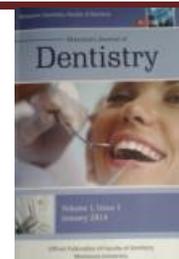




Viscosity and micro-tensile bond strength of total-etch adhesive system reinforced with hydroxyapatite nano-particles



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Abstract:

Objectives: the purpose of this study was to evaluate the effect of HA nanofiller addition to two steps etch and rinse adhesive resin on its viscosity and microtensile bond strength.

Methods: The HA nanoparticles were prepared using wet precipitation method by neutralization of calcium nitrate tetrahydrate [Ca(NO₃)₂·4H₂O] with ammonium dihydrogen orthophosphate [NH₄H₂PO₄]. The nano-particles were treated with silane coupling agent and then added to dental adhesive in different ratios producing four adhesive groups [group 1(control), group 2 (0.2 wt% HA), group 3 (0.5 wt% HA) and group 4 (1 wt% HA)]. The viscosity of the adhesives were measured with a Brookfield rheometer. Forty extracted human molars were ground flat to expose occlusal dentin. The exposed dentin surfaces were polished and rinsed, and the adhesives were applied. Composite buildups were performed with Glacier micro-hybrid composite. The specimens were sectioned perpendicular to the adhesive interface to produce dentin-resin composite sticks, with an adhesive area of approximately 1 mm². The sticks were subjected to tensile loading until failure occurred. The data were analyzed using One-way ANOVA and Least Significant Difference (LSD) tests.

Results: Highest mean viscosity value was (2.53 ± 0.43 centipoise) for group 4 adhesives and the lowest value was for group 2 (0.78 ± 0.13 centipoise). There was a significant difference in viscosity among the adhesive groups (P < 0.0001). Group 2 adhesives showed the highest microtensile bond strength value (35.39 ± 6.96 MPa), while group 4 showed the lowest value (28.82 ± 5.03 MPa). There was a significant difference in microtensile bond strength among the studied groups (P ≤ 0.05).

Conclusions: Modification of two-steps etch and rinse adhesive system with 0.2 wt% HA nano-fillers decreased viscosity and improved the microtensile bond strength. On the other hand, 1 wt% HA-modified adhesive showed increased viscosity and decreased microtensile bond strength.

Keywords: Viscosity, total-etch adhesive, hydroxyapatite, bond strength.

Introduction

The main goal of the adhesion of restorative materials to dental hard tissues is to obtain high bond strength and a satisfactory, long-lasting seal [1,2]. Dental adhesives are widely used in modern dentistry to provide retention of composite restorations. Current adhesive systems are classified into two major categories; etch-and-rinse and self-etch adhesives, based on the mechanisms by which they interact with the smear layer [3,4]. The difference between the two approaches is represented by the use of a preliminary and separate etching step for etch and-rinse systems (usually characterized by a gel of 35-37% phosphoric acid) that is later rinsed away.³ Conversely, the self-etch/primer agent is only air-dried, remaining within the modified smear layer, thus, it could be called an "etch-and-dry" approach. Etch-and-rinse adhesive systems can be either three- or two-step depending on whether primer and bonding are separated or combined in a single bottle. Similarly self-etch adhesives can be either two- or one-step systems depending on whether the etching/primer agent is separated from the adhesive or combined with it to allow a single application procedure [4].

Recently, nanoparticles have been used in the formulation of restorative composite systems [5]. With the

aim of improving physical and mechanical properties, nanoparticles have also been incorporated into the dental adhesives. Because of their very small sizes, nanoparticles are able to penetrate into the dentinal tubules along with the adhesive resin matrix providing a composite adhesive layer with improved properties (decreased polymerization shrinkage and increased the elastic modulus of the adhesive layer) [6,7].

Another potential area in which the performance of dental adhesives may be enhanced is bioactivity [8]. A bioactive dental material would have several benefits including remineralization of adjacent tooth substance, potential closure of gaps between material and tooth and potentially better bond strength over time (less degradation of bond). In general, today's dental restorative materials are not considered to be bioactive [9]. Currently, only a few attempts to develop bioactive dental materials have been published based on either calcium aluminate (ceramics) or via addition of calcium phosphates to resin-based materials [10,11].

Hydroxyapatite (HA) Ca₁₀(PO₄)₆(OH)₂, which is a form of calcium phosphate, is one of the most biocompatible materials [12,13]. The mineral phase of bone and teeth is mainly hydroxyapatite (HA). Therefore, synthetic HA would seem a good choice as inorganic filler

to be used in dental restoration or bone implants. The use of HA in restorative dentistry offers several promising advantages, including intrinsic radio-opaque response, enhanced polishability, and improved wear performance, because synthetic HA has a hardness similar to that of natural teeth. Finally, this material is less expensive than most of the inorganic fillers in use today [14].

In the present study, hydroxyapatite nanoparticles were synthesized and then added in three different ratios into unfilled two-step etch and rinse adhesive system (one-bottle dentin bonding system) to evaluate the effect of their addition on its viscosity and microtensile bond strength.

