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ORIGINAL RESEARCH

Comparative evaluation of probiotic solutions on surface roughness and microhardness of different restorative materials and enamel

Ozcan Karatas[1,]*, Ebru Delikan[2], Ayse Tugba Erturk Avunduk[3]

[1]Department of Restorative Dentistry, Nuh Naci Yazgan University, 38170 Kayseri, Turkey
[2]Department of Pediatric Dentistry, Nuh Naci Yazgan University, 38170 Kayseri, Turkey
[3]Department of Restorative Dentistry, Mersin University, 32133 Mersin, Turkey

***Correspondence**
okaratas@nny.edu.tr
(Ozcan Karatas)

Abstract

This research study aimed to investigate the impact of probiotic mouthwash and kefir on the surface characteristics, specifically surface roughness and microhardness, of different restorative materials, as well as permanent and deciduous tooth enamels. Thirty disc-shaped specimens were prepared from composite resin (G-ænial Posterior (GP)), polyacid-modified composite resin (compomer) (Dyract-XP (DXP)), and resin-modified glass ionomer cement (Ionoseal (IS)). Additionally, thirty specimens of enamel were obtained from permanent teeth (PT) and thirty from deciduous teeth (DT) by embedding buccal and lingual sections, acquired through vertical sectioning of 15 permanent and 15 deciduous human tooth crowns in the mesiodistal orientation within acrylic resin blocks. The specimens were then categorized into three distinct groups and immersed for 14 days in one of the following solutions: distilled water, kefir or probiotic mouthwash. The mean surface roughness values of all specimens were assessed using an atomic force microscope, while the mean surface microhardness was measured using a Vickers hardness measuring instrument. The results revealed a statistically significant difference in mean surface roughness among the various restorative materials ($p < 0.001$). Among the restorative materials, the IS material exhibited notably higher mean surface roughness values than other restorative materials and tooth enamel, while no significant differences were observed between the PT and DT groups. Importantly, the main effect of the solutions under investigation was not statistically significant ($p = 0.208$). No significant difference was found between the surface roughness values of specimens subjected to the different solutions. When evaluating the effects of materials and solutions on microhardness, the main effects of material and solution variables and the influence of material-solution interactions were statistically significant ($p < 0.001$). Taken together, these results indicate that consistent use of kefir or probiotic mouthwashes may impact the surface properties of various restorative materials and tooth enamel.

Keywords

Atomic force microscopy; Microhardness; Probiotic mouthwash; Surface roughness

1. Introduction

The primary objective when addressing dental caries-affected teeth is to restore both esthetics and functionality while achieving smooth, plaque-resistant restorations without porosity [1]. To this end, dental materials such as composite resin, glass ionomer cement (GICs) and polyacid-modified composite resin (compomers) are commonly used in contemporary pediatric dentistry practices to ensure the preservation of tooth structure integrity [2]. However, a meta-analysis reported a lack of conclusive evidence regarding the superiority of any specific restorative material used in dentistry [3]. Therefore, clinical decisions regarding material selection should take into account factors such as caries activity, the use of minimally invasive techniques, substrate type (enamel or dentin), cavity morphology, and esthetic requirements [4]. Composite resin has demonstrated a success rate of approximately 90% in restoring class I and II caries in deciduous teeth when applied under local anesthesia and with the use of a rubber dam [5]. Conversely, compomers, while exhibiting lower mechanical properties and wear resistance compared to composite resins, offer the advantage of sustained, rechargeable fluoride release [6]. Additionally, compomers are available in tooth-colored shades and six distinct attractive colors designed to enhance cooperation among pediatric patients with limited compliance. Resin-modified glass ionomer cements (RMGICs), similar to compomers, have shown efficacy in preventing secondary caries due to their fluoride-releasing properties [4]. However, they are associated with certain limitations, including reduced resistance to masticatory forces compared to composites [7], increased susceptibility to moisture compared to conventional GICs, and greater surface roughness [8].

The use of resin-based restorative materials has grown in popularity due to the increasing demand for minimally invasive treatments and esthetically pleasing dental restorations. However, the long-term performance of these materials in dental restorations depends on several critical factors, such as microleakage, water absorption, water solubility, polymerization shrinkage, cavity shape, application technique and surface topography [9, 10]. While research efforts in enhancing resin matrices primarily focus on achieving better physical, mechanical and esthetic properties [11], there are also efforts to reduce surface roughness to improve wear resistance and the ease of polishing through modifications in the structure of the inorganic fillers and achieve an optimally smooth surface for maintaining the overall quality of restorations. In contrast, a rough surface can lead to issues such as the accumulation of dental plaque, bacterial adhesion, discoloration, gingival problems and the potential development of secondary caries [12, 13]. Microhardness, which is closely linked to the material's rigidity and strength, plays a vital role in the long-term durability of restorations. Restorations with low surface hardness are more susceptible to scratches and abrasions and have a reduced long-term survival rate [14].

Probiotics, a topic of increasing significance, are defined as products containing a sufficient quantity of viable microorganisms capable of positively influencing the host's microflora to promote beneficial health outcomes [15]. They are commonly used in both children and adults to support and maintain a healthy balance of microorganisms in the oral and intestinal regions, although the initial intentional use of probiotics was for fermented foods [16]. Probiotic microorganisms are utilized in the production of widely consumed food items like cheese, yogurt and kefir. Kefir, in particular, a fermented product known for its mild acidity, natural carbonation and unique flavor, has attracted significant attention from researchers due to its distinct and complex probiotic properties. Kefir contains granules containing a specialized blend of symbiotic microflora, including lactic acid bacteria, acetic acid bacteria and yeast cells, surrounded by a matrix composed of casein, complex sugars and polysaccharides. It is frequently used to support both intestinal and oral health [17]. As parents often introduce such supplements or products to their children from an early age to nurture a healthy microbiota, children generally become accustomed to the taste of these products and have no issues with their consumption. In addition to traditional probiotic products like milk, yogurt, kefir and cheese, probiotic mouthwashes harness the potential of commensal bacteria to establish a natural defense mechanism against harmful bacteria [18]. A recent addition to this field is Armoral^{TM}, a drinkable mouthwash suitable for individuals of all age groups, including children. This low-pH herbal product is infused with live probiotics in liquid form, with each serving containing 5×10^9 live and active Lactobacillus plantarum cells. The recommended regimen involves swallowing one serving of the solution after a 2-minute mouth rinse [19]. It can be used twice a day for the first 15 days and optionally 1–2 times a day thereafter. In regard to probiotic health benefits, providing an appropriate probiotic dosage has been shown to be of paramount importance. However, determining the optimal probiotic dosage remains a subject of ongoing investigation, as it may vary depending on specific probiotic strains and health conditions [20].

