

# Color stability of relined dentures after chemical disinfection. A randomised clinical trial

# Eduardo Buozi Moffa, Eunice Teresinha Giampaolo\*, Fernanda Emiko Izumida, Ana Cláudia Pavarina, Ana Lúcia Machado, Carlos Eduardo Vergani

Department of Dental Materials and Prosthodontics, Araraquara Dental School, UNESP – Univ. Estadual Paulista, Rua Humaita, 1680 CEP: 14801-903, Araraquara, São Paulo, Brazil

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#### ABSTRACT

*Background*: This randomised clinical study evaluated the effect of chemical disinfection with sodium perborate or chlorhexidine on the color stability of a hard chairside reline resin during six months.

Methods: Hard chairside reline resin (Tokuyama Rebase Fast II) was used to reline complete dentures. After baseline color measurements, the patients were randomly divided into 3 groups (n = 15) and allocation was concealed with the use of the BioStat program. The dentures were cleansed according to three methods: CG (control group) – brushing with coconut soap and soft toothbrush, PG (Perborate group) – brushing according to previous methods and disinfection with warmed sodium perborate solution (Corega Tabs) for 5 min, once a day for 6 months and ChxG (Chlorhexidine Group) – brushing according to CG and disinfection with chlorhexidine digluconate 2% for 5 min once a day for 6 months. The data of  $\Delta E^*$ ,  $\Delta L^*$ ,  $\Delta a^*$  and  $\Delta b^*$  were analysed by 2-way repeated-measures ANOVAs and Tukey tests ( $\alpha = 0.05$ ).

Results: There were significant differences amongst groups for  $\Delta L$ ,  $\Delta a$  and  $\Delta b$ . The time had a significant effect on  $\Delta E$  and  $\Delta L$ , for all groups evaluated.

Conclusion: Changes in some color parameters ( $\Delta L$ ,  $\Delta a$  and  $\Delta b$ ) of the reline resin Tokuyama Rebase were observed when the dentures were disinfected by perborate and chlorhexidine digluconate 2% solutions. The color stability of was also influenced by time, regardless of disinfection or nondisinfection.

Clinical implications: Color stability of the denture materials is one variable to be considered when choosing disinfection methods. The data in this study will be useful to clinicians when they are selecting disinfectant solutions for disinfection of relined denture.

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## 1. Introduction

The alveolar bone resorption can negatively affect the original stability and retention of dentures. Direct relining of dentures with hard chairside reline materials is faster and easier to perform than laboratory relining,<sup>1</sup> and has been considered

\* Corresponding author. Tel.: +55 1633016410; fax: +55 1633016406. E-mail address: eunice@foar.unesp.br (E.T. Giampaolo). suitable for improving the fit of the denture bases to the residual ridges.<sup>2</sup>

During the last years, hard chairside materials have been developed to increase the durability of relined dentures.<sup>3</sup> Long-term color stability is an important clinical behaviour for reline resin since their color and appearance should be similar to denture base materials.<sup>4</sup> Color change in the intaglio is

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important because it may be an indicator of ageing or damage of the reline material and have a negative impact on patient satisfaction.<sup>5,6</sup> Moreover, color stability may provide important information on the serviceability of these materials.7 Although the properties of hard chairside relining resins have been improved, the amount of ageing and weathering to which they are subjected can lead to discoloration.<sup>2</sup> There is little information in the literature regarding the color stability of the hard direct denture reliners. Brauer et al.<sup>8</sup> compared the color stability of hard direct autopolymerising reliners. They tested six commercial brands and observed that only one of the products exhibited color stability. Color stability of hard direct reliners also was studied by Bunch et al.<sup>9</sup> Color changes ranged from 1.4 to 17.6  $\Delta E^*$  and occurred after only 24 h of accelerated ageing. Polyzois et al.<sup>10</sup> reported that different types of hard reliners exhibited perceptible color change after 7 days of immersion in staining solutions. Matsumura et al.<sup>11</sup> evaluated the clinical performance of a hard relining material and reported discoloration of the relining material after 1 year when compared to the baseline. Amongst the factors which may contribute to the discoloration of these materials after long-term use, are stain accumulation, dissolution of ingredients, degradation of intrinsic pigments, and the chemical composition of the monomer.<sup>12</sup>

