Original Article

Effect of Helium/Oxygen Plasma on the Shear Bond Strength of Self-adhesive Resin Cement on Coronal Dentin

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Abstract

Objectives: This study investigated the effects of helium/oxygen plasma on shear bond strength (SBS) when RelyX™ U200 self-adhesive resin cement was applied to coronal dentin, both when the dentin surface was re-wetted and when it was not re-wetted.

Materials and Methods: RelyX™ U200 self-adhesive resin cement was applied to coronal dentin, using the following five methods: dentin surface with no plasma treatment (control group); helium plasma jet (He); helium/oxygen plasma jet (He/O_2); helium jet with re-wetting (He+R); and helium/oxygen jet with re-wetting (He/O_2+R). The shear bond strength (SBS) was measured 24 hours after bonding. Water contact angles and SEM images of each group after surface treatment were studied, along with SEM images of cement/dentin interfaces. One-way ANOVA and Scheffe Post Hoc test statistics were used.

Results: Mean SBS values in both groups treated with plasma followed by re-wetting (He+R: 16.25±2.39 and He/O_2+R: 18.15±1.93 MPa) were higher than in the plasma-only (He: 13.26±1.52 and He/O_2: 12.98±1.94 MPa) and control groups (11.20±1.87 MPa) ($p<0.05$). The results for the two plasma systems were not significantly different from each other, with or without re-wetting ($p>0.05$). Water contact angle measurements in all plasma-treated groups showed significantly lower contact angle values. SEM evaluations showed that plasma treatments followed by re-wetting could partially remove the smear layer and open the dentinal tubules, resulting in resin tag formation at the interfaces.

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Conclusion: The use of helium/oxygen plasma jet followed by re-wetting enhances the bonding effectiveness of RelyX™ U200 self-adhesive resin cement to coronal dentin.

Keywords: cold plasma, helium/oxygen plasma jet, self-adhesive resin cement, coronal dentin, shear bond strength, rewetting

Introduction

Resin cements are widely used for cementation of indirect dental restorations. Recently, self-adhesive resin cements were developed to simplify the bonding procedures, saving time and, most importantly, shortening the "window of contamination", because they do not require any tooth surface pre-treatment processes, and their application is accomplished through a single clinical step.(1) In theory, the adhesion of self-adhesive resin cement relies on chemical interaction and micromechanical retention.(2) The chemical interaction forms ionic bonds with hydroxyapatite in dentin to provide a second means of retention.

However, many studies have found that self-adhesive resin cements often show lower bond strength than resin cements used with separate adhesive bonding systems.(3-5) The inferior bond strength may result from the limited demineralization capacity of their functional monomers, which were not able to dissolve the smear layer completely, and also from superficial interaction with the tooth structure.(6) Many studies have attempted to promote the bonding efficiency of the self-adhesive resin cements.(7-9) One technology of interest to the authors is cold atmospheric pressure plasma technology.

Cold atmospheric pressure plasma has garnered attention in the biomedical field, including dental applications.(10) Cold plasma jets both disinfect, and improve the bonding quality of, direct resin composite restorations(11-16) but the effects are dependent on plasma chemistry and energy levels. Adding oxygen to the discharge gas renders plasma more effective in eliminating micro-organisms(17), but free-radical scavengers such as oxygen may inhibit polymerization of the resin cements.(18) Therefore, the effects of plasma gas composition on the bond strength of resin cements are the focus of this study.

There is also controversy regarding whether the dentin surface should be re-wetted following plasma treatment, which can cause the dentin surface to lose moisture because of its air-drying effect.(12,15,19-21) Interestingly, there are still questions about the optimal method for plasma treatment of the dentin surface to enhance the bonding qualities of different adhesive bonding systems.

The aim of this study is to evaluate the effects of helium/oxygen plasma jets on the shear strength of the bond between RelyX™ U200 self-adhesive resin cement (3M ESPE, St. Paul, MN, USA) and differently-pretreated dentin surfaces after bonding for 24 hours. In addition, water contact angles on the differently-pretreated dentin surfaces were measured. Scanning electron microscope (SEM) examinations were used to analyze the dentin surface morphology and cement/dentin interfaces.