Materials and methods

Hydroxyapatite nanoparticles were synthesized using a wet method by dropping 0.387 M $(\text{NH}_4)_2\text{HPO}_4$ into 0.5 M $\text{Ca}(\text{NO}_3)_2$ solution with continuous stirring at room temperature and under N_2 atmosphere. After 30 minutes of mixing, ammonium hydroxide was dropped into the mixed solution to precipitate HA and the reaction was terminated at pH 11 which could produce the stoichiometric Ca/P ratio of 1.67. After further stirring for 30 minutes, HA precipitates were filtered and washed repeatedly to remove the unwanted ions. The precipitates were then re-suspended with distilled water into 200 ml Teflon container. After the mixture was stirred well, the container was tightly sealed in a stainless steel autoclave and heated at 120 °C for 6 hrs. The autoclave was cooled down and the products were filtered and washed with ethanol. Finally, the precipitates were filtered, dried at 70 °C for 16 hrs. The dried HAP powders were calcined at 200 °C for 4 hrs. The obtained white sample was pulverized, sieved and stored in a desiccators [15]. The nanofillers were surface treated with 0.3 wt% of silane coupling agent to improve their wetting property [16]. Finally, four adhesive groups were prepared (one adhesive contains zero nanofiller, three adhesives (Table 1)

A rheometer (Brookfield Rheometer Programmable Model DV-III, Brookfield Engineering Laboratories, INC, USA) was used to measure the viscosity of the different adhesives at a given shear rates. Cone and plate geometry was used. This consisted of rotating cone and a stationary plate (cone angle 5, cone diameter 40 mm and gap 0.2 mm) with the sample was filled in the gap between them. Two ml of each adhesive sample was placed in the plate, then the cone was brought down and the viscosities of the samples were measured in centipoise [17].

Forty intact human lower third molar teeth were selected and examined by visual inspection. The teeth were cleaned by removing the remaining soft tissues and debris. Plastic ring molds were then filled with an autopolymerizing resin; the roots of the teeth were embedded vertically in the acrylic resin, leaving the crowns exposed. The occlusal enamel of the teeth was removed perpendicular to the long axis of the teeth with diamond burs under water cooling to form flat superficial, coronal dentin surfaces [18]. Phosphoric acid etchant (Super-etch) was applied for 15 s on the flat dentin surface and rinsed for 15 s. The specimens were divided into four equal groups (ten teeth each). For each group, one type of the bonding agents was applied, unfilled adhesive resin, 0.2 wt%, 0.5 wt% and 1 wt% HA-filled adhesive resin, to the dentin surface. Then adhesive gently dried and light polymerized

for 20 s using a light curing unit (Demetron LC, Kerr, USA). The glacier composite was built up over the dentin surfaces of the teeth using a teflon mold and light cured for 40 s. Immediately after composite curing, the mold was removed and the specimens were stored in distilled water for 24 h. The specimens were sectioned perpendicular to the adhesive interface using a diamond saw of 0.5 mm thickness on a cutting machine (Ham Co. Machines, Inc, Rocher, USA) in bucco-lingual and mesio-distal directions to produce dentin-resin composite sticks [19]. Each tooth-composite specimen was sectioned to give four sticks. For each adhesive material, ten dentin-composite resin sticks were used. After measurement of the cross-sectional area of each microbar with a digital caliper (Iwanson. Martin, Germany), the sticks were then bonded with cyanoacrylate adhesive to a microtensile testing device. The applied tensile force resulted in debonding along the substrate-adhesive interface. The load (Newton) required for debonding of each beam was divided by the bond area to express bond in MPa [20].

Means and standard deviations for viscosity, microtensile bond strength were calculated for each group. The data were analyzed using One-way ANOVA and Least Significant Difference (LSD) tests.

Results

Means of viscosity (centipoise) and standard deviations for all groups are shown in Table 2. One-way ANOVA showed a significant difference in viscosity among the adhesive groups ($P < 0.0001$). LSD statistical test showed significant difference between group 4 and other groups or between group 3 and group 2. In addition, there was no significant difference between group 1 and group 3 adhesives.

Means of microtensile bond strength (MPa) and standard deviations for all groups are shown in Table 3. One-way ANOVA results of microtensile bond strength showed a significant difference in microtensile bond strength among the studied groups ($P \leq 0.05$). LSD statistical test showed significant difference between group 2 and both groups 3 and 4. On the other hand, no significant difference was shown between group 1 and other groups or between group 3 and group 4.

