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ORIGINAL RESEARCH

Effect of adding sodium fluoride and nano-hydroxyapatite nanoparticles to the universal adhesive on bond strength and microleakage on caries-affected primary molars

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Abstract

Evaluation of micro tensile bond strength (μTBS) and marginal leakage of sodium fluoride (NaF) and nano-hydroxyapatite (n-HA) modified universal adhesives (UAs) bonded using etch-and-rinse (ER) and self-etch (SE) bonding technique to the carious affected dentin (CAD). One hundred and twenty primary molars were prepared for CAD on the occlusal surface. The occlusal CAD surface was flattened and underwent a polishing procedure. The specimens were divided into six groups using a random allocation method based on the UAs applied and the mode of etching used (n = 20) Group A1: UAs (ER), Group B1: UAs (SE), Group A2: UAs (NaF) + ER, Group B2: UAs (NaF) + SE, Group A3: UA (n-HA) + ER and Group B3: UAs (n-HA) + SE. Composite restoration was placed and samples were thermocycled. Microleakage, μTBS, and failure mode assessment were performed using a dye penetration test, universal testing equipment, and stereomicroscope respectively. The μTBS and microleakage results (mean $\pm$ SD) were examined using analysis of the variance (ANOVA) and Tukey *post hoc* tests. Group B1 (UAs + SE) demonstrated the maximum scores of microleakage (25.14 $\pm$ 9.12 nm) and minimum recorded value of μTBS (14.16 $\pm$ 0.55 MPa). In contrast, Group A3 (UAs (n-HA) + ER) displayed a minimum value of marginal leakage (12.32 $\pm$ 6.33 nm) and maximum μTBS scores (19.22 $\pm$ 0.92 MPa). The outcomes of the intergroup comparison analysis showed that Group A2 (UAs (NaF) + ER), Group B2 (UAs (NaF) + SE), Group A3 (UA (n-HA) + ER) and Group B3 (UAs (n-HA) + SE) presented comparable outcomes of marginal seal outcomes and μTBS scores ($p > 0.05$). NaF and n-HA-modified UAs displayed favorable bond strength and minimum marginal leakage to the deciduous affected dentin surface.

Keywords

Universal adhesives; Etch and rinse; Self-etch; Nano-hydroxyapatite

1. Introduction

Dental caries or "tooth decay" is the most common oral health disease worldwide. Statistics revealed that half a billion children aged from 2 to 11 need dental caries treatment every year [1]. In pediatric dentistry, the growing interest in aesthetics and minimally invasive cavity preparation has led to the increased use of tooth color restorative material [2]. However, it is widely believed that various dissimilarities exist between primary and permanent teeth in terms of their chemical composition, physiological characteristics and micromorphology [3].

Two primary modalities that facilitate the adhesion of composites to tooth substrates are etch-and-rinse (ER) and self-etch (SE) adhesives [4]. ER denotes the conventional technique in which an etchant is initially applied to the surface of the tooth. Based on the indexed literature, it has been established that adhesive systems designed for primary dentition contribute to achieving favorable micro tensile bond strength (μTBS) and improved marginal seal [5]. Nonetheless, while it is the conventional and most widely used bonding strategy, it is difficult to employ this technique on an uncooperative child due to the involvement of multiple steps [6]. As a result, self-etch (SE) adhesives have been developed and utilized to simplify the bonding procedure. These adhesives employ a single agent that both etches and primes the tooth surface, eliminating the requirement for rinsing [7].

Universal or multimode adhesives (UAs) are novel bonding agents that provide dentists with procedural flexibility, enabling them to select appropriate application methods depending on specific clinical circumstances [8]. Furthermore, the use of UAs in pediatric dentistry has proved to be beneficial in managing an uncooperative child [9]. Recently, fluoride-releasing UAs have been recognized as a prospective remedy for mitigating the problem of demineralization and deterioration of restoration bonds to the tooth substrate [10]. The integration of nano-hydroxyapatite (n-HA) particles into the adhesive resin has the potential to boost the strength and structure of the tooth, as well as improve the mechanical properties of the resin adhesive [11, 12]. However, there is a limited body of data available regarding the influence of sodium fluoride (NaF) and n-HA-modified UAs on the μTBS and marginal leakage when applied to the CAD surface of deciduous teeth.

