

## ORIGINAL RESEARCH

# Effect of various pediatric probiotic drops on the discoloration of composite resin: *in-vitro* study

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**Abstract**

**Background:** Probiotic supplements are commonly used to boost immunity in children; however, these formulations may negatively affect the surface of dental restorative materials and cause discoloration. This study aimed to evaluate the effects of pediatric probiotic drops containing various microorganisms on the color of resin composites. **Methods:** 56 micro-hybrid composite discs (Herculite<sup>TM</sup> Classic, Kerr Co., CA, USA) were prepared using standard size mold. Every sample was tested for a baseline color measurement (T0) using the *Commission Internationale de l'Éclairage* (CIE) L\*a\*b\* system with Vita Easy Shade Compact. The samples were divided into 4 different groups; 3 different pediatric probiotic drops (Group 1—BiGaia, Group 2—Kaleidon, Group 3—NBL) and the control group were kept in distilled water. Over 30 days, 6 drops of each probiotic were introduced into the composite discs immersed in 1.5 mL of distilled water in Eppendorf tubes. After 5 minutes, the discs were placed in renewed distilled water. On days 7 (T1) and 30 (T2), color measurements were repeated using spectrophotometry, and the color changes ( $\Delta E^*$ ) were statistically analyzed with a significance level of 0.05. **Results:** At the  $\Delta E_1$ ,  $\Delta E_2$  and  $\Delta E_3$  time periods, statistically significant differences occurred on discoloration according to the experiment groups ( $p < 0.05$ ). After 30 days, the color change measurements  $\Delta E^*$  were  $4.51 \pm 1.27$  for G1,  $5.24 \pm 3.06$  for G2,  $4.34 \pm 2.37$  for G3, and  $2.86 \pm 0.7$  for G4. On the 30th day, the discoloration in the G2—Kaleidon and G1—BiGaia groups was statistically significantly higher than the control group G4—Distilled water ( $p = 0.034$  and  $p = 0.015$ ). **Conclusions:** Noticeable color changes in composite resin materials occur with regular use of pediatric probiotic drops. The type of probiotics affects the discoloration of composite resins.

**Keywords**

Probiotics; Microhybrid composite; Color stability; Spectrophotometry

## 1. Introduction

Probiotics are described by the World Health Organization and the Food and Agriculture Organization of America as living microorganisms that confer positive health effects when consumed in adequate quantities [1]. Bacteriotherapy is a replacement therapy where probiotics are consumed to keep the immune system healthy. Probiotics have been shown to be beneficial in preventing gastrointestinal illnesses, balancing the immune system, reducing allergy symptoms, and inhibiting the growth of harmful microorganisms [2]. Furthermore, they also demonstrate positive impacts on oral health. Probiotics can adhere to surfaces in the mouth and provide competition for food sources with other microorganisms. They secrete organic acids, hydrogen peroxide, carbon peroxide, diacetyl, bacteriocin and adhesion inhibitors with antimicrobial activity against oral pathogens. Probiotics can change the pH level of the oral environment with these active substances. In addition,

probiotics affect the environmental conditions necessary for bacterial survival by altering oxidation-reduction potentials and stimulating the formation of protective mucin [3–5].

Probiotics, which create competition for nutrients with cariogenic bacteria, decelerate the growth of cariogenic bacteria and may prevent the formation of dental caries [6]. Studies on probiotics have focused on addressing the microbial imbalance that underlies common oral diseases, aiming to reduce the presence of pathogenic strains and restore a healthy oral microbiome [7]. Probiotics, which have various benefits for both general health and oral health, are found in different foods such as yogurt, kefir, pickles, kimchi and soya beans. Probiotics can also be taken as supplements in the form of tablets, chewing gum, mouthwash, drops and lozenges [2].

Probiotic microorganisms are commonly referred to as bacterial strains, including *Lactococcus*, *Lactobacillus*, *Bifidobacterium*, *Streptococcus* and *Enterococcus*. Additionally, some probiotic products also incorporate yeast strains from the genus

*Saccharomyces* [8]. For several decades, *Lactobacilli* have been a focus of substantial interest in dental research. *Lactobacilli* demonstrate a high tolerance for acidic conditions and are capable of enduring pH levels as low as 3.5 [3]. Studies have shown that the use of drops containing *Lactobacillus reuteri* reduces the number of *Streptococcus mutans* in the oral microbiota [9], while drops containing *Lactobacillus rhamnosus* cause a significant reduction in caries development [10]. *Bifidobacteria*, the predominant anaerobic bacteria residing and play a crucial role in preserving the equilibrium of the microbiota [3]. Probiotic solution that containing *Bifidobacterium infantis*, *Bifidobacterium longum*, *Bifidobacterium bifidum* successfully inhibits the growth of pathogenic bacteria with bacteriocin production [11].

