Analysis of three disinfectants after immersion of irreversible hydrocolloid and ZOE paste impressions.

Sheila R. S. Porta1* 
Vanderlei L. Gomes2* 
Luiz A. Pavanin3* 
Carla C. B. Souza4* 
*Uberlândia Federal University, Minas Gerais, Brazil. 
1DDS, MSc, Professor, Postgraduate, Oral Rehabilitation Program, UFU School of Dentistry. 
2DDS, MSc, PhD, Professor and Chairman, Division of Removable Prothodontics and Dental Materials, UFU School of Dentistry. 
3MSc, PhD, Adjunct Professor, UFU School of Chemistry. 
4BSc, Fourth Year Chemistry Student, UFU School of Chemistry.

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Abstract
This investigation sought to analyze 3 disinfectants: 0.5% chlorhexidine, 2% glutaraldehyde, and 1% sodium hypochlorite after immersion of irreversible hydrocolloid and zinc oxide-eugenol paste impressions. Changes detected in the solutions could suggest alterations in the dental impressions, compromising their quality and negatively influencing treatment outcome. Forty-five irreversible hydrocolloid impressions (Jeltrate) made in aluminum stock impression trays (AG) as well as 45 irreversible hydrocolloid and 45 zinc oxide-eugenol paste (Horus) impressions made in auto polymerizing acrylic resin (AAR) trays (VipiFlash) were immediately immersed in 200mL of 0.5% chlorhexidine (Labfa), 2% glutaraldehyde (Glutaron II), or 1% sodium hypochlorite (Miyako) solutions for 10, 30, and 60 minutes. After immersion the solutions were analyzed by visible ultraviolet spectroscopy and turbidimetry to verify possible alterations. Turbidimetry (registered in nephelometric turbidity units -NTU) results were subjected to Kruskal-Wallis and Mann-Whitney tests for statistical analysis (α=.05). An increase of absorption levels of all disinfectants was found after immersion of irreversible hydrocolloid impressions. This increase was proportional to time of immersion and absorption was higher for 0.5% chlorhexidine for the 3 time periods analyzed. Only 1% sodium hypochlorite presented statistically significant alterations after immersion of zinc oxide-eugenol paste impressions.

Key Words: 
disinfection, disinfectants, dental impression materials
Introduction
Disinfection of dental impressions is part of the daily routine in a dental office. After disinfection, it is important that impressions remain accurate and stable in reproducing the oral structures. Disinfection should not compromise the integrity of the impressions and is a necessary component for successful treatment. Various solutions and methods of disinfection have been analyzed and it has been found that immersion of irreversible hydrocolloid in 0.5% chlorhexidine for 30 seconds did not provoke superficial wrinkling or porosity of the impressions and an hour of immersion did not cause significant dimensional change. As for irreversible hydrocolloid impressions immersed in 2% glutaraldehyde, a study revealed less dimensional changes when compared to impressions immersed in 1% sodium hypochlorite, even though other materials showed significant dimensional alterations. However, for Durr and Novak the use of 2% glutaraldehyde in impression disinfected optimized their surface quality. The immersion of impressions in this solution, for one hour, seems to be an efficient method of disinfection. Zinc oxide-eugenol (ZOE) paste is compatible with this procedure while irreversible hydrocolloid is not.

The use of sodium hypochlorite for disinfection of irreversible hydrocolloid impressions appears to reduce the effects of syneresis and consequently results in casts with better surface quality than impressions rinsed only with running water. Blair and Wassel have suggested that all impressions, regardless of the type of material, should be immersed in 1% sodium hypochlorite for 10 minutes. This time is considered to be the minimum required for the effective use of all disinfectants.

The use of glutaraldehyde or iodophor is indicated for ZOE paste disinfection. For irreversible hydrocolloid impressions, immersion in hypochlorite, iodophor or glutaraldehyde with phenol buffer is recommended. Although studies that evaluate the effect of disinfection on dimensional stability and impression surface quality exist, there is no universally accepted protocol on the matter. Furthermore, no studies that include a chemical analysis of disinfection agents were identified. A qualitative change in disinfection solution would suggest an action of the solution on impression material, impression tray material, or both. Consequently, disinfection effectiveness and/or the surface quality of the dental cast may be compromised. The present study aimed to evaluate chemical changes in the disinfectants 0.5% chlorhexidine, 2% glutaraldehyde, and 1% sodium hypochlorite after immersion of irreversible hydrocolloid and ZOE paste impressions. Analysis was performed through visible ultraviolet spectroscopy seeking to assess chemical interaction between the materials, by absorption bands indicative of chemical specimen formation with light absorption. The method also suggests an erosion process in the impression materials, due to increase in solution absorbancy (background absorption) caused by suspension particles and making the passage of light difficult. Data were complemented with turbidity measures of the solutions in nephelometric turbidity units (NTU) as to assess increase of numbers of suspension particles.

