Influence of Herbal Mouthwashes and Chlorhexidine Mouthwash on the Physical Characteristics of Orthodontic Acrylic Resin

Farzaneh Golfeshan,[1] Shabnam Ajami,[1] Yasaman Khalvandi,[2] Seyed Ali Mosaddad,[3] Haniye Nematollahi[2]

[1]Orthodontics Research Center, Department of Orthodontics, School of Dentistry, Shiraz University of Medical Sciences, Shiraz; [2]School of Dentistry, Shiraz University of Medical Sciences, Shiraz; [3]School of Dentistry, Shiraz University of Medical Sciences, Shiraz, Iran

Abstract

The present study, intended to compare the effects of immersion in herbal mouthwashes and the Chlorhexidine mouthwash on orthodontic acrylic resin, specifically with regard to alterations in its hardness, roughness and color. Seventy-two specimens of Orthodontic self-cured acrylic resin were used for the experiments. After preparation, specimens were placed in three types of mouthwash including Chlorhexidine 0.2 % (CHX), Persica and Matrica. The changes in microhardness (ΔKNH), Surface roughness (ΔRa) and color (ΔE) were evaluated prior to and following the immersion in mouthwashes. In order to analyze the data, we made use of SPSS version 22. One-Way ANOVA test was used to find the differences between groups. The Tukey test was conducted in the final stage. It was revealed that acrylic resins had lower microhardness when 12 hours after immersion had taken place. There were significant differences after 12h, 24h and 7 days between the groups in the softening of acrylic resins. The reduction in microhardness of acrylic resin with herbal mouthwashes in the three-time intervals was higher than CHX. With regard to increasing surface roughness, the disparity between herbal mouthwashes was insignificant. However herbal mouthwashes significantly increased the value of roughness more than CHX. Changes in color were significantly higher in all mouthwashes. Herbal mouthwashes caused more color variation than CHX. The color, roughness and hardness of acrylic resin undergo changes as a result of being immersed in the mouthwashes. However, the effect of herbal mouthwashes was more than that of CHX.

Correspondence: Seyed Ali Mosaddad, School of Dentistry, Shiraz University of Medical Sciences, Shiraz, Iran
Tel.: +989175827063;
E-mail: mosaddad.sa@gmail.com

Key words: Orthodontic appliances; Mouthwashes; Hardness; Acrylic resins.

Acknowledgments: The authors thank the vice-chancellery of Shiraz University of Medical Sciences for supporting the research (Grant# 98-01-37-19703). Also, the authors thank Dr. Vosough from the Dental Research Development Center for the statistical analysis.

Conflict of Interest: The authors declare no potential conflict of interests.

Received for publication: 6 March 2020.
Accepted for publication: 28 April 2020.

Journal of Biological Research 2020; 93:8949
doi:10.4081/jbr.2020.8949

Introduction

Orthodontic removable appliances have diverse uses in contemporary orthodontic treatment.[1] They can be used in myofunctional therapies, small tooth movements or the retention of orthodontic treatments.[2] The different components of removable appliances such as the retentive sites of clasp, springs and especially acrylic base plates are suitable environments for microbial colonization because of their subsurface porosity.[3, 4] It was reported that gingivitis, halitosis and dental caries is more common among children in treatment with removable orthodontic appliances.[5] Therefore, good hygiene of orthodontic removable appliances is necessary to reduce these side effects[6] because biofilms on tooth surface may lead to dental caries, and supra- and subgingival plaques along and below the gingival crevice may cause periodontal disorders.[7]

The studies showed that the use of water alone or the brushing of removable appliances cannot completely remove microorganism biofilms from retentive sites and the microporosity of removable appliance.[8] Today, a combination of mechanical and chemical methods is routinely recommended.[9] The chemical solutions which are used for the disinfection of removable appliances should not have a negative impact on the physical properties of the acrylic base plate and the other components of these appliances.[10] For instance, immersion in some chemical solutions may cause the plasticization of polymer chains in acrylic base plates, leading to material degradation due to the increase in water sorption and solubility.[11] Chlorhexidine (CHX) is one of the most commonly used chemical solutions for biofilm control in this regard. However, it was reported that CHX negatively affects some properties of acrylic resin such as transverse strength,[12] color stability,[13] roughness and hardness.[14]

