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Research

The effect of water and sodium hypochlorite disinfection on alginate impressions

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ABSTRACT

Introduction: The control of cross-infection is an imperative issue when dealing with dental impression materials in Dentistry and the lack of procedures for its control is currently a real problem. The aims 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: Thirteen students voluntarily participated in the present study. The inclusion criteria were age between 21 and 24 years, inexistence of smoking habits and systemic and salivary gland pathologies, DMFT index (decay/missing/filled teeth) ≤ 5 and tooth brushing with right hand. 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 1.59 ± 2.79 to $2.68 \times 10^3 \pm 6.19 \times 10^2$ CFU/mm^2. It was verified that after water wash the microbial count decreased to 48.5% while after sodium hypochlorite disinfection microbial count decreased to 99.99%.

Conclusion: Dental impression materials can act as vectors transmitting a significant amount of microorganisms. Sodium hypochlorite disinfection is an efficient disinfection method for alginate impressions. Tap water rinsing reduces microbial load but does not eliminate the cross-infection potential of alginate.

A eficácia da água e do hipoclorito de sódio na desinfeção de impressões em alginato

Palavras-chave:
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RESUMO

Introdução: O controlo da infeção cruzada dos materiais de impressão em Medicina Dentária é de extrema importância e a falta de procedimentos para o seu controlo constitui

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Água

atualmente um problema real. Os objetivos do presente trabalho consistiram na avaliação da eficácia da lavagem com água e da desinfeção com hipoclorito de sódio na redução da carga microbiana do alginato após o contacto com a cavidade oral.

Material e métodos: Treze estudantes participaram voluntariamente no estudo. Os critérios de inclusão foram a idade entre 21-24 anos, ausência de hábitos tabágicos e de patologias sistémicas e das glândulas salivares, índice CPO (dentes cariados, perdidos e obturados) ≤ 5 e escovagem com a mão direita. Uma impressão em alginato da arcada mandibular foi obtida de cada participante. As amostras foram submetidas aos métodos de lavagem/desinfeção e, posteriormente, à análise microbiológica. Os testes ANOVA para comparações múltiplas e o teste t de student foram utilizados como ferramentas para a análise estatística.

Resultados: O alginato após o contacto com a cavidade oral apresentou uma carga microbiana de $2{,}68 \times 10^3 \pm 6{,}19 \times 10^2$ CFU/mm^2. A simples passagem por água corrente diminui a carga microbiana em 48,5%, enquanto que a desinfeção com hipoclorito de sódio diminui a carga microbiana em 99,99%.

Conclusão: Os materiais de impressão podem ser veículos de transmissão de microrganismos. O hipoclorito de sódio é um método de desinfeção eficaz para as impressões em alginato. Apesar da lavagem com água corrente reduzir significativamente a carga microbiana, não elimina o potencial de infeção cruzada das impressões em alginato.

Introduction

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.[1–3]

Increasing concern over the transmission of infectious diseases in dental office occurred in the eighties with the outbreak of Acquired Immunodeficiency Syndrome (AIDS).[4] This prompted the adoption of preventive routine procedures for the disinfection of dental impressions. 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.[5–7] However, the majority of professionals who work in hospitals, private clinics, dental schools and prosthetic laboratories do not follow the published recommendations.[2,3,8–10]

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.[11] 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.[11,12] 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).[11,13]

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.[14,15] According to the Guidelines previously mentioned, the products recommended for the disinfection of impression materials are chlorhexidine, sodium hypochlorite, glutaraldehyde and iodine agents.[5–7] Sodium hypochlorite is the elected disinfecting solution for alginate.[1] 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.[16]

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.[10,17,18] Spraying has a lower probability of distortion than the other technique, but it may not reach all surfaces.[10,19] Yet, the antimicrobial activity of both techniques is considered similar.[19–21] 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.[2,10,21,22]

Before disinfection, a pre-wash of the material with running water is also recommended to remove all debris, blood and saliva.[5,7]

Given the above stated facts, the goal of the present study was to evaluate the efficiency of water wash and sodium hypochlorite disinfection of alginate impression. With that purpose we aimed to: (1) evaluate the microbial load of alginate without mouth contact; (2) evaluate the number of microorganisms transferred to the alginate after the dental impression; (3) evaluate the reduction of microbial load after water pre-wash and (4) evaluate the disinfecting efficiency of sodium hypochlorite.

Materials and methods

Thirteen students, 6 men and 7 women from the 4th year of Bachelor plus Master degree of Faculty of Dental Medicine

of Porto University were invited to voluntarily participate in the present study. Inclusion criteria included age between 21 and 24 years, tooth brushing with left hand, inexistence of smoking habit, 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, Zhermack clinical, 84702, 2012-02, Rovigo, Italy) was performed at the mandibular arch. Informed consent forms, approved by Faculty of Dental Medicine ethical committee, were signed and obtained from each participant.