Material and Methods
A stainless steel cast, simulating an edentulous maxillary dental arch was used to make both irreversible hydrocolloid (Jetrate, Dentsply Ind. and Co. Ltda. Petrópolis, Rio de Janeiro, Brazil) and zinc oxide-eugenol (ZOE) paste (Horus; Herpo Produtos Dentários Ltd, Rio de Janeiro, Brazil) impressions. Aluminum stock impression trays (AG, Dental AG Ltd, and Sao Paulo, Brazil) and auto polymerizing acrylic resin (AAR) trays (VipiFlash, Dental Vipi Ltda, and Pirassununga, Brazil) were used for the irreversible hydrocolloid impressions. AAR trays were used for the ZOE impressions. Two disinfectants, 2% glutaraldehyde (Glutaron II, Indústria Farmaceutica Rioquimica Ltda, Sao Paulo, Brazil) and 1% sodium hypochlorite (Miyako, Miayako do Brazil, Sao Paulo, Brazil), recommended by the ADA, were used. Additionally, based on the works of Rowe and Forest and Bergman et al, 0.5% chlorhexidine, a common dental antiseptic, (Labfa, Uberlandia, Brazil) was evaluated to verify its feasibility in disinfecting irreversible hydrocolloid and ZOE impressions. After molding, the impressions were immediately immersed in 200mL of the disinfectant solutions. Tests were run at a temperature of 24°C to 26°C. The time periods for immersion were 10, 30 and 60 minutes. A visible ultraviolet (visible UV) spectroscopy was performed to verify changes in the disinfectant solutions using a PC compatible Vectra XM-diode arrangement HP8453 spectrophotometer. Turbidimetry (turbidimeter nephelometric model Ap-1000 810 series, 1987, Polilab Instrumentos técnicos Científicos Industria e Comercio Ltda, Brazil), with the values expressed in Nephelometric Turbidity Units (NTU), was also performed, to complement analysis. Maximum capacity of the device used in the tests was 1000 NTU.

UV spectra from a water specimen used for a 60 minute immersion of untreated impression and impression trays were obtained. The spectrum is a graph of relative absorption as the ordinate, plotted versus wavelengths (in nanometer) as the abscissa, with the short wavelengths to the left of the scale. Since all study disinfectants contained water in their composition, this analysis permitted the evaluation of the interaction of the disinfectant with the impression materials, independent of the water in the solution. All solutions were homogenized, manually, for 10 seconds immediately before being analyzed. Analysis of the disinfectants, before immersion of the impressions was used as a control for
spectrometry and turbidity. To analyze the effect of the disinfectants on irreversible hydrocolloid, 45 (15 for each disinfectant and 5 specimens for each immersion period) samples were made, using metallic trays. Likewise 45 other samples were obtained using AAR trays. Pre-tests were performed to determine number of samples to be used in immersion time interval. Five samples for each time were considered to be a sufficient amount for confident analysis.

To make the impressions, one part hydrocolloid powder (10g) to one part water (19 mL) was mixed in a rubber bowl manually, for one minute, with a metal spatula according to manufacturer’s recommendations. Impression material was then placed in the impression trays and a stainless steel cast, imitating an edentulous maxillary dental arch was molded.

Analysis of the effects of the disinfectants on ZOE paste was performed with a total of 45 samples, using only AAR impression trays: 15 for each disinfectant, 5 specimens, for each immersion period. A flexible steel cement spatula was used to mix the pastes of equal lengths (5 cm) on a mixing pad until a homogeneous mix was obtained, as recommended by the manufacturer. The material was placed in the AAR tray and when the material set, it was immersed in 200 mL of disinfectant.

After immersion, all disinfectants were analyzed to check the existence of chemical interaction between the materials. Analysis was performed through visible and ultraviolet spectroscopy and turbidimetry. Aiming to verify the existence of statistical differences in the obtained data in relation to the 3 time periods adopted and 3 disinfectants used, turbidimetry results were assessed using Kruskal-Wallis and Mann-Whitney tests (α = .05).

Results and Discussion

This study verified the chemical changes in 0.5% chlorhexidine, 2% glutaraldehyde and 1% sodium hypochlorite after immersion of irreversible hydrocolloid and ZOE paste impressions, for 10, 30 and 60 minutes. For chemical analysis, spectroscopy in the UV-visible region and turbidimetry were used.

