

# Evaluation of a Realistic Cleansing Protocol for Preventing Discoloration of Denture Resins

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## Keywords

Color stability; denture cleansers; denture resins; discoloration prevention.

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## Abstract

**Purpose:** To evaluate the efficacy of a realistic staining/cleansing protocol for long-term prevention of discoloration of denture base resins.

**Materials and Methods:** Sixty discs (20 × 2.5 mm) of auto- and heat-polymerizing denture acrylic resins were fabricated following manufacturer's instructions, polished on one side and stored in water (37°C) for 24 hours before they were assigned randomly into 6 groups (3 for each material, n = 10) to receive 220 cycles of three immersion protocols. Group A was immersed in the following baths: (1) filtered coffee at 45°C for 2 minutes, (2) tap water at 24°C for 20 seconds, (3) as bath 1, (4) as bath 2, (5) Corega Extradent cleansing solution at 45°C for 3 minutes, and (6) water at 24°C for 20 seconds. Group B was immersed in the four first baths of group A (without cleansing action) and group C in bath 1 for the cumulative action of 20 cycles (80 minutes in 45°C coffee) and bath 2 for 40 seconds. A noncontact optical interferometric profilometer was used for qualitative evaluation of the specimens and a contact colorimeter to estimate color change values ( $\Delta E^*ab$ ) at baseline and every 20 cycles in the baths for a total of 220 cycles. Data were statistically analyzed using a 2-way-repeated measures ANOVA with Bonferoni multiple comparisons and trend analysis at  $\alpha = 0.05$  level of significance.

**Results:** Data indicated changes in color from 1.7 to 14.2  $\Delta E^*ab$  units. The highest values were related to the number of cycles and group B immersion protocol. Significant differences were found among immersion protocols ( $p < 0.001$ ) and immersion cycles ( $p < 0.001$ ) with a significant interaction between protocols and cycles ( $p < 0.001$ ) but not between denture resins ( $p = 0.991$ ).

**Conclusion:** The evaluated protocol A was found effective in preventing color changes in both denture resins even after a long period of action. Protocol C had a much lower staining effect on both resins than protocol B and behaved similarly to protocol A until 140 to 160 cycles. Therefore, it is probably not appropriate for use in short-term color stability experiments.

The elderly population has been growing larger for years, and the population of the world is growing older at an unprecedented rate. In 2015, 8.5% of the global population was aged 65 and over; however, the proportion of this group was projected to double to 17% by 2050.<sup>1</sup> The need for removable dental prostheses (RDPs) is also increasing, together with life expectancy. Therefore, RDPs are expected to serve longer, as are the denture base material maintaining its mechanical and physical properties over time. Denture bases are fabricated mainly from heat-assisted or autopolymerizing acrylic resins; however, repairs

are mostly done using the latter. These denture base materials should match the color and appearance of the underlying tissues and retain that property over time.<sup>2</sup>

Color change is considered a proof of damage or aging for a given material.<sup>3</sup> Several factors, including stain accumulation, water sorption, dissolution of the ingredients, and surface roughness, may contribute to the discoloration of denture base materials after long-term use. For denture base resins, sorption is defined as a process of absorption and adsorption of liquids. They gradually absorb liquid over a period of time and undergo

**Table 1** Denture base resins, cleansing, and staining mediums used

	Manufacturer	Composition	Batch No
Triplex Cold (Autopolymerizing)	Ivoclar Vivadent AG, Schaan, Liechtenstein	PMMA (acrylic)	Powder D02359 Liquid D05718
Triplex Hot (Heat-polymerizing)	Ivoclar Vivadent AG, Schaan, Liechtenstein	PMMA (acrylic)	Powder 805469 Liquid 807944
Corega Extradent (Denture cleanser)	Stafford-Miller Ltd, Dungarvan, Waterford, Ireland	Peroxide cleanser	ST13272
Jacobs Krönung (Filtered coffee)	Jacobs Douwe Egberts, DE GmbH, Amsterdam, The Netherlands	Ground coffee grains	0XB0650943

staining through the intake of fluids and foods.<sup>4,5</sup> Beverages such as tea, coffee, wine, and some artificial food dyes may lead to a discoloration of denture base polymers.<sup>6-8</sup>

