Effect of using Different Remineralizing Agents on Micro-shear Bond Strength of Nanohybrid Composite Resin

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Abstract

AIM: This study was conducted to evaluate the effect of different remineralizing agents on micro-shear bond strength (SBS) of nanohybrid composite resin to dentin.

METHODS: Thirty-six human molars were divided into four main equal groups (nine teeth each) according to the type of remineralizing agent used; nanohydroxyapatite, sodium fluoride, fluorohydroxyapatite, and control without remineralizing agent. Each group was divided into three equal subgroups (three teeth each) according to the storage time: 1 day, 1 month, and 3 months. Specially fabricated cylindrical plastic mold was made, and teeth were embedded vertically in the mold to the level of cementoenamel junction of the tooth leaving the occlusal surface projecting above the surface of the mold. Cylindrical fissure carbide bur was used in teeth preparation. Teeth were trimmed perpendicular to the long axes of them. Demineralizing agents (Nano hydroxyapatite, sodium fluoride, and fluorohydroxyapatite) were applied then adhesive system applied then composite resin was applied using five sections of a pediatric intravenous tube to act as molds for composite specimen then the specimens stored in artificial saliva at 37°C for different storage times in an incubator. The micro-SBS was assessed using universal testing machine. Then, the mode of failure for each group was determined using stereomicroscope device. Then, the obtained data were tabulated and statistically analyzed. One-way ANOVA was used to compare between more than two non-related samples. The significance level was set at p ≤ 0.05. Statistical analysis was performed with IBM SPSS Statistics.

RESULTS: There was statistically significant difference between the four studied groups regarding bond strength at different storage times. Fluorohydroxyapatite had the highest bond strength mean values followed by nanohydroxyapatite, while the lowest value was the sodium fluoride group.

CONCLUSION: Fluorohydroxyapatite and nanohydroxyapatite had a positive effect on micro-SBS to dentin, but sodium fluoride had a negative effect. The storage time increase had a positive effect on the micro-SBS of dentin with fluorohydroxyapatite and nanohydroxyapatite, also it had adverse effect on the dentin bond strength with sodium fluoride and control groups.

Introduction

Remineralization is defined as the process whereby calcium and phosphate ions are supplied from a source external to the tooth to promote ion deposition into crystal voids in demineralized enamel to produce net mineral gain [1]. The availability of calcium and phosphate ions is the limiting factor for net remineralization to occur [2]. Several investigators have worked toward developing the ideal remineralizing agent, which diffuses into the subsurface or delivers calcium and phosphate into the subsurface [3]. The structure of enamel is too complex to be remodeled and the basic enamel building blocks are generally 20–40 nm hydroxyapatite (HA(Ca$_x$(PO$_y$(OH))$_z$) nanoparticles. Therefore, the remineralization of enamel minerals using synthetic apatite or HA that resembles enamel HA may be beneficial [2]. Hydroxyapatite is one such material exhibits excellent bioactive properties and striking similarities to dental hard tissues. Approximately 97% of tooth enamel and 70% of dentin are composed of hydroxyapatite [4]. Nanostructured HA crystals exhibit high levels of biomimetic properties due to their composition, structure, morphology, bulk, and surface physical–chemical properties [5]. Fluoride is the most commonly used remineralizing agent [6], [7]. Fluoride has a high affinity to calcified tissues [8]. Topical and/or systemic fluoride exposure is very common. Therefore, it is important to evaluate the influence of fluoride on remineralization which can affect tooth quality. Tooth quality is related to the tooth’s ability to fulfill its function, including withstand occlusal forces, and may be evaluated by the measurement of a tooth’s material, structural, and mechanical properties [7]. Nowadays, the request for esthetic restorations has been dramatically increased. Adhesive systems are available to attach the restorations to dental tissue. The efficiency and quality of adhesive systems are important in producing a stable connection between composite and dental structure. Shear bond strength (SBS) is an important factor in efficiency and quality of adhesive systems because of its essential role in bonding composite to dental tissue. Inadequate SBS causes early failure of restoration in the face of minimum masticatory forces [8].

