Published in final edited form as:

Clin Plasma Med. 2015 June 1; 3(1): 10–16. doi:10.1016/j.cpme.2015.05.002.

Plasma treatment of dentin surfaces for improving self-etching adhesive/dentin interface bonding

Xiaoqing Dong^a, Hao Li^a, Meng Chen^b, Yong Wang^c, and Qingsong Yu^{a,*}

^aCenter for Surface Science and Plasma Technology, Department of Mechanical and Aerospace Engineering, University of Missouri, Columbia, MO 65211, USA

^bNanova, Inc., Columbia, MO 65203, USA

^cCenter for Research on Interfacial Structure & Properties, School of Dentistry, University of Missouri-Kansas City, Kansas City, MO 64108, USA

Abstract

This study is to evaluate plasma treatment effects on dentin surfaces for improving self-etching adhesive and dentin interface bonding. Extracted unerupted human third molars were used after crown removal to expose dentin. One half of each dentin surface was treated with atmospheric non-thermal argon plasmas, while another half was untreated and used as the same tooth control. Self-etching adhesive and universal resin composite was applied to the dentin surfaces as directed. After restoration, the adhesive-dentin bonding strength was evaluated by micro-tensile bonding strength (μ TBS) test. Bonding strength data was analyzed using histograms and Welch's t-test based on unequal variances. μ TBS test results showed that, with plasma treatment, the average μ TBS value increased to 69.7 ± 11.5 MPa as compared with the 57.1 ± 17.5 MPa obtained from the untreated controls. After 2 months immersion of the restored teeth in 37 °C phosphate buffered saline (PBS), the adhesive-dentin bonding strengths of the plasma-treated specimens slightly decreased from 69.7 ± 11.5 MPa to 63.9 ± 14.4 MPa, while the strengths of the untreated specimens reduced from 57.1 ± 17.5 MPa to 48.9 ± 14.6 MPa. Water contact angle measurement and scanning electron microscopy (SEM) examination verified that plasma treatment followed by water rewetting could partially open dentin tubules, which could enhance adhesive penetration to form thicker hybrid layer and longer resin tags and consequently improve the adhesive/dentin interface quality.

Keywords

bonding strength; plasma treatment; self-etching adhesive; resin restoration; dentin surface

*Corresponding author. Address: Department of Mechanical and Aerospace Engineering, University of Missouri, E3411 Lafferre Hall, Columbia, MO 65211, USA, Telephone: 1-573-882-8076, yuq@missouri.edu.

Publisher's Disclaimer: This is a PDF file of an unedited manuscript that has been accepted for publication. As a service to our customers we are providing this early version of the manuscript. The manuscript will undergo copyediting, typesetting, and review of the resulting proof before it is published in its final citable form. Please note that during the production process errors may be discovered which could affect the content, and all legal disclaimers that apply to the journal pertain.

1. Introduction

Polymer-based dental composites have received widespread clinical acceptance because of its safety and natural tooth-like appearance. However, dental composite restoration has been shown to be less efficient, higher failure rate, and shorter lifespan compared with traditional amalgam [1, 2]. The adhesive-dentin interface has been well recognized as the weaker area for dental composite resin restoration [3]. Nowadays improving adhesive-dentin bonding strength is still one of the hot topics of current research in dentistry. Manufacturers and researchers have developed various types of adhesive systems and techniques to provide strong adhesive-dentin bonding and improve long-term durability of dental composite restoration.

Etch-and-rinse adhesive systems and self-etching systems are the most often reported adhesive systems [4]. In etch-and-rinse adhesive systems, an etching reagent is used to remove smear layer and smear plugs prior to adhesive application. This step exposes dentin collagen fibrils and opens dentinal tubules, and thus allow resin monomers to encapsulate collagen fibrils and penetrate into tubules to form the so-called “hybrid layer” [5] and “resin tags” respectively. On the other hand, etch-and-rinse adhesive system is well known as being time-consuming and technique sensitive. Over-drying of dentin before adhesive application could cause collapse of the exposed dentin collagen fibrils and result in insufficient penetration of dental adhesive monomers [6]. Over-etching could also lead to nanoleakage of the restoration since partial penetration of resin within given times [7].