While commercially available probiotic solutions are utilized for varying durations to address a range of conditions, including acute diarrhea prevention/treatment, allergic dermatitis, colitis and immune system modulation [16], traditional probiotic fermented milk and dairy products have been part of daily dietary practices for extended periods. These products, whether traditional or commercially produced, typically have a low pH and are regularly consumed over time, which may potentially influence restorative materials and dental enamel [21]. However, existing literature lacks comprehensive investigations into the long-term effects of probiotic-containing solutions on the mechanical properties of restorative materials and tooth enamel. To address this literature gap, the primary aim of this study was to evaluate the impact of kefir and probiotic mouthwash on the surface roughness and microhardness of different restorative materials, as well as on the enamel of permanent and deciduous teeth based on the following null hypotheses: (a) probiotic solutions have no effect on the surface roughness of different restorative materials or on permanent and deciduous teeth, and (b) probiotic solutions have no effect on the surface microhardness of different restorative materials or on permanent and deciduous teeth.

2. Materials and methods

The current study aimed to investigate the effects of probiotic solutions, namely kefir and probiotic mouthwash, in comparison to a control group using distilled water, on the surface roughness and microhardness of three commonly employed restorative materials (composite resin, compomer and RMGIC) and tooth enamel in clinical practice.

2.1 Specimen preparation

The study population was determined using the G*Power software (Version 3.1.9.4, Heinrich Heine, University of Düsseldorf, Düsseldorf, Germany), with a set significance level (α) of 0.05 and a desired statistical power (p) of 90%, following which the calculated minimum sample size was determined to be 128. To account for a potential dropout rate of 20%, the sample size was increased to 10 per group, resulting in a total of 150 subjects [22].

Fifteen permanent and 15 deciduous sound human molars, free from caries, white spots, cracks or other defects, were selected and stored in a sterile saline solution at room temperature. Prior to use, any remaining soft tissues and periodontal fibers on the root surface were meticulously removed using a slurry of pumice and a brush. These teeth were then kept in a sterile saline solution at room temperature until needed. Notably, the enamel surface was left unpolished. Subsequently, the roots were carefully extracted with ample water irrigation, and each tooth was vertically sectioned in the mesiodistal direction under continuous water cooling, employing a slow-speed diamond saw (Isomet, Buehler, Lake Bluff, IL, USA). The resulting buccal and lingual sections from permanent teeth (PT) and deciduous teeth (DT) were then embedded in acrylic resin blocks measuring 20 mm × 20 mm × 15 mm, exposing the outer enamel surfaces. As a result, a total of 30 specimens from permanent teeth and 30 specimens from deciduous teeth were obtained for further analysis.

In this study, we analyzed a nanohybrid composite resin (G-ænial Posterior (GP), GC, Japan), a polyacid-modified composite resin-compomer (Dyract-XP (DXP), Dentsply, DeTrey, Konstanz, Germany), and a resin-modified glass ionomer cement-RMGIC (Ionoseal (IS), Voco GmbH, Germany). For each group of restorative materials, we prepared thirty disc-shaped specimens (N = 30) with a diameter of 5 mm and a depth of 2 mm using Teflon molds. Table 1 provides information about the manufacturers, shades and composition of the restorative materials used in this study. To ensure uniformity, Mylar strips (SS White Co.; Philadelphia, PA, USA) were placed on top of the uncured restorative materials, and the mold was then gently compressed between glass plates to remove excess material and create a smooth surface. Even for Ionoseal, which is available in a flowable form, the same procedure was followed during placement [23]. After pressing the material, the glass plate was removed. All specimens were polymerized on the top surface using a light-emitting diode (LED, D-Light Pro, GC, Japan) light unit with an irradiance of 1200 mW/cm^2 for 20 seconds, following the manufacturer's recommendations. Prior to polymerization for each group, we verified the output intensity of the curing light using a radiometer. To standardize the distance between the light unit and the specimen, we employed a 1-mm Mylar strip band. A 1-mm metal ring was used to position the tip of the curing light unit, which was placed on the specimens before polymerization [24]. After being cured under Mylar strips, all the specimens were polished using the Super-Snap Rainbow Technique Kit (Shofu Inc., Kyoto, Japan) and the One Gloss Polishing Kit (Shofu Inc., Kyoto, Japan). A digital caliper gauge (N48AA, Maplin Electronics, Rotherham, UK) was used to confirm the final specimen thickness of 2 ± 0.1 mm. For post-polymerization, the specimens were stored in distilled water at 37 °C for 24 hours.

2.2 Cycling in the solutions

All specimens (teeth and restorative materials) were randomly divided into three groups (n = 10), namely the control (distilled water), kefir (İçim, Kırklareli, Türkiye) and probiotic mouthwash (Armoral, Kırklareli, Türkiye) groups. Each specimen was then immersed in a glass test tube containing 20 mL of solution. Detailed information regarding the manufacturers, properties and composition of the solutions are shown in Table 1. To ensure consistency in the staining process, the pH of the beverages was measured using a pH meter (Hanna instruments, Padova, Italy) before their use in this study (distilled water pH = 5.56, kefir pH = 4.2, probiotic mouthwash pH = 4.5).

Kefir and probiotic solutions, which are recommended to be used twice a day for children and adults, are in contact with teeth and restoration surfaces for approximately 2–4 minutes during each application. In our study, we immersed the specimens in these solutions (distilled water, kefir and probiotic mouthwash) for 8 hours, followed by placement in distilled water for the remaining 16 hours of the day at a constant temperature of 37 °C throughout the testing period. This daily routine was repeated over a 14-day storage period [22], and all solutions were changed daily. The immersion period used in our study, as described above, corresponds to roughly five years of real-world usage. At the end of the 14th day, we rinsed the specimens with distilled water and gently dried them before conducting surface roughness and microhardness measurements. Notably, the same specimens were used for both surface roughness and microhardness assessments.

2.3 Surface roughness measurements and topographic imaging

The middle enamel of PT/DT and the top surfaces of the restorative material specimens were examined for surface roughness and topography using atomic force microscopy (AFM). AFM analysis was conducted using an AL-coated silicon tip atomic force microscope (Multimode AFM, Veeco Instruments Inc., CA, USA) operating in tapping mode with a spring constant of 42 N/m. Scans were performed over 10 × 10 μm^2 areas at a scan rate of 1 Hz, generating 3D images with a resolution of 512 × 512 pixels. For each specimen, three randomly selected regions were measured, and both two-dimensional (2D) and three-dimensional (3D) AFM images were acquired. The mean surface roughness average (Sa) values of each specimen were calculated and recorded using the following equation (1):

$$Sa = \frac{1}{A} \int \int_A |z\,(x, y)|\, dxdy \qquad (1)$$

The function Z(x, y) represents the surface height in relation to the best-fitting plane, cylinder or sphere. In the integral expressions, "a" indicates that the integration is performed over the measurement area and subsequently normalized by the cross-sectional area "A" of the measurement. The terms "dx" and "dy" denote the specimen's "sampling distance" along the x and y axes [25].

