EFFECTS OF REPEATED LONG-TERM SODIUM HYPOCHLORITE DISINFECTION TREATMENT ON SURFACE HARDNESS AND ROUGHNESS OF SELF-POLYMERIZING RELINE ACRYLIC RESINS

ABSTRACT

INTRODUCTION: Denture stomatitis is the most common alteration on the palate of denture wearers and deficient denture hygiene is an important predisposing factor, because it facilitates both the presence of Candida albicans and bacteria in saliva and their colonization on the oral mucosa and denture surfaces. Sodium hypochlorite is an efficient chemical disinfectant to eliminate denture biofilm, but the effect of long-term disinfection on reline acrylic resins was not studied. PURPOSE: This study investigated the hardness and roughness of three self-polymerizing reline resins after repeated long-term sodium hypochlorite disinfections. MATERIAL AND METHODS: Forty round specimens (30 x 6mm) were made from each material: Jet, Kooliner and Tokuyama Rebase II Fast, and divided in 4 groups (n=10). The control group was stored in water and the others were disinfected with 1%, 2%, 5.25% sodium hypochlorite, respectively. The specimens were tested for knoop hardness (KHN) and roughness (Ra) before disinfection and after 30, 90 and 180 disinfection cycles. Data were analyzed by analysis of variance followed by the Tukey test at 5%. RESULTS: The hardness of Jet resin varied from 18.74 ± 0.47 to 13.86 ± 0.82 KHN, Kooliner varied from 14.09 ± 1.63 to 7.88 ± 0.88 KHN, and Tokuyama Rebase II Fast from 12.57 ± 0.94 to 8.28 ± 0.39 KHN. Statistically significant decrease in hardness of the three reline acrylic resins was observed early after the first 30 disinfection cycles. The roughness of Jet resin varied from 0.07 ± 0.01 to 0.24 ± 0.03 µm, Kooliner varied from 0.26 ± 0.05 to 0.37 ± 0.66 µm, and Tokuyama Rebase II Fast from 0.09 ± 0.03 to 0.24 ± 0.04 µm. CONCLUSION: Although the hardness and roughness value has showed a statistically significant increase after 90 immersion cycles in hypochlorite solution, this alteration was considered of little clinical relevance.

KEYWORDS
Sodium hypochlorite. Disinfection. Complete dentures.
INTRODUCTION

The use of complete dentures over the time carries normally tissue changes, causing denture mismatch which in most of cases indicates the necessity of reline by means of the use of hard or soft denture reline acrylics. The laboratory reline materials involve an extra patient visit as well as a laboratory fee, and involves the patient living without their denture for a period of time. The direct relining of the denture bases in the mouth with self-polymerizing acrylic resins, is not only faster than laboratory-processed reline systems, but can also reproduce the morphologic features of oral soft tissue directly on the denture base.

After relining, adjustments and polishing procedures are often required in order to provide better conditions of denture fit in relation to the alveolar bone. Polishing techniques produce differences in surface topography and affect the adhesion of microorganisms on the surface, with higher number of cells retained on rougher surfaces, creating an environment favorable for disease development.

Denture stomatitis is the most common alteration on the palate of denture wearers. Many factors can be associated with denture stomatitis, but oral and denture hygiene seem to be the most relevant. Deficient oral and denture hygiene is an important pre-disposing factor, because it facilitates both the presence of Candida albicans and bacteria in saliva and their colonization on the oral mucosa and dentures surfaces.

Studies in the literature show many chemical procedures that may be used for denture biofilm control. Some authors emphasized the use of oral hygiene products to decrease the quantity of microorganisms on the denture surface, but others consider that microorganisms can and do penetrate into the acrylic resin. Thus, it becomes important to ensure that disinfection procedures, using immersion disinfectant solutions, should effectively disinfect not only the external surface of the denture, but the interior of the dentures as well.

The hypochlorite is the oldest and most used chlorine compound in the field of chemical disinfection. They are powerful germicides, broad-spectrum antimicrobial agents, not harmful to humans at marketed concentrations without harmful residue or solution discoloration, because they are easy to use and more economical.

In dentistry, these solutions were introduced as antiseptics in 1835 and may be used at a concentration of 5.25%, which is a combination of chlorine activated with strong bases, or in lower concentrations of 2%, 1% or even diluted 0.5%. The immersion time is variable, according to the concentration used, it can vary between 5 and 30 minutes and is
not recommended to exceed more than 30 minutes\textsuperscript{14}.

