EFFECTS OF LONG-TERM REPEATED CHEMICAL DISINFECTION TREATMENT ON THE SURFACE HARDNESS SELF-POLYMERIZING RELINE RESINS

ABSTRACT

Denture hygiene techniques and procedures were developed in the 1960s and 1970s and most studies indicate the importance of mechanical biofilm removal by denture brushing associated with disinfection with chemical solutions. Studies in the literature show many chemical procedures that may be used for denture biofilm control. When the immersion procedure is used, the disinfectant should be selected with regard to its effectiveness in inactivating microorganisms without any adverse effects on the denture materials. PURPOSE: This study investigated the hardness of three self-polymerizing reline resins after long-term repeated chemical disinfections. MATERIAL AND METHODS: Sixty round specimens (30 x 6mm) were made from each material: Jet, Kooliner and Tokuyama Rebase II Fast, and divided in 6 groups (n=10). The control group was stored in water and the others were disinfected with 1%, 2%, 5.25% sodium hypochlorite, 2% glutaraldehyde, and 4% chlorhexidine gluconate, respectively. The specimens were tested for knoop hardness (KHN) 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 13.75 ± 0.95 KHN, Kooliner varied from 14.09 ± 1.63 to 7.52 ± 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. CONCLUSION: The hardness of the tested materials decreased after immersion in water and after long-term repeated chemical disinfections.

KEYWORDS

INTRODUCTION

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. Denture hygiene techniques and procedures were developed in the 1960s and 1970s\(^1\) and most studies indicate the importance of mechanical biofilm removal by denture brushing associated with disinfection with chemical solutions\(^2-4\).

When the immersion procedure is used, the disinfectant should be selected 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\(^5,6\) acrylic resin denture teeth\(^7\) and reline acrylic resins\(^8\), but none of these studies evaluated the effect after long-term repeated chemical disinfections. The surface hardness measurements of a denture base resin indicate to what extent the forces applied during mastication can be resisted, and their decrease indicates surface softening and degradation\(^9\). Furthermore, the effects of long-term immersion in disinfectant solutions and water on the hardness of reline acrylic resins should be investigated. There is no evidence in the literature that successive disinfection cycles could alter the acrylic resin surface. Many other studies have shown that there is a need to investigate long periods of immersion in disinfectant solutions to confirm whether this repeated procedure is really safe\(^5,7-9\). The hardness values of reline resins are lower than those of heat-polymerized resins, because polymerization at room temperature associated with the presence of oxygen inhibits or delays the polymerization and produces amounts of methyl methacrylate monomer that remain in the acrylic resin and facilitate microvoid formation between polymeric chains\(^10,11\). There is greater residual methyl methacrylate monomer release into water and water sorption in reline resins.

Water uptake in polymer network is related to resin polarity and chain topology\(^12,13\). Resin polarity influences the number of hydrogen bonding sites and the attraction between polymer and water molecules, while chain topology determines the spatial configuration of the molecular segments and the availability of nanopores within the polymer structure. Water sorption initially causes softening of the polymer resin component by swelling the network and reducing the frictional forces between the polymer chains. Water sorption may eventually cause irreversible damage to the material by microcrack formation 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. Since the polar sites in the polymer network become saturated with water, equilibrium is reached between bound and free sites and water sorption is stabilized, intermitting its absorption\textsuperscript{14}.

Thus, the aim of this study was to investigate the effect of long-term repeated chemical disinfection treatment and water on the hardness of three reline acrylic resins. The hypothesis to be tested was whether all solutions studied could cause adverse effect on the hardness of reline materials.