2.4 Microhardness measurements

The microhardness of the same specimens (polished top surface of each restorative material, middle enamel of PT/DT) was measured for the control, kefir and probiotic mouthwash groups using a Vickers microhardness (VHN) tester (Buehler MMT 3 digital microhardness instrument, Lake Bluff, IL, USA). Each specimen received three indentations: one at the center and two others around it, with a minimum distance of 0.5 mm between each indentation [26]. For the microhardness testing, the indentations were created under a 100 g force for 10 seconds. This specific load was chosen to ensure that the diagonal indentations would be as large as possible, maximizing the measurement resolution. The two diagonal

TABLE 1. The type, composition, manufacturer and LOT numbers of the materials used in the study.

Material	Type	Composition	Manufacturer	LOT	Filler ratio (weight)
G-ænial Posterior (GP)	Micro-filled hybrid resin composite	UDMA, Inorganic filler >100 nm Fluoroaluminosilicate inorganic filler <100 nm Fumed silica Pre-polymerized fillers (16–17 μm)-Silica-Strontium and lanthanoid fluoride	GC Corporation, Tokyo, Japan	1805231	65%
Dyract-XP (DXP)	Polyacid modified composite resin—Compomer	UDMA, TEGDMA, trimethacrylate and dimethacrylate resins, strontium-alumino-sodium-fluoro -silicate glass, strontium fluoride (0.8 µm, 47% wt, 50% vol. fillers)	Dentsply, Konstanz, Germany	2002001071	47%
Ionoseal (IS)	Resin-modified glass ionomer cement	Fluoroaluminosilicate, Bis-GMA, HEDMA, UDMA, camphoroquinone and amine	Voco, Cuxhaven, Germany	1928489	67%
Kefir	Probiotic beverage	Pasteurized cow's milk, kefir culture	İçim, Kırklareli, Türkiye	-	-
Armoral	Drinkable mouthwash with probiotic content	Water, Sugarcane molasses, Saccharomyces extracts, Natural mint flavor, Guarnam (E412), Live probiotic microorganism content (Lactobacillus Plantarum)	İçim, Kırklareli, Türkiye	-	-

Bis-GMA: Bisphenol A-glycidyl methacrylate; UDMA: Urethane dimethacrylate; TEGDMA: Triethylene glycol-dimethacrylate; HEDMA: 1,6-hexanediylbismethacrylate.

lines produced by each indentation were measured, and VHN values were calculated for the resulting indentations using the following equation (2):

$$VHN = (1.8544 \times P)/d^2 \quad (2)$$

In the equation, "VHN" represents the Vickers hardness expressed in kg/mm^2, "P" stands for the indenter load in kg, and "d" denotes the diagonal length of the indentation in mm. To calculate the VHN value, we took the average of three Vickers hardness measurements for each specimen, and any indentations that resulted in asymmetric diagonal lines, a jagged or chipped edge, or a noticeable shift in the location of the indentation tip were excluded from the analysis.

2.5 Statistical analysis

All statistical analyses were conducted using the Statistical Package for the Social Sciences (SPSS Statistic 20, SPSS Inc., Chicago, IL, USA) software. The hypotheses were evaluated at a significance level of α = 0.05. Descriptive statistics included calculating mean values and standard deviations. The Shapiro-Wilk test was performed to confirm that the data followed a normal distribution. To assess the significance of the Sa and VHN values, a two-way analysis of variance (ANOVA) was employed. For multiple comparisons, *post-hoc* Tamhane tests were applied considering the absence of homogeneous variances. Comparisons yielding p = 0.05 was considered to be statistically significant.

3. Results

3.1 Surface roughness and AFM examinations

3.1.1 Assessment of restorative materials/enamel

We assessed the main effects of material and solution variables on surface roughness. The results showed that the material variable had a statistically significant main effect ($p < 0.001$). The mean surface roughness values for GP, DXP, IS, PT and DT samples were 31.77, 43.33, 156.57, 43.37 and 40.80, respectively. Among these groups, GP exhibited the lowest surface roughness value, while IS had the highest. The surface roughness values of the IS group were significantly higher than those of the GP, DXP, PT and DT groups. However, no significant differences were observed between the surface roughness values of GP, DXP, PT and DT. When considering materials as two distinct groups, restorative materials (GP, DXP and IS) and enamel (PT and DT), IS was found to have a significantly higher surface roughness value compared to restorative materials. No significant differences were observed between the surface roughness values of PT and DT.

3.1.2 Assessment of solutions

The main effect of the solution variable was not found to be statistically significant ($p = 0.208$). The mean surface roughness values for the distilled water, kefir and probiotic mouthwash solutions were 61.90, 60.92 and 66.44, respectively. Despite the slightly higher surface roughness value in the probiotic mouthwash group compared to the other two solutions, this difference was not statistically significant. The main effect for the material variable, as indicated by the partial eta squared value, was 0.900, while the main effect for the solution variable was 0.023. These values suggest that the material has a greater influence on surface roughness compared to the solution variable.

When evaluating the surface roughness of the restorative material groups (GP, DXP and IS) and enamel groups (PT and DT), no significant differences were observed among the surface roughness values for the three solutions. However, for the PT and DT groups, we observed a tendency for surface roughness values to decrease when exposed to probiotic solutions, with the lowest values occurring in the kefir group. In contrast, within the restorative material groups (GP, DXP and IS), the surface roughness values tended to increase in probiotic solutions, with the highest surface roughness value observed in the probiotic mouthwash solution for the DXP and IS groups, and in the kefir solution for the GP group.

3.1.3 Assessment of restorative material/enamel and solutions interactions

The interactions between material and solution were also found to be statistically significant ($p < 0.001$). The lowest surface roughness value was observed in the distilled water-GP group, while the highest surface roughness value was consistently found in the IS material, irrespective of the solution. Notably, the surface roughness values of this particular restorative material significantly differed from those of the other solution-material combinations. When evaluating the enamel groups (PT and DT) within themselves, we observed no significant differences between the interaction groups in terms of surface roughness values. Details of other multiple comparison results are shown in Table 2.

3.1.4 Assessment of topographic imaging

The 2D and 3D AFM images of randomly selected specimens from each probiotic solutions group, including GP, DXP, IS, PT and DT are presented in Figs. 1,2,3,4,5. Upon closer examination of these AFM images, irregularities in surface topography were evident across various materials, including restorative materials and enamel, in all probiotic solution groups. When comparing all material-solution combinations with distilled water, it was observed that GP exhibited the lowest incidence of surface irregularities (Fig. 1). In contrast, irregular surface characteristics were notably prominent in the AFM images of IS (Fig. 3). Interestingly, both permanent and deciduous teeth displayed similar surface topography characteristics when exposed to the tested probiotic solutions, as illustrated in Figs. 4,5, respectively.

