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EFFECT OF ONE YEAR BRUSHING WITH NANO-HYDROXYAPATITE MODIFIED TOOTHPASTE ON SURFACE ROUGHNESS AND MICRO-SHEAR BOND STRENGTH OF ENAMEL TO RESIN COMPOSITE RESTORATION USING TWO ADHESIVE SYSTEMS: IN VITRO STUDY

Dalia M Abdel-Hamid [*], Norhan A El-Dokky [**] *and* Sherif B El-Tawil [**]

ABSTRACT

This study aimed at evaluating in vitro the effect of one year brushing with 10 wt. % nano-hydroxyapatite (n-HAp) modified toothpaste on surface roughness and micro-shear bond strength (μ-SBS) of enamel to resin composite restoration (Filtek Z350 XT Universal Restorative) using total-etch (TE), Adper Single Bond Plus Adhesive and self-etch (SE), Single Bond Universal Adhesive systems. Thirty human enamel/dentin slabs were prepared and divided according to the toothpaste into: control group (Signal Kids toothpaste) and test group (10 wt. % n-HAp ~20 nm modified Signal Kids toothpaste). Brushing was performed for one hour/day for 24 days. Roughness (Ra) was measured by the AFM for 10 brushed slabs. For μ-SBS evaluation, 20 brushed slabs were randomly subdivided into 2 subgroups/ group according to the adhesive system (control/TE, control/SE, test/TE and test/SE). Micro-cylinders of nano-filled resin composite were prepared. The μ-SBS test was performed by a universal testing machine. The mode of failure was evaluated by digital microscope and the morphological changes of the de-bonded surfaces were examined by the SE microscope. The data were statistically analyzed. The mean Ra (nm) values of the enamel brushed with unmodified toothpaste were not significantly different than those brushed with n-HAp modified toothpaste. Whereas, the mean μ-SBS (MPa) of the control (brushed with unmodified toothpaste) subgroups with either TE or SE were significantly lower than those of the test (brushed with n-HAp modified toothpaste) subgroups. Subgroups with SE adhesives exhibited predominantly adhesive failure mode, while predominance of mixed failures were detected for subgroups with TE adhesives. In conclusion, one year simulated brushing with 10 wt. % n-HAp modified toothpaste did not affect enamel roughness while it improved the bond strength to resin composite regardless of the adhesive system. However, the adhesive system type has an influential effect on the failure mode.

KEYWORDS: Brushing, Roughness, Nano-hydroxyapatites, Toothpaste, μ-shear bond strength, Adhesives, Enamel

* Assistant Professor of Dental Materials Science, Biomaterials Department, Faculty of Dentistry, Cairo University, Egypt.

** Assistant Professor of Pediatric Dentistry, Pediatric and Community Dentistry Department, Faculty of Dentistry, Cairo University, Egypt.

INTRODUCTION

Developments in science have greatly changed the methods of replacing any lost human structure with restorative materials [1]. Science had been directed towards using natural and biocompatible materials. Hydroxyapatite (HAp, $Ca_{10}(PO_4)_6(OH)_2$) is one of these materials. It is a major component of the hard tissues in the human body [2]. Consequently, the use of HAp toothpaste has captured a great deal of attention. Hydroxyapatite which is the main component of enamel gives the tooth its bright white appearance and eliminates the diffused reflection of light by filling up the fine pores of the tooth surface [3]. In addition, HAp acts as an abrasive in toothpaste that can eliminate tooth surface discoloration [4].

The production of nano-hydroxyapatite (n-HAp) followed the ongoing development in the field of nanotechnology. Nano-sized HAp particles (< 100 nm) have the great resemblance to the apatite crystal of tooth enamel in morphology and crystal structure [5]. In recent years, an increasing number of reports have shown that n-AHp containing dental products such as toothpastes and mouth washes have the potential to remineralize artificial carious lesions [6-11].

The combined importance of enamel remineralizing agents and techniques as a conservative approach for preventing and treating enamel incipient caries, and the popularity and evolution of tooth-colored bonded restorations, have raised the questions concerning the effects of these remineralizing agents on the surface roughness as well as the effectiveness of adhesive systems on enamel substrates.

Roughness is considered as a predisposing factor for bacterial adhesion and stain absorption. Precipitation of n-HAp layer on enamel surface in vitro has been reported by several studies concerned with bleaching and incipient caries managements [7,9-13]. However, there is limited information related to the effect of this newly precipitated layer on normal enamel surface roughness and bonding ability to different adhesive systems such as total-etch (TE) or self-etch (SE) adhesive systems.

Therefore, the aims of the current study were measuring the surface roughness and the micro-shear bond strength (μ-SBS) of enamel to resin composite restoration using 2 adhesive systems (total-etch and self-etch) after simulated one year brushing with a toothpaste modified by 10 wt.% nano-hydroxyapatite (n-HAp). Additionally, the mode of failure was evaluated by the digital microscope while the morphological changes of the de-bonded surfaces were examined by the scanning electron microscope (SEM).

The null hypotheses tested were as follows: simulated brushing with 10 wt.% nano-hydroxyapatite (n-HAp) modified toothpaste for one year, would not affect both the enamel surface roughness and the μ-SBS of enamel to resin composite restoration using 2 adhesive systems (total-etch and/or self-etch).

MATERIALS AND METHODS

This study was accomplished in accordance with the ethical guidelines in research with human participants. These ethical guidelines are in full agreement with the World Medical Association Declaration of Helsinki. The Research Ethics Committee of the Faculty of Dentistry, Cairo University (Code 16-9-18) approved this study.