Water sorption and solubility of autopolymerizing acrylic resins is higher than that of heat-assisted polymerizing, and therefore it is expected to stain more,<sup>9</sup> with a color stability that varies with the chemical composition of the monomer.<sup>6,10</sup> Denture bases manufactured from autopolymerizing resins could contain up to seven times the level of residual monomer found in heat-assisted polymerizing resins. The high level of residual monomer is associated with the observed color changes.<sup>11</sup> Hydrophilic materials exhibit greater color change than hydrophobic materials. This observation is related to the nature of the monomer contained on each denture base resin.<sup>5,12</sup> Autopolymerizing resins exhibit higher solubility and inferior color stability due to oxidation of the amine accelerator they contain.<sup>13,14</sup>

Surface roughness plays a substantial role in the staining procedures. Rough surface specimens stain significantly when compared with smooth surface specimens.<sup>15</sup> Irregularities and porosity could also lead to increased staining.<sup>16,17</sup> The absence of superficial porosity in heat-assisted polymerizing acrylic resins has already been described,<sup>18</sup> and since porosity has been associated with the degree of polymerization and the presence of residual monomer,<sup>19-21</sup> it can be inferred that autopolymerizing denture resins are more porous, and therefore stain more easily than heat-assisted polymerizing resins do.

Denture cleansers are widely used to remove stains, debris or deposits from denture surfaces and to prevent plaque formation or colonization of bacteria.<sup>22,23</sup> However their daily use may affect the denture base resin physical properties, such as surface roughness, hardness, gloss, or color.<sup>24-30</sup>

Studies investigating the effect of cleansers on the color stability of denture resins have used a variety of protocols.<sup>25,27,28,30-36</sup> They differ not only in the cleanser used but also in the immersion time and/or the duration of the whole experiment as well. 1, 3, 7, 30, 60, 90, or 180 days were the common time intervals at which resins were measured for changes in color, as a cumulative effect of the cleanser, where they were immersed continuously. Most of these protocols were not realistic, since this action does not simulate how these cleansers were used in real life. In a small number of studies, resin specimens were immersed in the cleanser not continuously but for 3, 10, or 20 minutes, or 2, 8, or 10 hours per day, and the cleanser's effect was measured at one or more of these time intervals.

Extensive studies were usually limited to 180 days.<sup>30,37</sup> but lately more studies extended their protocols even farther.<sup>27,32,35,38</sup>

Denture resins are attacked daily by a number of stains that may alter the action of the cleanser. Although staining test protocols are numerous,<sup>5,8,34,36,39</sup> in only three of them is a staining protocol used in conjunction with a cleansing one.<sup>34,36,39</sup> However, these do not simulate a daily use of a cleanser, as a prevention regimen against permanent discoloration. Staining solutions and foods do not continuously affect denture resins in the oral environment. Their action is short and possibly weak but sometimes strong and long in action, frequently neutralized by daily water intake or by overnight storage in water. Therefore, to be realistic, protocols should imitate such effects, and might also have different effects on denture base resins than those usually employed.

The purpose of this study was to measure the *in vitro* color stability of two denture resins subjected to a cleansing protocol that combines staining and cleansing action, over a long immersion period (220 cycles). A peroxide effervescent denture cleanser was used in combination with a staining solution of coffee and compared to the effect of the staining solution acting alone for a certain time per day or continuously. The null hypotheses tested were that color changes ( $\Delta E^*ab$ ) were not significantly different: (a) between denture resins, (b) among immersion protocols, and (c) among time intervals.

## Materials and methods

For the purpose of this study, one autopolymerizing (Triplex Cold) and one heat-assisted polymerizing denture base resin (Triplex Hot) were used (Table 1). The total sample size for 6 groups was a priori estimated for between-factors repeated-measures ANOVA, by G\*Power v3.1.9.2 (University of Kiel, Germany), using an effect size 0.40,  $\alpha = 0.05$ , and power = 0.85. Estimation indicated a sample size of 10 for each group with an actual power of 0.89. Thirty disks (20 mm diameter, 2.5 mm thick), were made from each of the two acrylic resins following the conventional flasking and pressure-pack technique and manufacturer's instructions. One side of the final discs was left as processed and the other side was finished using silicon carbide papers of 600, 800, 1000, and 1200 grit in polishing equipment (Ecomet III; Buehler Ltd, Lake Bluff, IL) under wet conditions and polished with a high shine polishing agent (KMG;

Candulor AG, Glattpark, Switzerland) on a cotton wheel. Polished specimens were stored dry in a dark box until the beginning of the experiment and assigned randomly to three groups ( $n = 10$ ), which received a different staining-cleansing protocol.

