ORIGINAL RESEARCH

Effect of Probiotic Mouthrinses on Surface Microhardness of Esthetic Restorative Materials

Bashaer Altwaim[1], Fouad Salama[2], Shahad Alogayyel[3]

Abstract

Aim: To assess the influence of three probiotic mouthrinses on the microhardness of three esthetic materials used for teeth restorations.

Materials and methods: Thirty specimens of each material: conventional glass ionomer cement (GIC), resin-modified glass ionomer, and resin composite were randomly assigned to three groups. Surface microhardness was measured at baseline. The specimens were immersed in probiotic mouthrinses, group I: (P2 probiotic power), group II: (Probioclean), and group III (BōKU natural). Microhardness was measured after 21 and 63 minutes of immersion which is comparable with 3 and 9 weeks of mouthrinse use every day, respectively. Measurements of microhardness were completed using Micro Vickers testing machine with a 200 g load applied for a duration of 15 seconds.

Results: The microhardness change of the three restorative materials reveled statistically significant differences in all mouthrinse groups ($p = 0.001$). BōKU natural mouthrinse decreased microhardness significantly in all restorative materials ($p < 0.05$). However, Probioclean mouthrinse was associated with an increase in microhardness in all restorative materials. The effect of P2 probiotic power mouthrinse varied depending on time cycles and the restorative material. The mean difference in mouthrinse groups of resin composite was highest in BōKU natural at immersion time of 63 minutes. While no mean difference was seen in P2 probiotic power group at immersion time of 21 minutes which had no effect on the microhardness of resin composite.

Conclusion: Surface microhardness was affected by immersion in probiotic mouthrinses. The BōKU natural mouthrinse had the highest reduction, while resin composite showed the least change in surface microhardness.

Clinical significance: There is lack of studies that investigated the effect of probiotics mouthrinses on the surface properties of restorative materials. This study showed evidence that some of the tested probiotic mouthrinses in this study decreased the microhardness of the tested tooth-colored restorative materials after immersion for 21 and 63 minutes which is equivalent to 3 and 9 weeks of everyday use.

Keywords: Laboratory research, Microhardness, Mouthrinse, Probiotic, Restorative materials.

The Journal of Contemporary Dental Practice (2020): 10.5005/jp-journals-10024-2809

Introduction

Surface quality is one of the important contributors to the esthetic requirements of the tooth-colored restorations. The surface properties of restorative materials play an important role in the clinical success.[1] Esthetic restorative materials are commonly used in dentistry as anterior and posterior restorations in which it requires minimal invasive techniques.[2] One of the important factors for any restorative material for its longevity is the microhardness.[2] The esthetic restorative materials have been widely investigated for their longevity and durability in oral environment.[3] As long as the microhardness is linked to rigidity and strength of the material,[4] it has a strong effect on the permanency of clinical restorations.[5] Surface microhardness has been used to measure the polymerization adequacy as an indirect method[6] for its simplicity and for presenting good association to the degree of polymerization.[7,8] With low surface hardness, the restoration will be vulnerable for scratches and aggravate failure of restorations.[9] Long-term survival of the dental restoration in the mouth is essential for the oral health and quality of life.[10] For this reason, meticulous maintenance is essential for tooth-colored restorations.[11] All individuals are required to remove the bacterial plaque from all surfaces of the dentition as a prevention.[12,13] As a matter of a fact, attaining satisfactory points of control of plaque is difficult with single by using manual methods such as toothbrushing; thus, mouthrinses usage is favorable and supported by dentists.[12,13] Several forms of mouthrinses have been used over the years.[14,15] Recently, a wide-ranging forms of mouthrinses are present in the market, and many of them have not been tested for their influence on the dental restorations.[16] Taking into consideration that frequent use of mouthrinses may have unfavorable effects on oral tissues and on the dental restorations.[17] The contents of mouthrinses include water, antimicrobial agents, salts, fluoride preventive anti-caries agents, and some types of three alcohols.[18,19] These contents comes into variable concentrations in mouthrinses, which can cause a change in pH of mouth[20] and change in the inorganic phase of the restorative material including the surface hardness.[21–23] Even alcohol-free mouthrinses might have an impact on the hardness of the restorative materials.[10] Currently, probiotics have been incorporated into contents of mouthrinses.

