The effectiveness of chemical denture cleansers and ultrasonic device in biofilm removal from complete dentures

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ABSTRACT

Adequate denture hygiene can prevent and treat infection in edentulous patients. They are usually elderly and have difficulty for brushing their teeth. Objective: This study evaluated the efficacy of complete denture biofilm removal using chemical (alkaline peroxide-effervescent tablets), mechanical (ultrasonic) and combined (association of the effervescent and ultrasonic) methods. Material and Methods: Eighty complete denture wearers participated in the experiment for 21 days. They were distributed into 4 groups (n=20): (1) Brushing with water (Control); (2) Effervescent tablets (Corega Tabs); (3) Ultrasonic device (Ultrasonic Cleaner, model 2840 D); (4) Association of effervescent tablets and ultrasonic device. All groups brushed their dentures with a specific brush (Bitufo) and water, 3 times a day, before applying their treatments. Denture biofilm was collected at baseline and after 21 days. To quantify the biofilm, the internal surfaces of the maxillary complete dentures were stained and photographed at 45°. The photographs were processed and the areas (total internal surface stained with biofilm) quantified (Image Tool 2.02). The percentage of the biofilm was calculated by the ratio between the biofilm area multiplied by 100 and the total area of the internal surface of the maxillary complete denture. Results: The Kruskal-Wallis test was used for comparison among groups followed by the Dunn multiple-comparison test. All tests were performed respecting a significance level of 0.05. Significant difference was found among the treatments (KW=21.18; P<0.001), the mean ranks for the treatments and results for Dunn multiple comparison test were: Control (60.9); Chemical (37.2); Mechanical (35.2) and Combined (29.1). Conclusion: The experimental methods were equally effective regarding the ability to remove biofilm and were superior to the control method (brushing with water). Immersion in alkaline peroxide and ultrasonic vibration can be used as auxiliary agents for cleaning complete dentures.

Key words: Denture. Biofilms. Denture cleansers. Peroxides. Ultrasonic.
INTRODUCTION

Several studies have mentioned the precarious conditions of oral health of denture wearers\textsuperscript{14}. Poor hygiene is associated with the lack of guidance, intrinsic characteristics of dentures and diminished manual dexterity of most denture wearers due to old age\textsuperscript{20}.

Poor denture hygiene allows the accumulation of biofilm, which is defined as a dense microbial layer, formed by microorganisms and their metabolites, consisting of more than 10\textsuperscript{11} microorganisms per gram of dry weight\textsuperscript{15}. When it is properly removed, the result is a reduction in organic material accumulation and proliferation of bacteria and fungi that can cause bad breath, acrylic resin pigmentation and staining, formation of calculus deposits and the development of chronic atrophic candidiasis, also known as denture stomatitis\textsuperscript{25}. Sometimes, microorganisms are spread and lung or gastrointestinal infections can also occur\textsuperscript{17}. Thus, it can be suggested that adequate denture hygiene can prevent some of the uncomfortable conditions and oral diseases associated with denture wearing.

Denture hygiene methods can be divided into mechanical or chemical procedures\textsuperscript{20,21}. Mechanical methods comprise brushing and ultrasonic treatments\textsuperscript{17}. Although brushing is the most widespread\textsuperscript{15,25}, simple, inexpensive and effective method\textsuperscript{20,21}, patients with motor incoordination find it difficult to perform\textsuperscript{15} and there is a possibility of acrylic resin wear\textsuperscript{9,16} and superficial damage to relining materials, therefore it is essential to use adequate brushes and auxiliary agents\textsuperscript{8}.

Ultrasonic devices are mechanical aids generally used by professionals\textsuperscript{5}. The mechanical cleansing activity of the device is complemented with the concomitant use of a chemical solution\textsuperscript{25}. Ultrasound has two mechanisms of action, the first being the movement of liquid resulting from sound waves transferred to the liquid (vibration), and the second, the collapse of bubbles formed by vibration of the unit\textsuperscript{22}.

Chemical methods are classified according to their composition and mechanism, i.e., hypochlorites, peroxides, enzymes, acids, crude drugs and mouth washes for dentures\textsuperscript{17}. Immersion of complete dentures in alkaline peroxide is a simple hygiene method. When these peroxides are dissolved in water, they become alkaline hydrogen peroxide, which decomposes and releases small oxygen bubbles with the mechanical action of detaching the biofilm from the denture surface\textsuperscript{6}. This type of solution can be used alone or in combination with a mechanical method\textsuperscript{17,20,26}.

Clinical experiments show varying results regarding the effectiveness of such agents, demonstrating superiority of the chemical method\textsuperscript{5,13}, brushing\textsuperscript{29}, or an association of the two methods\textsuperscript{28}.