The adhesion of Candida albicans on the fitting of the denture bases or lining materials appeared to be of critical importance for development and maintenance of denture stomatitis in continuous denture wearing. Thus, proper hygienic care of removable dentures is important for maintaining a healthy oral mucosa in denture wearers.13,14 Dentures can be cleaned mechanically, chemically, or by a combination of the two.15 Mechanical procedures to remove denture biofilm include brushing with soap or an abrasive paste and water and are the most used method amongst elderly patients for removing denture biofilm.<sup>13</sup> However, effective biofilm removal requires a degree of manual dexterity that is often lacking particularly amongst elderly individuals.<sup>15,16</sup> Thus, chemical cleaning, such as immersion in disinfectant solutions should be considered. The chemical agents of denture cleansing solutions should be simple to use, effectively remove organic and inorganic matter from denture surface, and have bactericidal, antifungal and biocompatibility properties.<sup>17</sup> Denture cleansers can be divided into five groups: alkaline peroxides, alkaline hypochlorites, acids, disinfectants, and enzymes.<sup>18</sup> The use of chemical cleansers is usually associated to mechanical methods and their efficacy in reducing biofilm formation on the surface irregularities of dentures have been reported.<sup>19</sup> Alkaline peroxide effervescent tablets have excellent biofilm removal properties.<sup>20</sup> When dissolved in water, the sodium perborate readily decomposes to form an alkaline peroxide solution which releases oxygen whilst in contact with water, thus enabling a mechanical cleaning by oxygen bubbles as well as chemical cleaning.<sup>21</sup> Chlorhexidine has been shown to be effective in the treatment of denture related stomatitis,22 reducing biofilm formation and improving the condition of the patient's mucosa. Ideally, a disinfection agent should be able to inactivate microorganisms, be biologically compatible and should not cause adverse effects on denture materials. In the literature, various in vitro studies have reported whitening or discoloration of acrylic

resin after prolonged exposure to chemical disinfection agents.<sup>10,22,23</sup> However, no clinical study had yet compared the effects of these agents on the color stability of the chairside hard reline materials. Considering that the capacity of a material to maintain its color when in function is one of the factors that determine their longevity, the aim of this randomised clinical study was to evaluate the effect of disinfection with sodium perborate or chlorhexidine on the color stability of relined dentures in different time intervals. The null hypothesis tested was that the disinfection solutions would have no effect on color stability of one reline material.

#### 2. Materials and methods

Forty-five patients, ranging in age from 50 to 75 years, who attended at the School of Dentistry of Araraquara/UNESP, were included in this study. The procedures carried out in this randomised clinical trial followed the criteria of Resolution 196/96 of the Brazilian Health Ministry, which regulates research involving human subjects. The project was approved by the Ethics Committee of the Araraquara Dental School, UNESP – Univ. Estadual Paulista (number 60/2009). All participants were made aware of the objectives of the study as well as probable risks and benefits. All subjects voluntarily entered the study and signed an informed consent form before their enrollment.

The subjects were healthy people and their maxillary dentures required relining. The hard chairside reline resin Tokuyama Rebase II (Tokuyama Dental Corporation, Tokyo, Japan) was used to reline complete maxillary dentures. From each denture, material from the tissue side and a little over the border was removed with a tungsten-carbide rotary cutting instrument to provide space for an adequate thickness of reline material. The intaglio surface of the denture base was cleaned and dried. A coat of a separating agent (Chemco Ind. e Comércio Ltda, Campinas, SP, Brazil) was applied over the polished surfaces and artificial teeth. The bonding agent provided by the reline resin manufacturer was applied with a brush on the intaglio surface of the denture base, and the reaction time (120 s) occurred before the resin was inserted. Tokuyama Rebase II was proportioned and manipulated according to the manufacturer's instructions. The material was applied in a uniform layer on the tissue side of the denture base and over the borders, and the denture was immediately placed in the patient's mouth in its terminal position. Afterwards, with the patient's mouth open, the cheeks were manipulated to turn the excess at the border and establish harmony with bordering attachments. Before initial hardening, the denture was removed from the mouth and gross excess material was removed with a scalpel blade. Thereafter, the denture was replaced in the patient's mouth in its terminal position, and the teeth were brought into occlusion until setting was complete. After final hardening, the occlusion was adjusted, and the denture was polished.