3.2 Microhardness

3.2.1 Assessment of restorative materials/enamel

Next, we analyzed the main effects of material and solution variables on microhardness, and the results indicated that the material variable showed a statistically significant main effect ($p < 0.001$). The mean microhardness values for GP, DXP, IS, PT and DT samples were 37.39, 38.33, 40.11, 395.56 and 339.54, respectively. Among these groups, GP had the lowest microhardness value, while PT had the highest. When categorizing the materials into restorative materials (GP, DXP and IS) and enamel (PT and DT), we found no significant differences in microhardness values between these subgroups. Additionally, there were no significant differences in microhardness values between the PT and DT groups.

3.2.2 Assessment of solutions

The main effect of the solution variable was also statistically significant ($p < 0.001$). Specifically, the mean microhardness value in the probiotic mouthwash group (151.54) was significantly lower compared to the distilled water (183.59) and kefir (151.76) groups. The highest microhardness values were observed in the kefir group. Regarding the main effects, the partial eta squared value for the material variable was 0.976, while the main effect for the solution variable was 0.222. These values indicate that the effect of material on microhardness was more pronounced compared to the effect of the solution variable.

Examining all restorative materials and PT/DT within their respective groups, we observed a decrease in microhardness values with the probiotic mouthwash solution compared to the control group. This reduction was statistically significant only in the DXP group. In contrast, microhardness values increased in the kefir solution compared to the control group, except for the PT group, and this increase was statistically significant only in the IS group.

3.2.3 Assessment of restorative material/enamel and solutions interactions

Material and solution interactions were also found to be statistically significant ($p < 0.001$). Among the restorative materials, the probiotic mouthwash-GP group had the lowest microhardness value (33.59), whereas the kefir-IS group had the highest microhardness value (46.07). The microhardness values of the PT and DT groups in the three solutions were significantly higher than those of the restorative material groups. However, the microhardness values of the PT/DT groups in the three solutions did not differ, except for the probiotic mouthwash-DT group.

Assessment of the restorative materials within themselves showed that the microhardness values of the kefir-DXP and kefir-IS interaction groups were significantly higher compared to the other interaction groups. Conversely, the microhardness value of the probiotic mouthwash-DXP interaction group was significantly lower than the other interaction groups. Within

TABLE 2. Descriptive statistics (mean ± standard deviation) and multiple comparison results of surface roughness average (Sa) values (nm) according to materials and solutions.

	Descriptive Statistics Material						Two way ANOVA Results			
	Restorative Material			Enamel		Total	Factor	*F*	*p*	Partial Eta Square
	GP (n = 10)	DXP (n = 10)	IS (n = 10)	PT (n = 10)	DT (n = 10)					
Solutions										
Distilled water	27.32 ± 9.40^{EF}	31.35 ± 6.34^{CDF}	145.67 ± 14.30^{B}	56.92 ± 15.62^{A}	48.26 ± 4.04^{ACF}	61.90 ± 8.29	Material	302.806	<0.001	0.900
Kefir	36.01 ± 7.39^{ADE}	47.96 ± 6.12^{ACF}	154.36 ± 28.13^{B}	32.51 ± 7.74^{ACF}	33.76 ± 5.36^{ACF}	60.92 ± 12.95	Solution	1.587	0.208	0.023
Probiotic mouth-wash	30.78 ± 7.50^{CE}	50.68 ± 12.74^{AC}	169.67 ± 36.71^{B}	40.69 ± 9.34^{ACF}	40.37 ± 9.28^{ACF}	66.44 ± 11.95	Material* Solution	4.030	<0.001	0.193
Total	31.77 ± 10.29^{a}	43.33 ± 14.80^{a}	156.57 ± 28.80^{b}	43.37 ± 15.10^{a}	40.80 ± 14.90^{a}	63.09 ± 50.31				

p values are based on the Two way ANOVA Test with Bonferroni correction test and Post-hoc Tamhane test.
R^2 = 0.902 (Adjusted R Squared = 0.892).
$^{(a)}$: Lower letters indicate the difference between materials and $^{(A-F)}$capital letters indicate the difference between material.
**solution interactions ($p < 0.05$), p values presented in bold font indicate statistical significance.*
GP: GC G-ænial Posterior (composite); DXP: Dyract-XP (compomer: polyacid-modified resin composite); IS: Ionoseal (resin-modified glass ionomer cement); PT: Permanent tooth; DT: Decideous tooth.

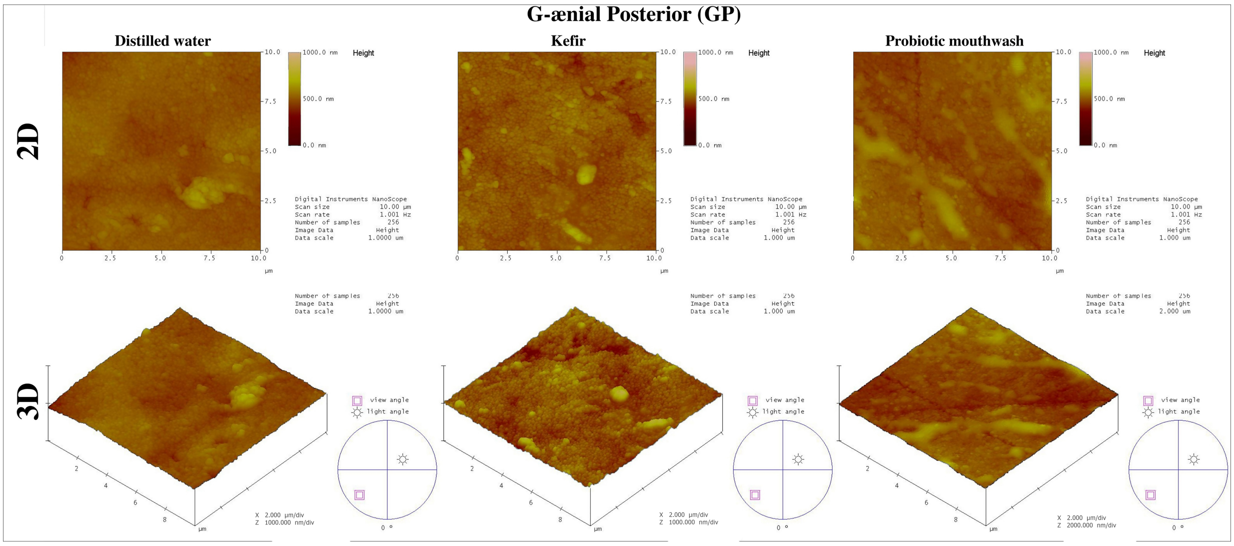

FIGURE 1. Representative AFM images of the G-ænial Posterior (GP).