**MATERIAL AND METHODS**

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

<table>
<thead>
<tr>
<th>Brand name</th>
<th>Liquid</th>
<th>Composition</th>
<th>Manufacturer</th>
<th>Powder/ 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>
<tr>
<td>Rebase II Fast</td>
<td></td>
<td></td>
<td></td>
<td></td>
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</tbody>
</table>

\textsuperscript{IBMA}=isobutyl methacrylate; PEMA=poly(ethyl methacrylate); PMMA=poly(methyl methacrylate); MAOP=\(\beta\)-methacryloyl oxyethyl propionate; 1,6-HDMA=1,6-hexanediol dimethacrylate

Sixty specimens of each resin were produced in molds prepared by investment of plastic discs (30mm x 6mm) 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). All materials were mixed with the correct powder/liquid ratios and following the manufacturers’ instructions (Table 1), inserted into the molds, and packed under 0.5kgf pressure for 10 minutes until complete polymerization. After deflasking, flash and excess resin were removed by hand polishing on both sides using 320-, 600-, 1200- grit silicon carbide paper and polishing with felt paper wet with diamond (Extec Corp., Enfield, USA) to obtain a smooth flat surface.

After polishing, all specimens were numbered, to allow comparisons during the
study, and then 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 for hardness testing. Thereafter, an initial hardness value of each specimen was measured using a Knoop Hardness Tester (HMV-2000/ Shimadzu Corporation, Tokyo, Japan). Four indentations were made at different points on each specimen, and the means of individual specimens were calculated.

The specimens of each resin were divided randomly into 6 groups (n=10) for immersion in one of the following solutions: water (control group); 1% sodium hypochlorite (H1%) (Pharmácia Específica Manipulação de Fórmulas, Bauru, SP, Brazil); 2% sodium hypochlorite (H2%) (Pharmácia Específica Manipulação de Fórmulas, Bauru, SP, Brazil); 5.25% sodium hypochlorite (H5.25%) (Pharmácia Específica Manipulação de Fórmulas, Bauru, SP, Brazil); 2% glutaraldehyde (G2%) (Pharmácia Específica Manipulação de Fórmulas, Bauru, SP, Brazil); and 4% chlorhexidine gluconate (CG4%) (Pharmácia 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 time 15-26. For Groups H1%, G2%, CG4% a protocol of immersion for 10 minutes, and for Groups H2% and H5,25% immersion for 5 minutes was adopted.

Specimens were submitted to the disinfection protocol 180 times to simulate 180 cycles of repeated disinfection. Between each cycle, the specimens were washed in deionized water for 3 minutes to simulate the clinical condition in which the patient put the denture into a disinfectant solution, washes it in water after disinfection, and re-uses it. After washing, the specimens were dried with paper towel, so that drops of water were not incorporated into the disinfectant solution.

Control specimens were kept in water for the time required to perform the disinfection procedures. Both disinfectants solution and water were used at room temperature throughout the experimental period.

Hardness measurements were made after 30, 90 and 180 disinfection cycles, in order to obtain comparison parameters between different evaluation intervals, to allow comparisons throughout the study period. Statistical analysis of data was performed using the factorial scheme (6 solutions x 4 evaluation intervals) and the means were analyzed by two-way analysis of variance (ANOVA) and the Tukey test to determine differences in the effect of disinfectant solutions on the self-polymerizing acrylic
resins studied. Differences were considered statistically significant at p<0.05.

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.