When the immersion procedure is used, the choice of disinfectant should be made with regard to its effectiveness in inactivating microorganisms without any adverse effects on the denture materials. Few studies were found in the literature correlating the action of disinfectant solutions on the mechanical properties of heat-polymerized acrylic resins\textsuperscript{15-16-17}, acrylic resin denture teeth\textsuperscript{15,18} and reline acrylic resins\textsuperscript{19}, but few of these studies evaluated the effect after repeated long-term chemical disinfections\textsuperscript{20-21}.

An important property, which turns possible the use of acrylics like denture base is their hardness, supporting adverse conditions present in the oral cavity like occlusion forces. Other characteristic that is taken into account in dental materials is their roughness, related to increase the adhesion of microorganism. It is established that in front of material roughness below 0.2µm, a significant decrease in bacterial adhesion could be expected\textsuperscript{3}. Many authors show the effect of a implementation of a protocol of cleanness in denture wearers using chemical solutions or microwave in soft denture reline acrylics\textsuperscript{10,12}, but not much is known about negative effects on hard denture reline acrylics. Measurements of acrylic hardness will indicate us the possibility of degradation of polymeric matrix which will produce decrease on acrylic hardness, increasing the possibility of fracture and diminishing the longevity of the denture base\textsuperscript{22} whereas surface roughness could predict an increase in microorganism retention on the acrylic surface.

Therefore, the effects of long-term immersion in the sodium hypochlorite solution and water on hardness and roughness of relining acrylic resins are topics for investigation. There is no evidence in the literature that successive disinfection cycles could alter surface of acrylic resin. Many other studies showed that there is a necessity to investigate long period of immersion in disinfectant solutions to confirm if this repeated procedure is really secure\textsuperscript{16,23-24}. Thus, the aim of this study was to investigate the effect of long-term sodium hypochlorite disinfection solution and water immersion on the hardness and roughness of three reline acrylic resins. The hypothesis to be tested was that all solutions studied could cause adverse effect on the hardness and roughness of reline materials.

**MATERIAL AND METHODS**

Three self-polymerizing acrylic resins used to relines were evaluated. The names of the resins, manufacturers and powder/liquid ratios are presented in Table 1.

Forty specimens of each resin were produced in molds prepared by the investment of plastic discs (30mmX6mm) in silicone
rubber (Zetalabor Hard 85 shore-A, Zhermack, Rovigo, Italy), further supported by dental stone (Gesso Pedra Herodent – Vigodent S/A Ind. e Com., Rio de Janeiro, RJ, Brazil) within the flask (Mac Artigos odontológicos e prótese Ind. e Com. LTDA, São Paulo, SP, Brazil). The liquid/powder ratio of the polymer dough for all materials was mixed according to the manufacturers’ instructions (Table 1), inserted into the molds, and packed under 0.5kgf pressure, during 10 minutes until complete polymerization. Any flash and excess was removed by polishing both sides of specimens using progressively finer grades of silicon carbide paper (320, 600, 1.200) and polished with felt paper wet by diamond (Extec Corp., Enfield, USA) to obtain a smooth flat surface.

<table>
<thead>
<tr>
<th>Brand name</th>
<th>Composition</th>
<th>Powder</th>
<th>Manufacturer</th>
<th>Liquid ratio (g/ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Jet</td>
<td>PMMA</td>
<td>PMMA</td>
<td>A.O. Clássico Ltda, São Paulo, SP, Brazil</td>
<td>16/8</td>
</tr>
<tr>
<td>Kooliner</td>
<td>IBMA</td>
<td>PEMA</td>
<td>GC América Inc. ALSIP, IL, USA</td>
<td>30/12</td>
</tr>
<tr>
<td>Tokuyama</td>
<td>MAOP and 1,6-HDMA</td>
<td>PEMA</td>
<td>Tokuyama Dental Corporation, Tokyo, Japan</td>
<td>18/12</td>
</tr>
</tbody>
</table>

IBMA=isobutyl methacrylate; PEMA=poly(ethyl methacrylate); PMMA=polymethyl methacrylate; MAOP=β-methacryloyl oxyethyl propionate; 1,6-HDMA=1,6-hexanediol dimethacrylate.

After polishing, all the specimens were numbered, to allow comparisons during the study, and one of the specimens side was marked to indicate the side used for hardness test. The other side, without mark was used for roughness test.