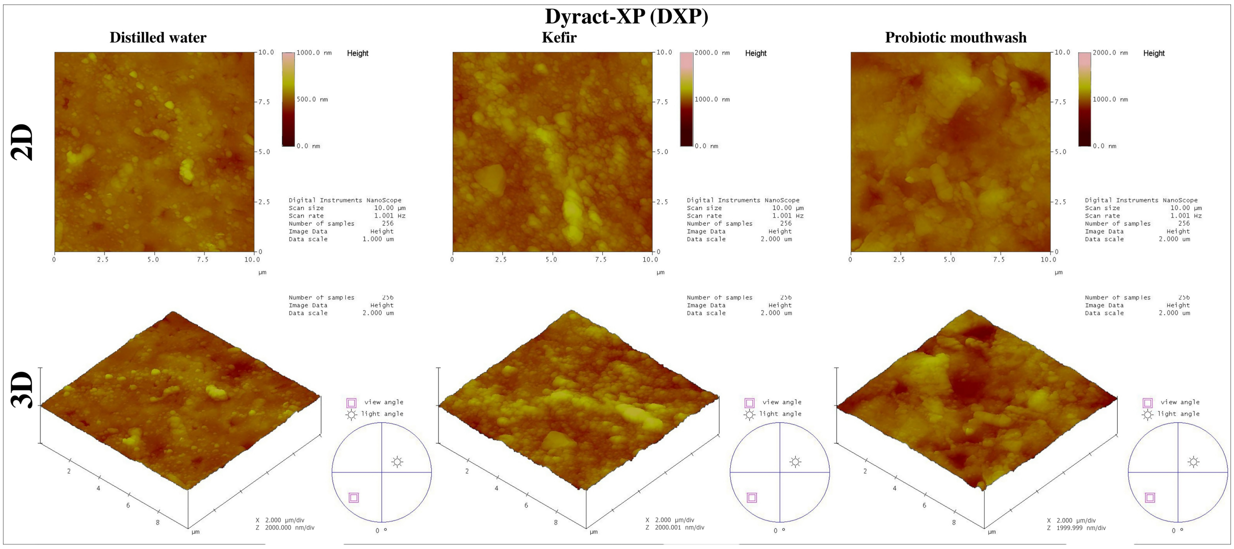

FIGURE 2. Representative AFM images of the Dyract-XP (DXP).

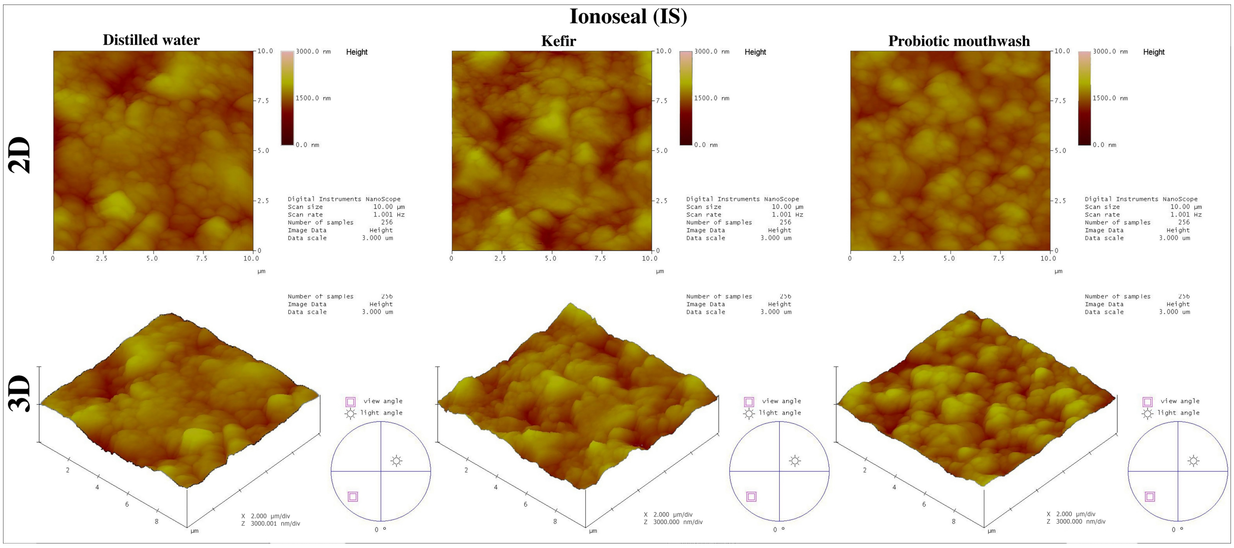

FIGURE 3. Representative AFM images of the Ionoseal (IS).

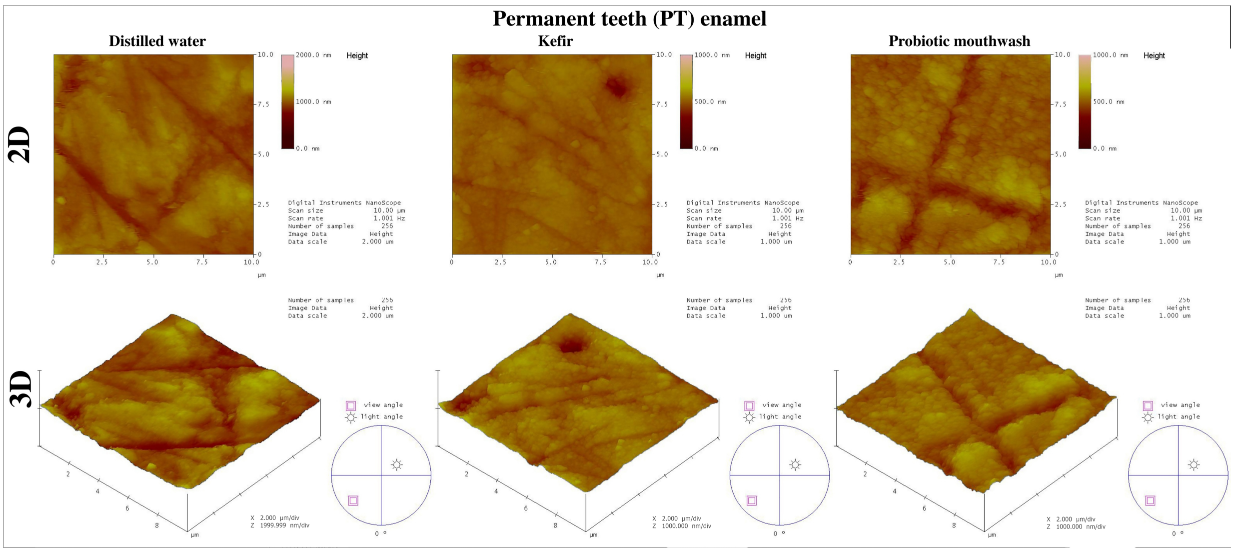

FIGURE 4. Representative AFM images of the permanent teeth (PT) enamel.