| Table 2: Effect of disinfection and water immersion on hardness of self-polymerizing reline resins. |
|-------------------------------------------------------|---------------------------------|---------------------------------|---------------------------------|---------------------------------|
| **Solution**                                          | **48 hours**                    | **30**                          | **90**                          | **180**                         |
|                                                      | **37 ± 2°C Water**              | **Disinfection cycles**         | **Disinfection Cycles**         | **Disinfection cycles**         |
| Water alone                                          |                                | 14.87 (0.56) \(^{a}\)           | 14.30 (1.10) \(^{ac}\)          | 13.75 (0.95) \(^{b}\)          |
|                                                      | H 1%                           | 14.34 (0.75) \(^{a}\)           | 15.22 (0.72) \(^{a}\)           | 15.26 (0.63) \(^{a}\)          |
|                                                      | H 2%                           | 14.29 (0.62) \(^{a}\)           | 14.23 (0.84) \(^{a}\)           | 14.58 (0.43) \(^{b}\)          |
|                                                      | H 5.25%                        | 13.86 (0.82) \(^{a}\)           | 15.11 (1.08) \(^{a}\)           | 15.00 (1.03) \(^{a}\)          |
| JET                                                  | G 2%                           | 14.34 (0.92) \(^{a}\)           | 14.51 (0.90) \(^{ac}\)          | 15.43 (0.39) \(^{a}\)          |
|                                                      | GC 4%                          | 14.71 (0.92) \(^{a}\)           | 14.44 (0.55) \(^{a}\)           | 14.89 (0.59) \(^{a}\)          |
|                                                      |                                |                                |                                |                                |
| Water alone                                          |                                | 14.09 (1.63) \(^{a}\)           | 9.90 (1.03) \(^{a}\)            | 10.13 (0.89) \(^{a}\)          | 9.02 (0.77) \(^{a}\)          |
|                                                      | H 1%                           | 8.96 (0.53) \(^{a}\)            | 7.88 (0.96) \(^{a}\)            | 7.70 (0.51) \(^{a}\)          |
| KOOLINER                                             | H 2%                           | 9.91 (0.53) \(^{a}\)            | 8.13 (0.96) \(^{a}\)            | 7.70 (0.51) \(^{a}\)          |
|                                                      | H 5.25%                        | 9.15 (0.83) \(^{a}\)            | 8.92 (0.70) \(^{a}\)            | 8.09 (0.90) \(^{a}\)          |
|                                                      | G 2%                           | 8.92 (0.67) \(^{a}\)            | 8.11 (0.76) \(^{a}\)            | 8.79 (0.93) \(^{a}\)          |
|                                                      | GC 4%                          | 8.72 (0.84) \(^{a}\)            | 8.92 (0.87) \(^{a}\)            | 7.52 (0.88) \(^{a}\)          |
|                                                      |                                |                                |                                |                                |
| Water alone                                          |                                | 12.57 (0.94) \(^{a}\)           | 9.45 (0.26) \(^{a}\)            | 9.91 (0.74) \(^{a}\)          | 8.47 (0.27) \(^{a}\)          |
|                                                      | H 1%                           | 9.07 (0.12) \(^{a}\)            | 8.91 (0.79) \(^{a}\)            | 9.10 (0.45) \(^{a}\)          |
| TOKUYAMA REBASE II FAST                              | H 2%                           | 9.06 (0.32) \(^{a}\)            | 9.15 (0.37) \(^{a}\)            | 9.49 (0.50) \(^{a}\)          |
|                                                      | H 5.25%                        | 8.28 (0.39) \(^{a}\)            | 9.60 (0.60) \(^{a}\)            | 9.24 (0.31) \(^{a}\)          |
|                                                      | G 2%                           | 9.34 (0.34) \(^{a}\)            | 8.57 (0.35) \(^{a}\)            | 8.90 (0.25) \(^{ac}\)         |
|                                                      | GC 4%                          | 9.13 (0.40) \(^{a}\)            | 9.32 (0.33) \(^{a}\)            | 9.53 (0.34) \(^{a}\)          |

The values are means; standard deviations are presented in parentheses. Horizontally, means with same letters were not significantly different from each other at p=0.05. No comparisons were made between solutions and self-polymerizing reline resins.
Analyzing each material alone, it can be noted that when subjected to disinfection with 1% and 2% sodium hypochlorite and 4% chlorhexidine gluconate, Jet 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 and 2% glutaraldehyde, a fluctuation in hardness values was noted. The mean values in the 30-cycle protocol were significantly lower in comparison with the initial assessment; however, in the following evaluation (90 cycles) an increase in hardness values was noted when compared with 30 cycles.

Kooliner specimens showed a significant decrease in hardness after 30 disinfection cycles, irrespective of the disinfectant solution used. However, the effect of the solutions was different; 1% and 2% sodium hypochlorite and 4% chlorhexidine gluconate caused significant decrease in hardness after 30 disinfection cycles, whereas for 5.25% sodium hypochlorite and 2% glutaraldehyde the hardness values were unchanged after 30 cycles.

The hardness analysis of Tokuyama Rebase II fast specimens showed a significant decrease in values after 30 disinfection cycles in any disinfectant tested. But in subsequent evaluations, a difference in behavior between the solutions was noted.

