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The Effect of poly Amido Amine Denderimer Pretreatment Alone or with Nano Hydroxyapatite Containing Adhesive on the Integrity of Resin Dentin Bond after PH Cycling

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ABSTRACT

Purpose: The purpose of the current study was to evaluate the effect of poly amidoamine dendrimer pretreatment alone and with Nanohydroxy apatite containing adhesive on the integrity of resin dentin bond as affected by PH Cycling. **Materials and Methods**: A total of eighty dentin samples were divided into four main (A) groups according to the material used where in (A1) group: (PAMAM $-NH_2$) alone, (A2) group: HA containing adhesive alone, (A3) group: Combination of (PAMAM-NH2 +HA adhesive), (A4) group: The control adhesive alone .And each of (A) groups were further subdivided into two (B) groups according to PH Cycling where (B1) exposed to PH Cycling while (B2) not exposed. After (21 days) the shear bond strength of the specimens was measured and the samples were examined by(ESM). **Results:** For both (B1) and (B2),the results revealed that the highest mean value was recorded in (A3B1) and (A3B2) group while the lowest mean value was recorded in (A4B1) and (A4B2). **Conclusion:** All the treatment materials used were effective in improving the bond strength and the PH Condition has a significant role in the efficacy of different materials in increasing the bond strength.

KEYWORDS

PAMAM-NH2,
Nanohydroxy apatite, SEM,
Bond strength, PH Cycling

INTRODUCTION

Remineralizing bonding techniques with therapeutic effect may help in increasing the strength of the hybrid layer, resulting in more gradual gradients of stiffness that interfere with localized stress concentrations. Recent innovative approaches in remineralizing the hybrid layer may improve the resistance of hybrid layers to different types of degradations overtime, and have an important role in caries prevention [(1)].

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Recently, nanoparticles have been used in the formulation of restorative composite systems[2]. Nanoparticles have very small size which enables them to penetrate with the adhesive resin matrix into the dentinal tubules resulting in a composite adhesive layer with improved characteristic [3,4]. using HA in dentistry provide many promising advantages, as intrinsic radio-opacity, improved polishability, and increased wear resistance , this is due to the similarity between the hardness of synthetic HA and that of natural teeth. Finally, HA is cheaper than most of the other used inorganic fillers [5]. Nano hydroxyapatite provides a large reservoir of free calcium ions. So it is suitable to be used for remineralization.

Dendrimers consist of many branches originating from one core molecule to external groups. These terminal groups are easily functionalized and have biomimetic properties, so different types of these molecules, are now widely used in biomimetic mineralization. Decalcified dentin is mainly consist of collagen fibrils, which can not induce HA nucleation and growth [6]. The dentin organic matrix have non collagenous proteins (NCPs), which are very essential in the mineralization of dentin. The growth of HA during the dentin mineralization is controlled by A series of NCPs which act as nucleation templates [7]. However, The ability of NCPs to induce remineralization is lost in the mature dentin,

Demineralized dentin remineralization need good nucleation templates that have the ability to bind with collagen fibrils. The production of new biomaterials that resemble the NCPs in their function in dentin remineralization is essential and promising. PAMAM dendrimer has multiple terminal reactive groups with well-defined size which make it suitable for mimicking natural NCPs[8]. The different types of functional groups present on the outer surface of PAMAM dendrimers can control HA nanostructures size and shape in vitro. PAMAM-NH2 has many amine groups on the outer surface and possess a multiple number of amide groups in its branches. The presence of these functional groups have the ability to attract more calcium ions during the process of remineralization. PAMAM-NH2 has been proved to be a good agent for inducing dentin remineralization[9,10].

MATERIALS AND METHODS

For Specimen preparation, the crowns of forty sound human premolar teeth were cut from the roots at about 1 mm beneath the (CEJ). Then the crowns were separated in two halves in a bucco-lingual direction [11]. After pulp remnants were removed, a flat and smooth dentin surface was obtained by grinding the inner surfaces of the crowns under constant water spray. Then self-cured acrylic resin was used for mounting the prepared dentin specimens using Teflon mold of 8mm height “2cm width and 2,8cm length. The specimens were mounted inside the mold with their flat dentin surface upward and in the same level with flat acrylic resin. the specimens were removed from the mold after curing of acrylic resin and stored in distilled water [12] .