### Immersion protocols

Each group was immersed in the baths according to the assigned immersion protocol for a series of 220 cycles. One cycle simulated a full day's staining action of two coffee drinks (2 minutes each), with or without the action of a cleanser. Specimens were hung in the baths, through a small hole in their periphery by a stainless steel orthodontic wire, from a wooden bar on top of the beaker, which contained 100 ml of the immersion solution.

#### Immersion protocol for group A (experimental)

Group A was initially immersed in a 45°C coffee bath for 2 minutes, and then placed and moved up and down for 20 seconds, in a 24°C distilled water bath, to remove any superficial stains. The procedure repeated with a new set of coffee and water solutions and then specimens were immersed in a 45°C denture cleansing solution for 3 minutes, followed by a 24°C water bath for another 20 seconds. Therefore, overall cycle duration was 8 minutes with a staining time of 4 minutes. Color changes were measured every 20 cycles after baseline measurement (at 0 cycles), until reaching 220 cycles.

#### Immersion protocol for group B (control)

Group B followed group A protocol without the last two steps (denture cleansing and water bath). It is actually a pure staining protocol with an overall cycle duration of 4 minutes and 40 seconds and a 4-minute staining action. Measurements were taken every 20 cycles, from 0 to 220 cycles.

#### Immersion protocol for group C (control)

Group C was immersed in a 45°C coffee bath for 80 minutes and then in a 24°C distilled water bath for 40 seconds. This protocol is also a pure staining protocol of 80-minute staining action on the materials, simulating the cumulative staining effect of 20 cycles. Coffee baths were agitated every 15 to 20 minutes, and discs were frequently removed and re-immersed in the bath to keep a homogeneous effect of the staining solution. Eleven cycles simulated the staining effect of 220 cycles in the other two protocols.

### Staining and cleansing baths details

Denture base resin staining was based on the action of a filtered brand of coffee (Jacobs Krönung, (Table 1) prepared by filtering 15 g coffee (3 spoons) with 2 cups (568 ml) of boiling water, which is half the recommended dose by the manufacturer for a strong drink. Denture resin cleansing was based on 3 minutes of action of 1/2 a denture cleansing tablet (Corega Extradent, Table 1), dissolved in 100 ml of warm (45°C) tap water, according to manufacturer's instruction (1 tablet dissolved in 200 ml of water).

### Color measurements

Before the start of the experiment, specimens were put in a 37°C distilled water bath for 24 hours and measured against a white background for their color parameters in the CIELAB system, using a contact portable colorimeter (Shade Eye NCC; Shofu Inc, Kyoto, Japan), with its 3 mm measuring window. Four measurements on the dried polished central surface area of the specimen at 90° were taken, and their mean was recorded as the specimens' baseline color.

After immersion of the groups in their immersion baths according to their protocols and every 20 cycles, disks were removed from the bath, dried on an absorbent soft paper, and their polished surface was measured as described above. Color measurements were focused on the tristimulus parameters  $L^*$  (lightness),  $a^*$  (green-red), and  $b^*$  (blue-yellow) of the CIELAB system, while color changes were calculated according to the formula:

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

A noncontact optical interferometric profilometer (Wyko NT1100; Veeco, Tucson, AZ) was also used to obtain 3D surface images of randomly selected specimens at the end of the immersion period. The instrument was operated in vertical scan image mode Myro lens ( $5 \times 2$  FOV) at 20.4x total magnification, 10 mm back scan length, 30 mm scanning length, and a modulation length of 2. One scan was taken per specimen surface, and  $S_a$  surface parameter (the average roughness in nm as evaluated over a complete 3D surface) was estimated.

### Statistical analysis

Color data obtained with the above measuring methods were analyzed statistically using 2-way repeated ANOVA and pairwise comparisons with Bonferroni correction at  $\alpha = 0.05$  significance level. The analysis included tests of between-subjects effects (denture resins, immersion protocols), tests of within-subjects effects (number of immersion cycles), and tests of within-subjects contrasts (trends of  $\Delta E^*_{ab}$  parameter with the immersion cycles) for locating differences between resins and among cycles or immersion protocols.