For 1% and 2% sodium hypochlorite and 4% chlorhexidine gluconate the hardness values remained unchanged after 30 disinfection cycles; 5.25% sodium hypochlorite caused a significant reduction in hardness after 30 cycles, however, these values increased again in the 90-cycle evaluation. There was no difference between hardness values found in 90 and 180 disinfection cycles.

When subjected to disinfection with 2% glutaraldehyde, Tokuyama Rebase II fast specimens showed a reduction in hardness after 30 disinfection cycles. There was also a significant reduction in these values between 30 and 90 disinfection cycle evaluations.

**DISCUSSION**

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

The analysis of these data shows that the storage in water caused a decrease in hardness of the three reline acrylic resins throughout the study period. Except for Tokuyama Rebase II fast, hardness values did not change after the 30th cycle.
The studies in the literature that evaluated the effect of storage in water on the surface hardness of reline acrylic resins are controversial. Some studies that exclusively evaluated the effect of immersion in water on the hardness of self-polymerizing acrylic resin for 24 hours, 7, 14, 32, 60 and 120 days observed an increase in hardness after immersion in water for 78, 27, 3228 and 60 days6. According to these authors the increase in hardness may be related to further polymerization and residual monomer release mechanisms, which probably overcame the plasticizing effect of water uptake.

Other authors, however, claim that when immersed in water, the reline acrylic resins release greater amounts of residual monomer, and absorb more moisture, promoting plasticizing of the surface layers10,11.

The results of the present study corroborate the hypothesis that water diffuses through the resin until it becomes saturated, which softens the surface in reline acrylic resins and decreases the hardness values. No increase in hardness was observed after long-term immersion in water; thus, according to present experimental conditions, there was no further polymerization of the residual monomer during this study.

The significant decrease in hardness observed for Tokuyama Rebase II Fast could be caused by water sorption of the material, which possibly did not reach the stabilization of water sorption at the same moment as the other materials evaluated, promoting a progressive decrease in hardness. Therefore, it is presumed that Tokuyama Rebase II Fast has nanopores within the polymer structure, or that this resin could be more hydrophobic, which impairs the water diffusion into the bulk. Thus, long-term storage may be necessary, for water to diffuse completely and reach saturation.

The effect of disinfectant solutions on each reline acrylic resin was evaluated separately, since the composition of the materials differs.

In general, all disinfectant solutions tested caused a reduction in hardness values, already noted in the 30-cycle assessment for all materials studied (Table 2). As in the case of water, these solutions may have been absorbed by the resin and may act as a plasticizer, thus altering the hardness when disinfection cycles are repeated many times.

Only the 5.25% sodium hypochlorite solution exhibited a small, but significant increase in mean hardness values. Once hypochlorite solutions are prepared, their stability is affected by organic contaminants, heavy metal ions, dilution, time, light and temperature. Because of its electronic configuration, chlorine has a tendency to acquire extra electrons. Chlorine in water reacts quickly with organic and inorganic...
reduced substances, which are powerful catalysts of decomposition, changing the solution from chlorine to inorganic chloride ions, and this reaction is greater at high concentrations\textsuperscript{29}. It may be possible that the reline acrylic resin absorbed sodium hypochlorite because a decrease in hardness was noted in the first period of time. In the present study, sodium hypochlorite was used at high concentration (5.25%), and it is possible that this solution decomposed to high concentration of chloride ions, which could alter the resin composition and consequently increase the hardness values.

These results corroborate the hypothesis that aqueous disinfectant solutions are absorbed and act as plasticizers, and suggest a possible alteration in the chemical structure of these resins when exposed to repeated disinfection cycles, causing the decrease in hardness values.

**CONCLUSION**

Within the limitations of this *in vitro* study, the following conclusions can be drawn:

1. For all reline acrylic resins tested, a decrease in hardness values during the experiment was noted.

2. The hypothesis tested was accepted because the studied solutions promoted adverse effects on reline acrylic resins.

**REFERENCES**