After relining, the baseline color of all dentures was determined in the spectrophotometer Color Guide 45/0 (BYK-Gardner, Santo André, SP, Brazil) according to the CIE (Commission Internationale de l'Eclairage)  $L^*a^*b^*$  system wich is most frequently used in dental research.<sup>24,25</sup> Color measure-

ments were made on the convex area of the intaglio surface of removable dentures through the sample sighting device, which has a circular hole 15 mm in diameter, provided by the spectrophotometer manufacturer. The color measurements were standardised by obtaining coincidence between a line made on the sighting device and the labial notch region, and the color stability was assessed by determining the color differences ( $\Delta E^*$ ) between CIE  $L^*a^*b^*$  coordinates after baseline, 7 and 15 days, 1, 3 and 6 months. Means were calculated for each evaluation. The total color change ( $\Delta E^*$ ) of each reline denture was then calculated using the relationship:

$$\Delta E^* = \left[ (\Delta L^*)^2 + (\Delta a^*)^2 + (\Delta b^*)^2 \right]^{1/2}$$

where

$$\Delta L^* = L_{interval}^* - L_{baseline}^*$$
  
 $\Delta a^* = a_{interval}^* - a_{baseline}^*$   
 $\Delta b^* = b_{interval}^* - b_{baseline}^*$ 

The data obtained from spectrophotometer must be manipulated and translated into a form useful for dental professionals.<sup>26</sup> Thus, the values of color stability was compared with the National Bureau of Standards (NBS)<sup>27</sup>. To match the color differences ( $\Delta E$ ) for the clinical environment, data were calculated according to the units of the National Bureau of Standards using the formula:

NBS Units =  $\Delta E \times 0.92$ 

After baseline color measurements, the patients were randomly divided into 3 groups (n = 15) and allocation was concealed with the use of the BioStat program. The dentures were cleansed according to three methods: CG (control group) – brushing with coconut soap and soft toothbrush, PG (Perborate group) – brushing according to previous methods and disinfection with warmed sodium perborate solution (Corega Tabs – GlaxoSmithKline Brasil Ltda, Rio de Janeiro – RJ, Brazil) for 5 min, according manufacturés instructions once a day for 6 months and ChxG (Chlorhexidine Group) – brushing according to CG and disinfection with chlorhexidine digluconate 2% (Arte & Ciência, Farmácia de Manipulação, Araraquara – SP, Brazil) for 5 min once a day for 6 months.

All study team members were blinded until study completion, except the clinician who performed the relining. The results of  $\Delta L$ ,  $\Delta a$ ,  $\Delta b$ , and  $\Delta E$  were evaluated statistically by 2-way repeated-measures analyses of variance (ANOVAs). The two factors analysed were group and time after relining. Tukey Honestly Significant Difference (HSD) post hoc test was used to determine differences between means ( $\alpha = 0.05$ ).

### 3. Results

Table 1 presents the mean values for  $\Delta a$  and  $\Delta b$  and the results of Tukey HSD post hoc test ( $\alpha = 0.05$ ). There were no statistically significant differences between CG and PG. However, ChxG produced higher mean values of color