Materials and Methods

2.1 Dentin and resin composite rod preparation: Caries-free human third molars were used in this study with the permission of the Human Experimentation Committee, Faculty of Dentistry, Chiang Mai University, Thailand (no.12/2018). The teeth were stored in 0.1% thymol solution for no longer than three months after extraction, then stored in distilled water for 24 hours before use. The roots and occlusal 1/3 of the crowns were removed using an IsoMet™ low speed saw (Buehler, Lake Bluff, IL, USA). Then all specimens were cut perpendicularly to the tooth axis 0.5 mm from the dentin-enamel junction (DEJ). The specimens were mounted in Teflon rings with self-cure resin acrylic. The flat dentin surface was then ground for 1 minute at 200 rpm with 600-grit silicon carbide paper on a grinding machine (MEGA Advance Co., Ltd., Shandong, China) under running water to create a standardized smear layer, cleaned in an ultrasonic cleanser (BioSonic® UC125: Whaledent Inc., Cuyahoga Falls, OH, USA) for 10 minutes, and gently air-blown for five seconds to maintain a moist dentin surface. Two-millimeter long cylindrical composite rods with a 3-mm diameter, were built in a split aluminium mould, using nano-filled hybrid resin composite (Filtex™ Z350XT, A1 color, 3M ESPE, St. Paul, MN, USA), then light-cured from the top aspect using an LED light unit (Bluephase® Style LED curing light, IvoclarVivadent, Schaan, Liechtenstein) for 40 seconds. The bonded surface of each composite rod was sandblasted with 50 μm aluminum oxide for 10 seconds at a distance of 10 mm, cleaned with distilled water in an ultrasonic cleanser, and air-dried. Before the bonding process, silane (Ceramic Primer, 3M ESPE, St. Paul, MN, USA) was applied at the bonded surface for 60 seconds, and air-dried.

2.2 Cold atmospheric pressure plasma jet treatment: In this study, a plasma jet device with a nozzle diameter of 3 mm, developed by Plasma and Beam Physics (PBP) research facility, Chiang Mai University, in cooperation with PhotoBioCare (PBC) Co. Ltd., Thailand, as described elsewhere[22] was utilized. All operating processes were conducted at the Research Center of the Faculty of Dentistry, in an air-conditioned room with an average room temperature of 23±1°C and a relative humidity of 53±4%. In the helium plasma treatment groups, helium as the principal gas at a flow rate of 1000 ml/m was used. In the helium/oxygen plasma treatment groups, helium at a flow rate of 1000 ml/m combined with oxygen at 3ml/m was used. The assigned plasma jet was exposed to the prepared dentin surface at a distance of 3 mm from the nozzle.

2.3 Bonding procedure: The prepared specimens were randomly divided into five groups (n=15): dentin surface treated with no plasma treatment (control group); helium plasma jet (He); helium/oxygen plasma jet (He/O_2); helium with re-wetting (He+R); and helium/oxygen plasma jet with re-wetting (He/O_2 +R). Before any treatment, adhesive tapes with a 3 mm diameter hole were placed on the dentin surfaces to localize the bonding area. In the plasma-only groups the dentin surfaces were treated with helium or helium/oxygen plasma jet for 30 seconds. In the plasma with re-wetting groups, after being treated with helium or helium/oxygen plasma for 30 seconds, the bond surfaces were re-wetted with de-ionized water droplets for one minute. The water droplets were then removed by gentle air-blowing for 5 seconds to obtain a visible moist surface, instead of blot-drying. In the cementation process, RelyX™ U200 resin cement was luted on the bonding area of the prepared composite rods according to the manufacturer's instructions (Table 1). A load of 1 kg was applied for 10 seconds and excess cement and adhesive tape were removed. The resin cement was then polymerized, using the LED light unit for 20 seconds from the top and from the four sides. All specimens were stored in distilled water at 37°C for 24 hours.

2.4 Shear bond strength (SBS) test: The bonded specimens were subjected to SBS testing using a Universal Testing Machine (Instron® 5566, Instron, Norwood, MA, USA) at a crosshead speed of 0.5mm/min until failure. All bond strength data were recorded in megapascals (MPa). The de-bonded surfaces

Table 1 *Components of, and instructions for, RelyX™ U200 resin cement*

Product	Components	Instructions
RelyX™ U200 (3M ESPE, St. Paul, MN, USA) Batch no. 662199	Base paste: Methacrylate monomers containing phosphoric acid groups, methacrylate monomers, silanated fillers, initiator components, stabilizer, rheological additives Catalyst paste: Methacrylate monomers, alkaline fillers, silanated fillers, stabilizer, initiator components, pigments, rheological additives	- Dispense cement onto mixing pad and mix cement 20 sec - apply on adhesive surfaces - allow to self-cure for 5 min. or light-cure for 20 sec. at each side (if preferred)

were observed using an inverted phase contrast microscope (CK 40 culture microscope, Olympus, Tokyo, Japan) at 40x magnification. The SBS data obtained from specimens that showed cohesive failure within dentin or resin composite were discarded.