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Materials and Methods

A total number of 36 freshly extracted, sound human molar teeth were used in this study. The teeth were stored in distilled water at 37°C. Distilled water was changed daily [9]. Teeth were divided into four main equal groups (nine teeth each) according to the type of remineralizing agent; group (A) with nanohydroxyapatite, group (B) with sodium fluoride, group (C) with fluorohydroxyapatite, and group (D) control without remineralizing agent. Each main group was divided into three equal subgroups (three teeth each) according to the storage time; (S1) 1 day, (S2) 1 month, and (S3) 3 months [10]. A specially fabricated cylindrical plastic mold with internal diameter 15 mm×20 mm in height was fabricated. The internal surface of the mold was coated with separating medium. The mold was filled with self-curing acrylic resin and the base of the mold rested on a glass slab to obtain a flat smooth surface. Each tooth was embedded vertically in the mold while the acrylic resin still in the dough stage to the level of cementoenamel junction of the tooth leaving the occlusal surface projecting above the surface of the mold [11]. Cylindrical fissure carbide bur in a high speed hand piece was used with copious air water spray was used in teeth preparation. Occlusal enamel was removed to expose flat dentin surfaces. Teeth were trimmed perpendicular to the long axes of them. The new exposed dentin surfaces were thoroughly washed with water to ensure removal of any cut debris. Nanohydroxyapatite and fluorohydroxyapatite powder had been embedded into PEO* gel (*Polyethylene oxide-based gel) separately with concentration 10 wt. % [12] to obtain gel forms of both. Nanohydroxyapatite gel was applied on all dentine surfaces using micro brush and left for 2 min. It was repeated for 5 consecutive days. During this time, the specimens were stored in artificial saliva at 37°C. In last time of application, NHa gel was washed with distilled water for 15 s to ensure complete removal of excess and then air dried. Self-etch adhesive system (OptiBond-All-in-One, Kerr) was applied, according to the manufacturer’s instructions, using disposable micro brush and rubbed for 20 s on the entire dentin surface. Subsequently, air thinning was done for approximately 5 s until the adhesive layer no longer moves, indicating the complete vaporization of the solvent. Fluorohydroxyapatite was added the same as mentioned in nanohydroxyapatite. Sodium fluoride gel is applied to dentin surface and left for 5 min then excess is removed with distilled water for 15 s then bonding agent applied according to the manufacturer instruction and no application of remineralizing agent in the control group then the specimens stored in artificial saliva at 37°C for different storage times as mentioned before and all were added in an incubator. Five sections of a pediatric intranervous tube of 3 mm length with 1 mm internal diameter were cut using sharp scissors to act as molds for the preparation of the nanohybrid composite (Harmonize, Kerr) specimens. The cut tubes were placed on the dentin surface and then light cured for 10 s keeping the curing light tip within about 2 mm from the surface, using LED curing device (Woodpecker light cure unit Led-F (LK-G38), China). Application of the nanohybrid composite (Harmonize, Kerr) in the cut pediatric tube parts was done using periodontal probe (1 mm diameter, by dent supply), then curing for 30 s, then the pediatric tubes were cut vertically and removed to obtain nanohybrid composite cylinders bonded to dentin surface. Each mold was horizontally secured with tightening screws to the lower fixed compartment of a universal testing machine with add of 5 KN and data recorded using computer software. A loop prepared from an orthodontic wire was wrapped around the bonded micro cylinder assembly as close as possible to the base of micro cylinder and aligned the loading axis of the upper movable compartment of the testing machine. A shearing load with tensile mode of force was applied through universal testing machine at across head speed of 0.5 min. The relatively slow cross head speed was selected to produce a shearing force that resulted in debonding of micro cylinder along the substrate adhesive interface. The load required to debonding was recoded in megapascals (MPa) [13]. After micro-shear bond testing all the fractured specimens, were examined using a stereomicroscope at ×25. Stereomicroscope device adjusted to allow for proper vision of the fractured area of the specimens to determine the type of failure mode which could be:

a. Cohesive failure = Failure within dentin or resin composite material.
b. Adhesive failure = Failure in which no resin composite was seen on dentin surface.
c. Mixed failure = Failure in which part of resin composite was seen retained on dentin surface.