Group			Time						
			7 days	15 days	1 month	3 months	6 months		
CG	ΔΕ	М	2.25 <sup>Aa</sup>	3.24 <sup>Ab</sup>	3.85 <sup>Abc</sup>	4.50 <sup>Acd</sup>	4.53 <sup>Ad</sup>		
		SD	1.46	1.35	2.47	3.57	2.31		
PG		М	1.64 <sup>Aa</sup>	2.22 <sup>Ab</sup>	2.77 <sup>Abc</sup>	3.32 <sup>Acd</sup>	3.67 <sup>Ad</sup>		
		SD	1.35	1.49	1.55	1.48	1.30		
ChxG		М	2.83 <sup>Aa</sup>	4.26 <sup>Ab</sup>	4.64 <sup>Abc</sup>	5.76 <sup>Acd</sup>	6.26 <sup>Ad</sup>		
		SD	1.73	2.43	2.01	2.84	2.82		
CG	$\Delta L$	М	1.11 <sup>Aa</sup>	1.36 <sup>Bab</sup>	2.16 <sup>Bab</sup>	3.19 <sup>Bb</sup>	2.43 <sup>Bab</sup>		
		SD	2.22	2.66	3.33	4.42	4.21		
PG		М	-0.77 <sup>Ab</sup>	-1.38 <sup>Aab</sup>	-1.73 <sup>Aab</sup>	-2.36 <sup>Aab</sup>	-2.85 <sup>Aa</sup>		
		SD	0.68	1.52	1.39	1.53	1.24		
ChxG		М	1.48 <sup>Aa</sup>	2.65 <sup>Bab</sup>	3.52 <sup>Babc</sup>	4.05 <sup>Bbc</sup>	4.79 <sup>Bc</sup>		
		SD	1.14	1.45	1.62	1.91	2.12		
CG	Δa	М	-0.41 <sup>Aa</sup>	-0.37 <sup>Aa</sup>	-0.35 <sup>Aa</sup>	-0.01 <sup>Aa</sup>	-0.34 <sup>Aa</sup>		
		SD	1.04	1.52	2.33	1.71	1.73		
PG		М	$-0.91^{Aa}$	-1.00 <sup>Aa</sup>	-0.83 <sup>Aa</sup>	-1.01 <sup>Aa</sup>	-1.35 <sup>Aa</sup>		
		SD	1.61	1.12	1.36	2.09	1.84		
ChxG		M	1.61 <sup>Ba</sup>	2.29 <sup>Ba</sup>	2.04 <sup>Ba</sup>	2.61 <sup>Ba</sup>	2.51 <sup>Ba</sup>		
		SD	1.87	2.61	2.18	3.12	3.14		
CG	$\Delta b$	M	-0.09 <sup>Aa</sup>	0.65 <sup>Aa</sup>	0.27 <sup>Aa</sup>	0.35 <sup>Aa</sup>	0.21 <sup>Aa</sup>		
		SD	0.44	1.20	0.84	1.11	0.78		
PG		M	-0.16 <sup>Aa</sup>	-0.12 <sup>Aa</sup>	-0.52 <sup>Aa</sup>	-0.41 <sup>Aa</sup>	-0.23 <sup>Aa</sup>		
		SD	0.37	0.93	1.65	0.46	0.80		
ChxG		M	0.33 <sup>Ba</sup>	0.86 <sup>Ba</sup>	0.60 <sup>Ba</sup>	1.61 <sup>Ba</sup>	1.32 <sup>Ba</sup>		
		SD	1.29	1.65	1.34	1.71	1.65		

Horizontally,  $\Delta E^*$ ,  $\Delta L^*$ ,  $\Delta a^*$  and  $\Delta b$  mean values designated with identical lower letters and vertically identical capital letters were not statistically different (P > 0.05).

Table 2 – Summary of 2-way ANOVA for the ( $\Delta E^*$ ).						
Effect	SM	DF	F value	P value		
Group	51.33	2	3.21	0.056		
Time after relining	32.01	4	19.78	0.000		
$\textbf{Group} \times \textbf{time after relining}$	0.92	8	0.57	0.803		
SM, mean square; DF, degrees of freedom.						

# Table 3 – National Bureau of Standards (NBS) system of expressing color differences.

Critical remark of color difference	NBS units	
Extremely slight change	0.0–0.5	
Slight change	0.5–1.5	
Perceivable change	1.5-3.0	
Marked change	3.0-6.0	
Extremely marked change	6.0-12.0	
Change to another color	12.0-	

changes being statistically different when comparing the CG and PG. Moreover, there was no significant difference over time for each group. The results of  $\Delta L$  are summarised in Table 1. For CG and ChxG, all specimen surfaces tended to become lighter over time. However, the surfaces of PG specimens tended to become darker. This lightening and darkening ( $\Delta L$ ) were significantly more pronunced at 3 and 6 months, respectively. Table 2 also shows the  $\Delta E^*$  mean values and standard deviations of all groups evaluated. No significant differences were found amongst the tested groups (p = 0.056), with a moderate statistical power, the findings should be used with discernment as the level of replication was probably not large enough to detect the differences amongst treatment groups.

The results also revealed that the treatments seem to undergo gradual color changes with time (p < 0.001; power = 1.0) with the effect being more pronounced at 15 days and 6 months. The critical remark of color change ( $\Delta E^*$ ) has been quantified by the National Bureau of Standards (NBS) with the NBS units of color difference as shown in Table 3. Color change values according to the National Bureau of Standards unit system are presented in Table 4.

### 4. Discussion

This in vivo study assessed the color stability of Tokuyama Rebase when immersed in two disinfectant solutions (chlorhexidine digluconate and Corega Tabs) as compared with immersion in water. The null hypothesis that there would be no effect of denture cleansers on color stability of the reline material was rejected. There were some significant differences in color between controls and disinfectant solutions. Also, the color of the reline resin changed with time for control and disinfectant groups.

