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ORIGINAL ARTICLE

Effect of Ozonated Water on the Color Stability of Denture Teeth and Heat Polymerized Acrylic Base Resin

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ABSTRACT

Objective: To determine the effect of ozonated water on the color stability of denture tooth and denture bases. **Material and Methods:** Thirty denture base discs consisting of 15 Acropars and 15 ProBase Hot specimens with the dimensions of 40 × 5 mm were prepared. Fifteen denture teeth in shade A1 (Ivoclar Vivadent, Schaan, Liechtenstein) were mounted in a specific acrylic jig. All specimens were immersed in three solutions (1% sodium hypochlorite, ozonated water, and distilled water) for four months (one year of clinical use). Color measurements were done with a spectrophotometer and assessed using the CIE L*a*b* colorimetric system (0, 4, 8, 12, and 16 weeks). Data were analyzed using the three-way ANOVA and Tukey test (α=0.05). **Results:** Tukey's post hoc test revealed a significant change in color in the Acropars denture base for the distilled water group compared to the ozonated water and 1% hypochlorite ($p<0.05$). Regarding the ProBase Hot denture base, significantly less color change was observed in the 1% hypochlorite group compared to the ozonated water and distilled water ($p\leq0.001$). For the denture teeth, significantly less color change was seen in the distilled water group than in the ozonated water ($p=0.015$) and 1% hypochlorite ($p<0.05$) groups. **Conclusion:** The color change of denture bases and denture tooth in ozonated water are acceptable. Ozonated water can be considered a good disinfectant for cleaning dentures.

Keywords: Denture Cleansers; Acrylic Resins; Ozone; Color.

Association of Support to Oral Health Research - APESB

Introduction

Denture use may change the oral environment and provide a suitable condition for the proliferation and colonization of microorganisms [1,2]. For example, *C. albicans*, as a commensal fungus that colonizes the human mucosal surfaces in the oral cavity, is present in 30-60% of healthy individuals and 60-100% of denture wearers [3]. Hence, efficient and regular hygiene is important for the long-term maintenance of complete and partial denture teeth and is essential for the general health of denture wearers [4]. Denture cleaners are used to remove debris, stains, and bacteria [5]. Conventional methods for cleansing denture teeth that are recommended in the literature include chemical and mechanical methods or a combination of these methods [6,7]. In the mechanical techniques involve the use of microwave ovens, brushes, and ultrasonic cleaners [8-11]. In mechanical cleaning method, brushing is a simple method of cleaning dentures, but it has several disadvantages, such as wear of the denture base and relining materials and this resulting irregularity on the surface of the denture and increase in surface roughness which help biofilm formation [12]. Potentially, improper brushing techniques increase the risk of damage to denture bases and denture teeth, which could affect the users' esthetic sense and self-confidence [13].

Chemical cleaners are made of sodium hypochlorite, peroxides, and neutral peroxides with enzymes or acids [11]. Several studies showed the effects of denture cleaners, such as Efferdent (Pfizer Consumer Health Care, Scarborough, Ontario, Canada), chlorhexidine, peroxide, and other chemical cleaners, on the color stability of dentures [14]. The ideal denture cleanser should be compatible with the denture base and not only has disinfectant properties, adequate organic and non-organic particles, and eliminate organic matter, but also, it should not change the color of the denture base and denture teeth [15]. However, there is no available material with the combination of all these properties [16].

Ozone (O_3), an allotrope of oxygen, is recognized as a strong oxidative antimicrobial agent [17]. Ozone also has a high potential to be used as an antimicrobial agent in endodontics, restorative dentistry, and implant surgery [18,19]. In addition, it has been shown that ozonated water is a strong and reliable substance for germ disinfecting [1,20]. Home ozone generators are portable, inexpensive, and user-friendly devices for producing ozone gas for home use, such as disinfecting water, vegetables, or air. It has also been proven that home-generated ozonated water can be applied to remove the *Candida albicans* attached to the surface of denture plates [1].