Another group of eight molars in acrylic blocks was ground to have a flat dentin surfaces as mentioned before and divided into four groups of two teeth each according to remineralizing agents (NHa, NaF, Fluoro Ha, and control) then application of nanohybrid composite (Harmonize, Kerr) on the all occlusal surfaces of specimens then stored in artificial saliva for 24 h. After storage, the crown of each specimen was sectioned using a slow speed diamond disk under water cooling fixed into IsoMet 4000 µ saw device, to produce multiple slaps of full-sized crown (composite and dentin) with thickness of 1 mm. Each specimen surface was immersed in 6 mm/l hydrochloric acid for 30 s to remove the smear layer then rinsed with distilled water and immersed in 5.25% sodium hypochlorite for 10 min to remove the free un-encapsulated collagen. Specimen was further rinsed with distilled water and dehydrated through immersion in 99.9% ethyl alcohol. Resin dentin interface of each disk sample was analyzed using environmental scanning electron microscope at ×1500. The mean and standard deviation values were calculated for each group. Data were explored for normality using
Kolmogorov–Smirnov and Shapiro–Wilk tests and showed parametric (normal) distribution. Independent sample-t test was used to compare between two non-related samples. Repeated measure ANOVA was used to compare between more than two related samples. One-way ANOVA was used to compare between more than two non-related samples. The significance level was set at $p \leq 0.05$. Statistical analysis was performed with IBM SPSS Statistics Version 20 for Windows.

Results

It was found that regardless of the storage times, fluorohydroxyapatite groups showed the highest bond strength mean value followed by nanohydroxyapatite group while the lowest was sodium fluoride group. There was statistically significant difference found between the four studied groups regarding bond strength.

| Variable          | Mean   | SD    | p-value |
|-------------------|--------|-------|---------|
| Treatment groups  |        |       |         |
| NHa               | 17.42  | 1.01  | 0.027   |
| NaF               | 15.07  | 2.74  |         |
| Fluoro Ha         | 19.01  | 2.10  |         |
| Control           | 16.52  | 0.88  |         |

SD: Standard deviation.

There was no statistically significant difference found between control group and NaF group, while there was statistically significant difference found between control group and fluoro Ha, NHa group, and also between fluoro Ha, NHa group, and NaF (Table 1 and Figure 1).

![Figure 1](https://www.id-press.eu/mjms/index)

Figure 1: Bar chart showing mean values of micro-shear bond strength (MPa) of different remineralizing agents regardless of different storage time

Furthermore, it was found that 3 months storage time group had the highest mean value followed by 1 month, and the lowest mean value was 1 day. There was statistically significant difference found between the three studied groups regarding bond strength at different storage times (Table 2 and Figure 2).

| Variable | Mean    | SD    | p-value |
|----------|---------|-------|---------|
| 1 day    | 15.0175 | 2.414 | 0.027   |
| 1 month  | 17.015  | 1.37  |         |
| 3 months | 17.565  | 1.265 |         |

SD: Standard deviation.

The failure mode of each dentin specimen was recorded after micro-shear test using stereo microscope.

- **Cohesive failure**: The highest frequency of the cohesive failure pattern was recorded with fluoro Ha at 3 months.
- **Adhesive failure**: The highest frequency of the adhesive failure pattern was record with fluoro Ha at 3 months.
- **Mixed failure**: The highest frequency of the adhesive failure pattern was recorded with control group at 1 day.

The results of scanning electron microscope revealed that the control group had the highest depth of penetration followed by nanohydroxyapatite group, while the shallowest depth of penetration was with sodium fluoride group.

![Figure 2](https://www.id-press.eu/mjms/index)

Figure 2: Bar chart showing mean values of micro-shear bond strength (MPa) of different storage times regardless of remineralizing agents