World Health Organization defined probiotics as "live microorganisms which when administered in adequate amounts

[1,3]Department of Dentistry, College of Dentistry, King Saud University, Riyadh, Kingdom of Saudi Arabia

[2]Department of Clinical Dentistry, School of Dentistry, University of Detroit Mercy, Detroit, Michigan, USA

Corresponding Author: Fouad Salama, Department of Clinical Dentistry, School of Dentistry, University of Detroit Mercy, Detroit, Michigan, USA, Phone: +1 313-494-6877, e-mail: salamafs@udmercy.edu

How to cite this article: Altwaim B, Salama F, Alogayyel S. Effect of Probiotic Mouthrinses on Surface Microhardness of Esthetic Restorative Materials. J Contemp Dent Pract 2020;21(5):543–548.

Source of support: Nil

Conflict of interest: None

confer a health benefit on the host."[24] Introduction of probiotic is considered an innovation for oral health maintenance along with being a natural defense against the harmful bacteria to the dentition and gingiva.[25] Studies are recently recommending that probiotics have the potential to change or inhibit the microbiology of oral flora, for example, dental caries and the periodontal diseases.[26] The results of studies on probiotic have indicated inhibition of *Candida albicans* and *Streptococcus mutans*.[24–27]

At a young age or adult, probiotic bacteria do not colonize continually the mouth.[24–27] The probiotic strains administration resulted in caries reduction.[24,25] However, there was a lack of studies that investigated the effect of probiotics containing mouthrinses on the mechanical properties of the tooth-colored restorative materials including the surface microhardness. *In vitro* testing of any new mouthrinse product is recommended although the effect pattern of the mouthrinses might not be the same on different restorative materials taking into account many factors that could not be replicated *in vitro*.[3] Therefore, the aim of this investigation was to assess and compare the influence of the three probiotic mouthrinses on surface microhardness of three esthetic restorative materials. This was studied by assessing the change in the microhardness of the restorative materials after 21 and 63 minutes cycles of four immersion in the probiotic mouthrinses. The null hypothesis is no difference of the effect of the tested probiotic mouthrinses on the microhardness of the three esthetic restorative materials.

Materials and Methods

This study was carried out as collaboration between College of Dentistry, King Saud University and School of Dentistry, University of Detroit Mercy, after obtaining the ethical approval. Three restorative materials were used in this study: conventional glass ionomer cement (GIC) (Ketac™ Universal Aplicap™; 3M ESPE, MN, USA), resin-modified glass ionomer (Photac™ Fil Quick Aplicap™; 3M ESPE), and resin composite (Filtek™ Z350 XT; 3M ESPE). The power sample size was 0.81 and level of significant $\sigma = 0.05$ with estimated standard deviation = 0.9, and the sample size should be at least nine in each group. Thirty specimens from each restorative material were fabricated and used in this study (Table 1). Samples were made using from each restorative material using silicon Teflon mold (8 mm × 2 mm), matrix strip, and glass slide. Where applicable each sample was polymerized on the top and bottom according to the instructions of the manufacturers using an light-emitting diode curing light (Bluephase®; Ivoclar Vivadent, Schaan, Liechtenstein). The samples were placed in distilled water at 37°C for 72 hours and then polished sequentially under running water with 240, 320, 400, and 600 silicon carbide paper. After that, the samples were stored in distilled water for 72 hours at 37°C and thermocycled (Thermocycler SD 5 Mechatronik; GmbH Dental Research Equipment, Germany) 1,500 times cycles in baths at 5 and 55°C, with 5 seconds transfer time and 30 seconds dwell times.