Currently, due to some undesirable side effects reported from CHX consumption, the tendency to use herbal solutions as an aid for the disinfection of removable appliances has been increased.[15-

[17] Persica is an herbal solution, containing an extract of *Salvadora persica* (*S. persica*). Benzyl Isothiocyanate is the major component of this herbal extract that prevents acid from being produced and *Streptococcus mutans* from growing. Besides antivirals and antifungals activity of this herbal extract have been reported.[16-19]

Matrica is another herbal solution that contains chamomile extract. Chamomile is a flower with the scientific name *Matricaria chamomilla* from the *Compositae* Family.[20] The reason for the heightened interest in the use of this herbal solution is that chamomile has anticancer, antifungal, anti-bacterial and anti-inflammatory characteristics, which make it particularly effective against *Candida albicans* and the herpes virus.[21, 22]

At present (or to date) the desire to use herbal mouthwashes as a disinfectant is increasing. Studies have shown that some herbal mouthwashes, especially those containing chamomile can have far more antifungal effects than CHX with fewer negative side effects.[23,24] An ideal disinfectant solution in addition to have good antibacterial and antifungal effects must not have destructive effects on the physical and mechanical properties of orthodontic acrylic resins. Since no studies have been performed to compare the effect of herbal mouthwashes with chemical ones on the physical and mechanical properties of orthodontic acrylic resins, the present study was designed. In this research, our intention was to carry out a comparison between and assessment on the way CHX, Persica, and Matrica influence acrylic resin in terms of its physical characteristics. These three types of mouthwash were chosen because CHX is the most common chemical mouthwash and Matrica and Persica are the most ones in the herbal mouthwashes with antifungal and antibacterial effects. It was held as the null hypothesis that herbal solutions do not alter the physical characteristics of acrylic resin as much as CHX.

Materials and Methods

This *in vitro* double blinded study was approved ethically (IR.SUMS.REC.1398.063). Chlorhexidine digluconate 0.2% (Behsa Co., Iran) and two other types of herbal mouthwashes including Persica 10% (Poursina Co., Iran) and Matrica 10% (Barij Essence pharmaceutical Co., Iran) were used in this study. As the mean wearing time of orthodontic appliances is 13.4 (±10.3) months,[25] in the present study the microhardness, roughness and colorimetric analysis were checked after 12h, 24h and 7 days for each mouthwash. These time intervals were established to replicate, and even extrapolate, situations in orthodontic patients' home disinfection practice. Before the resin samples were submerged in the solutions, the pH of the mouthwashes was measured. For this measurement, a digital pH meter was used[26, 27] (pH 21, Hanna Instruments Inc., Woonsocket, RI, USA) at room temperature.

Acrylic resin specimens

Seventy-two Acrylic resin specimens (10 mm diameter and 2.5 mm thickness) were prepared using cold cure acrylic resin (Ortho Clas®; Acropars). To produce a smooth surface, each coin was polished (Figure 1). Forty-five specimens were randomly divided into 9 groups (n=5) per time interval and mouthwash for microhardness and roughness tests. The remaining 27 were used to create 9 other groups (n=3) for each time interval and mouthwash for color analysis. After the initial measurements, the acrylic resin was immersed in 30 ml of mouthwash for 12h, 24h and 7 days. After mouthwash immersion, the specimens were washed with distilled water for 10 s and dried with compressed air for 1 minute. Then, the second measurement was done as described below.

Microhardness Test

A hardness tester machine (SCTMC Company, MHV_1000Z model, Chinese) was used to determine the surface Knoop microhardness (KHN) of specimens at the beginning and at the end of the experiment. A 25 g load was applied to surface of the specimens for 10 seconds in order to create 3 indentations. The value of KHN was measured according to the following formula:

$$KHN = [(14228\ c)/(d^2)]$$

Where: c = applied load in g; d = length of the longest indentation diagonal in mm; 14228 = constant number.

The KHN value of the specimen was determined by calculating the mean value of the three indentations. By comparing the Knoop microhardness at the beginning and the end, and by calculating the percentage of difference between the two (KHNF - KHNI), the softening of acrylic resin (ΔKHN) was determined (Figure 2).