Available indexed literature suggested that data is scarce regarding the effect of NaF and n-HA-modified multimode adhesives on bond integrity and marginal seal of composite bonded to the CAD surface of primary molars. Therefore, the present study aimed to assess the μTBS and marginal seal of modified adhesives applied using various etching modes on CAD substrates in primary dentition. The hypothesis posited that there would be no significant difference in μTBS and marginal leakage between NaF and n-HA-modified Universal Adhesives (UAs) compared to unmodified UAs when bonded to affected dentin surfaces of deciduous molars. Additionally, it was postulated that there would be no notable distinction in μTBS and marginal leakage between each adhesive when applied using either etch-and-rinse (ER) or self-etch (SE) techniques.

2. Materials and methods

One hundred and twenty primary molars were collected and disinfected by submerging in a chloramine trihydrate solution (Merck, #MFCD00149066, Frankfurt, FFM, Germany) for 48 h at a temperature of 10 °C. A radiographic examination was conducted to select only teeth where caries were still within the middle third of dentin International Caries Detection and Assessment system (ICDAS) criteria 3 and 4. The carious infected dentin was removed using round carbide bur (F0142, Dentsply Sirona, Bensheim, Bensh, Germany) in a slow-speed handpiece. CAD surface was then obtained by visual examination, degree of hardness, and use of caries-detecting dye. The occlusal CAD surface was flattened and underwent a polishing procedure using silicon carbide paper (Jeanwirtz GmbH & Co. Charlottestrabe Dusseldorf W. Germany) to achieve a consistent surface texture [13, 14]. The CAD surface was assessed independently by two examiners. The study demonstrated a Kappa value of 0.90 for inter-examiner reliability in categorizing the dentin type, indicating a substantial level of agreement between the examiners.

2.1 Synthesis of n-HA

Simple hydrothermal synthesis was used to produce the n-HA. The n-HA particles were combined with the adhesive SB at mass fractions of 1 wt%. n-HA were homogenized using a sonicator (SH80-2L, MTI Corporation, Pennsylvania, PA, USA) for 20 min [15]. The specimens were divided into six groups using a random allocation method based on the UAs applied and different etching modes n = 20 (Fig. 1).

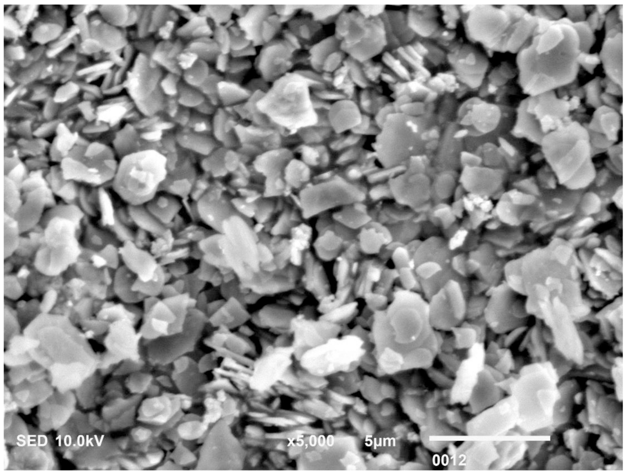

FIGURE 1. SEM image demonstrating hydroxyapatite nanoparticles that were synthesized had a morphology characterized by a heterogeneous rod structure with variability in sizes.

2.1.1 Group A1: UAs (ER)

In this group, single bond UA (3M ESPE, St. Paul, MN, USA, lot #471008) was applied in the ER technique. The CAD surface was first applied with etchant (Ultra-etch, # 685-1, Ultradent, Berlin, BE, Germany) for 15 seconds followed by rinsing and air drying. The surface was then scrubbed with the adhesive for 20 secs, air-thinned and light-cured for 10 secs using an 800 mW/cm^2 light emitting diode (LED) unit (Bluephase C8, Ivoclar Vivadent, Liechtenstein, LI, Austria).

2.1.2 Group B1: UAs (SE)

In this group, single bond UA (3M ESPE, St. Paul, MN, USA, lot #471008) was applied in the SE technique. The surface was scrubbed with the adhesive using a micro brush for 20 seconds. A controlled airflow was applied to the smear of the adhesive for approximately 5 seconds until its motion ceased and the solvent underwent complete evaporation. The light curing process was conducted for 10 sec using an 800 mW/cm^2 LED light unit (Bluephase C8, Ivoclar Vivadent, Liechtenstein, LI, Austria).