Table 1: The restorative materials and mouthrinses used in this study including numbers of specimens/readings

Restorative materials	*Mouthrinses*	*Numbers of specimens*	*Number of readings*
Conventional GIC	P2 probiotic power	10	30
	Probioclean	10	30
	BōKU natural	10	30
Resin-modified glass ionomer	P2 probiotic power	10	30
	Probioclean	10	30
	BōKU natural	10	30
Resin composite	P2 probiotic power	10	30
	Probioclean	10	30
	BōKU natural	10	30

The 30 specimens prepared from each material were randomly assigned to three groups of 10 each according to the assigned probiotic mouthrinses. Following the allocation of the specimens, the baseline of the surface microhardness was measured. After that, the specimens were immersed in the assigned probiotic mouthrinses (Table 1) according to the manufacturer's instructions for daily single use. The experimental groups included group I (I clean your teeth, P2 probiotic power, PA, USA—ingredients: a proprietary blend of Food and Drug Administration Generally Recognized as Safe (FDS GRAS) probiotics, enzymes, water, and organic spearmint. No alcohol, phthalates, triclosan, or sodium lauryl sulfate (SLS)), group II (Probiorinz, Probioclean, NY, USA—ingredients: water and proprietary stabilized beneficial organic probiotics), and group III (Mouth™, BōKU natural, CA, USA—ingredients: olive oil, silica, spearmint oil, menthol, natural plant saponins, monk fruit, vitamin E, vitamin C, peppermint, and probiotic K-12). The investigator who fabricated the specimens of the restorative materials and applied the probiotic mouthrinses was different from the investigator who measured the microhardness who was blind to each group. The first reading after immersion was done after 21 minutes of immersion which is equivalent to 3 weeks mouthrinse use. It was followed by a second measurement at 63 minutes of immersion time which is equivalent to 9 weeks of daily use. Following each immersion, the specimens in all groups were rinsed in running water for almost 5 seconds and then dried. Microhardness measurements were done using a Vickers diamond indenter (Innovatest, Micro Vickers tester, Micro-Met II; BUEHLER, IL, USA) with a 200 gm load applied for a duration of 15 seconds. The indentations were measured by a built-in graduated microscope with ×40 objective lens. Three indentations/readings were made for each specimen.

Two-way analysis of variance and Tukey's *post hoc* multiple comparison tests were utilized to compare the microhardness values between different groups. All statistical analyzes were set with a significance level of $p < 0.05$. The statistical analysis was performed using SPSS Version 20 (Statistical Package for the Social Science; SPSS Inc., Chicago, IL, USA).

Results

The means and standard deviations of microhardness values of the three restorative materials for each period of analysis are presented in Table 2. The microhardness change (Table 3) of the three restorative materials that tested reveled statistically significant differences in all mouthrinse groups (P2 probiotic power, Probioclean, and BōKU natural) ($p = 0.001$). A significant decrease in the microhardness was noted in the conventional GIC after the cycles of immersion (baseline and post-immersion cycles 21 and 63 minutes) in the P2 probiotic power and BōKU natural mouthrinse groups. While for Probioclean mouthrinse group, a significant increase was observed over time to the baseline. The mean difference in the mouthrinse groups of conventional GIC was

Table 2: Mean microhardness values and standard deviation of the three restorative materials at baseline and after each immersion cycle of 21 and 63 minutes

Restorative material	*Mouthrinse*	*Time of immersion*	*n*	*Mean*	*Standard deviation*	*p value*
GIC	P2	Baseline	30	95.37	0.81	0.001
		21 minutes	30	89.40	0.89	
		63 minutes	30	93.39	1.52	
	Probioclean	Baseline	30	95.51	0.90	0.001
		21 minutes	30	96.52	0.91	
		63 minutes	30	97.57	1.25	
	Bōku	Baseline	30	94.68	0.85	0.001
		21 minutes	30	76.66	0.87	
		63 minutes	30	83.53	2.05	
Resin-modified glass ionomer	P2	Baseline	30	68.01	0.90	0.001
		21 minutes	30	70.59	1.20	
		63 minutes	30	74.49	1.74	
	Probioclean	Baseline	30	67.67	1.11	0.001
		21 minutes	30	70.69	1.06	
		63 minutes	30	71.11	1.98	
	Bōku	Baseline	30	66.69	0.70	0.001
		21 minutes	30	69.78	0.91	
		63 minutes	30	65.50	2.41	
Resin composite	P2	Baseline	30	92.74	0.95	0.001
		21 minutes	30	92.74	3.17	
		63 minutes	30	90.57	0.95	
	Probioclean	Baseline	30	93.74	0.87	0.001
		21 minutes	30	98.60	0.95	
		63 minutes	30	96.75	0.76	
	Bōku	Baseline	30	91.43	0.74	0.001
		21 minutes	30	88.89	1.24	
		63 minutes	30	86.25	1.09	