Self-etching adhesives have overcome these drawbacks of etch-and-rinse adhesives through simultaneous demineralization and adhesive infiltration into the dentin. However, recent studies have suggested that most of the self-etching adhesives cannot provide as strong bonding strength as the conventional adhesive system [8]. The reasons include reduced conditioning of the enamel, water content of the adhesive layer and micro-bubbles formed during the etching process of the hybrid layer [9, 10]. Another problem was observed that combining the primer and adhesive resin into a single application step may reduce hybridization effectiveness [11]. Self-etching systems work on smear layer directly and have to penetrate beyond the smear layer into the tooth structure to form resin tags and hybrid layer [12]. In fact, thinner hybrid layer was observed in self-etching adhesive/dentin interface [13] and self-etching adhesive/intact enamel interzone [14]. Simple removal or modification of the smear layer on prepared dentin surface, without further demineralization of the dentin surface, will be beneficial to diffusion and penetration of self-etching adhesive monomers and possibly further increase dentin/adhesive bonding strength. However, it is impossible to remove the smear layer by routine methods such as air blowing, water spray even scrubbing because of its incorporation into the underlying tissue.

In the past two decades, low-temperature or non-thermal atmospheric plasmas have been rapidly developed as an effective surface modification technique in medicine and dentistry [15, 16]. Our recent research works have demonstrated that plasma treatment using a non-thermal atmospheric plasma brush is very effective in improving total-etch adhesive/dentin bonding by working on etched dentin surface. The effectiveness was achieved by changing exposed collagen structure and increasing dentin surface hydrophilicity using plasma

treatment [17–19]. It is considered that plasma treatment might also have the capability in enhancing self-etching adhesive/dentin bonding through removing or cleaning smear layers and surface modification of the dentin surfaces.

In this work, non-thermal atmospheric argon plasmas were applied on smear layer covered dentin surfaces to evaluate the effectiveness of plasma treatment on self-etching adhesive/dentin bonding. To eliminate the effect of difference in individual human teeth, the plasma-treated dentin surfaces were compared with their untreated same-tooth controls in terms of the surface topography, adhesive/dentin interface quality, and interfacial bonding strength.

2. Material and methods

2.1 Dentin preparation

Extracted unerupted human third molars were collected under a protocol approved by the University of Missouri-Kansas City Adult Health Sciences Institutional Review Board and after obtaining patient consent. All tissue samples were non-patient identified and all samples were handled and disposed according to the protocols suggested by Environmental Health and Safety Department at the University of Missouri. The teeth had no caries and were stored in the phosphate buffered saline (PBS, pH=7.4) with 0.02% sodium azide to inhibit bacteria growth. Isomet 5000 diamond saw (Buehler, Lake Bluff, IL, USA) was used to cut off the roots and enamels to expose dentin surface. The dentin surface was polished with 600-grit silicon carbide abrasive paper under wet condition and then then cleaned by copious water spray.

2.2 Tooth preparation for micro-tensile bond strength (μ TBS) test

After dentin preparation, excess water was gently blown away using dental airline to obtain a moist surface. Half of a tooth was treated with plasma brush while another half was covered using a thin blade. Except the electrical current, all the other plasma operating parameters, such as argon flow rate and treatment time, were kept the same as our previous work [19]. In this study, the current level was increased to 8 mA instead of the 6 mA used in our previous work. After plasma treatment, eight teeth were rewetted by applying deionized water droplets on dentin surface for 1 min. Then water droplets were removed by dental airline hand piece to obtain a visible moist surface. Dental adhesive (OptiBond All-In-One, Kerr, Romulus, MI, USA) was applied on the moist dentin surface and light-cured for 10 s using Spectrum 800 (Dentsply, Milford, DE, USA) by following the manufactures' instructions. Then three to four layers of dental composite (Filtek Z250, 3M ESPE, St Paul, MN, USA) were applied on top of the adhesive and light cured for 20 s after each layer application.