One of the most important factors in dentistry that should be considered in the elderly is the discoloration of denture teeth and denture bases [21]. Color stability of denture teeth and denture bases acts as an important role for the patient and prosthodontist in selecting materials for the preparation of removable prostheses [22]. In long-term use as a cleansing agent, whitening effects could be observed on the denture teeth and denture bases, and subsequently, they may be clinically visible [23]. Fernandes et al. have reported that disinfection with 1% sodium hypochlorite and peracetic acid can change the quality of the color of the resins [23].

While previous studies have investigated the effect of denture cleaners on the denture base and soft tissue denture lining resins to change color [23-25], no study has evaluated the effect of ozonated water on the color stability of denture bases and denture teeth. Hence, the present study aimed to determine the effects of ozonated water on the color stability of denture teeth and two heat-polymerized denture bases. The null hypothesis was that ozonated water does not affect the color stability of denture teeth and denture bases.

Material and Methods

Two heat-polymerized acrylic denture base resins: ProBase Hot (Ivoclar, Schaan, Liechtenstein) and Acropars (Marlik Co., Tehran, Iran), and one denture tooth in the shade A1 (Ivoclar Vivadent, Schaan, Liechtenstein) were evaluated. Thirty disc-shaped blocks (40 x 5 mm) of each denture base material were prepared according to the manufacturer's instructions. After polymerization, the specimens were placed in distilled water for 24 hours at 37°C. The surfaces of the specimens were polished with 1200-grit abrasive waterproof paper (Sankyo Rikagaku Co Ltd, Saitama, Japan) and then washed with tap water and air-dried. Fifteen denture teeth (maxillary central incisor) were mounted in a specific acrylic jig according to the size of the spectrophotometer caliber (Figure 1A). The materials used in this study are shown in Tables 1 and 2.

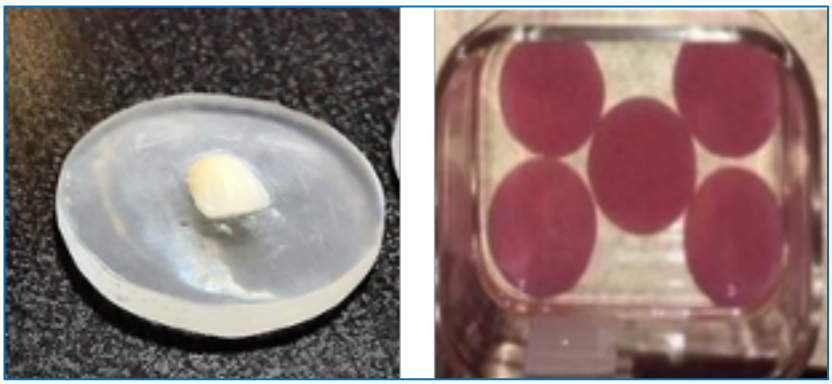

Figure 1. (A) Denture tooth in a specific acrylic jig. (B) The acrylic resin specimens were immersed in denture-cleansing solutions.

Table 1. The used specimens.

Material	Code	Shade	Manufacturer
ProBase Hot	PH	Live Pink	Ivoclar Vivadent, Schaan, Liechtenstein
Acropars	AP	Live Pink	Marlik. Co, Tehran, Iran
Denture tooth	DT	A1	Ivoclar Vivadent, Schaan, Liechtenstein

Table 2. The used denture cleansers.

Material	Code	Type	Manufacturer
Distilled water	DW	Control	
Ozonated water	OW	Home ozone generator	Arda, MHP 1H model, France
Home bleach	HB	1% sodium hypochlorite	Tehran, Iran

In this study, we used ozonated water that was made by a home ozone generator (Arda, MHP1H, Iran) as a disinfectant group, 1% sodium hypochlorite as a positive control group, and distilled water as the control group. In each denture cleanser, five specimens of each material were immersed for eight hours at room temperature, and then removed and washed thoroughly with tap water and air-dried. Next, they were plunged into a fresh solution (Figure 1B). This procedure was repeated for four months (three immersions per day), which is equivalent to one year in clinical use. Color changes of the specimen were calculated at 0, 4, 8, 12, and 16 weeks.