Before initial hardness and roughness test, specimens were stored in distilled water at 37°C for 48 ± 2 hours according to ADA (American Dental Association, 1975) and ISO (International Organization for Standardization Specification 1567, 1988) specifications. Thereafter, an initial hardness value of each specimen was measured using a Knoop Hardness Tester (HMV-2000/Shimadzu Corporation, Japan). The test involved the use of a lozenge pyramid-shaped diamond indenter point; a 30-gf load for 30-second. Four indentations were made at different points on each specimen, and the means of individual specimens were calculated.

The surface roughness (Ra, µm) was analyzed with a surface roughness profilometer (Hommel Tester T 1000 basic; Hommelwerke GmbH, ref. # 240851, Schwenningen, Germany) with a diamond spherical stylus end, that touches the surface.
connected to a unit that processes and analyzes the information by the software (Turbo Datawin-NT Version 1.34, Copyright © 2001). The measurements were obtained by the analyzing tip of the profilometer; skimming in a line 4.80mm of the surface.

The specimens of each resin were divided randomly into 4 groups (n=10) for immersion in one of the following solutions: water (control group); 1% sodium hypochlorite (H1%) (Farmácia Específica Manipulação de Fórmulas, Bauru, SP, Brazil); 2% sodium hypochlorite (H2%) (Farmácia Específica Manipulação de Fórmulas, Bauru, SP, Brazil); 5.25% sodium hypochlorite (H5.25%) (Farmácia Específica Manipulação de Fórmulas, Bauru, SP, Brazil). The disinfection protocol used for each solution, followed those of studies already published in literature, which demonstrated the effectiveness of disinfection according to concentration and immersion time²¹. For Groups H1%, a protocol of immersion for 10 minutes, and for Groups H2% and H5.25% immersion for 5 minutes was adopted.

Both for the hardness and roughness test, the specimens were divided in four quadrants, totalizing 4 measures for each specimen. The measurements were made randomly on the surface of each quadrant before disinfection and after 30 and 90 disinfection cycles in order to obtain comparison parameters between different evaluation intervals, to allow comparisons throughout the study period.

Statistical analysis of the data was performed using factorial scheme (4 solutions X 3 evaluation interval) and the means were analyzed by two-way analysis of variance (ANOVA) and Tukey test to determine differences in effect of disinfectant solutions on the relining acrylic resin studied. Statistical analyses were conducted at 95% confidence level.

RESULTS

Analysis by two-way ANOVA indicated that the solutions and evaluation intervals showed statistically significant differences (p<0.05) for all resins studied.

According to the data contained in Table 2, it was observed that immersion in water caused a decrease in hardness values of specimens of three reline materials studied, and so did the solutions used for disinfection.

Analyzing each material alone, it can be noted that when subjected to disinfection with 1% and 2% sodium hypochlorite, Jet and Tokuyama Rebase II Fast specimens showed a significant decrease in mean hardness values in the 30-cycle disinfection protocol, but in subsequent assessments, these values remained unchanged. When these specimens were disinfected with 5.25% sodium hypochlorite significant continuous decreasing
in hardness values was noted until the last evaluation period.

Table 2. Effect of disinfection and water immersion on Hardness (KHN) of Materials.

<table>
<thead>
<tr>
<th>Material</th>
<th>Solution</th>
<th>48 hours 37± 2°C Water</th>
<th>1-30 Disinfection cycles</th>
<th>31-90 Disinfection cycles</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>1D30-Disinfection-cycles</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Water-alone</td>
<td>18,05 ± 1,45ª</td>
<td>14,87 ± 0,56 b</td>
<td>14,30 ± 1,10 b</td>
</tr>
<tr>
<td>JET</td>
<td>H 1%</td>
<td>18,21 ± 1,76ª</td>
<td>14,34 ± 0,75 b</td>
<td>15,22 ± 0,72 b</td>
</tr>
<tr>
<td></td>
<td>H 2%</td>
<td>17,65 ± 1,63ª</td>
<td>14,29 ± 0,62b</td>
<td>14,23 ± 0,84 b</td>
</tr>
<tr>
<td></td>
<td>H 5,25%</td>
<td>18,74 ± 0,47ª</td>
<td>13,86 ± 0,82 b</td>
<td>15,11 ± 1,08 c</td>
</tr>
<tr>
<td></td>
<td>Water-alone</td>
<td>14,09 ± 1,63ª</td>
<td>9,90 ± 1,03b</td>
<td>10,13 ± 0,89 b</td>
</tr>
<tr>
<td>KOOLINER</td>
<td>H 1%</td>
<td>12,74 ± 1,06ª</td>
<td>8,96 ± 0,53b</td>
<td>7,88 ± 0,96 c</td>
</tr>
<tr>
<td></td>
<td>H 2%</td>
<td>11,9 ± 1,06ª</td>
<td>9,91 ± 0,53b</td>
<td>8,13 ± 0,96 c</td>
</tr>
<tr>
<td></td>
<td>H 5,25%</td>
<td>12,99 ± 0,96ª</td>
<td>9,15 ± 0,83b</td>
<td>8,92 ± 0,70 b</td>
</tr>
<tr>
<td></td>
<td>Water-alone</td>
<td>12,57 ± 0,94ª</td>
<td>9,45 ± 0,26b</td>
<td>9,91 ± 0,74 b</td>
</tr>
<tr>
<td>TOKUYAMA REBASE II FAST</td>
<td>H 1%</td>
<td>12,54 ± 0,80ª</td>
<td>9,07 ± 0,12b</td>
<td>8,91 ± 0,79 b</td>
</tr>
<tr>
<td></td>
<td>H 2%</td>
<td>11,66 ± 0,58ª</td>
<td>9,06 ± 0,32b</td>
<td>9,15 ± 0,37b</td>
</tr>
<tr>
<td></td>
<td>H 5,25%</td>
<td>11,27 ± 0,41ª</td>
<td>8,28 ± 0,39b</td>
<td>9,60 ± 0,60 c</td>
</tr>
</tbody>
</table>

