5% sodium hypochlorite could disinfect impression samples effectively (96.6%) in 10 minutes (Ghahramanloo *et al*., 2009). However, a partial disintegration of the alginate samples was observed suggesting that the quality of the impression could be compromised after sodium hypochlorite treatment. A number of materials are not compatible with some disinfectants, which may affect the accuracy of the impression, its texture or dimensional stability (Samra and Bhide, 2010). The simple rinsing of the impressions with tap water reduced the amount of microorganisms in the alginate's surface by 48.5%. This result is in accordance with the report of Al-Jabrah and colleagues (Al-Jabrah *et al*., 2007) that showed a reduction of microbial load ranging between 40 and 90%. In many dental settings, including dental medicine schools, the impressions are only washed with water (Egusa *et al*., 2008). The present work shows that, although this procedure reduces significantly the amount of microorganisms present in the impression, many thousands of other microorganisms remain. So, an accurate disinfection of dental materialto avoid cross-infection is imperative. The oral microbiota consists of a wide range of microorganisms, including bacteria, yeasts, protozoa and virus. A great number of oral bacteria are anaerobes, but only aerobes were evaluated in the present study due to the complexity and costs associated to anaerobic cultures. Brain heart infusion was the culture medium used for the growing of total aerobic mesophilic bacteria, although some fastidious bacteria as well as protozoa and virus were not able to grow. Viruses were not considered for this experiment because of the potential danger in its manipulation and the inexistence of required equipment. Given that the study was limited to aerobic mesophilic bacteria, the microbial load observed in alginate samples after mouth contact, is significantly lower than the real total microorganism load emphasizing even more the importance of cross-infection in dental impressions.

Conclusion

Dental impression materials can act as a transmission vehicle for oral microorganisms. Alginate appears to absorb more microorganisms than silicone. Dental impression water wash alone is insufficient for reducing the risk of cross-infection. However, the immersion of dental impressions in sodium hypochlorite (0.5%), is effective in reducing significantly the microbial load, so the immersion disinfection procedure should be mandatory.

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