2.5 Scanning electron microscope (SEM) examination: One prepared dentin surface from each group was dehydrated in ascending ethanol concentrations (25%, 50%, 75%, 80%, 95% and 100%) to observe the dentin surface micromorphology. One bonded specimen from each group was cross-sectioned to investigate the cement/dentin interfaces. The cross-sectioned surfaces of the bonded specimens were etched with 37% phosphoric acid for 60 seconds to remove the smear layer, washed, immersed in 5% NaOCl for 60 seconds to remove organic substances from the dentin, cleaned in the ultrasonic cleanser for one minute and then air-dried overnight at 37°C. All prepared specimens were mounted on aluminum stubs, sputter-coated with gold and examined under SEM (JEOL, JSM-5910LV, Tokyo, Japan) at 1,000x magnification.

2.6 Water contact angle measurement: Three treated dentin surfaces from each group were used for the water contact angle measurement. Approximately 0.5 μl of de-ionized water was dropped on the dentin surface, using a micropipette (Transferpette® Pipette, BRAND GMBH, Wertheim, Germany) from a constant distance of 10 mm. Photographic images of the water contact angles were immediately transferred to a computer for analysis, using contact angle measurement software (CTAMS V.1, Faculty of Science, Chiang Mai University, Chiang Mai, Thailand). Means and standard deviations of contact angle values for each group were calculated.

2.7 Data analysis: Normal distribution and homoscedasticity of the data was confirmed by Shapiro-Wilk test. One-way ANOVA was performed to compare SBS data and water contact angle values. The Scheffe test was used for post-hoc pairwise comparisons. The data analysis was performed at a significance level of α=0.05 using SPSS V17.0 software (SPSS Inc., Chicago, IL, USA).

Results

3.1 Shear bond strength: The He/O_2+R group showed the highest mean SBS values of 18.15±1.93 MPa, followed by the He+R group (16.25±2.39 MPa), the He group (13.26±1.52 MPa), the He/O_2 group (12.98±1.94 MPa) and the control group (11.20±1.87 MPa). One-way ANOVA indicated significantly higher SBS in the plasma treatment with re-wetting groups (He/O_2+R and He+R), than

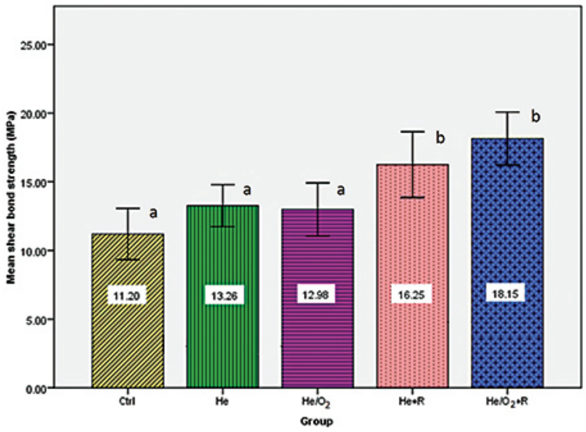

Figure 1 *Statistical comparison of SBS in all test groups. Numbers in boxes show the mean values in each group. Different letters in the plot indicate significant differences (p<0.05).*

in the plasma treatment only (He and He/O_2) and the control groups (p<0.05). Figure 1 shows mean and standard deviation data in the five experimental groups.

3.2 Scanning electron microscope (SEM) examination: SEM images of dentin surface micromorphology are illustrated in Figure 2. Less debris and more dentinal tubule morphology was seen in the plasma-only groups than in the control group. The plasma with re-wetting groups also showed partial removal of the smear layer and open dentinal tubules. SEM images of the cement/dentin interface are shown in Figure 3. No hybrid layer or resin tag formation was detectable in the control group, while the plasma-only groups showed some evidence of very short resin tag formation. The plasma with re-wetting groups showed intact cement/dentin interfaces with well-developed resin tag formations.