Values are means ± standard deviations. Horizontally, means with the same letter were not significantly different from each other at p=0.05 level. No comparison was made among materials and solutions.

It can be seen from Table 3 that at the end of this study, water promoted significant ($p < 0.05$) increase in roughness, only for Jet and Tokuyama Rebase II Fast specimens.

Jet specimens disinfected with 1% and 2% sodium hypochlorite showed continuous roughness increase during the evaluated period while 5.25% sodium hypochlorite promoted roughness increase after 90 disinfection cycles.
Table 3. Effect of disinfection and water immersion on Roughness (Ra µm) of Materials.

<table>
<thead>
<tr>
<th>Material</th>
<th>Solution</th>
<th>48 hours 37±2°C Water</th>
<th>1-30 Disinfection cycles</th>
<th>31-90 Disinfection cycles</th>
</tr>
</thead>
<tbody>
<tr>
<td>JET</td>
<td>Water alone</td>
<td>0.08 ± 0.04 b</td>
<td>0.12 ± 0.03 a</td>
<td>0.12 ± 0.02 a</td>
</tr>
<tr>
<td></td>
<td>H 1%</td>
<td>0.08 ± 0.02 c</td>
<td>0.13 ± 0.02 b</td>
<td>0.24 ± 0.03 a</td>
</tr>
<tr>
<td></td>
<td>H 2%</td>
<td>0.07 ± 0.01 c</td>
<td>0.12 ± 0.03 b</td>
<td>0.19 ± 0.03 a</td>
</tr>
<tr>
<td></td>
<td>H 5.25%</td>
<td>0.09 ± 0.03 b</td>
<td>0.11 ± 0.03 b</td>
<td>0.19 ± 0.03 a</td>
</tr>
<tr>
<td>KOOLINER</td>
<td>Water alone</td>
<td>0.36 ± 0.08 a</td>
<td>0.34 ± 0.04 a</td>
<td>0.34 ± 0.06 a</td>
</tr>
<tr>
<td></td>
<td>H 1%</td>
<td>0.29 ± 0.07 a</td>
<td>0.31 ± 0.07 a</td>
<td>0.37 ± 0.06 a</td>
</tr>
<tr>
<td></td>
<td>H 2%</td>
<td>0.36 ± 0.07 a</td>
<td>0.26 ± 0.05 b</td>
<td>0.29 ± 0.04 b</td>
</tr>
<tr>
<td></td>
<td>H 5.25%</td>
<td>0.31 ± 0.09 a</td>
<td>0.31 ± 0.08 a</td>
<td>0.33 ± 0.07 a</td>
</tr>
<tr>
<td>TOKUYAMA REBASE II FAST</td>
<td>Water alone</td>
<td>0.09 ± 0.03 b</td>
<td>0.12 ± 0.03 ba</td>
<td>0.11 ± 0.01 a</td>
</tr>
<tr>
<td></td>
<td>H 1%</td>
<td>0.10 ± 0.02 b</td>
<td>0.11 ± 0.03 b</td>
<td>0.24 ± 0.04 a</td>
</tr>
<tr>
<td></td>
<td>H 2%</td>
<td>0.15 ± 0.05 ab</td>
<td>0.11 ± 0.02 b</td>
<td>0.16 ± 0.01 a</td>
</tr>
<tr>
<td></td>
<td>H 5.25%</td>
<td>0.13 ± 0.04 b</td>
<td>0.12 ± 0.04 b</td>
<td>0.18 ± 0.04 a</td>
</tr>
</tbody>
</table>

Values are means ± standard deviations. Horizontally, means with the same letter were not significantly different from each other at p=0.05 level. No comparison was made among materials and solutions.