In the literature, the effectiveness of ultrasound is contradictory, as it is attributed to the mechanical action of the device\textsuperscript{23} or to the chemical solutions used\textsuperscript{18}. The combination of this method with brushing or with a chemical immersion method has been suggested as an effective alternative for cleaning complete dentures; however, this effectiveness has not been clinically tested. Although the comparison of chemical methods and brushing is relatively common\textsuperscript{15,20,21}, the comparison of soaking solutions, ultrasound and their association has not previously been described\textsuperscript{28}.

Literature reviews about the efficacy of denture cleansers, dating back to the study of Nikawa\textsuperscript{17} (1999), Shay\textsuperscript{25} (2000) up to the recent study of Souza, et al.\textsuperscript{28} (2009) have shown that there is no consensus about which is the best denture hygiene method, since it is difficult to make comparisons between different studies because of the use of different methodologies to evaluate denture hygiene methods. Therefore, the objective of this study was to evaluate the clinical effectiveness of chemical (sodium perborate-based effervescent tablets), mechanical (ultrasound unit) and combined methods (chemical + mechanical), when associated with brushing, for the removal of complete denture biofilm.

MATERIAL AND METHODS

Patient selection

After approval by the institutional Ethics Committee and signature of informed consent forms by the eligible participants, 80 patients aged 45-80 years (13 men and 47 women) were selected. These individuals needed denture replacements and were under treatment at the Department of Dental Materials and Prosthodontics of the Ribeirão Preto Dental School. The participants presented good general health and were wearing conventional maxillary and mandibular complete dentures. The complete dentures were fabricated from heat-activated acrylic resin, had been in use for periods of time ranging between 5 and 10 years, and received ≥1 scores according to the Additive Index of Ambjørnsen, et al.\textsuperscript{1} (1982).

Hygiene methods and experimental design

The experimental period lasted 21 days. Before the use of each method, the biofilm was eliminated by brushing with a specific brush for complete dentures (Denture - Condor S.A., São Bento do Sul, SC, Brazil) and liquid soap (JOB Química, Produtos para Limpeza Ltda., Monte Alto, SP, Brazil). During the 21-day period, all participants brushed their dentures 3 times a day. Brushing performed by
patients ended on the 21st day. In the morning of the 22nd day, they returned to the clinic without having brushed their dentures again and handed them over to the researchers.

The patients were randomly assigned to groups each using one of the following hygiene methods (n=20):

1) Control:
   a) Brushing the dentures 3 times a day, after each meal (breakfast, lunch and dinner) for 2 min using tap water and a specific brush for complete dentures (Bitufo, Itupeva, SP, Brazil); b) Rinsing the oral cavity with running water after brushing; c) Keeping the dentures immersed in water during sleep.

2) Experimental 1:
   a) Brushing the dentures 3 times a day, after each meal (breakfast, lunch and dinner) for 2 min using tap water, and a specific brush for complete dentures (Bitufo, Itupeva, SP, Brazil); b) Rinsing the oral cavity with running water after brushing; c) Soaking the dentures in a receptacle with warm water (37°C) and one effervescence tablet (Corega tabs – Block Drug Company, Inc., USA) for 20 min after dinner; d) Rinsing the dentures before insertion into the oral cavity; e) Keeping the dentures immersed in water during sleep.

3) Experimental 2:
   a) Brushing the dentures 3 times a day, after each meal (breakfast, lunch and dinner) for 2 min, with a specific brush for complete dentures (Bitufo, Itupeva, SP, Brazil) and tap water; b) Rinsing the oral cavity with running water after brushing; c) Keeping the dentures immersed in water during sleep; d) At the end of the experimental period (21 days), immersion of the dentures in a sterile beaker containing 250 mL of sterile water, and ultrasonic vibration (Ultrasonic Cleaner, modelo2840 D – Odontobrás Ind. e Com. Equip. Méd. Odont. Ltda, Ribeirão Preto, SP, Brazil) for 15 min, performed by a professional.

4) Experimental 3: combination of methods 2 and 3.

**Percentage of area covered with biofilm**

The internal surfaces of the upper dentures were disclosed by 1% neutral red solution. The surfaces were then photographed (digital camera: Canon EOS Digital Rebel EF-S 18-55; and flash: Canon MR-14 EX, Canon Inc., Tokyo, Japan) with standard film-object distance and exposure time. The camera was fixed on a stand (CS-4 Copy Stand, Testrite Inst. Co., Inc., Newark, NJ, USA). Photographs were transferred to a computer. Total surface area and areas corresponding to the stained region were measured using image processing software (Image Tool 2.02) (Figure 1). The biofilm percentage was calculated using the ratio between biofilm area multiplied by 100, and total surface area of the internal denture base. The researcher who measured biofilm coverage was blind to the group to which the dentures belonged, did not participate in the other experimental phases, such as giving instructions, delivering products to patients, or handling the dentures.