Four teeth prepared with plasma treatments and rewet processes were randomly selected for interface stability test. These teeth after restoration were stored in PBS (pH=7.4) with 0.02% sodium azide to prevent bacteria growth at 37°C for 2 months. All the other tooth-composite bonded samples were stored in distilled water at 37°C for 24 hours. After storage all teeth samples were sectioned and produced ~1 mm × 1 mm micro-bar specimens for micro-tensile bond strength (μ TBS) test, which was performed using micro tensile tester (BISCO, Schaumburg, IL, USA) at a 0.5 mm/min strain rate [19].

2.3 Water contact angle analysis

After dentin preparation, half of tooth was treated with plasma brush at 8 mA current level for 30 s whilst a blade was used to separate and protect another half side which was used as the untreated same-tooth control. Water contact angles were measured with a goniometer equipped with a special optical system and a CCD camera. A drop of water (approximately 0.5 μ l) was placed on dentin surface and the image was immediately sent via the CCD camera to the computer for analysis. The droplet images at different deposition times (5 s, 15 s, 30 s and 50 s) were captured by Windows Live Movie Maker. The image J software with drop analysis plugin was used to determine the contact angle of the water droplet. Means and standard deviation of water contact angles were measured to assess surface hydrophilicity change upon plasma treatment.

2.4 SEM analysis

The morphologies of dentin surface and adhesive/dentin interface were examined using a scanning electron microscopy (SEM) (Quanta 600, FEI, Hillsboro, OR, USA). Dentin slices and slices with plasma treatment and plasma treatment plus water rewet process were dehydrated in a series of ethanol/water mixtures with ascending concentrations, starting with 50%, 70%, 85% for 15 min each, and then with 90%, 95%, 100% for 30 min each. After μ TBS test, specimens with cohesive failure of dentin or composite were selected for adhesive/dentin interface observation. In order to examine hybrid layer and resin tags, acid-bleach treatment was used for SEM sample preparation as our previous work reported [18]. After drying in the vacuum overnight at room temperature, all prepared specimens were mounted on aluminum stubs and coated with 5nm thickness of platinum to observe using SEM at 10 KV.

2.5 Data analysis

The means and standard deviations of tensile strengths obtained from μ TBS test were used to evaluate the plasma treatment effects on adhesive/dentin bonding strength. The bonding strength data was analyzed using histograms and Welch's t-test based on unequal variances.

3. Results

The micro-tensile bond test employed in this study was developed by Sona et al in 1994 [20]. One of the advantages of the microtensile test method is that multiple specimens can be produced from a very few number of human teeth [21]. As shown in Table 1, 28 ~ 44 micro-bar specimens were prepared from teeth in each condition and undertaken μ TBS test. The μ TBS test results showed that, in comparison with their corresponding untreated controls, the plasma-treated specimens gave 22.1% and 30.1% higher mean bonding strength after the restored teeth were stored in 37 °C PBS for 24 hours and 2 months, respectively. It can be seen that, after 2 months storage in PBS, the adhesive/dentin bonding strength of the untreated controls decreased from 57.1 ± 17.5 MPa to 48.9 ± 14.6 MPa. In contrast, plasma-treated specimens slightly decreased from 69.7 ± 11.5 MPa to 63.6 ± 14.4 MPa. The reduction of the adhesive/dentin bonding strength with plasma treatment is much less than the untreated controls. Statistical analysis proved that the plasma-treated specimens had statistically significant increase ($p < 0.001$) in adhesive/dentin

bonding strength as compared with the untreated controls. It is noted that the bonding strength difference between plasma-treated specimens and the same tooth control specimens after 2 months storage ($p=0.00011$) became more significant than that with 24 hours storage ($p=0.00096$).