Regarding sample size calculation, power analysis for a 2 x 2 fixed effects analysis of variance was estimated. The first factor i.e. toothpaste (unmodified and n-HAp modified toothpaste) includes 2 levels and the second factor (adhesive system) includes 2 levels. Based upon the published results of *Weerasinghe et al.* (2005)[14] and *Beloica et al.* (2010) [15] , the effect size for μ-SBS was found to be (7.2) for the first factor and (7.32) for the second factor, using alpha (α) level of (5%) and beta (β) level of (10%) i.e. power = 90%; the study included 5 specimens per cell for a total of 20 specimens.

Sample size calculation was performed using IBM® SPSS® SamplePower® Release 3.0.1

Fifteen extracted human premolars were collected from the Pediatric and Community Dentistry Department, Faculty of Dentistry, Cairo University. The extracted teeth were obtained from the participants (10-14 years) whose orthodontic treatment plan required extraction. The teeth were collected with an informed consent signed by the parents of the participants and approved by the Research Ethics Committee (Faculty of Dentistry, Cairo University). The freshly extracted teeth were thoroughly cleaned and examined under a stereomicroscope (Leica applicator suite, version 3.1.0, Leica Microsystems, Switzerland). Teeth with observable cracks, hypoplasia or white spot lesions were excluded from the study. Selected teeth were stored in 4 °C physiologic saline solution. A diamond-coated band saw was used to separate the crowns from their roots under copious amount of water (Struers Minitom; Struers, Copenhagen, Denmark). Each premolar tooth provided 2 enamel/dentin slabs of 3mm x 3mm in dimensions (one from the buccal surface and one from the lingual surface). Each enamel/dentin slab was embedded in a chemically-cured polymethyl methacrylate (Acrostone, Egypt) blocks. In order to standardize the amount of enamel reduction, 0.5 mm depth orientation pits were prepared with air-rotor bur where superficial enamel was removed by a superfine diamond bur (SF 101CR, Shofu, Inc., Kyoto Japan) with a high speed hand piece followed by grinding with #600-grit SiC paper under water coolant. The teeth were then ultrasonically cleaned in distilled water for 5 minutes to remove any remaining SiC dust particles [14, 15]. The 30 enamel/dentin slabs obtained from the 15 teeth (buccal and lingual enamel/dentin slab/tooth) were randomly divided in to 2 groups, control and test groups according to the toothpaste (unmodified or modified with n-HAp) used. Each tooth half was allocated in one group. A computer generated random sequence table (randomn.org) was used by one of the authors (other than the one prepared the specimens) to determine the order of specimens allocation.

Commercially available toothpaste (Signal Kids, Unilever Mashreq-Personal Care Company, Egypt) was selected and used as a control group. For the test group, 10 wt. % nano-hydroxyapatite powder (n-HAp) with the size range of 10-20 nm and Ca/P ratio of 1.67 (Nanostreams Egypt, patch no NS 0012) was added to the selected commercial toothpaste [9,10,16]. Toothpastes/artificial saliva slurries were prepared according to the European standards for preparing artificial saliva/toothpaste slurries (EN ISO 11609) [17,18]. Brushing of the enamel/dentin slabs was carried out with an electric brush (Oral-B, Cross action Power Max with medium bristle stiffness) under a weight of 200g for 1 hour /day for 24 days simulating one year brushing in a tooth brushing holder device [16,19].

Enamel surface roughness measurement:

A total of 10 enamel/dentin slabs (5 control slabs brushed with unmodified toothpaste with their corresponding test slabs which were brushed with n-HAp modified tooth paste) were randomly selected from the totally prepared 30 slabs to determine the enamel surface roughness by the atomic force microscope (AFM) in a contact mode. The slabs were evaluated at the same scan size (49.5 x 49.5 μm^2) by triplicate in different areas, all of which were selected at random and the mean roughness (Ra nm) was obtained from 3 measurements for each slab [20]. Evaluation of enamel roughness was carried out at a scanning rate of 3.052 Hz by an AFM (Model, VEECO Dimension 3100 Scanning Probe Microscope, Germany) with Bruker, NanoScope V5.33R1S11 software.

Micro-shear bond strength (μ-SBS) test:

The restorative material and adhesive systems used in the current study with their chemical compositions and batch numbers as reported by the manufacturers are presented in table (1).

TABLE (1) Detailed description of the selected restorative material and adhesive systems as mentioned by the manufacturers

Material	Batch no.	Manufacturer	Composition
Filtek Z350 XT Universal Restorative	N612321	3M ESPE Dental Products, St. Paul, USA	**The resin** contains bis-GMA, UDMA, TEGDMA, and bis-EMA (6) resins. To moderate the shrinkage, PEGDMA has been substituted for a portion of the TEGDMA resin. **The fillers** are a combination of non-agglomerated/non-aggregated 20 nm silica filler, non-agglomerated/non-aggregated (4- 11nm) zirconia filler, and aggregated zirconia/silica cluster (0.6 to 10 microns), comprised of 20 nm silica and 4 - 11 nm zirconia particles). **Filler content:** 78.5 wt.%
Adper™ Single Bond Plus Adhesive 5th generation Total etch adhesive system	51202	3M ESPE Dental Products, St. Paul, USA	Bis-GMA, Dimethacrylate resins, HEMA, Vitrebond™ Copolymer, Fillers, Ethanol, Water, Initiators
CharmEtch-37	2115050	DENKIST Inc. 265, Dangjeong-dong, Gunpo-si, Gyeonggi-do 435-831, Korea	Contains 37% phosphoric acid Strong acid pH < 0.5 Low viscosity
Single Bond Universal adhesive 6 th generation that could be used in total etch or self-etch manner	578362	3M ESPE Dental Products, St. Paul, USA	Methacryloxydecyl phosphate (MDP Phosphate) monomer, Dimethacrylate resins, HEMA, Vitrebond™ Copolymer, Fillers, Ethanol, Water, Initiators , Silane Mild self-etch PH 2.7