Discussion

An absolutely integrated tooth-restoration junction is an important goal that is hardly achieved. Indeed, irregularities at the cavosurface junction and microgaps at the tooth-restoration interfaces are always present and induce bacterial accumulation leading to tooth decay. Therefore, the remineralization concept on the restorative tooth surface junction is a preventive method in restorative dentistry [14]. NHA is considered as one of the most biocompatible and bioactive...
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Materials used in dentistry in the recent years [15]. NHA at a concentration of 10% is capable of remineralization process on enamel surface [16], [17]. Dentin remineralization is more complex than enamel remineralization; even so dentine remineralization is clinically significant since it would contribute to the minimally invasive management of dentine caries, root caries, and dentine hypersensitivity [18]. The pivotal discovery of fluoride as agent that could prevent dental caries was one of the most important landmarks in dentistry [19]. Although traditional fluoride-based remineralization remains the cornerstone for caries management with the highest level of supporting evidence, additional remineralizing agents to enhance the fluoride effects are often needed in high caries risk individuals and population groups [20], [21]. New methods that could be used as alternatives to fluoride are synthetic hydroxyapatite materials (mixture of HA nanoparticles and sodium fluoride), due to the chemical similarity with natural HA crystals. Remineralization of enamel and dentin using synthetic hydroxyapatite was investigated in many dental researches [22]. In vitro dynamic pH-cycling experiments have shown that nHA had the potential to remineralize initial enamel lesions with a comparable or even superior efficacy to that of fluoride [11], [23], [24], [25]. Another in vitro study found that nHA gel had significant potential for enamel remineralization around restoration margins [26]. Li et al., 2008, [5] and Pepla et al., 2014, [17] stated that the mechanism of nHA biomimetic function is unclear and it may promotes remineralization through the creation of a new layer of synthetic enamel around the tooth or by depositing apatite nanoparticles in the enamel defects. However, another research by Huang et al., 2011, [23] have proposed that nHA acts as calcium phosphate reservoir maintaining a state of supersaturation with respect to enamel minerals, thereby inhibiting demineralization and enhancing remineralization. For more user-friendliness and less time effort, self-etch adhesive (OptiBond-All-in-One, Kerr) was used in this study as it can etch and prime simultaneously and dissolve the smear layer with acidic monomers, thus enabling a single application procedure of a so-called “all-in-one” adhesive. Self-etch adhesives demineralize and infiltrate into the dentin surface, theoretically preventing incomplete penetration of the adhesive into the exposed collagen network. These systems contain specific functional monomers such as 10-MDP, 4-MET, and phenyl-P with carboxylic and phosphate groups. These functional groups can ionically bond with calcium in hydroxyapatite providing satisfactory chemical bonding to dentin [27]. Nanohybrid composite resin (Harmonize, Kerr) was used as it offers the advantages of durability, low polymerization shrinkage, high polishability, easy handling, and superior esthetic properties due to the nano-sized filler particles and higher filler content [28]. Different mechanical tests have been proposed to assess the bonding performance of restorative materials. Testing SBS is a relatively simple, reproducible and widely accepted test. Recently, micro-SBS test is a new test method that use specimens with reduced dimensions and used as a substitute for the conventional shear test [29]. This test allows for testing a small areas and preparing multiple specimens from same tooth. It also provides better stress distribution that can be accomplished in smaller specimens, since the number of voids and stress-raising factors is lower than that possibly occur in larger areas such those used for shear or tensile bond strength tests [30]. Water storage is the most used artificial aging technique. The specimens were stored in fluid at 37°C for a specific period. This period may vary from a few months. Hence, distilled water was used currently as storage media for the specimens, for 24 h, 3 months, and 6 months [10]. The results of this study revealed that fluorohydroxyapatite group had the highest bond strength mean value (19.01 MPa), followed by nanohydroxyapatite (17.42 MPa), while the lowest bond strength was in sodium fluoride group (15.07 MPa) these results could be due to the fact that hydroxyapatite materials have the potential to biomimetically remineralize dentin by replacing matrix and water with apatite crystallites. This exchange would increase mechanical properties and inhibit water-related hydrolysis, thus enhancing the bond strength with time. The preservation of hydroxyapatite within the submicron hybrid layer may serve as a receptor for additional chemical bonding [31], [32]. The presence of calcium and phosphate ions correlates to this chemical bonding. Furthermore, fluoride component of the nanohydroxyapatite increases the mineral content of the dentin on the expense of proteinious material of dentin or its water content. These results are in agreement with Mahmoud [33], who found that synthetic compound of fluorohydroxyapatite increases bond strength to dentin rather than hydroxyapatite itself. This is also agreed by Garcia et al., 2015, [30], Chermont et al., 2015, [32] and Gupta et al., 2017, [34], who concluded that nanohydroxyapatite has a beneficial effect on the SBS to dentin. However, these results were not consistent with Saraç et al., 2009 [35], who concluded that NHa treatment of dentin decrease its bond strength this controversy may be due to differences in the materials or methods that were used. The bond strength values were the lowest among all groups in sodium fluoride. This is may be due to the fact that sodium fluoride did not form organometallic complexes with dentin, resulting in an interface that was quite weak and acid resistant causing decreased bond strength [36]. This was in agreement with Diamanti et al., 2010 [37], who concluded that sodium fluoride has a deleterious effect on SBS of dentin. Furthermore, the result of this study revealed that 3 months storage time had the highest bond strength mean value (17.565 MPa), followed by 1 month storage time (17.15 MPa), while the lowest mean value was 1 day storage time (15.01 MPa). The increase in bond strength with time may be explained by the impossibility for resin monomers to
completely displace water within the extrabril and particularly the intrafibrillar compartments of a demineralized collagen matrix, and infiltrate the collagen network completely but, relate that hydroxyapatite materials have the potential to biomimetically remineralize dentin by replacing matrix and water with apatite crystallites. This exchange would increase mechanical properties and inhibit water-related hydrolysis, thus enhancing the bond strength with time. The preservation of hydroxyapatite within the submicron hybrid layer may serve as a receptor for additional chemical bonding with time. The presence of calcium and phosphate ions correlates to this chemical bonding [31]. This was in agreement with Gupta et al., 2017, who found that increase in storage time of dentin treated samples with hydroxyapatite compounds up to 6 months leads to increase in micro-SBS. In the control group and NaF group with storage time increase, there is a decrease in bond strength, and this could be explained by the reduced bond strength after storage for 1 and 3 months could be attributed to the excessive hydrophilicity of self-etch adhesives. They act as semipermeable membranes, even after polymerization, permitting water movement from the substrate toward them [38], [39]. Consequently, small droplets can be found at the transition between the adhesive layer and the lining composite which also contribute to the hydrolysis of resin polymers and therefore, the degradation of tooth-resin bond over time [40], [41]. After polymerization, the hybrid layer provided micromechanical retention [42]. The rationale behind this is that as long as the dentin is kept fully hydrated, the dentin matrix does not collapse, and free spaces are available allowing resin infiltration and good adhesion [43]. Another factor could be related to the precence of the matrix metalloproteinase that affects the integrity of the interface between the composite and tooth structure and this is in agreement with Kato et al., 2014 [44], who concluded that there is decrease in bond strength to dentin with non-treated dentin samples and with fluoride treated samples by time.