The restorative treatment of caries is mostly done with resin materials similar to tooth color and this offers an aesthetic alternative. However, it is known that resin-based materials can be colored by beverages [12], medications [13–17] and supplements [14, 18]. There are studies investigating the effects of medications and supplements on the surface properties [17], microhardness [14], roughness [18] and discoloration [13–16, 18] of resin-based restorative materials. In particular, there is no study evaluating the effect of probiotics on the discoloration of restorative materials in the literature. This study aims to evaluate the effect of drop-form products containing different probiotic microorganisms on the color stability of micro-hybrid composite resin material. The main research questions to be answered in this study are whether the use of pediatric probiotic drops causes discoloration of composite resins, and if so, the possible reasons and the differences on the impact of various bacterial strains on discoloration.

The hypothesis of the study is as follows: There is no difference in the effect of pediatric probiotic drops containing different microorganisms on the color change of micro-hybrid composite resins.

## 2. Materials and methods

The pediatric probiotic drops and micro-hybrid composite resin used in the study are shown in Table 1. Power analysis was performed by the G\*Power 3.1.9.4 program (Heinrich Heine, University of Düsseldorf, Düsseldorf, NRW, Germany) to determine the number of samples to be used in the study and calculated as  $n = 56$  ( $\alpha = 0.05$ ,  $1 - \beta = 0.80$ , effect size of 0.4).

A total of 56 samples measuring 4 mm in diameter and 2 mm in height were created by micro-hybrid composite (Herculite<sup>TM</sup> Classic, Kerr Co., CA, USA) discs with a standard-sized mold. The composite specimens were applied as a single layer, then stripped and cured for 20 seconds using an light-emitting diode (LED) (Woodpecker LED-C, Woodpecker Medical Instrument Co., Guilin, Guangxi, China) in standard mode with an intensity of 1000 mW/cm<sup>2</sup>. Subsequently, all the specimens underwent polishing with aluminum oxide discs (Sof-Lex, 3M ESPE, St. Paul, MN, USA) before being stored in distilled water for a period of 24 hours. The production of the composite disks was carried out by a single researcher using the same process steps and times to minimize the influence of environmental factors. The use

of micro-hybrid composite as the restorative material in this study was chosen due to its versatility for application in both anterior and posterior regions, ease of manipulation, and its proven resistance to discoloration.

In each group, 14 composite discs were randomly distributed into Eppendorf tubes containing of 1.5 mL distilled water. Baseline (T0) color measurements were performed before immersed in probiotic drop solutions. Every day for 30 days, 6 drops of products containing different probiotic microorganisms according to the groups were added to the Eppendorf tubes. After waiting for 5 minutes, the distilled water in the Eppendorf tube was renewed. On the 7th (T1) and 30th day (T2), color measurements were repeated.

Before taking any measurements, the spectrophotometer was positioned in the center of each sample and calibrated using its calibration tools. Color measurements were conducted under standard D65 light conditions with a white background. The color measurements for each sample were performed based on the CIE ( $L^*$ ,  $a^*$ ,  $b^*$ ) system, repeated three times, and an average value was documented.  $L$ ,  $a$  and  $b$  values are derived based on the “Commission Internationale de l’Éclairage” system. The  $L$  value represents the brightness or lightness of the color, while the  $a$  value indicates its greenness or redness and the  $b$  value signifies its yellowness or blueness [19]. Any color change ( $\Delta E$ ) measurement equal to or greater than 3.5 indicates a clinically noticeable color change, while  $\Delta E$  values below 3.5 indicate clinically acceptable discoloration. The color measurement intervals, the formula used to determine the color change and the process of the test are shown in Fig. 1.

Mean, standard deviation, median, minimum and maximum were calculated using descriptive statistics. The assumption of normal distribution was checked by Shapiro Wilk test, homogeneity of variance by Levene’s test and sphericity assumption by Mauchly’s W test. Analysis of Variance (ANOVA) test was used to compare three or more independent groups with normal distribution and Kruskal Wallis test was used when there was no normal distribution. Repeated Measures ANOVA (Greenhouse-Geisser Statistics) test was used to examine the difference between the averages of three dependent groups where the normality assumption was met with the interaction effect, and Friedman test was used when there was no normal distribution. *Post Hoc* Bonferroni and Adjusted Bonferroni tests were used to reveal the group or groups that created the difference. Analyses were performed in IBM SPSS 25 program (IBM SPSS Statistics for Windows, Version 25.0. Armonk, NY, USA).