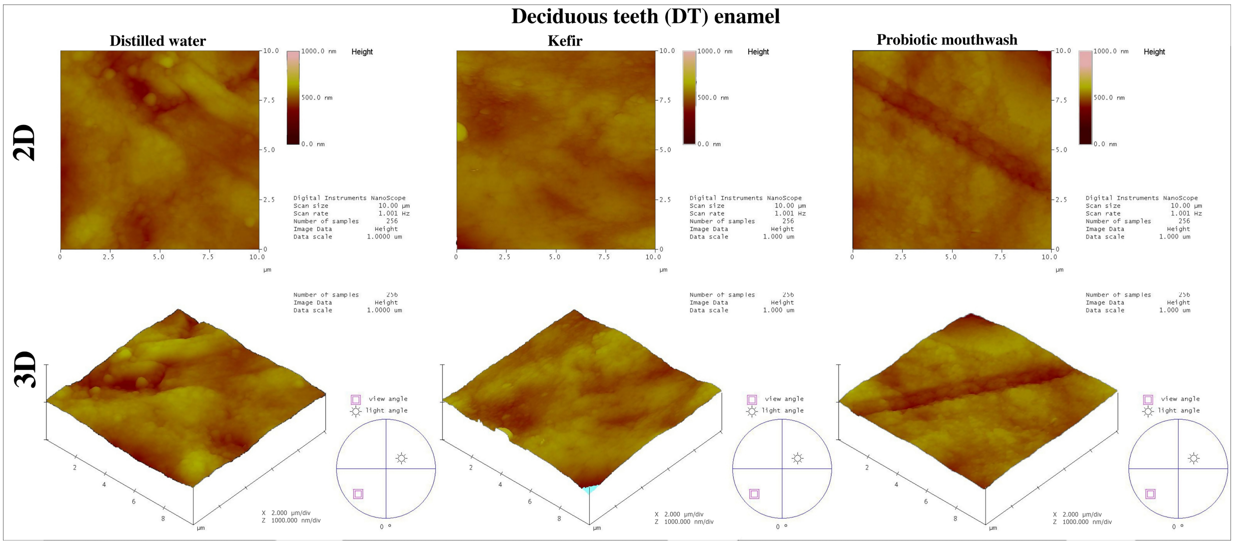

FIGURE 5. Representative AFM images of the deciduous teeth (DT) enamel.

the PT and DT enamel groups, the microhardness value in the probiotic mouthwash-DT interaction group was significantly lower than the other interaction groups, with no significant differences observed in the remaining interaction groups. Additional multiple comparison results can be found in Table 3.

4. Discussion

In pediatric dentistry, the selection of appropriate restorative materials is of paramount importance, particularly when dealing with young patients who may present challenges during treatments, as making the right choice in restorative materials can help avoid the need for more expensive and complex procedures [27]. Composite resins and compomers are among the most commonly used restorative materials for treating carious lesions that cannot be halted or remineralized, whether in permanent or deciduous teeth [28]. Additionally, RMGICs have shown favorable outcomes when used as intermediate base materials or for restoring cervical lesions in permanent teeth, as well as for permanent restorations in deciduous teeth [29]. In this study, we examined the changes in surface roughness and microhardness by utilizing a nanohybrid composite resin, compomer and RMGIC to provide insights into the suitability of these materials in pediatric dentistry.

The enamel surface of erupted and functional teeth can exhibit irregularities due to structural changes resulting from occlusal function or exposure to the oral environment, and it is worth noting that deciduous tooth enamel tends to have smoother surfaces compared to permanent tooth enamel [30]. Additionally, beverages with low pH values have been observed to cause irregularities on enamel surfaces and dental restorations, potentially resulting in decreased hardness of both restorative materials and hard tissues [31]. In light of these considerations, we aimed to investigate the impact of a novel probiotic mouthwash (pH = 4.5) on the surface characteristics, including roughness and microhardness, of various restorative materials and enamel from both permanent and deciduous teeth. To establish meaningful comparisons, we assessed the effects of this new mouthwash alongside kefir (pH = 4.2), a widely consumed probiotic beverage, and used distilled water as a control group.

Parents often use probiotics as a means to enhance their children's overall health, and it has been reported that a significant number of children are introduced to probiotics before the age of two [32]. Determining the appropriate duration for probiotic consumption depends on individual health needs and specific circumstances. The decision regarding the duration of probiotic usage should be customized, taking into account factors such as the intended purpose of using probiotics, the specific type or product of probiotics being used, and the underlying health conditions. Probiotics are used for short-term relief of acute digestive issues, such as diarrhea and antibiotic-associated diarrhea [33], as well as for long-term management of chronic digestive ailments, such as irritable bowel syndrome and inflammatory bowel disease [34].

A study conducted by O'Connor *et al*. [35] in the USA highlighted the increasing use of non-food prebiotics, probiotics, and synbiotics in both adults and children and indicated that the use of probiotics has tripled in recent years, with a significant portion of individuals initiating their use without a physician's recommendation. Furthermore, Ciprandi and Tosca [36], in their investigation of the effects of long-term (one-year) consumption of fermented milk in 187 preschool children (aged 2–5) with allergic asthma and/or rhinitis, concluded that while this product did not show a significant impact on asthma, it did reduce and shorten the occurrence of rhinitis and diarrhea attacks. Given the growing recognition of the role of probiotic foods and supplements in promoting both oral and intestinal microbiota health, adults now have the flexibility to incorporate these products into their and their children's routines without necessarily consulting a physician. Traditional probiotic foods like yogurt and kefir have been consumed daily for many years. However, when it comes to newer and more diverse probiotic supplements such as mouthwash or tablets, the optimal duration of usage remains uncertain. In this present study, a 5-year immersion period was primarily chosen because one of the probiotics we investigated was a long-term use solution, namely kefir.

TABLE 3. Descriptive statistics (mean ± standard deviation) and multiple comparison results of microhardness values (VHN) according to materials and solutions.

	Descriptive Statistics Material						Two way ANOVA Results			
	Restorative Material			Enamel		Total	Factor	F	p	Partial Eta Square
	GP (n = 10)	DXP (n = 10)	IS (n = 10)	PT (n = 10)	DT (n = 10)					
Solutions										
Distilled water	37.12 ± 2.80^{JEK}	38.93 ± 2.21^{EG}	37.60 ± 5.26^{DEF}	417.20 ± 18.06^{A}	345.18 ± 32.39^{AB}	175.21 ± 15.34^{d}	Material	1396.693	<0.001	0.976
Kefir	38.39 ± 4.56^{EHLM}	42.48 ± 3.31^{DGLHJ}	46.07 ± 3.38^{DH}	406.60 ± 36.26^{A}	384.43 ± 35.16^{A}	183.59 ± 12.24^{d}	Solution	19.286	<0.001	0.222
Probiotic mouth-wash	36.66 ± 2.40^{EI}	33.59 ± 3.15^{FMIK}	36.67 ± 2.43^{EF}	362.88 ± 32.66^{AC}	289.01 ± 30.29^{BC}	151.76 ± 18.34^{c}	Material* Solution	6.432	<0.001	0.276
Total	37.39 ± 3.54^{a}	38.33 ± 4.67^{a}	40.11 ± 5.70^{a}	395.56 ± 47.98^{b}	339.54 ± 55.73^{b}	170.19 ± 165.91				

p values are based on the Two way ANOVA Test with Bonferroni correction test and Post-hoc Tamhane test.
R^2 = 0.977 (Adjusted R Squared = 0.974).
*^{a,b}: Lower letters indicate the difference between materials and ^{c,d}: Lower letters indicate the difference between solutions and $^{A-M}$: capital letters indicate the difference between material. *solution interactions ($p < 0.05$), p values presented in bold font indicate statistical significance.*
GP: GC G-ænial Posterior (composite); DXP: Dyract-XP (compomer: polyacid-modified resin composite); IS: Ionoseal (resin-modified glass ionomer cement); PT: Permanent tooth; DT: Decideous tooth.