Discussion

Adhesive resin must be able to make intimate contact with the substrate and also be able to spread easily on it to be effective to produce good adhesion. Too high viscous adhesive is less likely to spread out on a surface due to its high resistance to flow. On the other hand, too low viscous adhesive is difficult to be controlled during its placement on the surface. Bonding resin must present proper fluidity to permit its infiltration and be able to fully penetrate into the etched substrate and polymerize in situ [21]. The results of the present study showed that viscosity values of filled adhesives decreased at lower concentrations of the filler (0.2 wt% HA and 0.5 wt%) and increased at higher concentration (1 wt% HA). This can be attributed to the fact that at a low filler concentration, silane coupling was strongly chemical-grafted on the surface of fillers, which improved dispersion and stability, and thus reduced viscosity of dental adhesives and improved flowability. On the other hand, at high filler concentration, particles were closer to each other and the probability of collision was higher under shear and caused aggregates [22,23]. These

results were comparable to that obtained by Luo et al. [23]. The researchers added different concentrations of surface modified fillers into dental adhesives, then viscosity of materials was measured. They found that viscosity of dental adhesives was decreased when the proportion of fillers was less than 1wt%, but was significantly improved when the proportion was more than 1wt% fillers. Results of the present study showed that incorporation of HA nanofiller increased bond strength to a certain degree and then decreased it. The highest value (35.39 ± 6.96 MPa) was obtained for 0.2 wt% HA-filled adhesive then decreased with 0.5 wt% HA-filled adhesive (29.35 ± 5.28 MPa) and 1 wt% HA-filled adhesive (28.82 ± 5.03 MPa). This can be explained by the fact that incorporation of fillers with larger dimensions than the interfibrillar space of the etched dentin increases viscosity of the adhesive and causes filler accumulation over the top of etched dentin surface. Therefore it reduces the adhesive penetration into the etched dentin and produce a defective hybrid layer and formation of flaws and cracks which decreases bond strength [20]. The present results are comparable to that obtained by Sadat-Shojai et al. [24]. They found that microtensile bond strength increased significantly to the maximum value for specimens containing 0.2 wt% HA

nanorods and then steeply decreased with increasing filler content. In case of μ TBS test specimens bonded with the adhesive loaded with 0.2 wt% nanofillers. Nanofillers were easily observed as primary clusters which were formed by the aggregation of nanofillers and ranged from several tens of nanometers to around 500 nm in diameter. They were dispersed evenly within the adhesive layer. Therefore, nanofillers can reinforce the adhesive layer at the resin-dentin interface. In contrast, in case of μ TBS test specimens bonded with adhesive loaded with more than 0.2 wt% nanofillers, the primary clusters failed to disperse in the adhesive layer and aggregated into larger spherical clusters of approximately 2 μ m in diameter. Such aggregation of the nanofillers accumulates on the top of etched dentin substrate reducing adhesive penetration into dentinal tubules and hybrid layer of etched dentin forming a defective adhesive layer.

Conclusion

Modification of one-component total-etch adhesive system with 0.2 wt% and 0.5 wt% HA nano-fillers decreased viscosity, while 1 wt% HA-modified adhesive showed increased viscosity and microtensile bond strength.

Table 1: Materials used in the study.

Materials	Composition	Batch number	Manufacturer
Materials used to prepare HA nanoparticles powder			
Calcium nitrate powder	Calcium nitrate tetrahydrate [Ca(NO ₃) ₂ ·4H ₂ O]	C1396	Sigma-Aldrich Chemie GmbH, Steinheim, Germany
Ammonium hydrogen phosphate powder	Ammonium dihydrogen orthophosphate [NH ₄ H ₂ PO ₄]	204005	
Ammonium hydroxide solution	Ammonium hydroxide [NH ₄ OH]	320145	
Silane coupling agent	3-(Methacryloyloxy) Propyl Trimethoxy Silane	14031	
Super etch Acid etchant for enamel and dentin	Phosphoric acid (37%)	111054	SDI limited, Bayswater, Victoria, Australia
Stae Dentin/enamel single component total-etch adhesive	Urethane dimethacrylate (UDMA), 2-hydroxyethyl methacrylate (HEMA), fluorides, CQ, stabilizer, acetone and water.	0406165	
Glacier Microfilled hybrid composite	Multifunctional methacrylate ester Inorganic filler	111174	

Table 2: Means, standard deviations and results of LSD test for viscosity (centipoise) of different groups.

Adhesive	Mean ± SD	P-value	LSD
Group 1 (control group)	1.75 ± 0.29 ^b	<0.0001	0.392
Group 2 (0.2 wt% HA)	0.78 ± 0.13 ^c		
Group 3 (0.5 wt% HA)	1.44 ± 0.24 ^b		
Group 4 (1 wt% HA)	2.53 ± 0.43 ^a		

Means with the same superscript letters are not significantly different.

Table 3: Means, standard deviations and results of LSD test for microtensile bond strength of all groups.

Adhesive	Mean ± SD	P-value	LSD
Group 1	32.52 ± 6.26 ^{ab}	0.05	5.381
Group 2	35.39 ± 6.96 ^a		
Group 3	29.35 ± 5.28 ^b		
Group 4	28.82 ± 5.03 ^b		

Means with the same superscript letters are not significantly different.

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