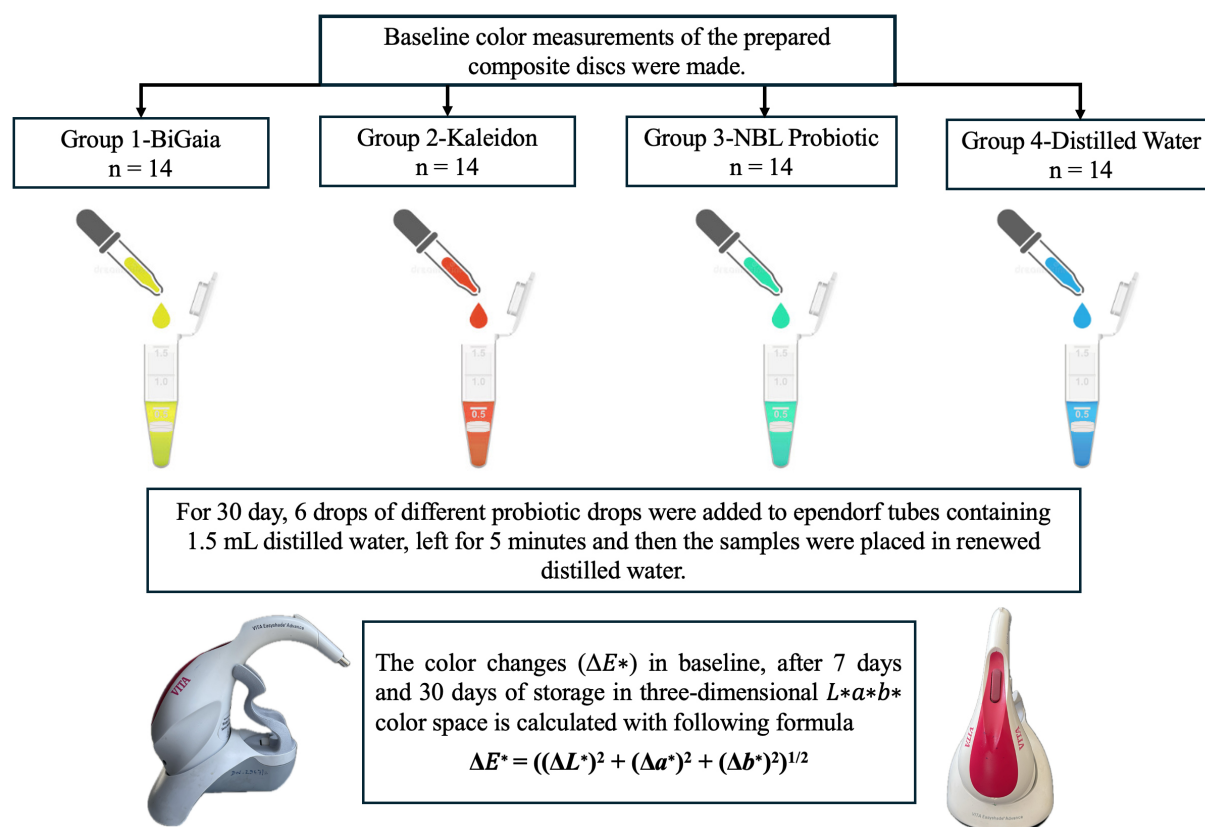
## 3. Results

The mean, standard deviation, minimum, maximum, and median as the descriptive statistics of the study groups according to time intervals were shown in Table 2. The distribution of coloration measurements according to study groups and time were given, ANOVA and Kruskal Wallis tests were performed for comparisons according to study groups and Repeated Measures ANOVA and Friedman tests were performed for comparisons according to time. Statistically significant differences were determined between the coloration measurements accord-