The experimental protocol was performed in a dental clinic atmosphere using sterilized materials. After opening, the alginate was sealed in a sterile bottle and stored in a dry and clean environment. After hand disinfection, the alginate was hand-mixed 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 2 min the impression was separated from the mouth. The selected impression area to study was dissected under aseptic conditions and consisted in the first and second right molars. These selected teeth were divided in 3 parts following buccal–lingual direction. Each sample was constituted by a pull of one third of each tooth (first and second molar) in order to minimize the difference of microbial colonization between teeth ($n = 13$). Each pull was submitted to one of the following treatments: (1) sample was left untreated, without any disinfection methodology; (2) sample was washed with running tap water during 15 s and (3) sample was disinfected by immersion in 0.5% of sodium hypochlorite (Hipoclorito 0.5% Solução de Dakin, AGA Álcool e Genéricos alimentares S.A., 4.144.51.23.15, Lisboa, Portugal) during 15 s followed by placing the sample in a gaze embedded with the same disinfectant for more 10 min.[5,23]

Following the exposure to treatment regimes, the microbiological analysis was performed. For that purpose, each sample was placed in sterile tubes containing 3 mL of 0.9% NaCl sterile solution and sterile glass beads. The tubes were then vortexed for 5 + 5 + 5 s 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.[24] The plates were incubated aerobically at 37 °C for 48 h. The colonies were counted and expressed as colony-forming units per square millimeter (CFU/mm^2). The tooth area was determined by mean values of the first and second right molars, described by Scheid and Woelfel.[25]

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.

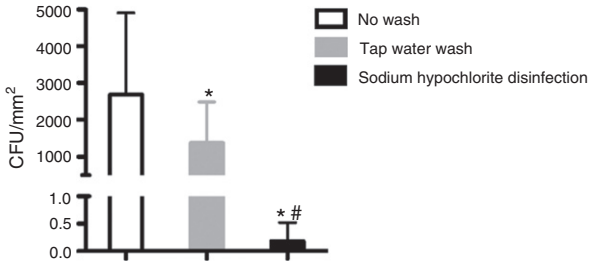

Fig. 1 – Alginate microorganisms' quantification after mouth contact, with no wash, after tap water wash or after sodium hypochlorite disinfection. * Significantly different from no washed alginate (Student's t-test for paired comparisons, $p < 0.01$) and # significantly different from tap water washed alginate (Student's t-test for paired comparisons, $p < 0.01$).

Results

The mean age of the participants was 21.9 ± 0.3 years. The participants mean DMFT index was 2.46 ± 0.63. In respect to oral hygiene habits, 84.6% of the participants used manual toothbrush whereas 7.7% used electric toothbrush and 7.7% used both. In addition, 46.1% used mouthwash.

As expected, alginate without mouth, water or disinfectant contact, presented very low microbial load, 1.59 ± 2.79 CFU/mm^2.

After mouth contact, alginate microbial load increased significantly to $2.68 \times 10^3 \pm 2.23 \times 10^3$ CFU/mm^2 (Student's t-test for unpaired comparisons, $p = 0.0019$).

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

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. In addition, the impression area chosen for analysis consisted in the first and second right molars given that all participants were right handed and thus presented greater difficulty to brush the right side of the oral cavity.[26] In addition, the first inferior molar was chosen because it is one of the first teeth to erupt, being exposed earlier to the oral environment, and presents anatomic features that facilitate biofilm adhesion.[25] The samples were extended to the second molar due to the need to gain a larger sample and because this is an adjacent and similar tooth.

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%. 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.[17,20] 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[19] 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.[27] 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 material to 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. Protozoa were also not evaluated due to its low prevalence in our society.[28] In a pilot experiment the possible presence of yeasts, particularly *Candida* was evaluated using the culture medium *Sabouraud* agar with chloranphenicol. However, this approach was abandoned due to absence of growth in the firsts experiments. A plausible explanation for this result is the low prevalence of yeasts in the young population or its low adherence to alginate.[29] 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. In addition, an interesting characterization, not performed due to funds limitations, would be the qualitative evaluation of the isolated microorganisms to identify and characterize the microorganisms implied in cross-infections and further understand its potential pathogenicity.

Conclusions

Dental impression materials can act as a vehicle for microorganisms' transmission, playing an important role in cross-infection. Sodium hypochlorite disinfection is an efficient disinfection method. Tap water rinsing can reduce microbial load but does not disinfect efficiently dental impression materials, so, additional methods should be used.

Ethical disclosures

Protection of human and animal subjects. The authors declare that the procedures followed were in accordance with the regulations of the relevant clinical research ethics committee and with those of the Code of Ethics of the World Medical Association (Declaration of Helsinki).

Confidentiality of data. The authors declare that they have followed the protocols of their work center on the publication of patient data and that all the patients included in the study received sufficient information and gave their written informed consent to participate in the study.

Right to privacy and informed consent. The authors have obtained the written informed consent of the patients or subjects mentioned in the article. The corresponding author is in possession of this document.

Conflicts of interest

The authors have no conflicts of interest to declare.

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