Roughness

In order to determine the mean surface roughness of each specimen a roughness surface analyzer was used (COMPANY TESA, RUGOSOR Model, Switzerland). This device benefited from a metal tip that could move up and down at an accuracy of ± 0.01 Micrometer (μm) . Furthermore, it was capable of precisely probing and recording 3 mm segments of each group in 15 seconds. The tip moved up and down upwards and downwards. This irregular pattern was plotted against the profile of the surface

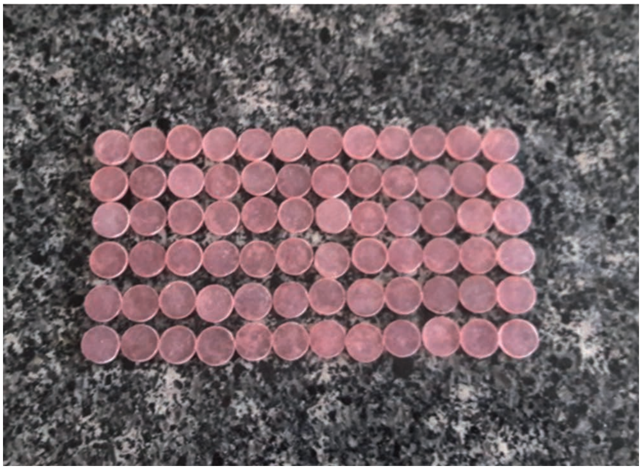

Figure 1. Preparation of acrylic resin specimens.

explored. In every scan, a length of 3 mm was analyzed. Three profilometric scans were performed at different parts of each wire section. The equipment automatically determined the profilometric mean roughness from the surface of each specimen (Figure 3A).

Colorimetric Analysis

Color changes were measured using a spectrophotometer (VITA Company**,** Easy shade **V** Model**,** Germany Figure 3B). Color changes were calculated according to the International Commission on Illumination.[20] The CIE Lab system allows color perception in three-dimensional space through a wavelength versus refraction index. Color evaluation took place under the following conditions: L*a*b* coordinates were calculated in daylight conditions (D65 CIE illuminant) from an observation angle of 10 degrees. In these calculations, "L" stands for luminosity on a scale from 0 to 100; "a" stands for the amount of red and green; and "b" stands for the amount of yellow and blue. In order to determine color changes from one point to another, the CIE Lab system utilizes the formula below:

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

Statistical Analysis

Data analysis was conducted with SPSS version 22. In order to determine discrepancies between the two groups, One-Way ANOVA test was utilized. In the final stage, the Tukey test was carried out. While analyzing the data, values greater than 0.05 were considered significant.

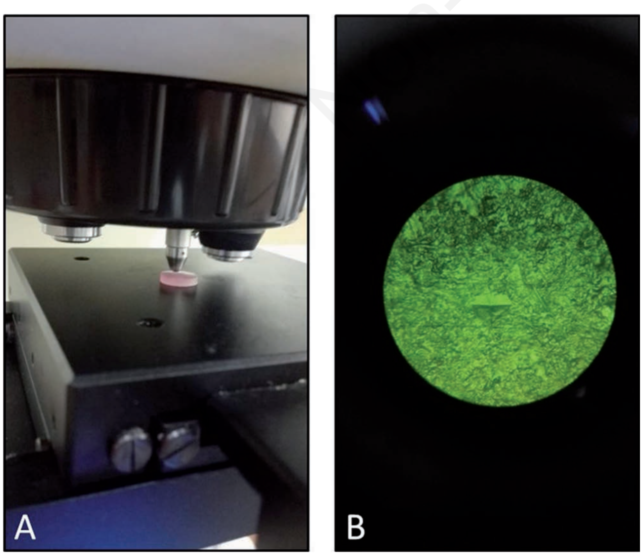

Figure 2. The microhardness tester machine (A) and the view of acrylic resin under microhardness tester machine after immersion in CHX for 12 h (B).

Results

pH of the Mouthwashes

The values of pH solutions varied from a minimum of 4.22±0.03 to a maximum of 7.3±0.05, which indicated that the differences among groups were significant. The minimum pH value was recorded for Persica at 4.22±0.03. The pH of Matrica was 4.33. Chlorhexidine showed the highest pH value (7.3±0.05).