Surface roughness is a crucial factor affecting various aspects of oral health, including plaque adherence, discoloration, caries development, restorative performance and periodontal health [37]. Polishing and achieving smooth surfaces are important considerations in studies evaluating material surface properties. A prior study had demonstrated that the smoothest surfaces for restorative materials were achieved when specimens were in contact with a celluloid strip matrix [38]. In our present study, the specimens of restorative materials were covered on both the top and bottom surfaces with a celluloid strip matrix and smoothed using a glass plate. While the restorative specimens underwent additional polishing, the enamel surface did not undergo this process. Surface smoothness can be assessed using a light microscope after the final polishing, and if necessary, the polishing process can be repeated using different finishing/polishing kits, while failing to adhere to this condition could be considered as one of the limitations of our present study. The study findings revealed that interactions of material-solution had a significant impact on the surface roughness of the materials under investigation. Specifically, among the different restorative materials studied, Ionoseal exhibited the highest levels of surface roughness, as outlined in Table 2, which aligns with the observations of Guler and Unal [22], who found that RMGICs displayed higher surface roughness compared to composite resins and could be attributed to the presence of glass particles within RMGICs. Moreover, the high roughness observed in RMGICs has been attributed to the dissolution of the siliceous hydrogel layer in the glass ionomer structure and the matrix surrounding the glass particles following exposure to acidic solutions [22]. In this current study, GP demonstrated a better performance relative to other restorative materials. The susceptibility of resin-based materials to roughness has been found to be closely related to the resin phase. Specifically, resin composites based on urethane dimethacrylate (UDMA) demonstrate lower surface hardness and reduced roughness compared to those based on bisphenol A-glycidyl methacrylate (Bis-GMA). The difference in filler content per volume of resin composite can be cited as the reason of GP exhibited lowest surface roughness values. Additionally, the incorporation of pre-polymerized fillers in the GP and the addition of fillers such as glass, ceramic, and zirconia may also be evidence for this finding.

Our research findings are consistent with a previous study [27], which reported that the mean surface roughness values of the restorative materials followed the order of composite < compomer < RMGIC. The differences in the mean surface roughness values between composite, compomer and RMGIC materials may be attributed to the glass particle content in compomer and RMGICs and the resin matrix structure of the restorative materials in our study. Our investigations showed that permanent tooth enamel exhibited slightly higher roughness compared to deciduous teeth, although the difference was not statistically significant. The results also led us to partially accept the first null hypothesis that "probiotic solutions do not affect the surface roughness of different restorative materials, or of permanent and deciduous teeth". The process of demineralization in tooth enamel begins with the partial centripetal loss of mineral/hydroxyapatite crystals. This process can lead to structural deterioration of the enamel surface and an increase in roughness [30]. Various researchers have reached conflicting conclusions regarding the differences between the demineralization process of permanent and deciduous tooth enamel [30, 39]. While one previous study suggested that deciduous teeth are as resistant to initial acid attacks as permanent teeth [40], another study reported that the demineralization process is faster in deciduous teeth compared to permanent teeth [39].

Research has consistently shown that the pH of solutions applied to restorative materials can have a significant impact on their surface roughness, with low pH (the pH values of the solutions used in these studies were between 1.2 and 3.76) solutions generally leading to an increase in surface roughness [41, 42]. In our current study, we did not observe a significant difference in surface roughness between the probiotic solutions, and all treatments resulted in surface roughness values that were relatively similar (Table 2), which contrasts with a previous study that reported the least change in surface roughness in restorative materials treated with kefir [22]. However, in our study, there were statistically significant differences in the interaction between material/probiotic solutions ($p < 0.001$, Table 2). The mean surface roughness values of the restorative material groups treated with probiotic mouthwash and kefir were higher than those of the control groups. Furthermore, the interaction of distilled water with GP resulted in the lowest surface roughness value, while the probiotic mouthwash with IS exhibited the highest values. In a study by Ozan *et al.* [8], they investigated the surface properties of various restorative materials treated with different probiotic solutions (probiotic sachet (pH = 3.0), kefir (pH = 4.4), and artificial saliva (pH = 7.0)), including two types of GIC, RMGIC, compomer, three bulk-fill composites, and one microhybrid composite. Their findings were consistent with the results of our study, as they also found that probiotics with low pH values increased the surface roughness values of RMGIC, compomer, and composite specimens. The low pH (4.2–4.5) of the probiotics used in our study may have contributed to an increase in surface roughness by causing deterioration of the surface matrix structures and dissolution of the restorative fillers.

In the current study, the effect of the control group on surface roughness varied among materials. While the control group generally exhibited lower roughness than the probiotic solution groups for all restorative materials, it resulted in increased roughness values for both permanent and deciduous tooth enamel. This discrepancy in the effect of distilled water on surface roughness could be attributed to several factors, including the degree of water absorption by the restorative material and the hydrophilic/hydrophobic nature of the resin matrix, as noted in previous research [43]. In contrast, our study found that the mean surface roughness values decreased in the permanent and deciduous tooth groups treated with kefir and probiotic mouthwash compared to the restorative materials (Table 2). Agents containing probiotics are known to possess anti-plaque and antibacterial properties by adhering to dental enamel [44]. Moreover, their high calcium content, ability to adhere to enamel surfaces, and capacity to penetrate microcracks on the enamel surface could contribute to increased surface smoothness. These findings align with a study by Saha *et al.* [45], who reported no significant difference in surface roughness and elemental composition of enamel when specimens were immersed in a probiotic solution. However, it is worth noting that these results contrast with studies by Ferrer *et al.* [46] and Angarita *et al.* [47], who found that probiotic consumption led to a "superficial loss of calcium and phosphorous from the enamel surface, resulting in increased roughness".