3.3 Water contact angle measurement: Water contact angle values are presented in Table 2. After plasma treatment for 30 seconds, the plasma-only groups showed the lowest contact angle values. Plasma-treated dentin surfaces in the re-wetting groups showed significantly lower water-contact-angle values than those in the control group (p<0.05).

Discussion

This study aimed to investigate the effects of different plasma treatments on the SBS of RelyX™ U200 self-adhesive resin cement when applied to dentin. The results show significant increases in shear bond strength in the plasma treatment with re-wetting groups (He+R and He/O_2+R). In this study, after dentin was treated by plasma and re-wetting, partially-opened dentinal tubules and thin smear layers were observed in SEM images (Fig 3). These results can be explained by the cleaning effect of plasma and modification of the contacted surfaces[10,23,24] as plasma contains large amounts of reactive particles, radicals and electronically excited atoms. The reactive oxygen species, generated from plasma through ambient air, react with the organic compounds and chemically transform organic substances on the dentin surface into volatile compounds and this leads to the enlargement of dentinal tubules.[23] This partial

Table 2 *Means and standard deviations of water contact angle values in all test groups*

Group	Contact angle
Control (no plasma treatment)	51.60±2.76[a]
He plasma	5.76±1.87[c]
He/O_2 plasma	5.94±1.74[c]
He plasma with re-wetting	10.70±2.00[b]
He/O_2 plasma with re-wetting	9.57±1.60[b,c]

Different superscript letters indicate significant differences (p<0.05).

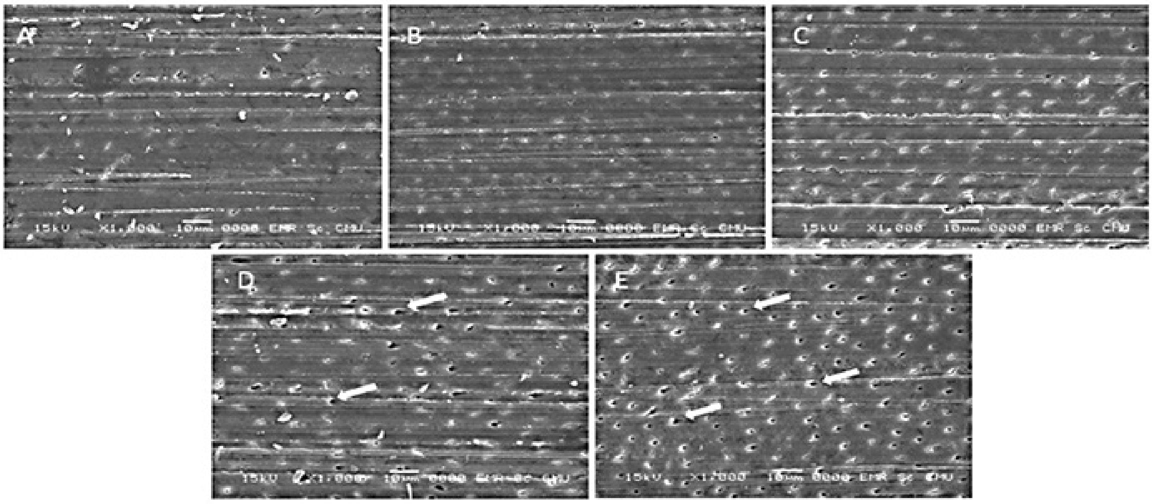

Figure 2 *Representative SEM images (1,000x)of dentin surface morphology produced by different procedures: (A) Control group, (B) He group, (C) He/O_2 group, (D) He+R group, (E) He/O_2+R group. Plasma with re-wetting procedures (D, E) show partial removal of smear layer and open dentinal tubules. White arrows indicate opening of dentinal tubules.*