The sample sizes for statistics analysis, mean bonding strength and fracture location of each tooth are summarized in Table 1. Being consistent with our previous work [19], individual tooth effect on the resulted adhesive/dentin bonding strength was also found in this study. It was noted that, for all the teeth used in this study, plasma-treated specimens gave much higher mean bonding strength than that of their corresponding untreated same-tooth controls. It was also noted that plasma-treated specimens showed less frequent interface failure and more cohesive failure than their untreated same-tooth control specimens. After 2 months storage of the restored teeth in PBS, the control specimens showed more interface failure than that after 24 hours storage. There were also more cohesive failures in plasma-treated specimens than control specimens after 2 months storage.

Histograms statistics for comprehensive microtensile strength of untreated controls and plasma-treated specimens after 24 hours storage of the restored teeth were shown in Fig. 1. Histograms of bonding strength showed that, with plasma treatment, the bonding strength distribution curve was much taller and narrower. All plasma-treated specimens showed much stronger a bonding strength, which are higher than 50 MPa. In contrast, bonding strength distribution curve of the untreated control specimens was much shorter and wider. Many of the untreated control specimens showed very weak adhesive/dentin interfacial bonding strength (20–50 MPa). This result is consistent with the variance presented in Table 1.

Fig. 2 shows the representative SEM images of dentin surface treated with plasma, plasma plus rewetting process, and untreated control. As seen from the images, the dentin surface treated with plasma only didn't show much difference from the untreated control. However, the dentin surface treated with plasma plus rewetting showed some partially opened dentin tubules as indicated by the arrows shown in Fig. 2C.

Fig. 3 shows the water contact angle data measured on dentin surface after phosphoric acid etching, plasma treatment, and plasma treatment plus rewet process. Since dentin surface composition and properties vary from tooth to tooth, dentin slices from the same tooth was used for water contact angle measurements. Plasma-treated dentin surface showed the lowest contact angle (less than 10°). Plasma-treated surface was measured only once for each data point because the plasma-treated surface was completely wet after placing the water droplet on the surface for water contact angle measurement. Therefore, there is no standard deviation shown with the contact angle curve measured from plasma-treated dentin surface. After water rewetting and then dried using air-blowing, plasma-treated dentin surfaces showed water contact angles that are about the same as the smear layer covered or phosphoric acid etched dentin surfaces. It was noted that, on both the plasma treated and phosphoric acid etched dentin surface, water contact angles decreased with the water drop sitting time because the water droplets gradually spread out along the dentin surface and penetrated into the dentin.

Fig. 4 shows the representative SEM images of specimen interfaces which were prepared by the acid-bleach technique. Compared with the untreated same-tooth controls, thicker hybrid layer (HL) and longer resin tags were clearly observed from the adhesive/dentin interfaces of plasma-treated specimens. Based on 6 measurements of the hybrid layer thickness using Image J software, the hybrid layer thickness of the plasma-treated specimens is $2.75 \pm 0.40 \mu\text{m}$, while the untreated control has a hybrid layer thickness of $1.05 \pm 0.25 \mu\text{m}$.

4. Discussion

Smear layer on dentin surfaces after mechanical drilling in dental restoration process is known as an unstable layer, which comprises of organic and inorganic debris and gets incorporated into the underlying tissue. It is impossible to remove it by using water spray or even scrubbing. Chemomechanical caries removal technique was reported to partially open tubules and leave less smear layer [22]. However, additional chemical agent and special design hand-held instrument will be needed with this technique. Plasma is a quick, effective, inexpensive and environmentally friendly technique for surface cleaning, which was implemented already in several industries such as semiconductor fabrication process, microchip fabrication, food packaging *et al* [23, 24].