Microhardness

The microhardness of the acrylic resin specimens was decreased after 12 h of immersion in all the mouthwashes. There were significant differences between CHX and Persica, and between CHX and Matrica in the reduction of microhardness after 12 h, 24 h and 7 days ($p<0.05$). Between Persica and Matrica, there were no significant differences in the reduction of hardness in these three time intervals. Reduction in the microhardness of

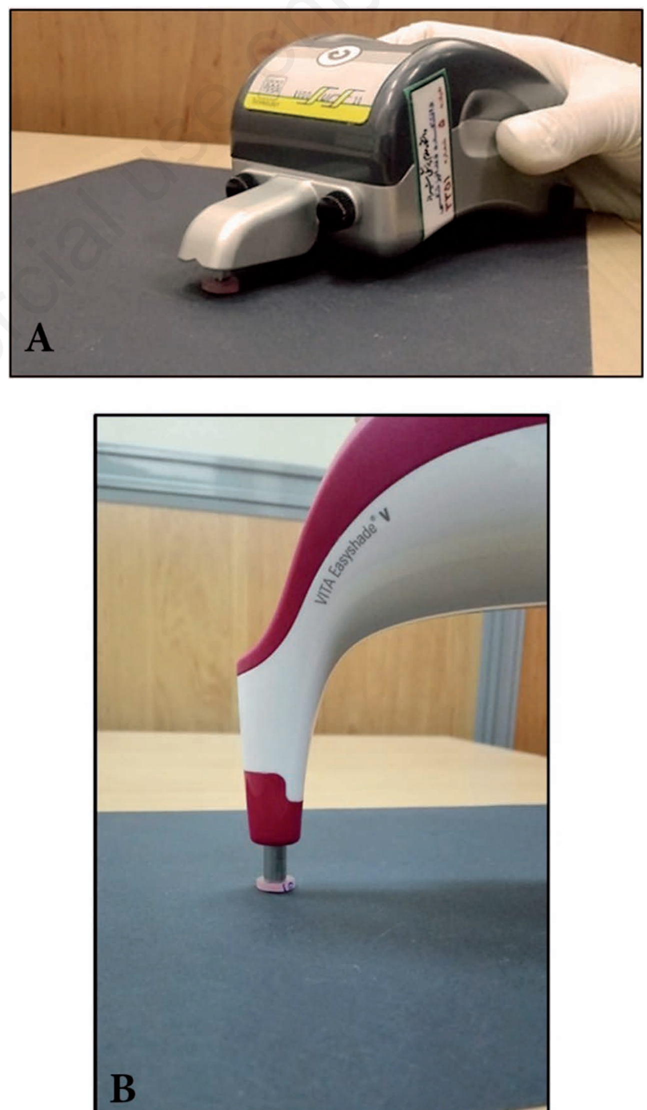

Figure 3. The surface roughness analyzer machine (A) and the spectrophotometer machine (B).

acrylic resin with herbal mouthwashes was higher than CHX in all the three time periods tested in this study (Table 1).

Roughness

Surface roughness increased in all tested groups. There was no significant difference between the three types of mouthwash at 12h (P>0.05). Between CHX and Persica groups there were significant differences in the increase of roughness at 24h (P<0.05). Between CHX and Matrica groups and between Matrica and Persica groups, the differences in the increase of roughness were negligible at 24h (p>0.05). The differences between CHX, Persica, CHX and Matrica were significant after 7 days (P<0.05). Between the Persica and Matrica groups, the differences in the increase of roughness were negligible at 7 days (P>0.05). The increase of surface roughness with the Persica was greater than that of Matrica, but this increase was at its lowest with CHX mouthwashes (Table 2). Table 3 shows the mean value of roughness and Knoop hardness of specimens before and after immersion.

Colorimetric Analysis

The different mouthwashes in the study showed differences in their colorimetric analysis (using ΔE) after the different time periods used in the test. Between CHX and Persica groups and between CHX and Matrica groups there were significant differences in the increase of color variation at 12h (P<0.05). The differences in the increase of color variation were insignificant between Persica and Matrica groups at 12h (P>0.05). The differences between CHX and Persica groups in color variation were insignificant at 24h (P>0.05). Between CHX and Matrica groups and between Persica and Matrica groups there were significant differences in the increase of color variation at 24h (p<0.05). The differences in the increase of color variation after 7 days were significant between all groups (p<0.05) (Table 4).

Table 1. Variation of Knoop hardness values after and before immersion in mouthwashes. (Each two numbers with one common letter in each column, are not significantly different).