The measurement of surface roughness is a fundamental method for assessing surface alterations, particularly in dental research [48]. In dental literature, various instruments, such as profilometers and electron microscopes, have been conventionally used to measure surface roughness. In recent years, AFM has gained recognition as a valuable tool for this purpose as it offers several advantages over other methods, including higher resolution measurements at the nanoscale, the ability to generate 3D images, and more suitability for evaluating restorative materials and dental tissues [8]. Furthermore, AFM simplifies the evaluation process as it does not require additional coating or fixation of samples [49, 50]. On the other hand, a previous study reported that the value of the surface parameter is dependent on the size of the area examined, and lower surface roughness values can be obtained because the areas scanned by the AFM are small [51]. However, Kakaboura *et al.* [52] proved that AFM is a more reliable method for determining the surface quality of restorative materials. In addition, even though images with limited section areas at the nanoscale are obtained as a result of imaging the tested materials with AFM, this device can visualize the surface topography at high resolution [8]. Given these considerations, the present study used AFM as the instrument of choice to assess the roughness of dental restorative materials and tooth surfaces.

Surface microhardness is another critical parameter when evaluating the characteristics of restorative materials. It plays a significant role in various aspects of dental materials, including their resistance to chewing forces, susceptibility to wear, tendency to develop fractures and cracks, preservation of matrix structure, and potential for plaque accumulation [21, 53]. In the field of dentistry, the Vickers hardness tester is a commonly used instrument for measuring surface microhardness, and this choice is justified by the tester's short tip structure, which makes it easy to perform measurements and allows for the assessment of surface hardness across different materials [54]. Considering these advantages, we used a Vickers hardness tester in this study to assess the surface microhardness of both restorative materials and dental specimens in our study.

A number of research studies have presented divergent findings concerning the relationship between increased microhardness and the amount of inorganic fillers contained within resin composites. While some investigations have reported a positive association between increased microhardness values and higher levels of inorganic filler content in resin composites [55, 56], others reported no significant correlation between the amount of fillers present and the mechanical properties exhibited by these composite materials [57, 58]. Moreover, Yesilyurt *et al.* [59] highlighted that these materials can exhibit significant changes in hardness when exposed to different chemical environments. For instance, a study published in 2020 assessed the microhardness changes of resin-based esthetic restorative materials exposed to acidic beverages at 7-day and 15-day intervals [60] and reported a gradual decrease in microhardness values with longer exposure to the beverage. Altwaim *et al.* [61] emphasized that the extent of this effect may vary depending on both the specific solution and the material composition. In line with these findings, the results of the current study could not establish any relationship between the microhardness of the different restorative materials tested and the amount of inorganic filler. However, the results corroborate with the notion that probiotic mouthwashes with low pH levels can lead to a decrease in microhardness, which is consistent with the above-mentioned research results. Yanıkoğlu *et al.* [50] reported that the alteration in microhardness of resin-containing restorative materials could be a consequence of the acidic nature of the probiotic solution. The restorative material with the highest change in microhardness values in probiotic mouthwash was DXP, which has a low filler ratio (high polymer matrix ratio). Considering that individual matrix polymer components can significantly impact microhardness values [58], it can be inferred that the high polymer matrix ratio in DXP makes it more susceptible to the pH value of the probiotic mouthwash, leading to the substantial change in microhardness. The fact that the microhardness values of GP and IS materials were not as affected by the probiotic mouthwash as DXP further supports this interpretation.

Interestingly, when we examined the impact of kefir, another low-pH (pH = 4.2) solution used in this present study, we noticed an increase in the microhardness values of the restorative material groups. However, this increase did not reach statistical significance compared to the control group. In addition, it is worth noting that even though kefir and probiotic mouthwash have similar pH levels, the significant improvement in microhardness values observed in restorative materials exposed to kefir can be attributed to the formation of calcium fluoride (CaF) compounds on the material's surface, which is likely influenced by the presence of fluoride (F) within the restorative material and the higher calcium (Ca) content in kefir [62]. The most significant increases in microhardness values were observed in the F-containing restorative materials, particularly the IS and DXP groups, which further supports the hypothesis mentioned earlier.

In this study, although the average surface microhardness values of both permanent and deciduous tooth enamel were decreased when exposed to probiotic mouthwash compared to the control group, the difference was not statistically significant. The decrease in microhardness could be attributed to the low pH of the probiotic mouthwash, which may have led to enamel softening through the dissolution of inorganic tissue. Similar observations have been documented by Devlin *et al.* [63], who highlighted that while acidic solutions reduce the surface hardness of tooth enamel, they cause an increase in the surface energy of the enamel and thus reduce the buffering effect of the oral environment by preventing calcium and other ions in saliva from penetrating the tooth surface.

In contrast to these findings, the microhardness values of enamel influenced by low-pH kefir showed a slight decrease in the permanent tooth (PT) group and an increase in the deciduous tooth (DT) group when compared to the control group. However, this variation observed in both PT and DT groups did not reach statistical significance and could be attributed to the relatively higher permeability associated with deciduous teeth, stemming from their lower mineral density compared to permanent teeth [64]. The penetration of calcium ions from the kefir solution into the tooth's surface, potentially mitigating the impact of the low-pH solution, is a plausible explanation for the observed effects. Consequently, based on our results, the second null hypothesis, which posited that "probiotic solutions have no effect on the surface microhardness of different restorative materials or permanent and deciduous teeth", was rejected.

This *in-vitro* study also has other limitations. Firstly, the evaluation of surface properties was limited to surface roughness and microhardness of the specimens. Future studies could consider additional parameters, such as energy-dispersive X-ray analysis, to assess the impact of probiotics on the elemental composition of restorative materials or enamel. Secondly, the selection of restorative materials in this study was limited. Subsequent research could explore the effects of probiotics on a broader range of restorative materials. Lastly, this study was conducted using *in vitro* settings, and clinical studies are required to assess the effects of probiotics on different restorative materials and enamel in a real-world context.

5. Conclusions

In summary, this study revealed that the effects of probiotic solutions on the surface roughness and microhardness of restorative materials and tooth enamel are contingent on the specific combination of material and probiotic solution used. Notably, Ionoseal exhibited the highest surface roughness among the restorative materials tested, while permanent and deciduous tooth enamel exhibited similar roughness trends, with no significant differences observed between the tested probiotic solutions regardless of materials. Traditional probiotics like kefir and probiotic mouthwash had distinct and varying effects on the microhardness of different restorative materials and tooth enamel. These findings underscore the importance for clinicians to consider the potential impact of probiotics on dental restorations and tooth enamel when recommending or prescribing these products for daily use. However, it is also important to acknowledge that this study had some limitations, and further research, both *in vitro* and clinical, is necessary to gain a more comprehensive understanding of the intricate interactions between probiotics and oral health.

AVAILABILITY OF DATA AND MATERIALS

The datasets and materials used or analysed during the current study are available from the corresponding author on reasonable request.

AUTHOR CONTRIBUTIONS

ED and OK—performed material preparation, data collection and analysis. OK, ED and ATEA—wrote the first draft of the manuscript. All authors commented on the manuscript and read and approved the final version. All authors contributed to the study conception and design.

ETHICS APPROVAL AND CONSENT TO PARTICIPATE

Ethical approval was given to this study by Nuh Naci Yazgan University Ethics Committee with the decision number 2020/13.

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CONFLICT OF INTEREST

The authors declare no conflict of interest.

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