In this work, partially opened dentinal tubules and less smear layer were observed by SEM examination after plasma treatment followed by water rewetting, whilst an obvious smear layer covering the dentin surface and blocking tubules remained with the untreated control and plasma treated only dentin surfaces (Fig. 2). Water contact angle measurement results confirm that, after plasma treatment and water rewetting, penetration of water into dentin tubules was observed on dentin surface. When dentin surface was covered with thick smear layer and dentin tubules are blocked by smear plugs, water does not show contact angle change after 50s of retention time as observed with the untreated control dentin surfaces. This result is consistent with the most recent Lehmann's work [25], in which it was stated that plasma treatment might chemically translate organic substance on dentin surface into volatile compounds and lead to the enlargement of dentin tubules.

In partial opening dentinal tubules and removal smear layer process, the step of water rewetting after plasma treatment plays an essential role. It could be explained that gas plasma consisting of atomic and ionic excited state species, which behaves like molecular sandblast. In traditional plasma cleaning system, gas plasma dislodges contaminants which are swept away in a vacuum stream. However, the plasma treatment was performed under atmospheric pressure by using our plasma brush system. If without water rewetting process, dislodged contaminants at atmospheric pressure might remain on dentin surfaces and redeposit or recombine on the dentin surface. Our μTBS test results, which were not presented in this paper, indicates that without rewet process, plasma-treated test specimens didn't show significant difference in adhesive/dentin bonding strength from the untreated control specimens ($p>0.05$). However, with water rewetting then air-blowing process, plasma dislodged contaminant could be blown away with excess water. Partially opened tubules were thus observed as shown in Fig. 2C.

Unlike etch-and-rinse adhesives, self-etching adhesives are applied directly on the smear layer of dentin surface. When self-etching adhesives are used, etching and subsequent penetration of monomers into the demineralized dentin is carried out as one step, which is achieved by incorporating polymerizable acidic monomers into its formula. These polymerizable acidic monomers can react with the other resin monomers in the adhesive formula to produce effective bonding using. Meanwhile, acid groups in these monomers are capable of etching mineral debris, removing smear layers, and helping resin monomers to penetrate into tubules [26].

Effective removal of smear layers is the key for achieving high adhesive/dentin bonding strength in dental composite restoration. The adhesive/dentin bonding strength mainly depends on the quality of smear layers and acid monomer concentration. The effect of the thickness and quality of the smear layer on the adhesive/dentin bonding strength for using self-etching systems has been widely studied [12, 27, 28]. Results by Chan *et al* showed that less smear layer was helpful to increase bonding strength and form thick hybrid layer [29]. On the other hands, some researchers reported that smear layer thickness has no significant effect on the resulted adhesive/dentin bonding strength [30]. These conflicting results might be attributed to the different self-etching adhesive systems that the researchers adopted in their study. Kenshima et al pointed out that the strong self-etching systems used in these researches could overcome the effects of the smear layer thickness effect [30].

Strong self-etching adhesives with high concentration acidic monomers could be effective to remove smear layer no matter how thick the smear layer is. However, the strong self-etching adhesives often cause postoperative sensitivity because of residual unreacted acidic monomer [31]. Strong self-etching adhesive are also widely recognized as a cause of hydrolytic instability to the methacrylate monomers in adhesive and resin components [26]. Mild self-etching adhesive could overcome these drawbacks. Yoshida *et al* also reported that mild self-etching adhesive may establish chemical bonds between acid groups of monomers and residual hydroxyapatite crystals, which are still present on the dentin collagen scaffold due to the mild aggressiveness of the acid phase [32]. However shallow depth of penetration is a major drawback of mild self-etching adhesives [27].

In this work, OptiBond All-In-One adhesive was selected as a model mild self-etching adhesive because it is a widely used self-etching adhesive in clinical application. More importantly, it is one of the mildest self-etching adhesive (pH = 2.5–3) [33]. Unlike short resin tags normally obtained by mild self-etching adhesives, after plasma treatment and rewetting process, partial opened dentinal tubules and removal smear layer help mild adhesive to penetrate into dentin and dentinal tubules and form thicker hybrid layer and longer resin tags, which were clearly observed in adhesive-dentin interface as shown in Fig. 4. Significantly enhanced adhesive/dentin interfacial bonding strength could be reasonably attributed to this quality adhesive/dentin interface.