Table 3: Mean difference between the baseline and after each immersion cycle of 21 and 63 minutes

Restorative material	*Mouthrinse*	*Time of immersion*		*Mean difference (I–J)*	*Standard error*	*p value*
GIC	P2	Baseline	21 minutes	5.97	0.29	0.001
			63 minutes	1.98	0.29	0.001
	Probioclean	Baseline	21 minutes	−1.01	0.27	0.001
			63 minutes	−2.05	0.27	0.001
	Bōku	Baseline	21 minutes	18.02	0.36	0.001
			63 minutes	11.15	0.36	0.001
Resin-modified glass ionomer	P2	Baseline	21 minutes	−2.58	0.34	0.001
			63 minutes	−6.48	0.34	0.001
	Probioclean	Baseline	21 minutes	−3.02	0.37	0.001
			63 minutes	−3.45	0.37	0.001
	Bōku	Baseline	21 minutes	−3.09	0.40	0.001
			63 minutes	1.19	0.40	0.01
Resin composite	P2	Baseline	21 minutes	0.00	0.51	1.00
			63 minutes	2.16	0.51	0.001
	Probioclean	Baseline	21 minutes	−4.85	0.22	0.001
			63 minutes	−3.00	0.22	0.001
	Bōku	Baseline	21 minutes	2.54	0.27	0.001
			63 minutes	5.18	0.27	0.001

highest in BōKU natural at immersion time of 21 minutes compared with the baseline which significantly decreases the microhardness of the conventional GIC. While the lowest mean difference in mouthrinse groups of conventional GIC was seen in Probioclean at immersion time of 21 minutes compared with the baseline which significantly increases the microhardness of conventional GIC (Tables 2 and 3).

A significant increase in the microhardness was observed in resin-modified glass ionomer groups after the cycles of immersion (baseline and post-immersion cycles) in the mouthrinse groups except in BōKU natural in which after 63 minutes, there was a significant reduction in microhardness compared with baseline of immersion time ($p < 0.00$) (Tables 2 and 3). The mean difference in mouthrinse groups of resin-modified glass ionomer was highest in P2 probiotic power at immersion time of 63 minutes compared with the baseline which increased significantly the microhardness of resin-modified glass ionomer. While the lowest mean difference in mouthrinses groups of resin-modified glass ionomer was seen in BōKU natural at immersion time of 63 minutes which shows a significant decrease in the microhardness (Table 3). BōKU natural mouthrinse group showed a significant reduction in microhardness of resin composite after the cycles of immersion. Moreover, a significant reduction in the microhardness was observed after immersion time of 63 minutes in P2 probiotic power, but no change was observed after 21 minutes of immersion. In contrast, Probioclean mouthrinse group revealed a significant increase in the microhardness of resin composite after the cycles of immersion (Table 2).

The mean difference in mouthrinse groups of resin composite was highest in BōKU natural at immersion time of 63 minutes compared with the baseline which significantly decreases the microhardness of the resin composite. While no mean difference was seen in P2 probiotic power group at immersion time of 21 minutes which had no effect on the microhardness of resin composite (Table 3).

Discussion

The null hypothesis of this study was rejected, as there was difference in the effect of the tested probiotic mouthrinses on the microhardness of the three esthetic restorative materials. In general, to prevent oral disease and maintain good oral hygiene, chemical anti-plaque products are used frequently to improve the effect of toothbrushing on plaque but not replace it.[27] Several types of mouthrinses are available to consumers, such as chlorhexidine, herbal, alcohol-containing, and alcohol-free mouthrinses.[3,5,10,28] Recently, new formulations of mouthrinses have been introduced to the market containing probiotics as an active ingredient.[29] The probiotic mouthrinse can perform an anti-plaque effect in numerous methods by decreasing bacterial adhesion to the surface of teeth, hindering them from growing and proliferation, preventing the establishment of intercellular plaque matrix, decreasing the formation of cytotoxic products by altering plaque biochemistry, and lessening the pathogenic flora by modifying plaque ecology.[30] The effect of mouthrinses on tooth-colored restorative materials has been evaluated.[3,5,10,28] Up to our knowledge, there is a lack of studies that evaluate the effect of probiotic mouthrinse on mechanical properties of tooth-colored restorative materials. In this study, P2 probiotic power, Probioclean, and BōKU natural were used as probiotic mouthrinses. These mouthrinses were examined for their effect on the microhardness of three tooth-colored restorative materials including conventional GIC, resin-modified glass ionomer, and resin composite.