The remaining 20 brushed enamel/dentin slabs (10 control slabs brushed with the unmodified tooth paste and their corresponding test slabs which were brushed with the n-HAp modified toothpaste) were utilized to determine the μ-SBS of enamel to nano-filled resin composite restorative material (Z350, 3M, ESPE, USA). Each main control and test group (n= 10) was subdivided into 2 subgroups (n = 5) according to the type of the adhesive system used as follows: ***Sub-group I*:** The brushed enamel surface was ultrasonically cleaned and then etched with CharmEtch etchant gel (Dentkist, Korea) containing 37% phosphoric acid for 15 seconds, and rinsed with water jet for 10 seconds. Then the excess water was blotted using a cotton pellet leaving a glistening enamel surface. Immediately after blotting, 2-3 successive coats of the total-etch (TE) adhesive (Adper Single Bond Plus Adhesive) were applied for 15 seconds with gentle agitation using a fully saturated applicator then gently air thinned for 5 seconds to evaporate solvent. Finally, the coatings were light-cured for 10 seconds according to the manufacturer's instructions with LED curing unit (Coxo®, DB-685-SUPER-LUX, wavelength of 420-480 nm, light power ≥1200mw/cm^2, China). ***Sub-group II*:** The brushed enamel surfaces were ultrasonically cleaned and then treated with the Single Bond Universal Adhesive (3M, ESPE, USA) in self-etch mode (SE) for 15 seconds with gentle agitation using a fully saturated applicator then gently air thinned for 5 seconds to evaporate solvent. Finally, the coatings were light-cured for 10 seconds according to the manufacturer's instructions.

Teflon mold with a metallic ring and a split Teflon cover was prepared. The split Teflon cover contains 2 central holes to prepare 2 cylindrical

resin composite specimens (0.8 mm in diameter and 1 mm in height) on the center of the enamel/dentin slab surfaces. Each enamel/dentin slab within the acrylic resin block was inserted in the Teflon mold with its metallic ring after the application of the adhesives. In order to the confine the bonding areas, the split Teflon mold with its 2 central holes was placed over the enamel/dentin slabs before curing of the adhesive coats as mentioned in subgroups I and II.

Nano-filled resin composite (Filtek Z350 ^{XT}, 3M ESPE) was packed into the 2 central holes of the split Teflon mold, topped with matrix strip, and gently pressed to obtain flat surface. Then the resin composite was light-cured for 20 seconds according to the manufacturer's instructions. Afterwards, the Teflon mold was disassembled. Using a blade, any excess resin composite was gently removed and the obtained cylinders (0.8 mm in diameter and 1 mm in height) were additionally light cured for 20 seconds to ensure optimum polymerization of the resin composite. The specimens were stored in distilled water at 37 °C for 24 hours in the incubator (Cbm. Torre Picenardi (CR), Model 431/V., Italy) prior to testing.

Before μ-SBS testing, all specimens were examined under the optical microscope (magnification = 30x) for any defects. Specimens that exhibited interfacial gap formation or air bubble inclusion were omitted from the study and replaced with other specimens.

Each acrylic embedded enamel/dentin slab with its bonded resin composite micro-cylinders was secured with tightening screws to the lower fixed compartment of a universal testing machine (Model 3345; Instron Industrial Products, Norwood, MA, USA) with a load cell of 5 kN. Data were recorded using computer software (Instron® Bluehill Lite Software). A loop of an orthodontic wire (0.2 mm in diameter) was wrapped around the bonded micro-cylinder as close as possible to its base and aligned with the loading axis of the upper movable part of the testing machine. A shearing load was applied at a crosshead speed of 0.5 mm/min until failure occurred. The load at failure was recorded in Newton (N) and divided by the bonded area to calculate the μ-SBS (MPa) according to the following equation [21]:

$$\tau = P/ \pi r^2$$

Where: τ = micro-shear bond strength (MPa), **P** =load at failure (N), π =3.14, **r** = radius of the micro-cylinder (mm).

Failure mode evaluation and morphological examination of the de-bonded surfaces:

After μ-SBS testing, each specimen was photographed using USB Digital microscope with a built-in camera (Scope Capture Digital Microscope, Guangdong, China) connected to an IBM compatible computer using a fixed magnification of 65x. A digital image analysis system (Image J 1.43U, National Institute of Health, USA) was used to evaluate the failure mode. Failure modes were classified as one of three types: adhesive (failures occurred exclusively at adhesive interfaces), cohesive (failures occurred exclusively within enamel or the resin composite and mixed (adhesive and cohesive failures of the adjacent substrates). The percentage of the failure modes was calculated. Representative specimens were selected to study the morphology changes of the de-bonded surfaces within each subgroup. Gold coating of the specimens was performed for 1.5 minutes. Microscopic examination of the specimens was done by the scanning electron microscope (SEM), (Model Quanta 250 FEG (Field Emission Gun) with accelerating voltage 30 KV. FEI Company, Netherlands).