1- Preparation of hydroxyapatite nanorods:

Hydroxyapatite Nanorods were produced by wet chemical reaction method as it is the most available method in Egypt. The synthesized nanorods were added with a concentration of 2 wt. % to the selected adhesive system [13].

2- Preparation of the bonding agent containing hydroxyapatite nanorods:

1- The selected adhesive system (2.5ml) was modified by mixing it with 2 WT% hydroxyapatite nanorods according to the equation [14].

$$\text{Amount of added material} = \frac{\text{Amount of solution} \times \textbf{weight} \text{ percent of the material}}{100\% \text{ Weight percent}}$$

2- The adhesive was put in empty tube that was wrapped with dark adhesive tape to protect it from exposure to the light. They were withdrawn by a syringe wrapped with dark adhesive tape.

3- 0.05 gm. of HA nanorods was weighed using digital balance sensor.

4- The adhesive and HA nanorods were sonicated together[13] within the adhesive bottle for 10 min using an autoclavable ultrasonic tip of the high speed ultrasonicator (100B-HB ultrasonic processor, 25000 revolutions per minute), to obtain a homogenous mix in a light proof environment at room temperature.

5- Immediately after the incorporation, the adhesive bottle was recapped securely again until use.

3 -Application of the etchant and adhesive;

The dentin surface was etched by the etchant gel for 15 seconds After that the water of a three way syringe was used for 10 seconds to remove the acid then excess water was dried.

The specimens were divided according to the material used into four groups:

A1: The pure G3.0 PAMAM dendrimer cover each demineralized dentin specimen for 30 seconds and then washed with deionized water [15]. The control adhesive was used according to the manufacturing instructions .The adhesive was rubbed using a disposable micro brush , thinned gently by compressed air for 5 seconds to completely evaporate the solvent. Then LED light curing unit used for 10 seconds to cure the adhesive .Then the second layer was applied in the same manner and cured.

A2: The adhesive containing Nano hydroxyapatite alone was applied in the same manner as group A1.

A3: The specimens were coated with pure PAMAM for 30 seconds then washed with deionized water then the adhesive containing Nano hydroxyapatite was applied.

A4: The control adhesive alone was applied without any previous treatment.

4- Composite resin packing;

A transparent silicone cylinder (2mm in diameter and 2 mm in height) was used for the production resin cylinders. After filling the cylinders with A4 shade of resin composite they were applied on to the pretreated surface of dentin then the resin composite was covered with a piece of clear matrix band and glass slap of 8 ml thickness then the excess resin composite was removed with the tip of the explorer . After the removal of glass slap the resin composite material was cured for 20 seconds with LED curing device .After curing the transparent cylinder was removed.

5- PH cycling:

Each of the treated (A) groups was further subdivided into (2 subgroups) according to PH cycling;

B1; exposed to PH cycling.

B2; not exposed to PH cycling.

- For (B1) groups ; Each sample of the aforementioned four groups was stored for 23 hours (h) in 1 mL of fresh artificial saliva, and then in 1 mL of the lactic acid solution for (1h) at 37°C every day for 21 days
- For (B2) groups; The specimens were stored in artificial saliva for 24 hours without any exposure to the lactic acid for 21 days [16].

6- Shear bond strength testing:

The seventy two samples were mounted on a universal testing machine with a 5 kN. load cell The required load for debonding was evaluated in Newton and the data were recorded using computer software.

7- Statistical analysis:

The shear bond strength results were tabulated and presented as mean and standard deviation. Regression model using (ANOVA) was used for comparison of mean values among the groups,

Tukey's post-hoc test was used for pair-wise comparison when ANOVA was significant. Statistical analysis was performed with IBM® SPSS® Statistics version 20.

8- Scanning Electron Microscope examination:

One sample from each of the eight groups was examined. The specimens scanned using SEM attached with EDX Unit (Energy Dispersive X-ray Analysis), Images from the selected sample were obtained (at 2000x magnification).