Kooliner specimens did not present roughness changes, except for specimens disinfected with 2% sodium hypochlorite. These specimens showed roughness increase after 30 disinfection cycles but the roughness values remained unchanged after 90 disinfection cycles.

All sodium hypochlorite concentration caused roughness increase in Tokuyama Rebase II Fast specimens after 90 disinfection cycles.

DISCUSSION

The present study evaluated the effect of disinfectant solutions and water on the hardness and roughness of reline acrylic resins after long-term immersion. Data obtained under the present conditions confirmed the hypothesis that the hardness and roughness of reline materials could be affected by the type of disinfectant and the time of storage in water.

Table 3 shows a significant decrease in hardness levels independently of the disinfectant solution used or water as control.

Compared with heat-cured or light-cured resins, self-polymerizing acrylic resin has a higher level of residual monomers.
Hardness values of self-polymerizing resins are lower than heat-cured resins because polymerization in room temperature associated with oxygen presence inhibit or retard the polymerization and produce amounts of methyl methacrylate monomer that still remains in the acrylic resin and facilitate microvoid formation between polymeric chains\textsuperscript{25-26}. The residual methyl methacrylate monomer released into water and the water sorption are superior in self-polymerizing resins.

Water sorption initially caused a softening of the polymer resin component by swelling the network and reducing the frictional forces between the polymer chains.

The absorbed moisture also acts as a plasticizer, lowering the glass transition temperature (Tg) of the cured resins. Water sorption may eventually cause irreversible damage to the material by formation of microcracks through repeated sorption/desorption cycles. This is followed by hydrolytic degradation of the polymer with scission of the ester linkages and gradual deterioration of the infrastructure of the polymer over time. Once the polar sites in the polymer network become saturated with water, equilibrium is reached between bound and free sites and the water sorption stabilized intermittently its absorption\textsuperscript{26}. The results of our study corroborate the hypothesis that both water alone and water contained into hypochlorite solution, diffuse through the resin until saturation, which softens the surface in self-polymerizing resins and decreases the hardness values.

Although the results of this study reveal a small, but significant decrease on all the materials after 90 repeated immersion cycles of disinfections; we considered these findings clinically not significant. The hardness exhibited by all the hard relining materials before and after 90 cycles of disinfection procedure are acceptable for a secure use by complete acrylic denture wearers; thus, for complete acrylic denture wearers with stomatitis, it is possible an implementation of a daily immersion protocol with any of this chemical disinfection solutions, with these concentrations and immersion time, for complement mechanical cleanses.

The roughness exhibited in all the materials, including the initial results and those after 90 immersion cycles of disinfection was lower than the results reported by the literature\textsuperscript{27}. The results of the present study revealed that after 90 cycles of a simulated denture disinfection procedure in chemical solutions, the surface roughness was close to or over the threshold surface roughness for bacterial retention (Ra = 0.2 µm); an increase in bacterial colonization would be expected on surface roughness over 2.2 µm. A previous study showed that the same acrylic resins maintained low surface roughness levels, when
submitted to 30 cycles of disinfection. That collaborates to low plaque accumulation and enables effective mechanical and chemical denture hygiene. The present study results corroborate to these findings and show that the tested hypochlorite solutions can be safely used on self-polymerizing reline acrylic resins through 90 immersion cycles, without drastic changes on its superficial roughness.

Therefore, for the complete interpretation of these results, further studies, such as increased periods of time, must be conducted to verify the effect of chemical disinfecting solutions on hardness and surface roughness of hard reline acrylic resins.

CONCLUSION

The hypothesis tested was accepted because the studied solutions promoted adverse effects on reline acrylic resins. Nevertheless the tested disinfection protocols were considered safe to use in self-polymerized acrylic resins. Although the hardness and roughness value has showed a statistically significant increase after 90 immersion cycles in hypochlorite solution, this alteration was considered of little clinical relevance. Other studies are necessary for an evaluation of the effects of degradation on acrylic resins by using sodium hypochlorite disinfection.

REFERENCES