2.1.3 Group A2: UAs (NaF) + ER

In this group, modified UA was prepared by adding 20 mg (5000 ppm) NaF in 4 mL single Bond UAs (3M ESPE, St. Paul, MN, USA, lot #471008). ER technique mentioned in group A1 was followed to apply the adhesive on the CAD surface.

2.1.4 Group B2: UAs (NaF) + SE

In this group, modified UA was prepared by adding 20 mg (5000 ppm) NaF in 4 mL Single Bond UAs (3M ESPE, St. Paul, MN, USA, lot #471008). SE technique mentioned in group B1 was followed to apply the adhesive on the CAD surface.

2.1.5 Group A3: UA (n-HA) + ER

The n-HA particles modified Single Bond UAs were applied to the CAD surface in the ER technique mentioned in group A1.

2.1.6 Group B3: UAs (n-HA) + SE

The n-HA particles modified Single Bond UAs were applied to the CAD surface in the SE technique mentioned in group B1.

2.2 Placing composite restoration

After adhesives application, the CAD surface was restored using shade A1 composite (Z250, 3M, ESPE, USA) using a cylindrical plastic mold. The material was then cured for 20 seconds by holding an LED unit at a distance of one millimeter perpendicular to the surface.

2.3 Microleakage assessment

To evaluate the marginal seal of sixty samples ten from each investigated group, a layer of nail varnish was uniformly applied to the whole tooth surface except for the restoration. All the samples were then submerged in a solution containing 0.5% methylene blue dye for 8 hours. The teeth were extracted from the dye, nail varnish was removed followed by rinsing and drying. The specimens were then sectioned in a bucco-lingual plane utilizing a water-cooled diamond saw. The interfaces were meticulously examined under a stereomicroscope at a magnification of 40× by two examiners [16, 17].

The degree of microleakage at CAD restoration margins was evaluated using a standard microleakage evaluation scale in nanometer (nm) [18].

0 = No dye penetration.

1 = Dye penetration up to 1/3 of cavity depth.

2 = Dye penetration up to 2/3 of cavity depth.

3 = Dye penetration up to the cavity floor.

When different scores of marginal leakage were observed among the two examiners, agreement was obtained on a single score.

2.4 μTBS and failure mode assessment

CAD teeth were vertically sectioned along both the mesial-distal and buccal-lingual axes using a slow-speed diamond saw (Isomet 1000, Buehler, Plymouth, MN, USA). From each sample, three stick-shaped tensile specimens, each measuring 1 mm^2, were obtained. These specimens were affixed to micro-tensile testing (BISCO; Schaumburg, IL, USA) and subjected to tensile stress at a crosshead speed of 1 mm/min until failure. The resulting tensile bond strength was quantified and expressed in megapascals (MPa). After debonding, the failure mode was analyzed under a stereomicroscope (Nikon Model C DSD230, Nikon Co. Tokyo, Japan) at ×40 magnification [19, 20].

2.5 Statistical analysis

Data was entered and analyzed in Statistical Package for Social Sciences (SPSS version 26.0, IBM, Chicago IL, USA). The data exhibited a normal distribution as evidenced by Bonferroni's correction to see the normality of the data. The marginal leakage values of modified adhesives that were bonded to the CAD surface of deciduous molars by using ANOVA, Tukey *post hoc* test ($p < 0.05$). The mean and standard deviation (SD) of μTBS and microleakage were compared using the Kruskal Wallis test followed by the Tukey *post hoc* test ($p \leq 0.05$). The failure analysis was assessed in percentages. $p < 0.05$ was taken as the level of significance.

3. Results

3.1 Microleakage evaluation

Table 1 presented the marginal leakage values of modified adhesives that were bonded to the CAD surface of deciduous molars. ANOVA, Tukey *post hoc* test ($p < 0.05$) was used to analyze outcomes and showed that the specimens from Group B1 (UAs + SE) discovered the maximum scores of microleakage (25.14 ± 9.12 nm). In contrast, Group A3 (UAs (n-HA) + ER) established the minimum value of marginal leakage (12.32 ± 6.33 nm). The outcomes of the intergroup comparison analysis directed that Group A2 (UAs (NaF) + ER) (13.89 ± 7.62 nm), Group B2 (UAs (NaF) + SE) (14.11 ± 7.63 nm), Group A3 (UA (n-HA) + ER) (12.32 ± 6.33 nm) and Group B3 (UAs (n-HA) + SE) (13.21 ± 6.56 nm) presented comparable outcomes of marginal seal outcomes ($p > 0.05$). Nonetheless, it was also witnessed that Group A1 (UAs + ER) (23.11 ± 11.41 nm) and Group B1 (UAs + SE) (25.14 ± 9.12 nm) displayed significantly lower scores of marginal leakage ($p < 0.05$).