It is known that inadequate or weak bonding interface could allow water or bacterial enzymes to further degrade the adhesive/dentin interface and the tissue, consequently cause the failure of composite restoration. The quality adhesive/dentin interface with thicker hybrid layer and longer resin tags resulted from plasma treatment could prevent

microleakage and prolong restoration time. Our μ TBS test results in Tables 1 also demonstrated that, after 2 months storage of the restored teeth in PBS, the plasma treated test specimens showed higher adhesive-dentin bonding strength than the untreated controls.

5. Conclusions

The experimental results obtained from this study demonstrate that non-thermal argon plasma treatment is very effective in improving adhesive/dentin interface bonding for durable composite restoration using mild self-etching systems. Such results can be attributed to that plasma treatment followed by rewetting process could partially open dentin tubules, and thus enhance adhesive penetration to form thicker hybrid layer and longer resin tags and consequently improve the adhesive/dentin interface quality. The better interface quality leads to the long-term durability of adhesive-dentin bonding.

Acknowledgments

This study was supported, in part, by the US National Institute of Health (NIH) under grant numbers of 5R01DE021431 and 5R44DE019041. The authors would thank Drs. Thomas E. Coyle D.D.S., John A. Johnson D.D.S., and Timothy T. Coyle, D.D.S., M.D. from Coyle & Johnson Oral and Maxillofacial Surgery for providing the extracted human teeth. The composite used in this study were generously sponsored by 3M ESPE.

References

- Bernardo M, Luis H, Martin MD, Leroux BG, Rue T, Leitão J, DeRouen TA. Survival and reasons for failure of amalgam versus composite posterior restorations placed in a randomized clinical trial. *J Am Dent Assoc.* 2007; 138:775–783. [PubMed: 17545266]
- Soncini JA, Maserejian NN, Trachtenberg F, Tavares M, Hayes C. The longevity of amalgam versus compomer/composite restorations in posterior primary and permanent teeth. *J Am Dent Assoc.* 2007; 138:763–772. [PubMed: 17545265]
- Spencer P, Ye Q, Park J, Topp EM, Misra A, Marangos O, Wang Y, Bohaty BS, Singh V, Sene F, Eslick J, Camarda K, Katz JL. Adhesive/Dentin interface: the weak link in the composite restoration. *Ann Biomed Eng.* 2010; 10:1989–2003. [PubMed: 20195761]
- Van Landuyt KL, Snauwaert J, De Munck J, Peumans M, Yoshida Y, Poitevin A, Coutinho E, Suzuki K, Lambrechts P, Van Meerbeek B. Systematic review of the chemical composition of contemporary dental adhesives. *Biomaterials.* 2007; 28:3757–3785. [PubMed: 17543382]
- Nakabayashi N, Nakamura M, Yasuda N. Hybrid Layer as a Dentin-Bonding Mechanism. *J Esthet Restor Dent.* 1991; 3:133–138.
- da Silveira Pereira GD, Paulillo L, De Goes MF, dos Santos Dias CT. How wet should dentin be? Comparison of methods to remove excess water during moist bonding. *J Adhesive Dent.* 2001; 3:257–264.
- Paul SJ, Welter DA, Ghazi M, Pashley D. Nanoleakage at the dentin adhesive interface vs microtensile bond strength. *Oper Dent.* 1999; 24:181–188. [PubMed: 10530280]
- Bouillaguet S, Gysi P, Wataha JC, Ciucchi B, Cattani M, Godin C, Meyer JM. Bond strength of composite to dentin using conventional, one-step, and self-etching adhesive systems. *J Dent.* 2001; 29:55–61. [PubMed: 11137639]
- De Munck J, Vargas M, Iracki J, Van Landuyt K, Poitevin A, Lambrechts P, Van Meerbeek B. One-day bonding effectiveness of new self-etch adhesives to bur-cut enamel and dentin. *Oper Dent.* 2005; 30:39–49. [PubMed: 15765956]
- Van Landuyt KL, De Munck J, Snauwaert J, Coutinho E, Poitevin A, Yoshida Y, Inoue S, Peumans M, Suzuki K, Lambrechts P, Van Meerbeek B. Monomer-solvent phase separation in one-step self-etch adhesives. *J Dent Res.* 2005; 84:183–188. [PubMed: 15668338]