Statistical analysis

Numerical data were explored using Kolmogorov-Smirnov and Shapiro-Wilk normality tests. All data revealed parametric distribution. Data were presented as mean values and standard deviation. Student's t-test was used to compare between enamel surface roughness (Ra nm) of the

control and test groups (brushed with unmodified or n-HAp modified toothpastes respectively). Two-way ANOVA test was used to study the effect of the toothpaste (unmodified or n-HAp modified), adhesive system (TE and SE) as well as the interaction between the 2 variables on the μ-SBS of enamel to resin composite restorations. Tukey's post-hoc test was used for pair-wise comparisons when ANOVA test was significant. Failure mode data were presented as frequencies (n) and percentages (%). Fisher's Exact test was utilized to compare between the different groups and subgroups. The level of significance was set at $P \leq 0.05$. Statistical analysis was accomplished with IBM® SPSS® Statistics Version 20 for Windows.

RESULTS

Surface roughness (Ra)

The results revealed that the mean enamel Ra (nm) of the group brushed with the unmodified tooth paste (control group) was not significantly different than those brushed with the n-HAp modified tooth paste (test group) as seen in table 2. The AFM images of the control and test groups show the enamel surface topography at the same location of the tooth surface, but one represents a 2-dimensional image (Fig. 1A) and the other represents a 3-dimensional (Fig. 1B) image.

TABLE (2) Enamel roughness mean values (Ra nm) and standard deviation of the 2 groups

Tooth paste	Mean (nm)	Standard deviation	*P*-value
Control group (brushed with unmodified toothpaste)	332.6	122.0	0.627
Test group (brushed with n-HAp modified toothpaste)	361.1	31.9	

: Significant at P ≤ 0.05

Micro-shear bond strength (μ-SBS)

The Two-way ANOVA results showed that the toothpaste (unmodified or n-HAp modified) had a statistically significant effect on the mean μ-SBS (MPa). On the other hand, the adhesive system as well as the interaction between the 2 variables had no statistically significant effect on the mean μ-SBS (MPa). Since the interaction between the variables is not statistically significant, therefore, the variables are independent from each other (Table 3).

Regardless of the adhesive system used, the control group (brushed with the unmodified tooth paste) revealed statistically significant lower mean ***μ*-SBS** (MPa) than the test group (brushed with the n-HAp modified toothpaste) as presented in table 4. Therefore, using either TE or SE adhesive system with the control subgroups produced statistically significant lower mean ***μ*-SBS (MPa)** values than the corresponding test subgroups (Table 5, Fig. 3).

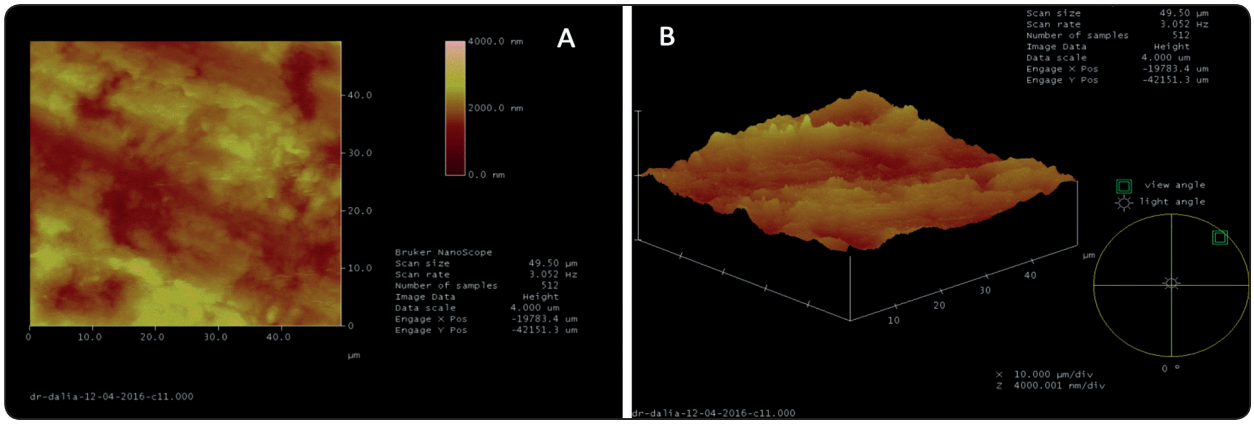

Fig. (1) Atomic force microscopy (AFM) contact mode images of the control enamel/dentin slab brushed with unmodified tooth paste (top view): A) 2-D image revealing the enamel smooth homogenous surface, B) 3-D image of the same location.

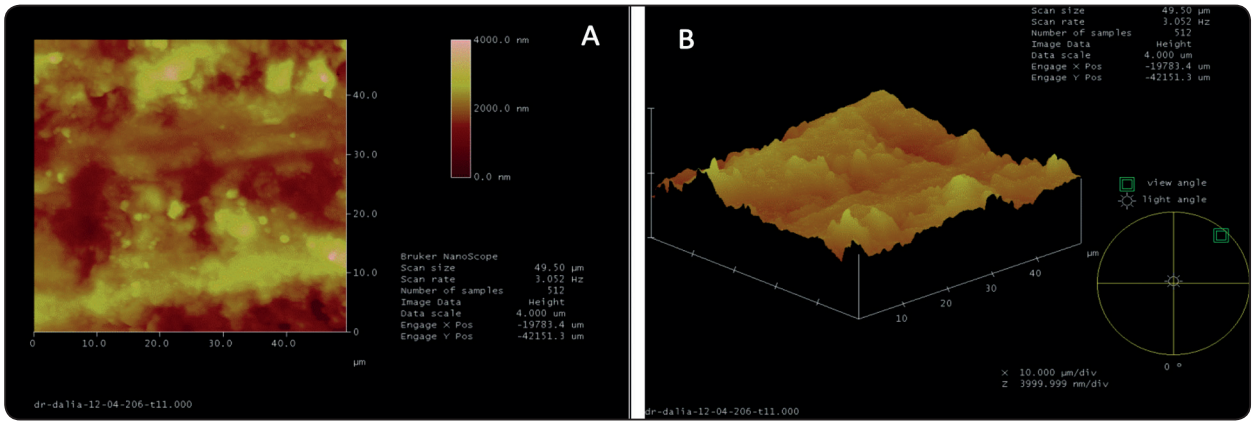

Fig. (2) Atomic force microscopy (AFM) contact mode images of the test enamel/dentin slab brushed with the n-HAp modified tooth paste (top view): A) 2-D image revealing the distribution of numerous closely packed nano-hydroxy appetite precipitates. B) 3-D image of the same location.