TABLE 1. Microleakage scores of different modified universal adhesives bonded to carious affected dentin (CAD) on primary molars.

Investigated groups	Mean (nm)	SD (nm)	p-value
Group A1: UAs (ER)	23.11^b	11.41	
Group B1: UAs (SE)	25.14^b	9.12	
Group A2: UAs (NaF) + ER	13.89^a	7.12	0.020
Group B2: UAs (NaF) + SE	14.11^a	7.63	
Group A3: UA (n-HA) + ER	12.32^a	6.33	
Group B3: UAs (n-HA) + SE	13.21^a	6.56	

UAs: Universal adhesives; ER: Etch and rinse; SE: Self-etch; NaF: Sodium Fluoride; n-HA: Nano-hydroxyapatite; SD: standard deviation.

Different superscript characters denote statistically significant difference.

3.2 μTBS assessment

Table 2 presented the means and SD of modified multimode adhesives μTBS bonded to the CAD surface of primary molars. The samples from group A3 (UA (n-HA) + ER) unveiled the maximum bond scores (19.22 ± 0.92 MPa). In contrast, group-B1 (UAs + SE) exhibited the lowest value of μTBS (14.16 ± 0.55 MPa). Bonferroni's correction was used to see the normality of data, Kruskal-Wallis test and *post hoc* test, ($p <$ 0.05) showed the results of the intergroup comparison that the bond strength scores. Group-A2 (UAs (NaF) + ER) (18.75 ± 0.77 MPa), group-B2 (UAs (NaF) + SE) (18.22 ± 0.59 MPa), group mA3 (UA (n-HA) + ER) (19.22 ± 0.92 MPa) and group-B3 (UAs (n-HA) + SE) (18.66 ± 0.85 MPa) were comparable ($p > 0.05$). However, it was also observed that group-A1 (UAs + ER) (15.28 ± 0.63 MPa) and group-B1 (UAs + SE) (14.16 ± 0.55 MPa) displayed significantly lower scores of μTBS ($p < 0.05$).

TABLE 2. Means and SD for Micro tensile bond strength (μTBS) of different modified universal adhesives bonded to carious affected dentin (CAD) on primary molars.

Investigated groups	Mean ± SD (MPa)	p-value
Group A1: UAs (ER)	15.28 ± 0.63^b	0.031
Group B1: UAs (SE)	14.16 ± 0.55^b	
Group A2: UAs (NaF) + SE	18.75 ± 0.77^a	
Group B2: UAs (NaF) + SE	18.22 ± 0.59^a	
Group A3: UA (n-HA) + ER	19.22 ± 0.92^a	
Group B3: UAs (n-HA) + SE	18.66 ± 0.85^a	

UAs: Universal adhesives; ER: Etch and rinse; SE: Self-etch; NaF: Sodium Fluoride; n-HA: Nano-hydroxyapatite; SD: standard deviation.
Different superscript characters denote statistically significant difference.

3.3 Failure mode assessment

Fig. 2 displayed the failure mode (adhesive, cohesive and admixed) among the investigated groups. The study discovered that groups A2, B2, A3 and B3 exhibited cohesive and admixed failure patterns predominantly. Nevertheless, it was observed that group A1 and B1 displayed the highest occurrence of adhesive and admixed failures.

4. Discussion

The existing study was based on the hypothesis that there will be no significant difference in the μTBS and marginal leakage of NaF and n-HA modified UAs bonded to the affected dentin surface of deciduous molars in comparison to the unmodified UAs. Furthermore, it was also hypothesized that there would be no significant difference in the μTBS and marginal leakage of each adhesive bonded using either ER or SE technique. Results indicated that the first stated hypothesis was completely rejected. However, the second hypothesis was completely accepted. The rationale behind using a bond strength test in the present study was based on the fact that adhesives with higher bond integrity would exhibit greater stress resistance and hence result in durable restorations. However, it does not always imply good clinical outcomes due to wide variations in the oral environment. Moreover, the evaluation of the marginal seal was performed using dye penetration as it is the most widely employed test in previous research owing to its ease of use and quickness [21, 22].