Color changes were measured with a spectrophotometer (Minolta Chroma Meter CR-400 with a data processor DP-300; Konica Minolta, Osaka, Japan). The spectrophotometer was calibrated according to the manufacturer's instructions as follows. Before each measurement period, we used the white pad that was supplied by the manufacturer. The color differences were assessed using the CIE L*a*b* colorimetric system [24,26]. Describing the color is dependent on three criteria of L*, a*, and b*. L* shows lightness, a-values show the

position on the red or green (+a = red, −a = green) axes, and b-values demonstrate the position on the yellow or blue (+b = yellow, −b = blue) axes [27]. The color change (ΔE) of each sample was calculated as follows [23]:

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

While a* and b* are on the chromatic scale and represent red as +a* and green as -a*, yellow corresponds to +b* and blue to -b*. Delta L*, Δa*, and Δb* explain the differences measured in L*, a*, and b* values before and after immersion for the different time intervals [28].

To link the ΔE to a clinical criterion, the National Bureau of Standards (NBS) index was used, which was obtained using the following formula (Table 3) [24]:

$$\text{NBS unit} = \Delta E \times 0.92$$

Table 3. NBS critical remarks of color differences.

Critical Remarks of Color Differences	NBS Unit
Trace	0-0.5
Slight	0.5-1.5
Noticeable	1.5-3.0
Appreciable	3.0-6.0
Much	6.0-12.0
Very much	12.0

With respect to the Hong et al. [24] study, with an alpha error of 0.05 and a test power of 95%, the sample size was calculated as 4 specimens per group. The mean and standard deviation of the color were determined. The three-way analysis of variance (ANOVA) was used to determine whether there were statistically significant differences among materials, different cleansers, and immersion times. The differences in color change values were evaluated with Tukey's multiplex test (Tukey's post hoc test). All data were analyzed at an alpha level of 0.05. Statistical analyses were performed using SPSS software version 23 (for the Windows operating system; SPSS Japan, Inc, Tokyo, Japan).

Results

The mean and standard deviation of the indices of L *, a*, and b* before immersion are demonstrated in Table 4.

Table 4. The mean and standard deviation of the indices of L*, a*, and b* before immersion.

Material	Indices	ProBase Hot		Acropars		Denture Tooth	
		Mean	SD	Mean	SD	Mean	SD
Ozonated Water	L*	43.79	0.96	50.80	0.39	73.83	0.76
	a*	22.78	0.24	19.19	0.23	0.26	0.09
	b*	5.25	0.34	4.30	0.16	12.07	0.70
Home Bleach	L*	45.38	1.42	50.81	0.25	79.39	0.92
	a*	22.77	0.17	19.05	0.20	0.28	0.049
	b*	5.57	0.63	4.3	0.24	12.13	0.32
Distilled Water	L*	44.59	0.79	51.06	0.40	73.55	0.92
	a*	22.28	0.43	19.09	0.24	0.23	0.11
	b*	4.95	0.47	4.39	0.42	12.19	0.48

In Table 5, the mean and standard deviation of the ΔE and NBS indices were recorded for all specimens at different times of immersion in different denture cleansers. The mean value of color change (ΔE) of all

specimens in all three solution types augmented with time. The maximal color change was seen in the AP denture base and the minimal color change was seen in the DT.

Table 5. The mean and standard deviation of the ΔE and NBS indices at different times of immersion in different denture cleansers.

Solution	Time	ProBase Hot			Acropars			Denture Tooth		
		Mean	SD	NBS	Mean	SD	NBS	Mean	SD	NBS
Ozonated Water	4 week	1.48	0.33	1.36	1.93	0.16	1.77	2.39	0.27	0.36
	8 week	1.72	0.31	1.58	2.57	0.16	2.36	0.55	0.31	0.50
	12 week	1.85	0.41	1.70	3.00	0.36	2.76	0.76	0.21	0.70
	16 week	1.94	0.39	1.78	3.61	0.46	3.32	0.89	0.23	0.82
Home Bleach	Week 4	0.78	0.11	0.72	1.62	0.16	1.49	0.48	0.11	0.44
	Week 8	1.08	0.17	0.99	2.40	0.22	2.21	0.58	0.12	0.54
	Week 12	1.35	0.26	1.24	2.87	0.32	2.64	0.71	0.15	0.65
	Week 16	1.44	0.32	1.32	3.02	0.35	2.78	0.77	0.22	0.71
Distilled Water	Week 4	1.29	0.20	1.19	2.42	0.43	2.23	0.31	0.12	0.29
	Week 8	1.73	0.35	1.59	3.27	0.39	3.01	0.40	0.11	0.36
	Week 12	1.81	0.39	1.67	3.63	0.36	3.34	0.46	0.16	0.43
	Week 16	1.93	0.366	1.78	3.94	0.30	3.62	0.58	0.12	0.54