### Results

Color data, obtained by the above measuring methods, were used to estimate differences in color from baseline ( $\Delta E^*_{ab}$ ) of the two different denture resins, at 11 intervals of 20 cycles each. Estimated marginal means and their 95% CI of auto- and heat-polymerizing denture resins for all groups are presented in Table 2, and at all immersion intervals in Figure 1.

Test of between-subjects effects indicated no significant differences between denture resins ( $p = 0.983$ ), but significant differences among immersion groups ( $p < 0.001$ ). Pairwise comparisons with Bonferroni correction indicated significant difference between groups B and A or C ( $p < 0.001$ ), with no difference between groups A and C ( $p = 0.130$ ).

Tests of within-subjects effects indicated significant differences among cycles with significant interactions of cycles x immersion group ( $p < 0.001$ ), cycles x immersion group x

**Table 2** Estimated marginal  $\Delta E^*ab$  means and their lower (LB) and upper bounds (UB) of 95% CI of auto- and heat-polymerizing denture resins for all groups (n = 10)

	Autopoly- merized mean	95% CI		Heat-poly- merized mean	95% CI	
		LB	UB		LB	UB
Group A	3.121 <sup>a</sup>	1.993	4.249	2.466 <sup>a</sup>	1.338	3.594
Group B	7.833 <sup>b</sup>	6.705	8.961	8.677 <sup>b</sup>	7.549	9.805
Group C	4.038 <sup>a</sup>	2.910	5.166	3.878 <sup>a</sup>	2.75	5.006

Note: Group A received a staining/cleansing protocol, groups B and C received two different staining protocols. Same superscript letters indicate no significant difference ( $p > 0.05$ ) based on vertical pairwise comparisons with Bonferroni adjustment.

denture resin ( $p = 0.004$ ), but no significant interaction of cycles x denture resin ( $p = 0.209$ ). Pairwise comparisons with Bonferroni correction revealed that a significant difference from the first 20 cycles was evident only after 140 cycles to 160 cycles ( $p < 0.05$ ), for both denture resins. Sphericity value for the 2-way repeated-measures ANOVA was estimated at 0.280, and therefore the probability values above were based on Greenhouse-Geisser correction.

Test of within-subjects contrasts (trends) indicated a significant quadratic regression of  $\Delta E^*ab$  values with the number of cycles ( $p < 0.001$ ). Coefficient of determination values ( $R^2$ ) were found as 0.78, 0.83, and 0.85 for autopolymerizing, and 0.27, 0.83, and 0.91 for heat-assisted polymerizing resins for the groups A, B, and C, respectively (Fig 1). Based on quadratic equation, estimation of immersion cycles thresholds for a color difference of 3.0 (considerable or appreciable in plain NBS units) and 6.0 (very or much in plain NBS units)<sup>40</sup> are presented in Table 3. Differences among immersion groups are

**Table 3** Estimated immersion cycle thresholds of all groups, for a color difference 3.0 and 6.0  $\Delta E^*ab$  units with their lower and upper bounds of 95% CI

Groups	3.0 $\Delta E^*ab$ units			6.0 $\Delta E^*ab$ units		
	Threshold	LB	UB	Threshold	LB	UB
A-A	103.8	62.1	145.4	313.8	282.4	345.2
A-B	31.9	0	73.7	80.3	39.8	120.8
A-C	90.3	64.3	116.3	177.8	153.9	201.6
H-A	86.0	33.7	138.3	467.4	429.7	505.0
H-B	31.0	3.3	58.6	74.8	47.8	101.8
H-C	89.1	66.5	111.6	194.3	174.2	214.5