The property of marginal sealing is of utmost importance when considering the application of adhesive restoration [23]. Previous research has demonstrated that microleakage at the resin-dentin interface is inversely proportional to the μTBS [24]. Outcomes of the present study proclaimed that n-HA and NaF-modified multimode adhesives displayed higher bond integrity and lower microleakage scores than the unmodified UAs when used to restore the CAD surface of deciduous dentition. The justification for the highest bond value and lowest microleakage scores seen in the n-HA modified UAs group can be attributed to the stimulation of epitaxial growth of HA crystals in dentin by HA microfillers. This leads to the remineralization of the hybrid layer region [16, 25]. In a recent study conducted by Al-Hamdan *et al.* [26], it was proposed that using smaller n-HA particles with heterogenous rod structures increases the surface area available for the attachment and release of minerals. It also improves the adhesive resistance to degradation by decreasing hydrophilicity, mitigating enzymatic collagen degradation, and reducing stress contraction [27, 28]. Similarly, the lowest marginal leakage scores observed in the n-HA group are in agreement with the findings of the study conducted by Taha and coworkers [29]. Low microleakage scores in n-HA-modified UAs can be ascribed to enhanced marginal adaptation, improved tissue response and upgraded mechanical properties [26, 30]. Consequently, there is a call for further in-depth investigations to address the existing gaps in knowledge and provide a more comprehensive understanding of the impact of Nano-hydroxyapatite -modified dental adhesives on adhesive properties and clinical outcomes.

The outcomes of the current investigation have also demonstrated that adhesives that release fluoride have shown satisfactory micro tensile bond strength (μTBS) scores and marginal seal [31]. Fluoride-releasing adhesives offer a multifaceted approach to reducing the risk of secondary caries. They create an acid-resistant zone in the tooth, inhibiting the activity of enzymes that could otherwise degrade collagen and ester bonds in the adhesive. This dual action helps maintain the structural integrity of the hybrid layer, ensuring the longevity of dental restorations minimizing the chances of secondary caries occurrence, and decreasing microleakage [32].

The results of the current study also revealed that the etching mode (ER and SE) did not have a significant effect on the bond strength and microleakage scores for both the modified and unmodified multimode adhesives when applied to CAD in primary dentition. Previous studies in the available literature have supported the findings of this investigation, suggesting that the bond strength of universal adhesives on enamel is improved when phosphoric acid etching is employed before bonding [8, 33]. However, it's important to note that this effect was not observed in the case of dentin. Research has also indicated that the bonding performance of universal adhesives is influenced by the material used, regardless of the etching technique applied [34, 35], However, enough data is not available regarding the impact of bonding mode on the μTBS and microleakage of UAs to the CAD surface of deciduous dentitions thus necessitating further inquiry. Regarding failure mode, it was witnessed that samples showing higher bond strength scores exhibited cohesive failure predominantly. The occurrence of cohesive failure is often associated with high