After the use of each method and quantification of the biofilm, it was eliminated by brushing with a specific brush for complete dentures (Denture - Condor S.A., São Bento do Sul, SC, Brazil) and liquid soap (JOB Quimica, Produtos para Limpeza Ltda., Monte Alto, SP, Brazil).

**Data analysis**

The variable “percentage area” was submitted to
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Table 1- Percentages of biofilm after the use of each method tested for 21 days

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Control (Corega)</th>
<th>Chemical (Corega)</th>
<th>Mechanical (Ultrasound)</th>
<th>Combined (Corega + Ultrasound)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>41.10</td>
<td>5.48</td>
<td>41.64</td>
<td>16.07</td>
</tr>
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<td></td>
<td>29.46</td>
<td>21.73</td>
<td>7.16</td>
<td>2.96</td>
</tr>
<tr>
<td></td>
<td>54.62</td>
<td>26.61</td>
<td>10.36</td>
<td>18.70</td>
</tr>
<tr>
<td></td>
<td>36.78</td>
<td>56.36</td>
<td>24.69</td>
<td>41.80</td>
</tr>
<tr>
<td></td>
<td>27.97</td>
<td>14.03</td>
<td>0.00</td>
<td>12.46</td>
</tr>
<tr>
<td></td>
<td>13.74</td>
<td>25.76</td>
<td>7.65</td>
<td>28.43</td>
</tr>
<tr>
<td></td>
<td>35.17</td>
<td>41.98</td>
<td>4.28</td>
<td>4.55</td>
</tr>
<tr>
<td></td>
<td>15.63</td>
<td>13.58</td>
<td>13.98</td>
<td>27.15</td>
</tr>
<tr>
<td></td>
<td>50.83</td>
<td>42.71</td>
<td>8.06</td>
<td>0.00</td>
</tr>
<tr>
<td></td>
<td>40.40</td>
<td>15.21</td>
<td>39.92</td>
<td>52.53</td>
</tr>
<tr>
<td></td>
<td>43.44</td>
<td>7.78</td>
<td>38.96</td>
<td>8.67</td>
</tr>
<tr>
<td></td>
<td>12.88</td>
<td>7.40</td>
<td>16.42</td>
<td>0.00</td>
</tr>
<tr>
<td></td>
<td>62.20</td>
<td>6.66</td>
<td>15.97</td>
<td>0.50</td>
</tr>
<tr>
<td></td>
<td>24.64</td>
<td>24.98</td>
<td>12.45</td>
<td>0.00</td>
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<td></td>
<td>79.27</td>
<td>39.89</td>
<td>15.92</td>
<td>1.26</td>
</tr>
<tr>
<td></td>
<td>18.92</td>
<td>3.89</td>
<td>7.76</td>
<td>1.21</td>
</tr>
<tr>
<td></td>
<td>22.07</td>
<td>1.12</td>
<td>12.05</td>
<td>15.68</td>
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<td></td>
<td>88.64</td>
<td>1.76</td>
<td>10.81</td>
<td>18.29</td>
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<td></td>
<td>72.78</td>
<td>6.05</td>
<td>20.89</td>
<td>6.29</td>
</tr>
<tr>
<td></td>
<td>16.52</td>
<td>5.18</td>
<td>5.53</td>
<td>6.14</td>
</tr>
</tbody>
</table>

Table 2- Mean ranks for the treatments and results for Dunn multiple comparison test

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Mean rank</th>
<th>Grouping*</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>60.5</td>
<td>A</td>
</tr>
<tr>
<td>Chemical</td>
<td>37.2</td>
<td>B</td>
</tr>
<tr>
<td>Mechanical</td>
<td>35.2</td>
<td>B</td>
</tr>
<tr>
<td>Combined</td>
<td>29.1</td>
<td>B</td>
</tr>
</tbody>
</table>

Identical letters denote no significant differences between the treatments.

RESULTS

The results of the biofilm percentage areas after the trial are presented in Table 1 and Figure 2. The Control treatment appeared to remove less biofilm than the other methods. The experimental methods appeared to be similar for biofilm removal. The Kruskal-Wallis test found significant difference among the treatments (KW=21.18; P<0.001). The experimental methods were similar, whereas the control group was significantly different.

Figure 2- Biofilm coverage area for each group following treatment (Exp. = Experimental)
from the former (Table 2). This implies that for
denture hygiene, brushing requires auxiliary agents
to achieve better results. However, the tested
agents - peroxide solution, ultrasound or their
combination - attained similar outcomes.