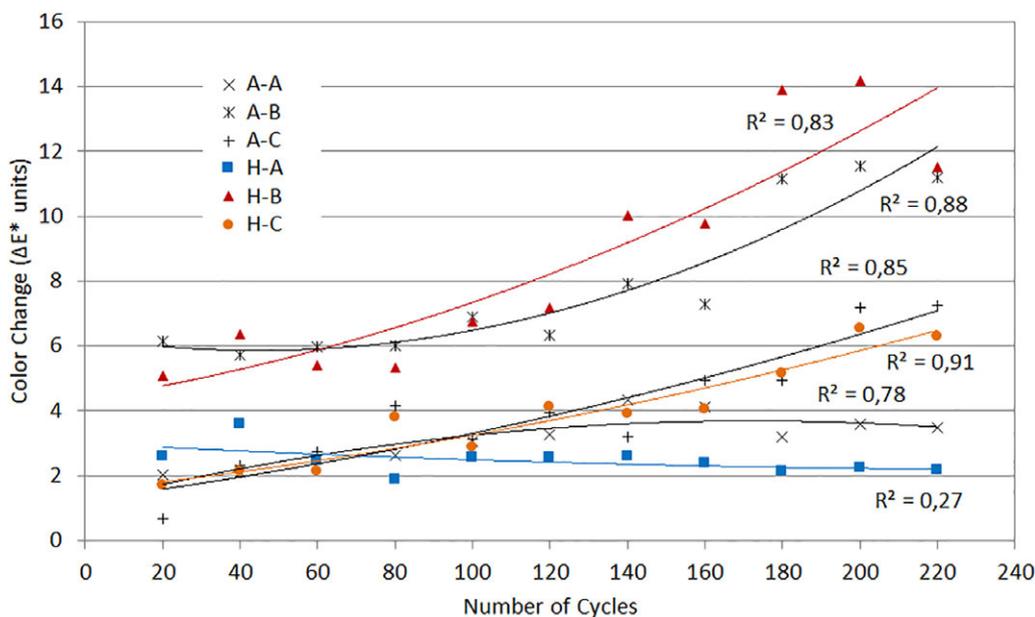
Note: Estimations were based on quadratic regression model of  $\Delta E^*ab$  data at  $a = 0.05$ . First letter is for material type (A for auto-, H for heat-polymerizing) and second letter for immersion group (A, B, or C).

more evident in the 6.0-unit threshold, as indicated by lower and upper bounds of 95% CI.

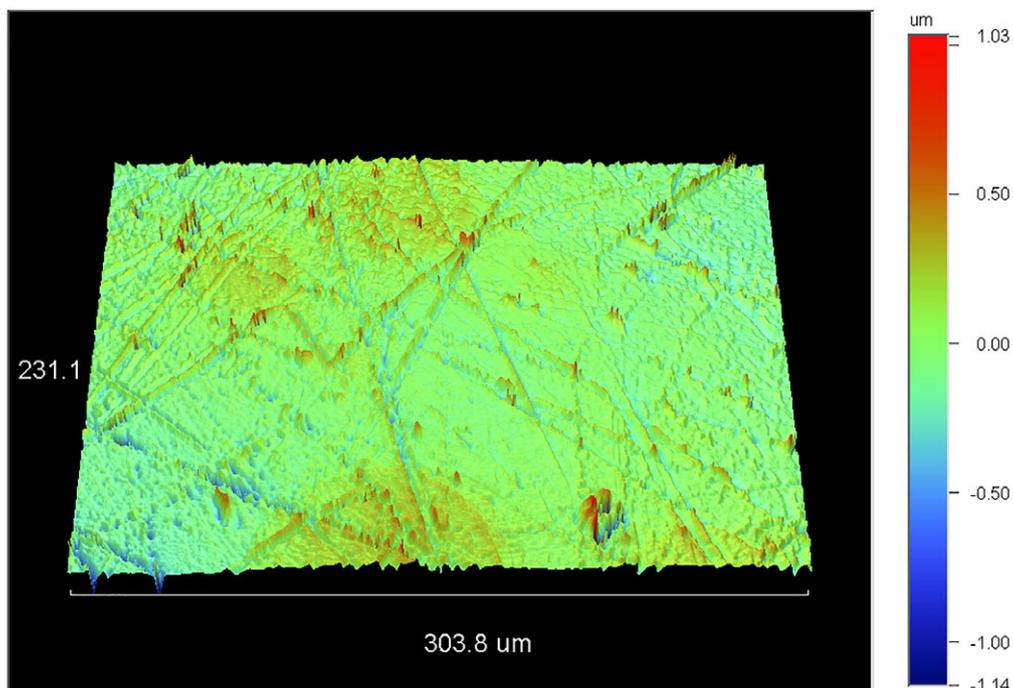
Optical profilometry provided 3D surface roughness, and two such images of auto- and heat-assisted polymerizing group A specimens are shown in Figures 2 and 3. The effect of immersion cycles on their surfaces is characteristic. PMMA beads were affected more than their matrix in autopolymerizing resins while the bead/matrix interface was more affected in heat-polymerizing resins.