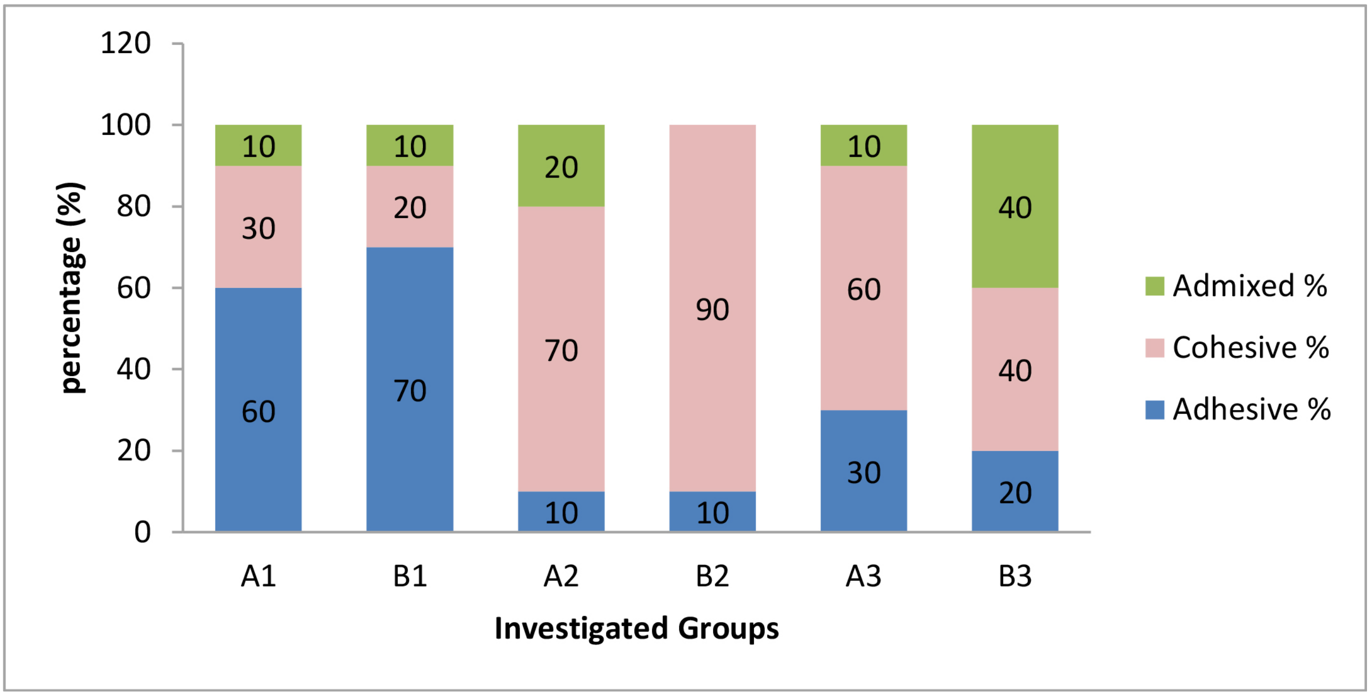

FIGURE 2. Percentage distribution of modes of failure. UAs: Universal adhesives; ER: Etch and rinse; SE: Self-etch; NaF: Sodium Fluoride; n-HA: Nano-hydroxyapatite.

bond strength, indicating that the adhesive material itself is robust and can withstand significant stress before breaking internally [36]. Factors such as the type of adhesive used, the bonding conditions, and the materials being bonded can influence the likelihood of cohesive failure [37, 38]. However, unmodified UAs displayed mostly adhesive fracture patterns. Adhesive failure can be indicative of a weaker bond at the adhesive-substrate interface. Factors such as improper adhesive selection, inadequate curing, thermal expansion mismatch and inadequate surface preparation all contribute to adhesive failure patterns [39].

Similar to other studies in the field of biomedical sciences, the contemporary study presents certain limitations. Since oral cavity conditions are invariably distinct from the *in vitro* settings significant effort was expended to reproduce them *in vitro*. However, it is impossible to simulate such an environment outside the mouth, which could have led to inevitable bias. Moreover, future research is recommended to analyze bond strength and microleakage concerning time. In addition, another available universal adhesive should have been modified to clearly understand the role of the material used. Scanning electron microscopy (SEM) and atomic force microscopy (AFM) should have been conducted to assess the surface changes that occurred after the application of modified multimode adhesives.

5. Conclusions

NaF and n-HA-modified UAs display reasonable micro-tensile bond strength and minimum microleakage to the deciduous caries-affected dentin and have the potential to be used in clinical practice. However, more clinical studies are recommended to extrapolate the findings of the present study.

AVAILABILITY OF DATA AND MATERIALS

The data presented in this study can be made available on request.

AUTHOR CONTRIBUTIONS

FAAA, AMA and MAK—designed the research study. NL—methodology, software validation and performed formal analysis. RKA, AS and GHA—investigation, resources and data curation. FHN, FAAA and NL—writing-original draft preparation, writing-review and editing; performed visualization.

ETHICS APPROVAL AND CONSENT TO PARTICIPATE

This study was approved by the Ethical Committee of Dar Al Uloom University COD/IRB/2022/05. The consent was taken by the child's guardian regarding tooth usage for experimentation purposes.

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CONFLICT OF INTEREST

The authors declare no conflict of interest.

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