**TABLE 1. Pediatric probiotic drops and micro-hybrid composite used in the present study.**

Probiotic Drops	Lot Number	Manufacturer	Ingredients
BiGaia <sup>TM</sup>	23EB094	BioGaia Production AB, Eslöv, Sweden	Oil-acid-rich sunflower oil, medium-chain triglycerides (Medium chain triglycerides, from palm kernel oil), probiotic microorganism ( <i>Lactobacillus reuteri</i> DSM 17938), anti-clumping (silicon dioxide), cholecalciferol (Vitamin D3)
Kaleidon	L3260	Menarini Group, Istanbul, Turkey	Corn oil, probiotic microorganism ( <i>Lactobacillus rhamnosus</i> GG-ATCC 53103), emulsifier: Mono and diglycerides of fatty acids
NBL Probiotic Drop	2218330001	Nobel İlaç, Istanbul, Turkey	Medium chain triglycerides, <i>Bifidobacterium infantis</i> , <i>Bifidobacterium breve</i> , <i>Bifidobacterium longum</i> , <i>Bifidobacterium bifidum</i>
Restorative Material	Lot Number	Manufacturer	Composition
Herculite <sup>TM</sup> Classic (Shade A1)	9804243	Kerr Co, Orange, CA, USA	Filler: Borosilicatealuminum glass (approximately 78% by weight or 59% by volume) Base resin: BIS-EMA, BIS-GMA, TEGDMA, UDMA

DSM: Deutsche Sammlung von Mikroorganismen; ATCC: American Type Culture Collection; BIS-EMA: Bisphenol A ethoxylate dimethacrylate; BIS-GMA: bisphenol A diglycidyl methacrylate; TEGDMA: Triethylene Glycol Dimethacrylate; UDMA: Urethane Dimethacrylate.

**FIGURE 1. Follow chart of the study.**

ing to the study groups at  $\Delta E1$ ,  $\Delta E2$  and  $\Delta E3$  time intervals ( $p < 0.05$ ).

For  $\Delta E1$  ( $T1 - T0$ ), Bonferroni tests revealed statistically significant differences between Group 4—Distilled Water with Group 3—NBL and Group 2—Kaleidon ( $p < 0.001$  and  $p < 0.001$ ). Group 3—NBL and Group 2—Kaleidon discoloration measurements were higher than those of Group 4—Distilled Water.

$\Delta E2$  ( $T2 - T0$ ), Bonferroni tests revealed statistically significant differences between Group 4—Distilled Water with Group 2—Kaleidon and Group 1—BiGaia ( $p = 0.034$  and  $p = 0.015$ ). The measurements of Group 2—Kaleidon and Group 1—BiGaia group were higher than the measurements of Group 4—Distilled Water.

According to Bonferroni tests for  $\Delta E3$  ( $T2 - T1$ ), statistically significant differences were found between Group 1—

**TABLE 2. Comparison of discoloration measurements by time for different groups.**

Groups	$\Delta E1$		$\Delta E2$		$\Delta E3$		According to time	
	Min.–Max.	Mean $\pm$ S.D. (Median)	Min.–Max.	Mean $\pm$ S.D. (Median)	Min.–Max.	Mean $\pm$ S.D. (Median)	Test Statistics	<i>p</i>
BiGaia	0.3–4.9	3.04 $\pm$ 1.23 (3.20)	1.7–6.6	4.51 $\pm$ 1.27 (4.50)	0.2–3.7	1.69 $\pm$ 1.13 (1.47)	20.855	<0.001*
Kaleidon	1.9–8.5	4.38 $\pm$ 1.83 (3.85)	1.5–10.6	5.24 $\pm$ 3.06 (4.45)	0.1–8.8	5.21 $\pm$ 2.39 (5.75)	0.802	0.459
NBL	2.6–10.9	4.69 $\pm$ 2.37 (3.50)	1.7–8.6	4.34 $\pm$ 2.37 (3.40)	1.3–8.4	3.72 $\pm$ 2.23 (3.15)	1.857†	0.395
Distilled Water	0.5–3.3	2.02 $\pm$ 0.79 (2.05)	1.6–4.2	2.86 $\pm$ 0.70 (2.75)	0.3–2.0	0.96 $\pm$ 0.47 (0.90)	38.457	<0.001*
Test Statistics	21.579‡		11.277‡		19.989			
<i>p</i>	<0.001*		0.010*		<0.001*			

\*Statistically significant association ( $p < 0.05$ ), ‡: Kruskal Wallis test; †: Friedman test. Min.: Minimum; Max.: Maximum; S.D.: Standard Deviation.

$\Delta E1 = T1 - T0$ ,  $\Delta E2 = T2 - T0$ ,  $\Delta E3 = T2 - T1$ .