TABLE (3) Two-way ANOVA results for the effect of different variables on the mean μ-SBS (MPa)

Source of variation	**Type III Sum of Squares**	**df**	**Mean Square**	**F-value**	***P*-value**
Toothpaste (unmodified or n-HAp modified)	1287.7	1	1287.7	12.6	0.001*
Adhesive system (TE or SE)	74.3	1	74.3	0.7	0.400
Toothpaste x Adhesive system interaction	0.04	1	0.04	0.0001	0.985

df: degrees of freedom = (n-1), *: Significant at P ≤ 0.05

TABLE (4) Mean and standard deviation values of enamel/resin composite µ-SBS (MPa) of the 2 groups regardless of the adhesive systems

Enamel brushed with unmodified toothpaste (Control group)		**Enamel brushed with n-HAp modified toothpaste (Test group)**		***P*-value**
Mean (MPa)	Standard deviation	Mean (MPa)	Standard deviation	
30.2	8.0	41.5	11.6	0.001*

****: Significant at P ≤ 0.05***

TABLE (5) Mean and standard deviation values of enamel/resin composite µ-SBS (MPa) of the subgroups with each adhesive system

Adhesive system	**Enamel brushed with unmodified toothpaste (Control group)**		**Enamel brushed with n-HAp modified toothpaste (Test group)**		***P*-value**
	Mean (MPa)	Standard deviation	Mean (MPa)	Standard deviation	
TE (Total-Etch)	31.6	8.7	42.9	11.7	0.017*
SE (Self-etch)	28.8	7.3	40.2	11.9	0.016*

****: Significant at P ≤ 0.05***

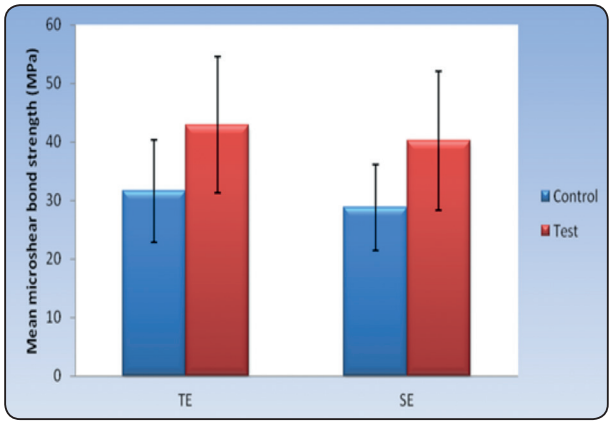

Fig. (3) Mean values of enamel/resin composite μ-SBS (MPa) of the subgroups with each adhesive system

Failure mode and morphological examination of the de-bonded surfaces:

There was a statistically significant difference between the failure modes in the different subgroups (P-0.033). The control/SE subgroup showed the highest prevalence of adhesive failures between the enamel and the adhesive system (50 %) while the test/TE subgroup presented the highest prevalence of cohesive failures within resin composite (10 %). On the other hand, the control/TE and the test/TE subgroups revealed the highest prevalence of mixed mode of failures (90%) where failures occurred adhesively at the enamel/adhesive interface and cohesively within the resin composites (Fig. 4).

The SEM micrographs of the de-bonded surfaces after μ-SBS testing are presented in Fig. 5 (A-D). The control/TE and the test/TE subgroups revealed mixed mode of failure as seen in Fig. 5,A and B respectively, where the de-bonded surface revealed enamel and resin composite islands separated by the adhesive system used. On the other hand, the control/SE and the test/SE subgroups exhibited adhesive mode of failure (Fig. 5,C and D respectively), where the de-bonded surface revealed homogenous enamel surface without any remnants of the adhesive systems in both the control (brushed with the unmodified toothpaste) and the test (brushed with the n-HAp modified toothpaste) subgroups. In addition, at higher magnification (4000x), the morphological changes of the enamel surface after de-bonding within each subgroup were observed (Fig. 6, A-D). In Fig. 6, A and B, the control/TE and the test/TE subgroups revealed more prominent enamel prisms with pronounced surface irregularities as compared with the corresponding subgroups treated with SE adhesive (Fig. 6,C and D).