### Discussion

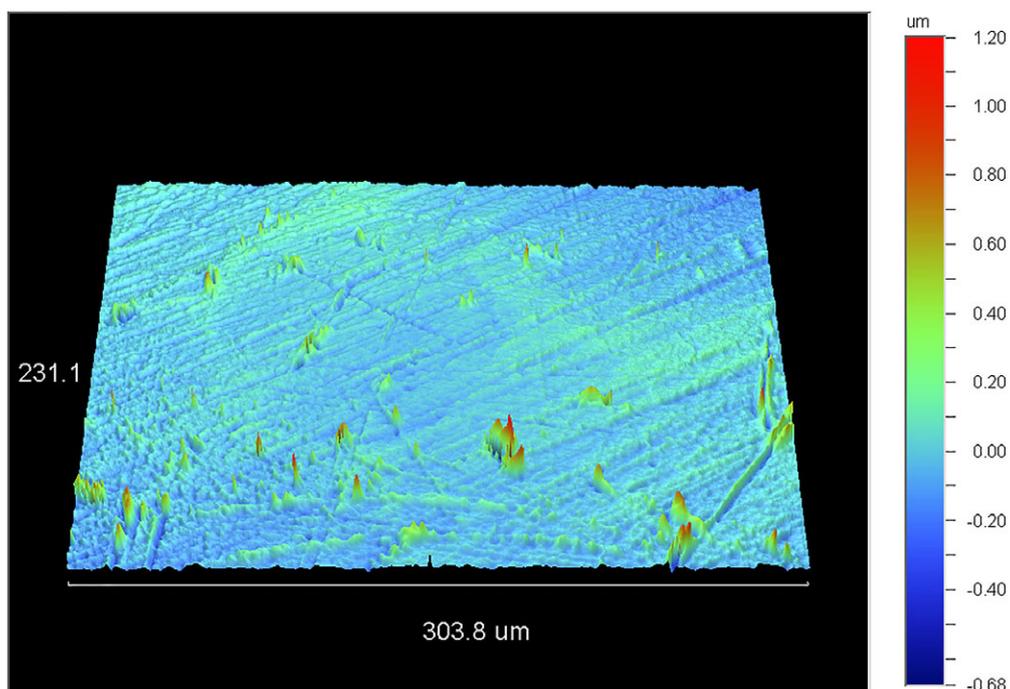
The findings of this study rejected the hypotheses of no differences among immersion protocols and cycles, and accepted the hypothesis of no differences in color changes between the two denture resins (auto- and heat-polymerizing PMMAs). Differences among immersion cycles were expected, since



**Figure 1** Quadratic trends of color changes ( $\Delta E^*ab$  units) for auto- and heat-polymerizing denture resin groups ( $R^2$  in parentheses).



**Figure 2** 3D interactive display of a group A, autopolymerizing PMMA specimen, after 220 cycles ( $S_a = 101.22$  nm).



**Figure 3** 3D interactive display of a group A heat-polymerizing PMMA specimen, after 220 cycles ( $S_a = 50.35$  nm).

coffee solution is a strong staining medium for most dental materials functioning in an oral environment.<sup>36,41</sup> Overall color change ( $\Delta E^*$ ) was evident for both denture resins even from the first 20 cycles and continued to increase as cycles increased in number; however, the increase was slow, and a

difference (from the first 20 cycles) was evident only at 140 to 160 cycles, indicating that to test color stability of materials, the staining solutions must have acted for a reasonable period of time, allowing them to build a significant effect on the test materials.