BiGaia with Group 2—Kaleidon and Group 3—NBL ( $p = 0.001$  and  $p = 0.039$ ). Group 2—Kaleidon and Group 3—NBL measurements were higher than those of Group 1—BiGaia group. Statistically significant differences were observed between Group 2—Kaleidon and Group 3—NBL with Group 4—Distilled Water ( $p = 0.001$  and  $p = 0.003$ ). Group 2—Kaleidon and Group 3—NBL measurements were higher than those of the Group 4—Distilled Water.

According to Bonferroni tests for Group 1—BiGaia, statistically significant differences were determined between  $\Delta E2 - \Delta E1$  and  $\Delta E2 - \Delta E3$  time periods ( $p = 0.001$  and  $p < 0.001$ ).  $\Delta E2$  time measurements are higher than  $\Delta E1$  and  $\Delta E3$  time measurements. No statistically significant differences were obtained between the coloration measurements according to time periods ( $\Delta E1 - \Delta E2$ ,  $\Delta E1 - \Delta E3$ ,  $\Delta E2 - \Delta E3$ ) in Group 2—Kaleidon and Group 3—NBL groups ( $p > 0.05$ ).

## 4. Discussion

Probiotics are effective in preventing allergic diseases in children, reducing the duration of rotavirus-induced diarrhea, strengthening the immune system and regulating the digestive system [20, 21]. It is known that probiotics have positive effects on children's oral health, reduce early childhood caries, slow down caries formation and compete with caries-causing pathogens [3, 6, 10]. Children can consume probiotics through food as well as supplement forms daily. The use of probiotic supplements has demonstrated effectiveness in providing short-term symptomatic relief for acute gastrointestinal disorders, including diarrhea and antibiotic-associated diarrhea [22], as well as long-term management of chronic digestive conditions, such as irritable bowel syndrome and inflammatory bowel disease [23]. The dosage and duration of usage of probiotics may vary depending on the consumption forms and factors such as the age of the child. The recommended dosage of probiotics varies depending on the specific strain [24], but

typically ranges between  $10^7$  to  $10^8$  colony-forming units (CFU) per gram of product per day [25]. In present study, 5 drops of BiGaia contained  $10^8$  CFU of *Lactobacillus reuteri*, 5 drops of Kaleidon contained  $5 \times 10^9$  CFU of *Lactobacillus rhamnosus* and 6 drops of NBL contained  $5 \times 10^{10}$  CFU of *Bifidobacterium infantis*, *Bifidobacterium breve*, *Bifidobacterium longum*, *Bifidobacterium bifidum* were used. Probiotic drops are generally used in the form of 3–6 drops per day in children over 6 years of age, while those in the form of a sachet are used in 1–2 sachets per day in children over 2 years of age. Probiotic supplements have different forms as tablets, chewing gum, mouthwash, drops and lozenges [24]. The purpose of this current study is to assess the impact of routine use of pediatric probiotic drops on the color and aesthetic properties of composite resin restorative materials. The research findings revealed that pediatric probiotic drops containing diverse microorganisms exhibited varying effects on the color changes of micro-hybrid composite resins, leading to the rejection of the study's hypothesis. Observations of discoloration in the obtained data could potentially address a gap in the existing literature. When we scanned the literature, we came across the fact that there are very limited studies evaluating the effect of regular use of probiotic supplements in different forms on hard tissues and restorative materials in the mouth. There is only one study in the literature investigating the changes caused by probiotics in the roughness, microhardness and elemental structure of the enamel surface. The crowns of extracted human teeth were kept in a probiotic solution containing *Lactobacillus rhamnosus* ( $3.605 \times 10^8$  CFU), *Lactobacillus acidophilus* ( $6.625 \times 10^8$  CFU), *Bifidobacterium longum* ( $8.05 \times 10^7$  CFU), and *Saccharomyces boulardii* ( $1.375 \times 10^8$  CFU) for 6 days. With the probiotic solution, lactic acid concentration increases and accordingly the pH of the environment decreases. Therefore, the microhardness of tooth enamel was found decreased, surface roughness was increased



and alters elemental composition [26].

There is another study evaluating the effect of kefir and probiotic mouthwash on the surface roughness and micro-hardness of deciduous/permanent human molars and restorative materials such as composite resin, compomer and resin modified glass ionomer cement. Considering that the pH of kefir was 4.2 and the pH of probiotic mouthwash was 4.5, it was found that probiotic products with acidic content caused changes on the enamel surface and on the surface of different restorative materials [27]. There are no further studies in the literature showing the changes caused by probiotic ingredients on the surface of restorative materials, and there are no studies evaluating the aesthetically important color change, and so this present study was conducted to fill this gap in the literature.