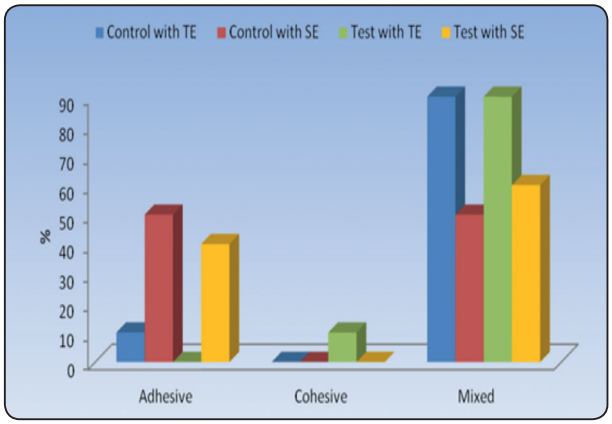

Fig. (4) Failure modes in the different subgroups with each adhesive system. *(Adhesive failure: At the enamel/ adhesive system interface, Cohesive failure: Exclusively within resin composite, Mixed failure: Both adhesive failure at the enamel/adhesive system interface and cohesive failure within the resin composite failure within the resin composite).*

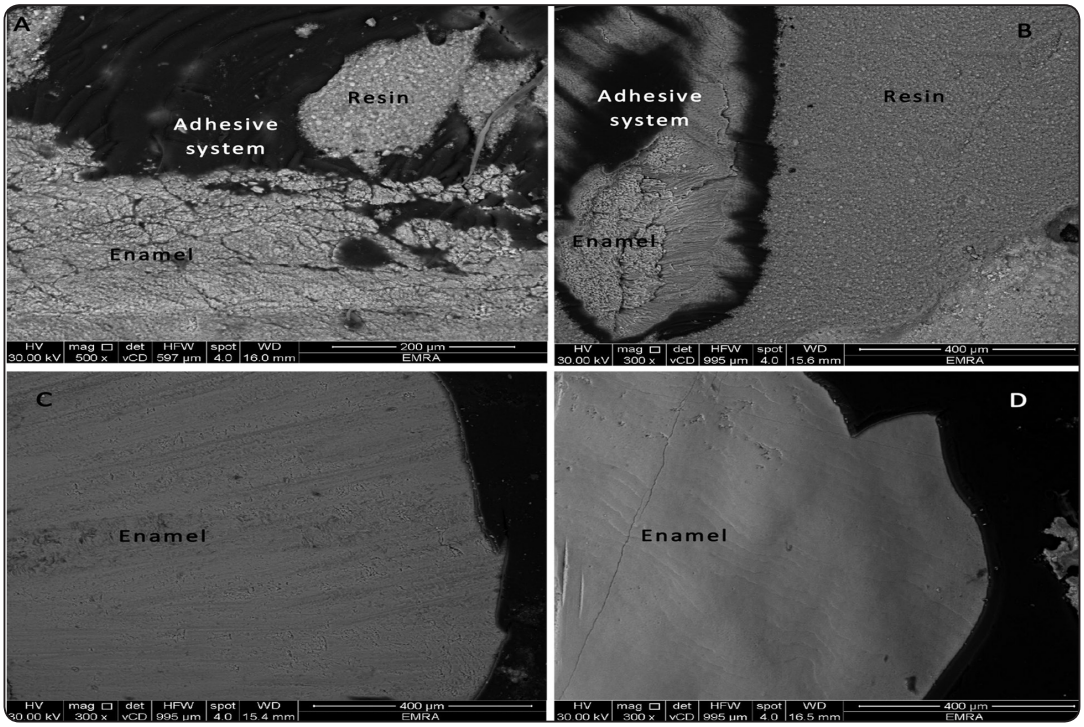

Fig. (5) SE micrographs representing the failure modes observed in the μ-SBS test (300 x) (A-D). The control/TE and the test/TE subgroups revealed mixed mode of failure (A and B respectively). The control/SE and the test/SE subgroups revealed adhesive mode of failure at the enamel/adhesive system interface (C and D respectively).