Mouthwashes	H (Mean±SD)		
	12h	24h	7 days
CHX	0.726±0.503[a]	1.771±0.248[a]	3.244±0.458[a]
Persica	1.15±0.885[b]	4.786±1.311[b]	6.556±0.634[b]
Matrica	3.284±0.763[b]	4.531±0.897[b]	6.922±1.004[b]

Table 2. Variation of roughness value, in µm, after and before specimen immersion in mouthwashes. (Each two numbers with one common letter in each column, are not significantly different).

Mouthwashes	R (Mean±SD)		
	12h	24h	7days
CHX	0.033±0.024[a]	0.21±0.027[a]	0.24±0.08[a]
Persica	0.159±0.104[a]	0.397±0.208[b]	0.521±0.211[b]
Matrica	0.099±0.033[a]	0.233±0.072[ab]	0.292±0.061[b]

Table 3. The mean value of roughness and Knoop hardness of specimens before and after immersion.

	Roughness (Mean value)			Knoop hardness (Mean value)		
	12 hours	24 hours	7 days	12 hours	24 hours	7 days
Mouth washes	Before After	Before After	Before After	Before After	Before After	Before After
CHX	0.847 0.880	0.554 0.765	0.580 0.822	13.246 12.520	13.911 12.140	14.164 10.919
Persica	0.837 0.997	0.700 1.098	0.580 1.101	11.382 10.232	11.502 6.715	11.602 5.045
Matrica	0.791 0.890	0.693 0.927	0.762 1.055	13.807 10.523	11.346 6.815	12.628 5.706

Table 4. Variation of color (ΔE), after and before specimens' immersion in mouthwashes. (Each two numbers with one common letter in each column are not significantly different).

Mouthwashes	E (Mean±SD)		
	12h	24h	7 days
CHX	7.891±0.836	7.310±2.522	3.240±1.01
Persica	14.790±1.478	13.539±1.021	18.452±0.704
Matrica	15.135±0.609	17.8±0.21	26.689±1.839

Discussion

In this *in vitro* study, the effects of the most commonly used chemical mouthwashes (CHX) and two types of herbal mouthwashes (Persica and Matrica) were evaluated on some of the physical properties of self-cured orthodontic acrylic resin. We evaluated the changes in microhardness, roughness and the color of acrylic resin specimens in three time intervals including 12h, 24h and 7 days. According to a systematic review done by Mavreas *et al.*,[25] the mean wearing time of orthodontic removable appliances was 13.4 (±10.3) months. Patients are usually instructed to immerse their appliances 1 hour a day for disinfection. Accordingly, these three periods of immersion in mouthwashes were defined to simulate the meantime that these appliances may be immersed in mouthwashes during orthodontic treatment. Twelve and 24 h could replicate the average time of immersion of removable orthodontic appliances used approximately for 1 month during orthodontic treatment. Seven days could replicate the mean immersion time for wearing 6 months of removable appliances. Our null hypothesis was that using the herbal mouthwashes might have a lesser effect on acrylic resin properties since they are alcohol-free, unlike the CHX mouthwash. The other reason why we decided to compare herbal mouthwashes with CHX was that they had some additional advantages reported in the studies.[23,24] Most of chemical mouthwashes contain alcohol and fluoride which can be toxic at high dose. Therefore, most herbal mouthwashes are safer in this regard to pregnant women, diabetic patients, and children. Besides in comparison with chemicals, herbal mouthwashes could have additional anti-inflammatory and antioxidant effects, which could be beneficial for gingival health. In several studies it was reported that herbal mouthwashes containing chamomile are more effective against *Candida* species than CHX. Also, desquamation and irritation of oral mucosal cells are less common with herbal mouthwashes.[28]