Furthermore, the result of the present study revealed that the frequency of the cohesive and mixed failure increase with fluoro Ha and NHa than adhesive failure this may be due to the higher bond strength values with these two materials and not attributed to mechanical properties of composite because of the higher mechanical properties of nanohybrid composite [45] this was in agreement with Pei et al., 2013 [46], and Gupta et al., 2017 [34], who found that there was a higher percentage of cohesive and mixed failure rather than adhesive failure with NHa compounds treated samples. On the other hand, in control and NaF groups there was increase in the adhesive failure than cohesive and mixed type and this is may be due to decreased bond strength values with these two groups and higher mechanical properties of the composite and these results agreed with Saraç et al., 2009 [35], and Devabhaktuni and Manjunath 2011 [36], who found that there was increase in adhesive failure with fluoride treated dentin samples. The results of scanning electron microscope examination revealed that the deepest penetration of the dentinal tubules was recorded in control group, followed by NHa treated group, while the shallowest penetration found in NaF group and this can be explained as NaF forms organometallic compounds with tooth substrate that is acid resistant so decrease the depth of penetration of adhesives within dentinal tubules, and this was in agreement with Devabhaktuni and Manjunath 2011 [36], who concluded that NaF forms organometallic with dentin that decrease acid effect and decrease diffusion into dentinal tubules.

**Conclusion**

Floro hydroxyapatite and nano hydroxyapatite had a positive effect on micro shear bond strength to dentin, but sodium fluoride had a negative effect. The storage time increase had a positive effect on the micro shear bond strength of dentin with Floro-hydroxyapatite and nano hydroxyapatite, also it had adverse effect on the dentin bond strength with sodium fluoride and control groups.

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