 $\Delta L$  exhibited a significant increase over time for CG, which indicates a bleaching (whitening) effect of the reline material. Discoloration of resin-based materials may be caused by intrinsic or extrinsic factors. Intrinsic factors are related to internal alterations in the material resulting from physicochemical reactions or residual monomer oxidation.<sup>28,29</sup> Thus, the initiator, quantity and type of monomer and the polymerisation efficiency may affect the color stability of resin-based materials.<sup>28</sup> Polymerisation may have a significant influence on color change, since the greater the degree of conversion the lower the quantity of residual monomer and, consequently, the formation of colored degradation products.<sup>28</sup> Furthermore, extrinsic factors that may influence the color change include adsorption and absorption of pigments resulting from patients' dietary habits, cigarette smoking and biofilm accumulation.<sup>30,31</sup> These results suggest that the color stability of the reline resin evaluated still needs to be improved if this material is to be used for long term clinical applications.

As observed for CG, the analysis of  $\Delta L$  showed that ChxG had a whitening effect on the reline material. It was also observed that the mean values of the color parameters  $\Delta a$  and  $\Delta b$  of ChxG group were consistently higher than those of control, regardless the time of measurement. This qualitatively indicates a color change towards orange (yellow and red mixture) after immersion of the reline resin in chlorhexidine. The staining potential of chlorhexidine gluconate has already been reported in the literature.<sup>23,32</sup> Amongst the factors that interfere in the prevalence and severity of color change are the concentration, immersion time and volume of chlorhexidine that was being used. Thus, lower concentrations, in larger volumes may cause less staining whilst maintaining similar

Table 4 – Color change values according to the National Bureau of Standards unit system.							
Time	CG		PG		ChxG		
	$\Delta E$ mean	NBS	$\Delta E$ mean	NBS	$\Delta E$ mean	NBS	
7 days	2.25	2.07***	1.64	1.50**	2.83	2.62***	
15 days	3.24	2.98***	2.22	2.04***	4.26	3.91***	
1 month	3.85	3.52***	2.77	2.54***	4.64	4.26	
3 months	4.50	4.14****	3.32	3.05****	5.76	5.29****	
6 months	4.53	4.16****	3.67	3.37****	6.26	5.75****	

Critical remarks of color difference:

\*Extremely slight change (0.0-0.5).

<sup>\*</sup> Slight change (0.5–1.5).

Perceivable change (1.5–3.0)

Marked change (3.0–6.0).

effectiveness. Furthermore, the time of immersion must be considered, since prolonged immersion times may influence and alter the polymer structure and thus cause greater color change.33,34 It has been hypothesised that chlorhexidine would participate as catalyser in non enzymatic browning reactions (Maillard Reactions), in which glycoproteins present in the acquired pellicle (80% proteins and 20% carbohydrates) participate as substrate for a series of condensation and polymerisation reactions, leading to the formation of browning substances called melanoidins.<sup>35</sup> The capacity of chlorhexidine to promote protein denaturing, and then produce staining by the formation of ferric and stannous sulphite, composes another theory of the staining resulting from chlorhexidine.<sup>36</sup> Finally, there is a third hypothesis, in which the formation of staining would occur by the precipitation of chromogens from the diet directly onto the tooth surface, such as coffee, tea and red wine, forming colored products.<sup>37</sup> However, the latter two hypotheses, in part, present similar mechanisms as high concentrations of iron (Fe) are found in red wine. It is important to consider that most of these findings have been obtained from in vitro investigations. It is

known that many factors that could not be replicated in an in vitro study such as salivary pellicle, foods and beverages consumed may affect the pattern of staining on denture materials in clinical conditions.<sup>38</sup> Watts and Addy<sup>39</sup> reported that there is great individual variation in the degree of staining from person to person, and this makes explanation more difficult as it may be caused by intrinsic factors, differences in extrinsic factors or both. Therefore, further in vivo studies are necessary to evaluate the contribution of the discussed mechanisms on the staining process of reline resins.