The results from the present study indicated that immersion influenced acrylic resin regardless of the type of mouthwash solution used, but this influence was even greater when herbal mouthwashes were used. Therefore, our null hypothesis was rejected. The harder removable appliances are the longer they will resist abrasion, so hardness directly influences their longevity. Decreasing hardness during treatment with removable appliances may make the appliance prone to fracture.[29] In our study, the microhardness of Acrylic resin was reduced as a result of immersion in all mouthwashes even after 12h. The effects of herbal mouthwashes on the reduction of microhardness were higher than CHX in the three time intervals. As we mentioned previously, the pH of herbal mouthwashes used in this study was lower than that of CHX. This acidity might be responsible for the greater softening of acrylic resin. Acid solutions weaken polymer molecule bonds, which makes them softer and more easily degraded.[29] Jyothi *et al.*[30] analyzed the influence of different mouthwashes on the microhardness of restorative materials in an *in vitro* study. The conclusion of their research was the fact that the lowest pH in a mouthwash led to the greatest decrease in microhardness. The CHX mouthwash also decreased the microhardness value in our study. The alcohol content of this mouthwash could explain the changes on the acrylic surface. This conclusion supports the former discovery that ethanol softens acrylic resins.[12] Apparently, both ethanol and water can separate polymer chains from each other by driving them apart and allowing them to slide with greater ease (they will plastically deform).[12] Ethanol penetrates the matrix and expands the space between the chains[31], which results in greater plasticization due to lower VHN values for PMMA.[32] Penugonda *et al.*[33] reported that the alcohol in mouthwashes affects the hardness of acrylic resin in the dentures base and that the softening effect is directly related to the percentage of alcohol in the mouthwashes. The surface roughness of acrylic resins is fundamentally important as it directly affects the health of oral tissues in direct contact with the base plate of appliances.[34] According to Quirynen[35] and Bollen,[36] if the base of removable appliances has a rough surface, it accumulates and retains more dental plaque than smooth surfaces. The increased roughness of the surface area may prevent bacteria from being removed either naturally or intentionally using hygiene instructions. Furthermore, Quirynen[35] and Bollen[36] demonstrated that bacteria are capable of remaining alive for extended periods when attached to rough surfaces or places that allow them to remain stagnant. According to research, at 0.2 mm roughness is necessary for the bacteria to be able to remain attached to the surface.[37] In this study, all mouthwashes increased surface roughness more than 0.2 mm, with more increase in herbal mouthwashes than CHX. Persica and Matrica are alcohol-free mouthwashes that have been recently introduced. This increase in surface roughness can be attributed firstly to the low pH of the mouthwash and secondly to the hygroscopic nature of resin-based materials.[38] The degradation of the upper layer of acrylic resin has probably been caused by the higher acidity of the mouthwashes in question, which has resulted in greater roughness. When acrylic resin is in contact with the mouthwash for a long period of time, the top layer is removed and a subsurface area is exposed to the mouthwash. This might be the reason for greater roughness when acrylic resin is immersed in Persica and Matrica from 24h to 7 days. Similarly, Sadaghiani *et al.*[39] did an *in vitro* study to check the effect of different mouth- rinses on the surface roughness of resin-modified restorative materials and concluded that the mouthwashes with lowest pH resulted in the greatest increase in the surface roughness. Kamna Gorka in 2016 checked the effect of CHX with herbal mouthwash on the roughness of resin-modified glass ionomer restorative material and concluded that herbal mouthwash increased the roughness of resin-based materials more than CHX.[38] ABO and Yousef did an *in vitro* study to assess the various restorative materials after exposure to CHX and concluded that exposure to CHX for 1 month showed a rise in mean surface roughness values in restorative materials.[40] The alcohol content of CHX may affect the surface integrity of acrylic resins. Absorption of alcohol molecules into acrylic resin could result in the softening of acrylic resin surface.[41] This could explain the increased roughness of specimens after immersion in CHX.

Colorimetric analysis using the ΔE as a parameter showed that the color of acrylic resin was changed as a result of exposure to different solutions. Persica and Matrica experienced the most noticeable color alteration even after the 12h of the immersion period. Acrylic resins may absorb water or aqueous solutions, and pigments dissolved in these solutions may be carried into the body of the resin.[42] Since the herbal mouthwashes used in this study were darker in comparison to CHX, they caused more color alteration. Factors such as surface roughness, oxidation, dehydration, water absorption, product degradation, and chemical degradation might also contribute to color instability in acrylic resin.[43] These factors could explain the color changes induced by CHX and herbal mouthwashes.

As patients do not immerse their appliances in mouthwashes for a continuous period of time, the main limitation of our study is the continuous immersion time intervals. This continuity may cause the greater degradation of acrylic resin by ethanol or the acidic content of the mouthwashes. Nonetheless, the long period of immersion in this study might be compared to what would actually happen over the course of several months of daily immersion of an acrylic device by the user. To sum up, all mouthwashes used in this study including CHX, Persica, and Matrica had a negative influence on the hardness, roughness and color stability of orthodontic acrylic resin but the effect of herbal mouthwashes on these physical properties was higher than CHX.

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