11. Van Meerbeek B, Yoshida Y, Snauwaert J, Hellemans L, Lambrechts P, Vanherle G, Wakasa K, Pashley DH. Hybridization effectiveness of a two-step versus a three-step smear layer removing adhesive system examined correlatively by TEM and AFM. *J Adhesive Dent.* 1999; 1:7–23.
12. Tay FR, Sano H, Carvalho R, Pashley EL, Pashley DH. An ultrastructural study of the influence of acidity of self-etching primers and smear layer thickness on bonding to intact dentin. *J Adhesive Dent.* 1999; 2:83–98.
13. Kaaden C, Powers JM, Friedl K-H, Schmalz G. Bond strength of self-etching adhesives to dental hard tissues. *Clin Oral Invest.* 2002; 6:155–160.
14. Kanemura N, Sano H, Tagami J. Tensile bond strength to and SEM evaluation of ground and intact enamel surfaces. *J Dent.* 1999; 27:523–530. [PubMed: 10507209]
15. Fridman G, Friedman G, Gutsol A, Shekhter AB, Vasilets VN, Fridman A. Applied plasma medicine. *Plasma Proces Polym Plasma.* 2008; 5:503–533.
16. Kong MG, Kroesen G, Morfill G, Nosenko T, Shimizu T, Van Dijk J, Zimmermann J. Plasma medicine: an introductory review. *New J Phys.* 2009; 11:115012.
17. Ritts AC, Li H, Yu QS, Xu C, Yao X, Hong L, Wang Y. Dentin surface treatment using a non-thermal argon plasma brush for interfacial bonding improvement in composite restoration. *Eur J Oral Sci.* 2010; 118:510–516. [PubMed: 20831586]
18. Yu, QS.; Li, H.; Ritts, AC.; Yang, B.; Chen, M.; Hong, L.; XU, C.; YAO, X.; WANG, Y. Nonthermal atmospheric plasma treatment for deactivation of oral bacteria and improvement of dental composite restoration. In: Machala, Z.; Hensel, K.; Akishev, Y., editors. *Plasma for bio-decontamination, medicine and food security.* Springer; Netherlands: 2012. p. 215–228.
19. Dong XQ, Ritts AC, Staller C, Yu QS, Chen M, Wang Y. Evaluation of plasma treatment effects on improving adhesive–dentin bonding by using the same tooth controls and varying cross-sectional surface areas. *Eur J Oral Sci.* 2013; 121:355–362. [PubMed: 23841788]
20. Sano H, Shono T, Sonoda H, Takatsu T, Ciucchi B, Carvalho R, Pashley DH. Relationship between surface area for adhesion and tensile bond strength - Evaluation of a micro-tensile bond test. *Dent Mater.* 1994; 10:236–240. [PubMed: 7664990]
21. Pashley DH, Carvalho RM, Sano H, Nakajima M, Yoshiyama M, Shono Y, Fernandes CA, Tay F. The microtensile bond test: a review. *J Adhes Dent.* 1999; 1:299–309. [PubMed: 11725659]
22. Li H, Wang WM, Yu SL, Wen Q. Morphological and microtensile bond strength evaluation of three adhesive systems to caries-affected human dentine with chemomechanical caries removal. *J Dent.* 2011; 39:332–339. [PubMed: 21333713]
23. Chang JS. Recent development of plasma pollution control technology: a critical review. *Sci Tech Adv Mater.* 2001; 2:571–576.
24. Belkind A, Gershman S. Plasma cleaning of surfaces. *J Vacuum Tech Coating.* 2008; 1–11.
25. Lehmann A, Rueppell A, Schindler A, Zylla IM, Seifert HJ, Nothdurft F, Hannig M, Rupf S. Modification of Enamel and Dentin Surfaces by Non-Thermal Atmospheric Plasma. *Plasma Proces Polym.* 2013; 10:262–270.
26. Moszner N, Salz U, Zimmermann J. Chemical aspects of self-etching enamel–dentin adhesives: a systematic review. *Dent Mater.* 2005; 21:895–910. [PubMed: 16038969]
27. Tay FR, Pashley DH. Aggressiveness of contemporary self-etching systems: I: Depth of penetration beyond dentin smear layers. *Dent Mater.* 2001; 17:296–308. [PubMed: 11356206]
28. Reis A, Grandi V, Carlotto L, Bortoli G, Patzlaff R, Rodrigues Accorinte MdL, Dourado Loguercio A. Effect of smear layer thickness and acidity of self-etching solutions on early and long-term bond strength to dentin. *J Dent.* 2005; 33:549–559. [PubMed: 16005794]
29. Chan KM, Tay F, King N, Imazato S, Pashley D. Bonding of mild self-etching primers/adhesives to dentin with thick smear layers. *Am J Dent.* 2003; 16:340. [PubMed: 14677614]
30. Kenshima S, Reis A, Uceda-Gomez N, Tancredillo LL, Nogueira F, Loguercio A. Effect of smear layer thickness and pH of self-etching adhesive systems on the bond strength and gap formation to dentin. *J Adhesive Dent.* 2005; 7:117.
31. Unemori M, Matsuya Y, Akashi A, Goto Y, Akamine A. Self-etching adhesives and postoperative sensitivity. *Am J Dent.* 2004; 17:191. [PubMed: 15301216]