This study showed a statistically significant reduction in the microhardness of conventional GIC restorative material group when it was immersed in P2 probiotic power and BōKU natural mouthrinses ($p = 0.001$). A previous study by Gürgan et al. reported that the microhardness of GIC and resin composite was affected in a similar way, after immersion in both alcohol-containing and alcohol-free mouthrinses.[10] The authors mentioned that the effect of these mouthrinses might be altered by several elements which cannot be[9] simulated in an *in vitro* setting such as saliva and salivary pellicle, taking into consideration that alcohol cause softening of the restorative material.[10] The microhardness of the glass ionomer restorative material might be affected by the water component of the mouthrinse.[31] It was shown that the chemical composition of the glass ionomer restorative material might facilitate the water sorption and binding water molecules of the ion leachable glasses and polycarboxylic acids.[3] Water has resulted in unfavorable consequence on the microhardness of GIC which is increased after 24 hours causing hydrolysis and dissolution of some of the components by eroding the surface of the cement.[32] In contrast, a statistically significant increase in the microhardness of the same conventional GIC restorative material occurred when immersed in Probioclean mouthrinse ($p = 0.001$). Moreover, in our study, there was a statistically significant decrease in the microhardness of resin-modified glass ionomer restorative material group when it was immersed in BōKU natural for 63 minutes. Furthermore, there was a statistically significant increase in P2 probiotic power, Probioclean, and BōKU natural mouthrinses after 21 minutes of immersion. The effect of these mouthrinses on the resin composite restorative material group was shown as a statistically significant reduction when it was immersed in BōKU natural at all cycles of immersion times (21 and 63 minutes) and in P2 probiotic power only after 63 minutes ($p = 0.001$). As mentioned previously, both alcohol-containing and alcohol-free mouthrinses have detrimental effect on the microhardness of the resin composite.[10] Similarly, another study found that the chlorhexidine mouthrinse has reduced the microhardness of the resin composite which might be due to the acidic pH of the mouthrinse that caused acid erosion which begins by acid etching and leaching the principal matrix forming cations.[28] It has been reported that low pH mouthrinses have a detrimental effect on the microhardness of the resin composites.[5,10] However, a statistically significant increase was resulted in the microhardness of the same restorative material when immersed in Probioclean ($p = 0.001$). However, P2 probiotic power mouthrinse showed no statistically significant effect on resin composite when it was immersed for 21 minutes ($p = 1.00$).

Additionally, considering that one of the mouthrinses that was used in this study contain essential oil, which is BōKU natural that showed mostly a reduction in the microhardness of the used restorative materials. The literature showed that the harmful effect of essential oil alcohol-containing mouthrinses on the microhardness of the restorative material depends on the type of the material, study design, and method of analysis.[33,34] Furthermore, in this study, some of the groups, as mentioned previously, have showed an increase in the microhardness of the restorative material after immersion for a specific time in one of our probiotic-containing mouthrinses. An increase in the microhardness was also reported for the resin composite restorative materials after immersion in distilled water which might be resulted from post-

irradiation and polymerization of unreacted components of resin composites.[35] It has been reported that differences in the results among the restorative materials originated from the structure of the materials themselves rather than the examined mouthrinses.[3] Further investigation is needed to understand the mechanism leading to the effect of the mouthrinses on microhardness and how it can be modified to improve clinical outcomes.

This study has some limitations including *in vitro* setting which may not reflect the more complex oral environment produced *in vivo*.[36] *In vitro* studies are unable to simulate the oral environment and other factors that could have an influence on the microhardness. However, in this *in vitro* study, standardization of experimental conditions was advantage, and the results demonstrated a clear correlation between[11] surface microhardness of the tested restorative materials and the probiotic mouthrinses. In addition, we only used three probiotic mouthrinses and three restorative materials.