Spectra of 0.5% chlorhexidine after immersion of irreversible hydrocolloid may be analyzed on Fig 1. Lack of absorption bands UV-visible regions indicates there is no chemical interaction between molding material and disinfectant. There is, however, a significant increase in the absorbance (background absorption) proportional to time of immersion, indicating that the solutions were quite concentrated, making the passage of light difficult in those wavelengths. Solutions were analyzed using turbidimetry, complementing the results presented by the spectroscopic analyses. Analyzing Table 1, it can be verified that the turbidity measurements reveal high values, even after only 10 minutes of immersion. This increase was proportional to the time in which the irreversible hydrocolloid/aluminum impression tray remained immersed. Alterations were more significant during the interval of 10 to 30 minutes, than during the interval of 30 to 60 minutes.

The spectra of irreversible hydrocolloid/AAR impression tray are quite similar to those obtained for the aluminum impression tray. The effect of erosion of the impression was detected by the increase of the number of particles in suspension, making light passage difficult and provoking an increase in absorption of the spectrum as a whole (background absorbancy). This increase in background absorbancy is clear until 30 minutes of immersion. Turbidity values (Table 1) are also similar to those presented for the aluminum impression tray. These data confirm that 0.5% chlorhexidine has no action on the impression tray materials; however, it does affect the impression material. These data can be explained by the chemical nature of chlorhexidine which is basic and that of irreversible hydrocolloid which is acidic.

![Fig. 1 - Absorption spectrum of 0.5% chlorhexidine after immersion of irreversible hydrocolloid with metal tray. With the increase of immersion time, the solution becomes more concentrated, until the point where the spectrum appears to be “burst”.

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more soluble in basic medium. Soon after the first minutes of immersion, increase in turbidity was clinically visible, which did not happen in the rest of the tested disinfectants. The results shown in this study suggest that the casts will present significant dimensional alterations, which conflicts with previous results reported by Bergman et al.\(^3\)

No absorbance peaks in the visible UV region could be observed in the spectrum shown in Fig. 2. Different from 0.5% chlorhexidine, there is uniformity in resulting spectra after the 3 periods of immersion tested. Although there is a slight increase in the background absorbancy, time does not appear to be a critical factor regarding the action of 2% glutaraldehyde on irreversible hydrocolloid (Table 2). Turbidity analysis (Table 1) shows much less alterations than those of chlorhexidine. However, there is an increase in turbidity after 10 minutes of immersion. These results are in agreement with the work of Durr and Novak\(^5\), where significant dimensional alterations were observed after 10 minutes of immersion in 2% glutaraldehyde. These same authors, however, allude to the fact that the use of this disinfectant produced casts with better surface quality than those produced with the use of 1% sodium hypochlorite. Yet in Table I an even more distinct increase in turbidity is seen during the interval of 10 to 30 minutes. A smaller value in

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**Table 1** – Average and standard deviation of the turbidity analysis (in NTU)

<table>
<thead>
<tr>
<th>Disinfectant</th>
<th>Impression material</th>
<th>Irreversible Hydrocolloid</th>
<th>ZOE</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Tray</td>
<td>Aluminum</td>
<td>AAR</td>
</tr>
<tr>
<td>Chlorhexidine</td>
<td>Time</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>10 min</td>
<td>20 min</td>
<td>30 min</td>
</tr>
<tr>
<td>Average</td>
<td>609.0</td>
<td>845.0</td>
<td>952.0</td>
</tr>
<tr>
<td>Standard deviation</td>
<td>159.9</td>
<td>130.4</td>
<td>66.9</td>
</tr>
<tr>
<td>Glutaraldehyde</td>
<td>Average</td>
<td>36.2</td>
<td>48.3</td>
</tr>
<tr>
<td>Standard deviation</td>
<td>13.7</td>
<td>1.0</td>
<td>22.2</td>
</tr>
<tr>
<td>Sodium hypochlorite</td>
<td>Average</td>
<td>35.9</td>
<td>48.2</td>
</tr>
<tr>
<td>Standard deviation</td>
<td>9.5</td>
<td>10.4</td>
<td>5.5</td>
</tr>
</tbody>
</table>

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*Fig. 2* - Absorption spectrum of 2% glutaraldehyde after immersion of irreversible hydrocolloid with aluminum tray. Little variation in relation to time of immersion on the spectra can be seen.
Table 2 – Probabilities found when Kruskal-Wallis test was applied to results obtained in the three time intervals, in each of the groups