The mean  $\Delta L$  values exhibited a significant variation over time for all immersion solutions, and therefore, influenced the enhancement of color change observed through the analysis of  $\Delta E$ . The results of  $\Delta E$  showed that time was a critical factor for color stability for all the evaluated groups. In the CG, color change was more pronounced at 15 days, and gradually increased up to 6 months. These findings are in agreement with the study from Matsumura et al.<sup>11</sup>, who, in a direct evaluation, observed staining on the surface of a relining material after one year of denture wearing. According to the authors, the color stability of the relining material could be affected by extrinsic factors such as saliva, foods and cigarettes. Although the mechanism of color change is not completely known, a combination of several factors such as saturation of the resin during the course of use, material surface roughness,40 chemical degradation, oxidation of the carbon chains<sup>41</sup> and the continuous formation of color degradation by products,<sup>42</sup> may explain the phenomenon of color change in the CG. Another factor that may be involved is the type of polymerisation of relining materials. Due to the lower degree of conversion during the polymerisation process, it is known that autopolymerised resins exhibit higher residual monomer content, relatively to the heat-polymerised materials. Thus, a high quantity of residual monomer could have interacted with the pigments of the Tokuyama Rebase and caused color deterioration.43 Moreover, relining resins have porosities<sup>44</sup> that may favour the absorption of pigments, consequently generating color change.

The results of this study also revealed that the PG quantified by NBS units, was classified as "slight" change for 7 days, "perceptible" change for the periods of 15 days and 1 month, and "marked" change for 3 and 6 months. Although not statistically significant, the numerical values of PG were lower when compared to other group, including CG. A contributory factor to these results could have been the use of warm water (50 °C) during denture immersion. The heat generated by the water on the resin mass may have promoted greater diffusion and release of the monomer to the material surface. Thus, the reduction in residual monomer content may have resulted in lower oxidation rates of the pigments in the resin, decreasing the intrinsic chromatic changes<sup>45</sup> and the formation of colored degradation products.<sup>28</sup> This finding contradicts the results of Ünlü et al.<sup>36</sup> that investigated four different types of denture cleanser (Polident, Efferdent, Blend-A-Dent and Corega) on discs of autopolymerising and heat-polymerising acrylic resin. They found that the greatest whitening effect was seen using Corega on autopolymerising acrylic resins. This difference in the results may be explained by the fact that these authors tested the product simulating 30 nights of immersion, whilst in present study, Corega Tabs immersion was performed for 5 min, once a day, for 6 months. In addition, the product used by them contained an oxygen releasing agent with enzymes. The product used in the present study did not contain enzymes, thus supporting the hypothesis that oxidation in combination with a strong alkaline solution may have been deleterious to color stability.<sup>36</sup>

When  $\Delta E$  was examined for GChx, the means color change showed gradual increase over the course of time. According to the NBS units, the change that occurred in the periods of 7 and 15 days was considered "perceptible" and the periods of 1, 3 and 6 months, showed "marked" change. Thus, although staining continues to be a subject that has not yet been completely explained, the reaction of chlorhexidine with dietary products, especially chromogens, appears to be the most accepted and scientifically proved hypothesis in both in vitro and in vivo studies. The results of present study are in agreement with these findings. The chlorhexidine stained the Tokuyama Rebase material more than the CG and PG. The increase of color change of the Tokuyama Rebase could be attributed to the concentration of chlorhexidine digluconate used in this study. Therefore, further studies should consider the evaluation of other concentrations. The susceptibility to color change of the resin Tokuyama Rebase evaluated in this study could be attributed to both intrinsic and extrinsic factors. The degree of water absorption and hydrophilic of the relining material may explain their lower color stability as a function of time. Although all the experimental groups presented marked changes at the end of the period of 6 months, PG showed color alteration values closest to perceivable change. In the same period, ChxG presented the highest NBS unit values indicating that the color change would be characterised as extremely marked change (NBS unit). Although the disinfectant solutions used in this study have been indicated for denture biofilm control, patients should be advised of the possibility of reline materials to staining in long-term, regardless of the use or not of disinfectant solutions.

### 5. Conclusions

- Changes in the color parameters  $\Delta L$ ,  $\Delta a$  and  $\Delta b$  of the reline resin Tokuyama Rebase were observed when the dentures were disinfected by perborate and chlorhexidine digluconate 2% solutions.
- The color stability of the reline resin was influenced by time, regardless of disinfection or nondisinfection.
- The perborate group exhibited color change classified as slight at 7 days, perceivable at 15 days and 1 month, and marked at 3 and 6 months (NBS units).
- The chlorhexidine group showed perceivable color change for the periods of 7 and 15 days and marked for the periods of 1, 3 and 6 months, (NBS units).

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