Conclusion

Under the experimental conditions and limits of this investigation, we concluded the following: the consequence of probiotic mouthrinses varied depending on the restorative material and time cycles (21 and 63 minutes which is equivalent to 3 and 9 weeks of daily use). BōKU natural mouthrinse had the highest reduction of microhardness in all restorative materials. While Probioclean mouthrinse was associated with an increase in microhardness in all restorative materials.

References

1. Cazzaniga G, Ottobelli M, Ionescu A, et al. Surface properties of resin-based composite materials and biofilm formation: a review of the current literature. Am J Dent 2015;28(6):311–320.
2. Roopa KB, Basappa N, Prabhakar AR, et al. Effect of whitening dentifrice on micro hardness, colour stability and surface roughness of aesthetic restorative materials. J Clin Diagn Res 2016;10(3): ZC06–ZC11. DOI: 10.7860/JCDR/2016/15700.7350.
3. Gürdal P, Akdeniz BG, Hakan Sen B. The effects of mouthrinses on microhardness and colour stability of aesthetic restorative materials. J Oral Rehabil 2002;29(9):895–901. DOI: 10.1046/j.1365-2842.2002.00924.x.
4. Anusavice KJ, Shen C, Rawls HR. Phillips' science of dental materials. Elsevier Health Sciences; 2012.
5. Diab M, Zaazou M, Mubarak E, et al. Effect of five commercial mouthrinses on the microhardness and color stability of two resin composite restorative materials. Aust J Basic Appl Sci 2007;1(4): 667–674.
6. Yanikoglu N, Duymus ZY, Yilmaz B. Effects of different solutions on the surface hardness of composite resin materials. Dent Mater J 2009;28(3):344–351. DOI: 10.4012/dmj.28.344.
7. DeWald JP, Ferracane JL. A comparison of four modes of evaluating depth of cure of light-activated composites. J Dent Res 1987;66(3):727–730. DOI: 10.1177/00220345870660030401.
8. Rueggeberg FA, Craig RG. Correlation of parameters used to estimate monomer conversion in a light-cured composite. J Dent Res 1988;67(6):932–937. DOI: 10.1177/00220345880670060801.
9. Claydon NC, Moran J, Bosma ML, et al. Clinical study to compare the effectiveness of a test whitening toothpaste with a commercial whitening toothpaste at inhibiting dental stain. J Clin Periodontol 2004;31(12):1088–1091. DOI: 10.1111/j.1600-051x.2004.00611.x.
10. Gürgan S, Önen A, Köprülü H. In vitro effects of alcohol-containing and alcohol-free mouthrinses on microhardness of some restorative materials. J Oral Rehabil 1997;24(3):244–246. DOI: 10.1111/j.1365-2842.1997.tb00321.x.
11. Goldstein RE, Garber DA, Schwartz CG, et al. Patient maintenance of esthetic restorations. J Am Dent Assoc 1992;123(1):61–67. DOI: 10.14219/jada.archive.1992.0027.
12. Devore LR. Antimicrobial mouthrinces: impact on dental hygiene. J Am Dent Assoc 1994;125(8):23S–28S. DOI: 10.1016/S0002-8177(94)14004-5.
13. Fischman SL. A clinician's perspective on antimicrobial mouthrinses. J Am Dent Assoc 1994;125(8):20S–22S. DOI: 10.1016/S0002-8177(94)14003-3.
14. Adams D, Addy M. Mouthrinses. Adv Dent Res 1994;8(2):291–301. DOI: 10.1177/08959374940080022401.
15. Mandel ID. Antimicrobial mouthrinses: overview and update. J Am Dent Assoc 1994;125(8):2S–10S. DOI: 10.1016/S0002-8177(94)14001-X.
16. Jyothi K, Crasta S, Venugopal P. Effect of five commercial mouth rinses on the microhardness of a nanofilled resin composite restorative material: an in vitro study. J Conserv Dent 2012;15(3):214–217. DOI: 10.4103/0972-0707.97938.
17. Almeida GS, Poskus LT, Guimarães JG, et al. The effect of mouthrinses on salivary sorption, solubility and surface degradation of a nanofilled and a hybrid resin composite. Oper Dent 2010;35(1):105–111. DOI: 10.2341/09-080-L.
18. Mitra SB, Wu D, Holmes BN. An application of nanotechnology in advanced dental materials. J Am Dent Assoc 2003;134(10):1382–1390. DOI: 10.14219/jada.archive.2003.0054.
19. Sarrett DC, Söderholm KJ, Batich CD. Water and abrasive effects on three-body wear of composites. J Dent Res 1991;70(7):1074–1081. DOI: 10.1177/00220345910700071201.
20. Miranda Dde A, Bertoldo CE, Aguiar FH, et al. Effects of mouthwashes on knoop hardness and surface roughness of dental composites after different immersion times. Braz Oral Res 2011;25(2):168–173. DOI: 10.1590/S1806-83242011000200012.
21. Awliya WY. Surface hardness and weight change of some esthetic restorative materials. J Pak Dent Assoc 2005. 84–89.
22. Cavalcanti AN, Mitsui FH, Ambrosano GH, et al. Effect of different mouthrinses on knoop hardness of a restorative composite. Am J Dent 2005;18(6):338–340.
23. Örtengren U, Andersson F, Elgh U, et al. Influence of pH and storage time on the sorption and solubility behaviour of three composite resin materials. J Dent 2001;29(1):35–41. DOI: 10.1016/S0300-5712(00)00055-5.
24. Jindal G, Pandey RK, Agarwal J, et al. A comparative evaluation of probiotics on salivary mutans streptococci counts in Indian children. Eur Arch Paediatr Dent 2011;12(4):211–215. DOI: 10.1007/BF03262809.
25. Harini PM, Anegundi RT. Efficacy of a probiotic and chlorhexidine mouth rinses: a short-term clinical study. J Indian Soc Pedod Prev Dent 2010;28(3):179–182. DOI: 10.4103/0970-4388.73799.
26. Allaker RP, Ian Douglas CW. Non-conventional therapeutics for oral infections. Virulence 2015;6(3):196–207. DOI: 10.4161/21505594.2014.983783.
27. Shah SS, Nambiar S, Kamath D, et al. Comparative evaluation of plaque inhibitory and antimicrobial efficacy of probiotic and chlorhexidine oral rinses in orthodontic patients: a randomized clinical trial. Int J Dent 2019. 1964158. DOI: 10.1155/2019/1964158.
28. Dalmia S, Aher G, Gulve M, et al. Comparative evaluation of the effect of chlorhexidine based mouthwash and herbal mouthwash on the microhardness of two different composite resins – an in vitro study. Int J Adv Res 2018;6(3):306–311. DOI: 10.21474/IJAR01/6679.
29. Shimazaki Y, Shirota T, Uchida K, et al. Intake of dairy products and periodontal disease: the Hisayama study. J Periodontol 2008;79(1):131–137. DOI: 10.1902/jop.2008.070202.
30. Sarvari F, Mortazavian AM, Fazei MR. Biochemical characteristics and viability of probiotic and yogurt bacteria in yogurt during the fermentation and refrigerated storage. Appl Food Biotechnol 2014;1(1):55–61. DOI: 10.22037/afb.v1i1.7125.