Tukey's post hoc test revealed a significant change in color in the AP denture base for the distilled water group compared to the ozonated water and 1% hypochlorite ($p<0.05$). Regarding the PH denture base, significantly less color change was observed in the 1% hypochlorite group compared to the ozonated water and distilled water ($p\leq0.001$). For the DT, significantly less color change was seen in the distilled water group compared to the ozonated water ($p=0.015$) and 1% hypochlorite ($p<0.05$) groups.

The three-way ANOVA results (Table 6) showed that the main factors affecting the color change of the samples were the type of solvent and acrylic material as well as time. The interaction effect between the type of solution and type of material and also the type of material and time was statistically significant ($p<0.001$). The color changes of the two types of denture bases in the distilled water were higher than the ozonated water, which contained more than 1% hypochlorite.

Table 6. Three-way ANOVA for color change of materials.

Source of Variation	Sum of Squares	DF	Mean Squares	F	p-value
Corrected Model	193.598	35	5.531	69.496	<0.001
Intercept	492.7930	1	492.790	6191.408	<0.001
Materials	157.573	2	78.787	989.872	<0.001
Solutions	4.974	2	2.478	31.245	<0.001
Immersion Periods	17.012	3	5.671	71.246	<0.001
Materials x Solutions	6.901	4	1.725	21.675	<0.001
Materials x Immersion Periods	6.514	6	1.086	13.640	<0.001
Solutions x Immersion Periods	0.215	6	0.036	0.450	0.844
Materials x Solutions x Immersion Periods	0.410	12	0.034	0.429	0.950
Error	11.461	144	0.080		
Total	697.850	180			
Corrected Total	205.060	179			

Discussion

C. albicans, as a commensal fungus that colonizes the human mucosal surfaces in the oral cavity is present in 30-60% of healthy individuals and 60-100% of denture wearers [3]. The aim of this study was to evaluate the effect of ozonated water on the color stability of two denture bases and denture teeth. Ozonated water is an easy and inexpensive way to disinfect denture teeth while providing acceptable color change. However, to the best of

our knowledge, no study was found on the color change of denture bases and denture teeth during immersion in ozonated water.

In the present study, the null hypothesis that denture cleaners have no effect on acrylic base color was rejected. This finding is in agreement with other studies [29,30]. Sarac et al. [31] and Purnaveja et al. [32] have reported that denture cleansers can cause whitening or bleaching, loss of soluble components, or water absorption in denture materials.

In this study, the mean value of color change (ΔE) of all specimens in all three solutions increased over time. However, this color change was maximum in the AP denture bases and minimal in the DT.

We used the NBS unit to measure the amount of color change clinically. The NBS of the PH denture base in sodium hypochlorite was slight after some time; however, in ozonated water and distilled water, it was slight only at the first time point, and at other times, it was noticeable. The NBS of the AP denture base in all denture cleaners was noticeable or considerable at all times. In a study by Fernandes et al. [23], the highest NBS unit in the three types of denture bases was trace. The NBS unit of our study was higher than the mentioned study because our immersion time was higher.

At many time points, NBS for the two types of denture bases were highest in distilled water and lowest in sodium hypochlorite, which was similar to Hong et al.'s study [24]. Hong et al. have reported that the leaching of colored materials in the hypotonic environment of distilled water could cause this action [24]. Previous studies have shown that color variations in denture bases are likely to be due to changes in the matrix and effects of external pigments, solubility, and water absorption, leakage, surface irritation, and chemical decomposition [33-35]. Other studies have shown that one of the side effects of denture disinfectant is whitening or bleaching [32]. This can be attributed to the fact that L * a * b * acrylic resins increased or reduced during immersion [24].