The results of the study showed that all probiotic drops caused some degree of discoloration in the micro-hybrid composites. There are no similar studies to compare these discoloration values between the time periods. When the reason for the statistically significant color change obtained in this study simulating the use of probiotic drops for 30 days is examined, we believe that the content of probiotic drops and the changes they cause on the surface are effective. Probiotics secrete organic acids with antimicrobial activity, hydrogen peroxide and bacteriocin, which are thought to cause acidic changes on the surface of the restorative material. *Lactobacilli* and *Bifidobacteria* produce lactic acid as a principal metabolic end-product from the fermentation of carbohydrates [28]. Composite resins exhibit enhanced resistance to acidic assaults compared to other restorative materials, but their surface properties can still be altered in acidic environments. Research has demonstrated that the composite surface becomes more roughened under acidic conditions, consequently leading to more frequent observations of penetration of any color pigments. The bacterial strains present in probiotic drops generate lactic acid, which roughens the composite surface and facilitates the occurrence of discoloration.

The diversity of bacterial strains in the content of pediatric probiotic drops can lead to changes in the final product, variations in its acidity, and consequently, alterations in its effect on the surface of the restorative material. This study concludes that the impact of a probiotic drop on the surface of the restorative material and its indirect color stability can vary depending on the bacterial strain, bacterial count, the resulting acid product, the degree of acidity of this product, the form of the probiotic supplement, the lubricating agents in the supplement's content, and the duration of probiotic exposure. When evaluating the effect of pediatric probiotic drops on the surface of the restorative material and color stability, another factor to consider is the content and application method of the restorative material used. Composite resins demonstrate superior resistance to acids and wear compared to other restorative materials commonly used in pediatric patients, such as compomers and glass ionomer cements. These advantageous properties often lead clinicians to prefer composite resins for restorative procedures, and as such, leading to their selection for sample preparation in this study. This study only assessed discoloration effects of pediatric probiotic drops on composite material, and future studies evaluating the effects on various restorative materials, such as compomers, giomers, glass

ionomers, and resin-modified glass ionomers are obviously needed. Materials that are less resistant to acid attack are expected to cause more roughness in the acid products formed by probiotic bacteria on the surface of materials that are less resistant to acid attack and increase the coloration due to this, but future research with different restorative materials is needed to evaluate this prediction.

According to present study, we firmly consider that the discoloration observed milder due to the oils in the formulation of the probiotic drops, which act as encapsulation for the survival of the bacteria and providing a lubricant effect. Encapsulating probiotic bacteria within emulsion droplets has been proposed as a strategy to improve the survival of microbial cells under the challenging physiological conditions present in the stomach and intestine [29]. As reported by Hou and colleagues, encapsulating lactic acid bacteria cells within reconstituted sesame oil body emulsion droplets resulted in a substantial enhancement of their survival rate, approximately  $10^4$ -fold, when exposed to simulated gastrointestinal tract conditions, compared to the survival of free bacterial cells [30]. We assume that this encapsulation process not only keeps the bacteria functioning and active, but also creates a slippery layer on the composite surface, thus preventing extrinsic coloration. In a way, it balances the increase in the retention area of the roughness that lactic acid can create on the composite surface by making it slippery with its oil content.

Present study has several limitations by the *in-vitro* nature, as the inability to evaluate the effects of saliva's buffering capacity, flow rate and pH, the oral microbiome's interactions with bacteria species in pediatric probiotic drop. These limitations should be taken into account when interpreting the results. Building upon the novel findings of this investigation, future research endeavors should explore the impacts of various probiotic supplements, encompassing diverse bacterial strains and differing usage durations, on the physical, chemical and aesthetic characteristics of different restorative materials.

## 5. Conclusions

Prolonged pediatric probiotic drop usage can lead to observable discoloration of resin composite restorations. Given that the bacterial strains in the probiotic drops alter the oral pH, long-term administration should be advised with caution, as these changes may negatively impact the aesthetic qualities of dental restorative materials.

## AVAILABILITY OF DATA AND MATERIALS

The data that support the findings of this study are available from the corresponding author, Asst. Prof. Aslı Aşık, PhD, DDS, upon reasonable request.

## AUTHOR CONTRIBUTIONS

AA and SA—conceived the idea. AA—collected and analyzed the data. Both of authors led the writing and revised the manuscript and gave final approval of the version to be published and agreed to be accountable for all aspects of the

study.

## ETHICS APPROVAL AND CONSENT TO PARTICIPATE

Not applicable.

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## CONFLICT OF INTEREST

The authors declare no conflict of interest.

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