RESULTS

Comparisons of Shear Bond Strength (MPa) between materials exposed to pH cycling revealed that the mean value recorded in group A3 [Nano hydroxyapatite containing adhesive+ (PAMAM)] was the highest, while the least value was recorded in A4 (Control adhesive). ANOVA test showed that the difference between all groups was extremely statistically significant. Tukey's post hoc test revealed that the difference between A1 (PAMAM) and A2 (Nano hydroxyapatite containing adhesive) was insignificant.

Comparison of Shear Bond Strength (MPa) between materials not exposed to PH Cycling showed that the mean value recorded in group A3 [Nano hydroxyapatite containing adhesive + (PAMAM)] was highest, while the least value was showed in A4 (Control adhesive). ANOVA test revealed that the difference between all groups was highly statistically significant. Tukey's post hoc test revealed insignificant difference between A1 (PAMAM), A2 (Nano hydroxyapatite containing adhesive) and A3 [Nano hydroxyapatite containing adhesive + (PAMAM)], (Table 1).

Table (1) *The mean value and standard deviation of all experimental groups under different PH cycling conditions (independent t test)*

Material	B1 (exposed to PH Cycling)			B2 (Not exposed to PH Cycling)			T	P
	Mean	Std. Dev.	Std. Error	Mean	Std. Dev.	Std. Error		
A1 (PAMAM)	17.539	2.539	0.803	18.365	5.051	1.597	-0.46	0.65ns
A2 (HA containing adhesive)	17.068	1.796	0.568	17.881	4.809	1.521	-0.501	0.63ns
A3 (PAMAM + HA)	24.621	6.328	0.426	19.722	1.346	0.426	2.39	0.028*
A4 (Control adhesive)	12.719	2.153	0.681	14.590	2.243	0.709	1.9	0.073ns

*Significance level p<0.05, *significant, ns=non-significant*

DISCUSSION

The stability of resin dentin bond is influenced by the hybrid layer degradation. The resin dentin interface remains the weak link in the restoration. The minerals Re-incorporation into the HL is important because the nanoleakages in the HL can be repaired by the precipitated minerals. The minerals could also prevent MMPs from penetrating and degrading the collagen matrix [17]. In addition, the remineralization of HL increase the resistance to degradation in the oral environment and has the ability to resist and neutralize biofilm acids. Therefore, in recent years, to facilitate dentin remineralization two strategies were employed: coating the demineralized dentin by nucleation templates; and giving Ca and P ions [18].

G3-PAMAM-NH2 was used in this study as the results of previous studies confirmed that it has a good ability to bind to demineralized dentin and enhance the formation of needle-like crystals both on the dentin surface and in the dentinal tubules. The regenerated minerals have a good ability to resist acid challenge.

Nano hydroxyapatite was used in this study as Hydroxyapatite (HA) (Ca_{10} $(PO_4)_6$ $(OH)_2$), which is a form of calcium phosphate, is one of the most bioactive materials. Hydroxyapatite is considered the main mineral of teeth and bone. Therefore, synthetic HA is excellent inorganic material to be used as a filler in dental restoration. The use of HA in dental restoration provide several promising advantages, such as intrinsic radio-opacity , improved polishability, and increasing wear resistance Finally, Nanohydroxy apatite is cheaper than most of the inorganic fillers used in dentistry today.

In the current study, comparing the mean value of the shear bond strength of the tested groups exposed to PH Cycling (B1), the results revealed that the highest mean values were recorded in A3 group (PAMAM+HA containing adhesive) followed by A1 group (PAMAM) then A2 group (HA containing adhesive) while the lowest mean value was recorded in A4 group (control universal bond).The results showed insignificant difference between (A1) and (A2) groups. The same results were also observed when the tested materials were compared in group (B2) where the materials were not exposed to PH Cycling and were immersed in artificial saliva the whole time.The highest mean value of shear bond strength was observed when the PAMAM and nanohydroxyapatite were combined together, followed by PAMAM group and nanohydroxyapatite group. The results showed insignificant difference between (A1), (A2) and (A3) groups. The results of shear bond strength matched the SEM observation.