32. Yoshida Y, Nagakane K, Fukuda R, Nakayama Y, Okazaki M, Shintani H, Inoue S, Tagawa Y, Suzuki K, De Munck J, Van Meerbeek B. Comparative Study on Adhesive Performance of Functional Monomers. *J Dent Res*. 2004; 83:454–458. [PubMed: 15153451]
33. Van Landuyt KL, Mine A, De Munck J, Jaecques S, Peumans M, Lambrechts P, Van Meerbeek B. Are one-step adhesives easier to use and better performing? Multifactorial assessment of contemporary one-step self-etching adhesives. *J Adhesive Dent*. 2009; 11:175.

Figure 1. Histograms statistics for comprehensive micro-tensile strength of (A) untreated controls and (B) plasma-treated specimens after 24 hours storage of the restored teeth in 37 °C PBS.

Figure 2. Representative SEM micrographs of (A) untreated dentin control surface, (B) plasma-treated dentin surface, and (C) plasma-treated plus water rewetted dentin surface with white arrows pointing to some of the partially opened dentin tubules.

Figure 3.

Water contact angle of dentin surfaces (■) covered with smear layer, (●) after phosphoric acid etching treatment, (▲) after plasma treatment, and (▼) after plasma treatment plus water rewetting.

Figure 4.

Representative SEM images of acid-bleach treatment interface of (A and C) untreated the same-tooth control test specimens, and (B and D) plasma-treated test specimens shown after 24 hours storage of the restored teeth in 37°C PBS. HL represents hybrid layer.

Table 1

Mean micro-tensile test data, percentage increase in bonding strength and t-test analysis results obtained after 24 hours (1 day) and 2 months (60 day) storage of the restored teeth in 37 °C PBS.

Storage time	1 day		60 days	
	Untreated control	Plasma treated	Untreated control	Plasma treated
Mean bonding strength (MPa)	57.1±17.5	69.7±11.5	48.9±14.6	63.6±14.4
Percentage increase	---	22.1%	---	30.1%
Variance (MPa ²)	307.5	132.3	212.0	207.7
Number of teeth	4	4	4	4
Number of micro-bar specimens	39	35	28	44
Two-tailed <i>p</i> -value	0.00096		0.00011	