The NBS of DT in all solutions was trace or slight. NBS for DT was lowest in distilled water and highest in ozonated water, which may be due to the inactivation of distilled water versus ozonated water and sodium hypochlorite. Also, it can be related to the acrylic teeth that were denser and less leaching. We suggest further research in this regard.

Color stability of the PH denture base, AP denture base, and DT was different. The color change of the AP denture base was more than the PH denture base, and the PH denture base was more than the denture teeth. Hence, the color stability of the denture teeth was better than the denture bases. The color change of all specimens was also different after 4, 8, 12, and 16 weeks. Hence, the color changes increased over time.

In the present study, 1% sodium hypochlorite was used as a positive control. The color changes of all denture bases induced by sodium hypochlorite were less than in other solutions. Similar to the current study, many studies have reported no significant change in the clinical appearance of denture teeth while immersion in sodium hypochlorite [36]. Another study emphasized that after 30 to 60 minutes of immersion, color changes were not visible clinically [37]. According to another study [30], the probability of color changes of acrylic samples in sodium hypochlorite depended on the concentration of solution and immersion time. Thus, in the present study, the main factors influencing the color change of the samples were the type of solvent, type of acrylic material, and time.

In a study by Hong et al. [24], which was similar to the current study, it was shown that the type of cleanser affected the amount of discoloration. In addition, Hong et al. [24] reported that the type of polymerization could affect the amount of discoloration; however, in our study, this was not proven.

Since color changes due to immersion occur in all parts of the denture, it is very difficult to detect color changes clinically. However, when ΔE is greater than 3.7, the unacceptable level of color change is clinically

significant. On the other hand, when the NBS unit is less than 6 in the long immersion time, many patients are not able to detect color variations [24].

In the present study, ΔE was not greater than 3.7, except for the AP denture base after four months of immersion in distilled water, but the NBS unit of all samples was less than 6 at all time points. Therefore, it is advisable to reduce the use of AP denture bases, especially for patients who pay attention to aesthetic aspects.

In a study by Moon et al. [38], color changes had occurred in all specimens, but ΔE was not greater than or equal to 3.5. Therefore, clinically, the color changes of all specimens are acceptable after 48 weeks (about one year). It was also found that the dental brand and type of cleanser affected color changes; however, the shade was ineffective, while color change was more affected by the type of cleaner than its brand [38]. In the present study, in most denture bases, ΔE was lower than 3.5. Similar to the study by Moon et al. [38], color variations of denture base samples were much higher than denture teeth.

In the comparative research conducted in this study, the color change of denture bases in distilled water was more than in ozonated water, and in ozonated water was more than sodium hypochlorite, which was statistically significant. The NBS unit was less than 6 in the long immersion mean in the lower class [39]. In our study, the NBS unit, which is a criterion for the clinical evaluation of color change, was less than 6 in all samples when immersed in ozonated water. Thus, it can be said that the color of denture bases and denture teeth in ozonated water was clinically acceptable; thus, many patients were not able to detect color changes. Furthermore, the appropriate antiseptic effect of ozonated water was confirmed in a previous study [40]. Therefore, based on the results of this study, ozonated water could be considered an appropriate antiseptic.

Conclusion

The color change of denture bases and denture tooth in ozonated water are acceptable. Considering ozonated water as a good soluble disinfectant, ozonated water can be considered as a good soluble disinfectant and can be used for cleaning dentures.

Authors' Contributions

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HB	https://orcid.org/0000-0003-1269-7434	Methodology, Formal Analysis, Investigation, Data Curation, Writing - Original Draft and Writing - Review and Editing.
AR	https://orcid.org/0000-0002-6323-6112	Conceptualization, Methodology, Formal Analysis, Investigation, Data Curation, Writing - Original Draft and Writing - Review and Editing.

All authors declare that they contributed to critical review of intellectual content and approval of the final version to be published.

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Conflict of Interest

The authors declare no conflicts of interest.

Data Availability

The data used to support the findings of this study can be made available upon request to the corresponding author.

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