CONCLUSIONS

Under the condition of this in vitro study, it can be concluded that;

1. Modification of the adhesive with nanoparticles is an effective method in dentin remineralization and improving dentin bond strength.
2. The PAMAM and Nanohydroxyapatite combination has a higher efficacy in improving the bond strength in acidic condition than the neutral condition.
3. Various PH condition in the oral cavity play an important role in the efficiency of different materials in improving shear bond strength.

REFERENCES

1. Bertassoni LE, Habelitz S, Marshall SJ, Marshall GW. Mechanical recovery of dentin following remineralization in vitro–an indentation study. J Biomech 2011; 4:176–81.
2. Kalachandra S. Influence of fillers on the water sorption of composites. Dent mat1989;5:283–8.
3. Kim JS, Cho BH, Lee IB, Um CM, Lim BS, Oh MH. Effect of the hydrophilic nanofiller loading on the mechanical properties and the microtensile bond strength of an ethanol-based one-bottle dentin adhesive. Biomedical Materials Research Part B Applied Biomaterials 2005; 72: 284–91.
4. Bae JH, Cho BH, Kim JS, Kim MS, Lee IB, Son HH. Adhesive layer properties as a determinant of dentin bond strength. J Biomed Mater Res B Appl Biomater 2005; 74:822–8.
5. Omelon S.J, Grynpas M.D. Relationships between Polyphosphate Chemistry, Biochemistry and Apatite Biomineralization. Chem. Rev 2008; 108: 4694.
6. Tay F R, Pashley D H. Guided tissue remineralisation of partially demineralized human dentine. Biomater 2008; 29: 1127.
7. George A, Veis A. Phosphorylated Proteins and Control over Apatite Nucleation, Crystal Growth, and Inhibition. Chem. Rev 2008; 108: 4670.
8. Svenson S, Tomalia D A. Dendrimers in biomedical applications—reflections on the field. Adv. Drug. Deliver. Rev 2012; 64:102-115.

9. Liang K, Yuan H, Cheng L. Remineralization of Demineralized Dentin Induced by Amine-Terminated PAMAM Dendrimer. Macromolecular Science and Engineering 2015; 300:107-117.
10. Donners J, Nolte R. J. M, Sommerdijk N. Biomimetic Systems for Hydroxyapatite Mineralization Inspired By Bone and Enamel. Chem. Rev 2008; 108: 4754–4783.
11. Chaharom MEE, Abed Kahnamoii M, Kimyai S. Effect of sodium hypochlorite on the shear bond strength of fifth- and seventh-generation adhesives to coronal dentin. Afr. J. Biotechnol 2011; 10: 12697-12701.
12. Dontula BSK, Nagaraj B, Danda N. The effect of sodium hypochlorite on the shear bond strength of acetone based system to dentin – an in-vitro study. Annals&Essences Dentistry 2012; 4:17-23.
13. Leitune VC, Collares FC, Trommer RM, Andrioli DG. The addition of nanostructured hydroxyapatite to an experimental adhesive resin. J Dent 2013: 321 – 327.
14. WWW.Physiologyweb.com/calculators/percent-solution-calculator.html.
15. Wang T, Yang S, Wang L, Feng H. Use of Poly (Amidoamine) Dendrimer for Dentinal Tubule Occlusion: A Preliminary Study. PLOS ONE 2015:1-16.
16. Xiao S, Liang K, Weir M, Cheng L, Liu H, Zhou X. Combining Bioactive Multifunctional Dental Composite with PAMAM for Root Dentin Remineralization. Materials 2017; 10: 1-17.
17. Kim Y.K, Mai S, Mazzoni A, Liu Y, Tezvergil-Mutluay A, Takahashi K. Biomimetic remineralization as a progressive dehydration mechanism of collagen matrices-implications in the aging of resin-dentin bonds. Acta. Biomater 2010; 6:3729-3739.
18. Zhou Y.Z, Cao Y, Liu W, Chu C.H, Li Q.L. Polydopamine-induced tooth remineralization, ACS Appl. Mater. Int 2012; 4: 6901-6910.