Differences among immersion protocols showed that the evaluated protocol A (cleansing after staining) was significantly different than protocol B (staining control), presenting the lowest mean  $\Delta E^*_{ab}$  (of a noticeable level only, according to actual critical remarks of National Bureau of Standards-NBS) at all immersion intervals (Fig 1). This means that the cleansing action of Corega Extradent is effective for maintaining denture resins' staining at low levels even after a long period of immersion in a coffee solution. Protocol B presented the highest mean  $\Delta E^*_{ab}$  (of a very perceptible level), starting at 5.8 units at 20 cycles and reaching 12 to 14  $\Delta E^*$  units at 220 cycles. This shows that the protocol has a definite effect on denture resin materials, easily differentiated from other protocols, even at the beginning of the experiment, and makes even clearer the effect of the evaluated protocol A. Protocol C was used as another control protocol but with a continuous action of the staining medium, since it is preferred in most studies for measuring staining effects. When protocol A was compared to C, no differences were found. This means that C cannot be used as a control staining protocol because it underestimates the effect of the cleanser. Its lower-than-protocol-B staining action on both resins could be explained by the fact that in protocol C, coffee was changed every 20 cycles, while in protocol B, coffee was changed every new cycle, and therefore the effect was much stronger in protocol B; however, it is also possible that residual monomers that exist in both resins have more time in protocol C than in protocol B to react in certain sites of the polymer chain, filling the microvoids and excluding water uptake<sup>42</sup> and its carried colorants, contributing to a process that might be considered as a passivation.

Both auto- and heat-assisted polymerizing denture resins behaved similarly throughout the whole immersion period, although a higher color change for autopolymerizing resin was expected due to its inherent higher porosity. Since staining or cleansing action attacks mainly the surface rather than the bulk of acrylic resins, the above result can be explained by the fact that both materials were very well polished (Ra values were below 120 nm). Three-dimensional interactive displays of specimens at the end of the immersion period (Figs 2 and 3) showed that autopolymerizing surfaces were almost twice as rough as the heat-assisted ones, but more importantly, acrylic beads were rougher than matrix areas in autopolymerizing surfaces, the opposite of what was depicted by heat-assisted polymerizing. These differences could be responsible for the small but not significant differences shown between the two materials; however, a small difference starts to be evident after 80 to 100 immersion cycles, for almost all protocols, perhaps because the action of the bath needs time to build up a difference. This needs further investigation, such as where stains are bound, how strongly they are bound, what changes of the polymer surface other than roughness facilitate discoloration, etc.

The results of this study are not easily compared to the studies that used a protocol with a combined staining and cleansing action, as previously mentioned.<sup>34,36,39</sup> Differences in the staining protocols used and the method for measuring the discoloration of the denture resins are the primary reasons; however, our results are not in agreement with those of Hollis *et al*,<sup>36</sup> since they found that common commercial cleaners have no significant effect on denture resin stains, and with those of Haghi *et*

al<sup>34</sup> who found that the use of a cleanser had no effect on the color of denture resins stained with tea. There is an agreement with the results of Al-Huraishi *et al*<sup>39</sup> who showed that all tested cleansers had the capacity to remove stain from denture resins, with an inhibitory effect on staining.

The investigated protocol A can show the ability of a cleanser to prevent discoloration of denture resins, if it is compared to protocol B. Therefore, they should be used more often in color stability studies. The tested peroxide cleanser proved effective in keeping denture acrylic resins clean for long periods of time, as long as it is used according to manufacturer's instructions. Staining protocol C seemed to have a mild and unrealistic effect on the resins tested, and it is recommended not be used, especially in short-term studies, since its mild action can give misleading results on the efficacy of denture cleansers. Autopolymerizing acrylic resins are not stained or cleaned much differently than heat-polymerized ones, but their behavior in staining solutions needs more investigation.

The use of only one cleansing product or one staining solution and the use of a staining solution as the control group instead of a water bath could be considered limitations of this study; however, the selected cleanser was chosen because it is the most common type used and the latest product of peroxide type needing only 3 minutes to act, according to the manufacturer. Coffee was chosen as the staining solution because of its strong staining effect among many others. Finally, the use of a staining solution as the control group was necessary to reveal the cleanser's effect in inhibiting coffee stains of the experimental group. These do not reduce the need for further investigation on the combining effect of more than one staining solution with cleansers on the color stability of UDMA (urethane dimethacrylate) or amide besides PMMA, and simulation of daily food intake and hygiene practices employed by denture wearers in a more realistic manner.

## Conclusions

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

1. Immersion protocol A (which combines staining protocol B with a cleanser) was found effective in preventing color changes in both denture resins even after a long period of action.
2. Protocol C stained both resins in a much lower level than protocol B, and similar to the level of protocol A until 140 to 160 cycles. For this reason it is probably not appropriate for use in short term color stability experiments.

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