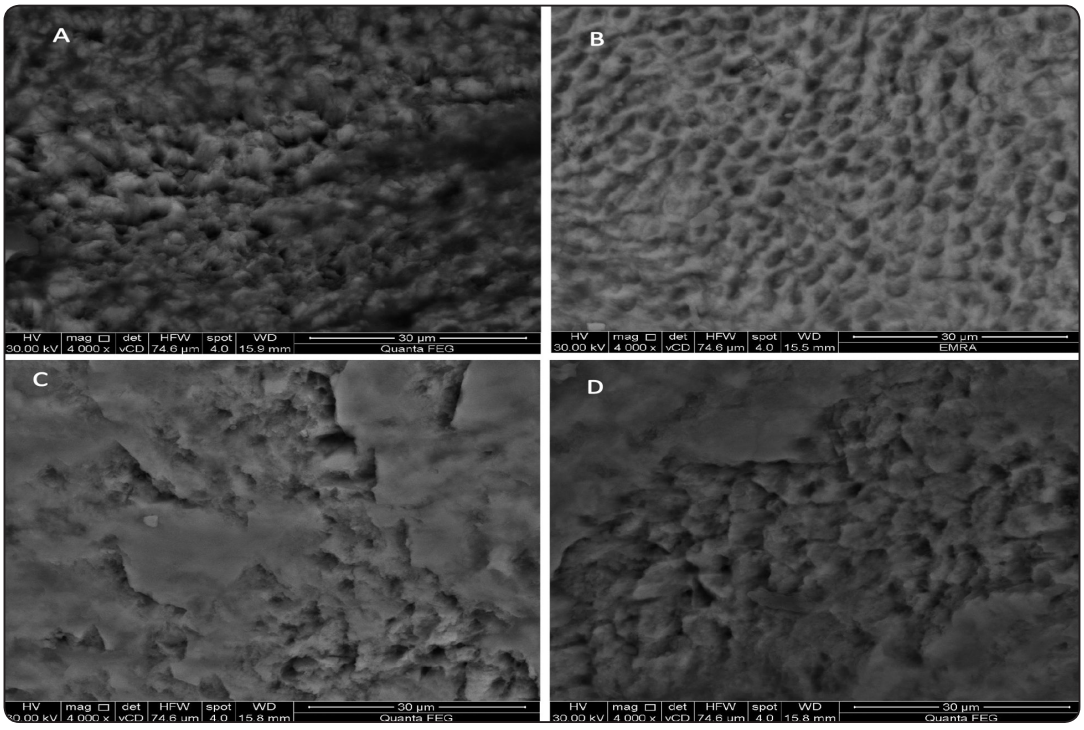

Fig. (6) SE micrographs revealing the morphological changes of the enamel surface after debonding under μ- shear stresses (4000x) (A-D). In A and B the control/TE and the test/TE subgroups, prominent enamel prisms with pronounced surface roughness can be seen. In C and D, the surface roughness of the enamel within the control/SE and the test/SE subgroups were shallower in depth with more intact enamel islands.

DISCUSSION

The improved oral care with the increased use of n-HAp containing products such as toothpastes in conjunction with the increased population awareness, have led to a reduction in caries incidence[6-11]. However, this has led to the development of certain clinical questions concerned with the effect of the prolonged use of such n-HAp products on enamel surface roughness and bonding ability to adhesive restorations. Therefore, the current study aimed at measuring the surface roughness (Ra) and the micro-shear bond strength (μ-SBS) of enamel to resin composite restoration using 2 adhesive systems (total-etch (TE) and self-etch (SE)) after simulated brushing for one year with a toothpaste modified by 10 wt.% nano-hydroxyapatite (n-HAp).

The first part of the null hypothesis was validated since the simulated brushing with n-HAp (10 wt. %) modified toothpaste for one year did not significantly affect the surface roughness (Ra) of enamel (Table 2). Nevertheless, the second part of the null hypothesis was rejected, as the simulated brushing with 10 wt. % n-HAp modified toothpaste for one year increased significantly the μ-SBS of enamel/resin composite with both adhesive systems (TE and SE) as shown in tables (3-5).

In the current in vitro study, brushing of the enamel surface with n-HAp modified toothpaste did not significantly alter its surface roughness (Ra) values as compared with those brushed with the unmodified toothpaste (Table 2). This could be related to the fact that the nano-size of the used HAp powder lacks the abrasive power that could significantly increases the surface Ra of brushed enamel. Regarding to the interaction between the n-HAp modified toothpaste and the enamel surface in vitro, n-HAp powder has a strong bonding ability to enamel surface. This ability is related to its nano-size, which considerably increases their surface energy and surface area/volume available for bonding [3]. This has been supported by the AFM images of the control group brushed with unmodified toothpaste and the test group brushed with the n-HAp modified toothpaste (Figs. 1, 2, - A, B respectively). The n-HAp present in the toothpaste precipitated as closely packed grins on the enamel surface during the simulated one year brushing as revealed by the AFM in the current study (Fig. 2 A, B) and confirmed by the SEM in a previous study[16]. Therefore, the n-HAp function is to protect the teeth with the formation of a new layer of synthetic enamel around the tooth rather than altering its surface roughness under conventional conditions [3,16]. It worth mentioning, that the obtained Ra (nm) mean values of enamel were slightly higher than those obtained from previous studies[13, 22-25]. This could be related to the topical application of the toothpaste instead of brushing and the method of specimen preparation. Additionally, the higher standard deviation value noticed among the control group could be related to the human variation among the teeth used.

Although the enamel surface roughness values obtained for both the control (brushed with unmodified toothpaste) and the test (brushed with the n-HAp modified toothpaste) groups (332.6± 122.0 and 361.1±31.9nm respectively) were higher than the reported critical threshold surface roughness for bacterial adhesion (200nm)[23], it should be noticed that this critical threshold was not obtained from enamel surface, but from resin strips and implants. These artificial materials are different in nature than enamel surface which is far more complex. The presence of waviness, pits, fissures and other enamel irregularities allows for easier protection of bacterial colonization[23,24]. Thus these straight rules may not be applied accurately from the clinical point of view.

The AFM provides a potent tool to investigate the surface morphology of a variety of biological samples with nanometer resolution. It is a useful

device to study site-specific structural topography of enamel and the changes in enamel after application of different materials[25]. Its main advantage over the other technologies is that it provides a comprehensive qualitative and quantitative data about Ra[26]. Generally, the advantages of this technique include: being nondestructive technique, do not require complex sample preparation and very sensitive. Meanwhile, the size of the scan area is frequently not greater than 100 μm x 100 μm [27]. Therefore, 3 scanning areas/slabs were used to calculate the average Ra of the enamel surface. Consequently, it is a time consuming technique and expensive[26,28]. The AFM images (Figs.1 and 2) in the current research were captured using the "contact" mode. Contact mode is most useful for hard surfaces and provides fast scanning for rough surfaces with higher resolution than other imaging modes (tapping and non-contact modes) [29,30].

Micro-shear bond strength (μ-SBS)

The bond strength test outcomes are affected by the substrate material, testing method and operator skills [31]. In the present study, human teeth were used for bond strength testing to simulate the clinical conditions. Although clinical trials produce the most reliable evidence, in vitro adhesion tests provide immediate data on the bonding efficiency of new materials and techniques [31]. The μ-SBS test was used in the current study instead of the of the macro-shear test to overcome its drawbacks that include: mixed loading mode, inhomogeneous stresses distribution over the area upon which the load is applied, and occurrence of premature failure in the dental substrate [32-34]. Additionally, the μ-SBS test allows testing of several specimens per tooth surface. Although, the micro-tensile bond strength (μ-TBS) test is a broadly used testing method for adhesive strength evaluation; as it is able to closely reflect the interfacial bond strength and offers uniform stress distribution, it possessed some limitations when testing the bond strength to enamel. High frequency of premature failures and complicated specimen production with high potential to induce micro-cracks at the interface were reported. This can affect bond failure, particularly in brittle materials like enamel [34, 35]. Therefore, when bonding to enamel is evaluated, the μ-SBS test method is considered to be more suitable method compared with the μ-TBS test method [35]. Consequently, in the current study the μ-SBS test was used.