<table>
<thead>
<tr>
<th>Analyzed variables</th>
<th>Impression Material</th>
<th>Disinfectant</th>
<th>Impression tray</th>
<th>Probabilities</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>Aluminum</td>
<td>0.011*</td>
</tr>
<tr>
<td>Irreversible Hydrocolloid</td>
<td>Chlorhexidine</td>
<td></td>
<td>AAR</td>
<td>0.007*</td>
</tr>
<tr>
<td></td>
<td>Glutaraldehyde</td>
<td></td>
<td>Aluminum</td>
<td>0.267</td>
</tr>
<tr>
<td></td>
<td>Sodium hypochlorite</td>
<td></td>
<td>AAR</td>
<td>0.064</td>
</tr>
<tr>
<td>ZOE</td>
<td>Chlorhexidine</td>
<td></td>
<td>AAR</td>
<td>1.000</td>
</tr>
<tr>
<td></td>
<td>Glutaraldehyde</td>
<td></td>
<td>AAR</td>
<td>1.000</td>
</tr>
<tr>
<td></td>
<td>Sodium hypochlorite</td>
<td></td>
<td>AAR</td>
<td>0.730</td>
</tr>
</tbody>
</table>

(*) $\alpha < .05$

Turbidity found in the solutions after 60 minutes of immersion may have resulted from the sedimentation of formed particles occurring from the erosion of the impression material, triggered by the disinfectant. Following this time of immersion, these particles should be bigger and sediment more quickly, resulting in lower turbidity values. In some groups, these particles were clinically visible without apparent changes in turbidity. It could be said that there was an erosion effect and not a dissolving effect of the impression material, that is, the spectrum analyses do not show bands that suggest the formation of new chemicals, thus there was no chemical reaction. In addition, the increase of the background absorption leads to the conclusion that the impression material was not dissolved in the disinfectant but remained floating in the solution, making the passage of the light beam difficult. The cast obtained after this period of disinfection will probably have its surface quality altered. Based on these data and different from those observed by Bergman et al., it can be concluded that immersion should not exceed 10 minutes so as not to negatively affect the quality of the impression and the outcome of treatment. When the action of 1% sodium hypochlorite on irreversible hydrocolloid/aluminum tray was analyzed, a slight increase in absorbancy was observed (Fig. 3). The absence of absorbance peaks in the visible UV region indicates that there was no chemical alteration between the disinfectant and the impression material. Table 1 reveals turbidity values of small variations related to the period of immersion. Proportionally, there is a greater variation related to 10 minutes. The measured turbidity after the 3 periods of immersion presented values that differed slightly among themselves. In comparison to 0.5% chlorhexidine, immersion in 1% sodium hypochlorite seems to result in better quality impressions, since the increase in absorbancy and turbidity which suggests erosion of the impression surface is significantly lower (Tables 1, 2). Also, there is no significant difference when comparing the use of 1% sodium hypochlorite and 2% glutaraldehyde. The values of turbidity (Table 1) and the spectra obtained after immersion of the irreversible hydrocolloid/AAR show values that are similar to those obtained using aluminum. These data confirm that there is no chemical interaction between impression material and disinfectant. They also confirm a distinct action, statistically significant between 10 and 60 minute time periods (Table 1), of the disinfectant on the impression material that resulted in erosion. In all of the differences found in the results, values after 10 minute immersion were always significantly less high. These data suggest that this time period should not be gone over in disinfecting irreversible hydrocolloid impressions.

By analysis of Figures 4, 5, and 6, the absorbance in the visible and UV regions spectra of the disinfectant solutions after immersion in the ZOE paste/AAR impressions can be evaluated. Contrary to the previous spectra, variation in absorbance, in relation to time of immersion was very small. This made it difficult to visualize each spectrum separately and indicates that the time factor did not interfere in the results. The spectra of 0.5% chlorhexidine after immersion in the ZOE paste/AAR impressions reveal that the time of immersion did not interfere with the results as there was no increase in the background absorbancy (Fig. 4 and Table 1) and no alteration in the turbidity. These data indicate that the 0.5% chlorhexidine did not present any undesirable action on the
zinc eugenol oxide impressions (Horus paste) regardless of the time used for immersion.

Analysis of the 2% glutaraldehyde spectrum registered after immersion in the ZOE paste/AAR impression tray (Fig. 5) did not reveal any absorbance peaks. There were no other variations related to the time of immersion. Turbidity of the 2% glutaraldehyde (Table 1) presented the same values, before and after immersion. These results indicate that the disinfectant triggered no undesirable effect on the ZOE paste/AAR impression during the intervals of time analyzed. This result confirms the compatibility between ZOE paste and 2% glutaraldehyde, reported in the literature12-13.
In the spectrum in Fig. 6, registered after the immersion of ZOE paste/AAR impression in 1% sodium hypochlorite, no absorbance peaks can be observed. No variation related to time of immersion in the spectroscopic, however, a small difference in the turbidimetric analysis was observed when compared to the other disinfectants (Table 1). As these changes were not clinically perceivable, other studies should be performed to evaluate alterations on impression surfaces and the quality of resulting molds.

References