DISCUSSION

The amount of denture biofilm is associated with
the presence of oral lesions\textsuperscript{14}. Thus, its quantification
can be regarded as a good measurement for
denture hygiene outcome. In the present study,
the photographic method associated with a
computerized method of biofilm quantification
was chosen. The first method mentioned above
was chosen because quantification by visual
inspection\textsuperscript{1} is known to be difficult and the second
method mentioned was chosen because it relies
on calibration\textsuperscript{19}.

Previous analyses by the photographic method,
used for comparing total surface areas (internal
and external) of complete dentures, showed high
correlation coefficients\textsuperscript{19}. According to the study
of Paranhos and Silva-Lovato\textsuperscript{19} (2004), biofilm
quantification performed by photographic and
computerized methods, offer objective and accurate
results, and should therefore be the methods of
choice in clinical experiments for the evaluation
of complete denture cleansers. It is important to
emphasize that although the computerized method
was shown to be reliable for measuring biofilm on
complete dentures, it has the important limitation of
requiring a longer amount of time spent on making
the measurements. Thus, in the present study,
the photographic method was associated with the
computerized method to evaluate the effectiveness
of chemical denture cleansers and an ultrasonic
device for biofilm removal from complete dentures.

The mechanical method of performing denture
hygiene is very popular among elderly complete
denture wearers, however, the immersion of
dentures in effervescent alkaline peroxide solutions
is also widely used\textsuperscript{12,25}. However, lack of access to
chemical materials, the cost, and even the patient’s
lack of adequate information about them limit
their use. An alternative method of hygiene is the
ultrasonic device, which is considered a fast and
effective means of cleaning instrumental devices\textsuperscript{18},
and is an important aid in the control of cross
contamination. Such devices are commonly found
in hospitals, asylums and dental schools\textsuperscript{25}.

As in previous studies\textsuperscript{5,24}, the results
demonstrated that brushing with water favored
biofilm accumulation (Figure 2), emphasizing the
need to incorporate an auxiliary agent to assist
brushing. Microbiologic assays and scanning
electron microscopic images have demonstrated
that using a denture brush with water is ineffective
for removing an unacceptably large proportion
of adherent microorganisms\textsuperscript{25}. The difference
observed between the control and chemical
methods (Table 1), indicated that the combination
of immersion in the alkaline peroxide solution and
brushing improved the effectiveness of hygiene,
even within a short period of immersion (5 min).
These results are in agreement with Sheen and
Harrison\textsuperscript{26} (2000) and Paranhos, et al.\textsuperscript{20} (2007),
who found the chemical methods effective in
standardized clinical trials, when compared with
control groups using water.

Similarly, there was a significant decrease in
the biofilm levels with the use of ultrasound
(Figure 2), demonstrating the effectiveness of
the method, even when it was used only once
during the experiment. This effectiveness can be
attributed exclusively to the ultrasonic cavitation,
as no chemical substance was incorporated to the
apparatus.

The statistical equality between the chemical
and mechanical methods indicated that the use
of ultrasound alone achieved the same results as
those obtained by using the tablets daily. Previous
studies demonstrated the superiority of ultrasound,
in comparison with the alkaline peroxide solutions,
even when used with water\textsuperscript{11,18,23}, however, the
ultrasound unit was used daily.

Although the use of the combined method
provided greater reduction in biofilm levels, the
means obtained (Table 1) were statistically equal
to those of the groups in which the methods were
used alone. Paranhos, et al.\textsuperscript{20} (2007) found greater
effectiveness with the combined method; however,
for the mechanical method, specific brushes and
toothpastes were used to clean the complete
dentures, products that proved to be effective in
removing biofilm\textsuperscript{11,24,27}.

As regards the mechanical method, experiments
incorporating antiseptic solutions such as sodium
hypochlorite are important, since studies have shown
that the effectiveness of these solutions is superior
when compared with peroxides\textsuperscript{13,15}. Microbiological
comparative studies have concluded that effective
hygiene is obtained by the combination of chemical
(immersion) and mechanical (brushing) methods\textsuperscript{5}.

This way, microbiological experiments that
evaluate the effectiveness of the combination of the
immersion with the ultrasound should be conducted
in future studies. With regard to the chemical
method, studies should be conducted using the
products in prolonged periods of immersion, since
previous studies indicated that short periods are
less effective in comparison with prolonged periods
of immersion (overnight)\textsuperscript{4}. Future comparisons
conducted in a clinical trial design could assess
the use of different chemicals within the ultrasonic
device, i.e., surfactants or antimicrobial agents,
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since an important limitation of this study was that the peroxides were not used as conducting fluid in the ultrasonic device.

CONCLUSION

The three methods used (chemical, mechanical and combined) were equally effective with respect to the ability to remove biofilm and were superior to the established control method (brushing with water), contributing to the maintenance of oral health care of the complete denture wearers.

ACKNOWLEDGEMENTS

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REFERENCES