In the present study, the control (brushed with unmodified toothpaste) group showed statistically significant lower mean μ-SBS (30.2±8.0 MPa) than the test (brushed with n-HAp modified toothpaste) group (41.5 ±11.6 MPa) as seen in table (4) regardless to the adhesive system used. Furthermore, the μ-SBS of the enamel/resin composite with either TE or SE adhesive system among the control subgroups produced statistically significant lower mean μ-SBS (MPa) than the corresponding test subgroups (Table 5, Fig. 3). These findings could be attributed to the differences in the enamel substrate rather than the effect of the adhesive system type (TE or SE).

After simulated one year brushing with the 10 wt.% n-HAp modified toothpaste, precipitation of the n-HAp layer (~5μm thickness) has been confirmed chemically and microscopically in a previous study conducted under the same testing conditions [16]. This precipitated layer differs than the biologic enamel in terms of; composition, surface energy, surface area and wettability [3,5,6]. Concerning the composition, it is formed of inorganic phase only while the biologic enamel is a composite structure of organic (~4 wt.%) and inorganic (96 wt.%) phases [36]. Furthermore, owing to the nano-scale effect, the precipitated n-HAp powder would increase the enamel surface energy, wettability and surface area available for bonding. Consequently, this could enhance the μ-SBS of enamel to resin composite restorative material within the test (brushed with n-HAp modified toothpaste) subgroups as compared to the control (brushed with unmodified toothpaste) subgroups. Moreover, the μ-SBS results of the control subgroups in the present study are in agreement with previous studies [21, 37].

Concerning the mode of failure, it has been noticed that both the control and test subgroups with the SE adhesive system behaved in the same manner; exhibiting the highest prevalence of adhesive mode of failure (50 and 40 % respectively). On the other hand, both the control and test subgroups with the TE adhesive system presented with 90% mixed mode of failure as seen in Figs. 4 and 5 (A-D). This could be explained in relation to the differences in the chemical composition and mechanism of action of both adhesive systems used.

Generally, the basic principles of acid etching wither in TE or in SE adhesive systems are: to transform enamel surface from a low surface energy into a higher surface energy, selectively dissolve and demineralize the inorganic matrix, creating micro-pores and micro-grooves to improve mechanical retention. The average etching depth as reported in earlier studies is ranged from 1-5 μm according to the degree of acidity and time of application of the used etching agent [38, 39]. In the TE adhesive system, 37 % phosphoric acid (for 15 seconds) was used to modify the enamel surfaces. Phosphoric acid is considered as a strong acid with an approximate pH value < 0.5 (Table 1). On the other hand, in the SE adhesive systems, the acidity arises from the acidic monomers (MDP). Typically, these compounds bear carboxylic or phosphoric acid groups that demineralize and penetrate into the enamel surface simultaneously. It is not rinsed away just air dried and then light cured. Thus, creating mechanical retention pattern by etching the tooth structure, and chemical bonding by complex formation with the calcium ions present at the tooth. However, the pH value of the used SE adhesive system was 2.7 which is considerably higher than that of phosphoric acid (pH< 0.5). Therefore, such reduced acidity (higher pH) in the SE adhesive system may results in less pronounced enamel etching pattern compared to enamel etched with phosphoric acid in the TE adhesive system. This has been supported by the SEM examination of the de-bonded surfaces under μ-shear stresses at higher magnification (4000X)where islands of enamel surfaces were detected (Fig. 6, A-D). In the SEM micrographes (Fig. 6,A and B), the control (brushed with unmodified toothpaste/TE) and the test (brushed with the n-HAp modified toothpaste / TE) subgroups revealed selective demineralization of enamel prism peripheries and cores. Prominent surface roughness was observed with subsequent increase in the surface area available for bonding. On the other hand, in the SEM micrographs presented in Fig. 6,D and C, the enamel surface of the control/SE and the test/SE subgroups resembles the pattern of surface roughness appeared in Fig. 6, A and B respectively, but with shallower depth and more intact enamel areas.

In other words, phosphoric acid (TE adhesive system) is more effective in dissolving the minerals of the enamel to expose the prismatic crystal structure in biological enamel (control subgroup) and to dissolve the purely inorganic layer of n-HAp (test subgroup) as compared to the acidic monomer in the SE adhesive system treated subgroups. These findings are in agreement with previous morphological study of etched enamel surfaces [40]. Additionally, it has been reported that bonding to enamel is mainly attributed to the ability of the resin to penetrate between the enamel crystallites and rods [41-43] which are in accordance with the findings of the current study.

CONCLUSIONS

From the obtained results, it was concluded that simulated one year brushing with 10 wt. % n-HAp modified toothpaste did not affect the enamel surface roughness. However, it has a significant positive effect on the μ-SBS regardless to the adhesive system used. On the other hand, the type of the adhesive system wither total-etch or self-etch appears to have a principal effect on the mode of failure rather than on the bond strength to the brushed enamel surface.

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CONFLICTS OF INTEREST

The authors declare no conflicts of interest. The authors alone are responsible for the content and writing of the paper.

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