31. Geurtsen W, Leyhausen G, Garcia-Godoy F. Effect of storage media on the fluoride release and surface microhardness of four polyacid-modified composite resins ("compomers"). Dent Mater 1999;15(3):196–201. DOI: 10.1016/S0109-5641(99)00034-2.
32. Hotta M, Hirukawa H, Aono M. The effect of glaze on restorative glass-ionomer cements. J Oral Rehabil 1995;22(3):197–201. DOI: 10.1111/j.1365-2842.1995.tb01564.x.
33. Yap AU, Tan BW, Tay LC, et al. Effect of mouthrinses on microhardness and wear of composite and compomer restoratives. Oper Dent 2003;28(6):740–746.
34. Festuccia MS, Garcia LdF, Cruvinel DR, et al. Color stability, surface roughness and microhardness of composites submitted to mouthrinsing action. J Appl Oral Sci 2012;20(2):200–205. DOI: 10.1590/S1678-77572012000200013.
35. Badra VV, FaraoniJ J, Ramos RP, et al. Influence of different beverages on the microhardness and surface roughness of resin composites. Oper Dent 2005;30(2):213–219.
36. Zachrisson B, Vanarsdal R. Bonding in orthodontics graber. Current Principles and Techniques. Philadelphia, USA: Elsevier; 2011.