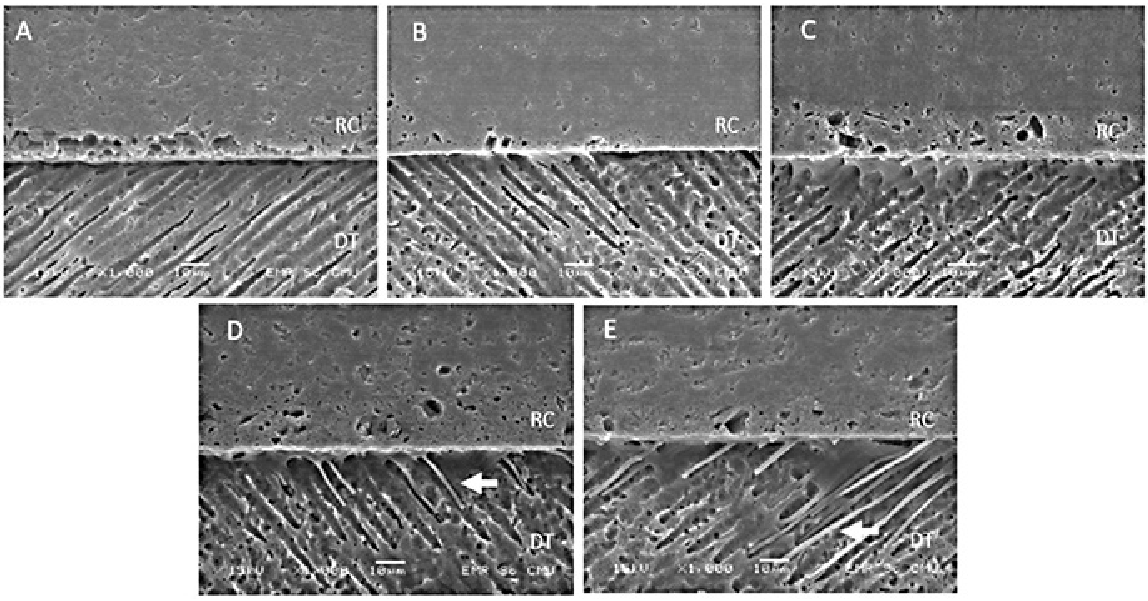

Figure 3 *Representative SEM images (1,000x) of cement/dentin interfaces produced by different procedures: (A) Control group, (B) He group, (C) He/O_2 group, (D) He+R group, (E) He/O_2+R group. In plasma treatment with re-wetting groups (D, E), well-developed resin tags were observed as a result of improved penetration of resin cement into dentinal tubules. White arrows indicate resin tag formation. (RC = resin cement; DT = dentin)*

removal of smear plugs from the tubules in this study is likely to have promoted the penetration of resin cement, providing a higher mechanical retention.

The re-wetting procedure also had an important effect. Plasma treatment, even for a few seconds, can cause the dentin surface to lose moisture.[20] As the basic components of RelyX™ U200 self-adhesive resin cement are methacrylate monomers containing phosphoric acid groups, when this cement contacts moisture on the tooth surface or water generated during neutralization of the functional group, the pH of the cement decreases and ion-releasing filler bodies react to demineralize and infiltrate dentin.[25] Hence, bond strength of this cement may depend on the moisture status of the dentin surface. One study showed lower bond strength when self-adhesive resin cement is applied to dry dentin[26], conforming with another study which observed increased bond strength when self-adhesive resin cement is applied to moist dentin under stimulated pulpal pressure.[8] In addition, remaining moist dentin surfaces after re-wetting, may have been an another important factor effect in increasing bond strength. In primary plasma cleaning systems, excited atoms and reactive particles dislodge contaminants which are removed in a vacuum system, but when using atmospheric pressure plasma jets, these contaminants may be redeposited on the dentin surface. With the re-wetting and air-blowing process, they are washed away with the excess water.[11] In our study both the dehydration of the dentin surface and the remaining debris effects resulted in there being no significant difference in the shear bond strength between the control group and the plasma treatment without re-wetting groups (p>0.05).

In this study, the contact angles of water on all plasma-treated dentin surfaces were found to be dramatically lower than in the control group. This result may be explained by the plasma modification effects on the surfaces. Many studies have revealed dentin surfaces treated with cold atmospheric pressure plasma showed greater surface energy values than did untreated surfaces, mainly by increasing the polar component, thus making the surface more hydrophilic.[21,27] Moreover, immediately after mixing the RelyX™ U200, the cement is very acidic and has a hydrophilic property. This phenomena can create good adaptation to the hydrophilic tooth surface, which is an advantage during the very first step of the clinical procedure. As seen in this study's SEM images of the dentin/cement interfaces, the plasma-only groups showed some evidence of very short resin tag formation. In the rewetting groups, the hydrophilic monomers in resin cement easily penetrate into open dentinal tubules dentin, forming well-developed resin tags, thus enhancing adhesion (Fig 3). Voids could be seen in the resin cement layers as cement was manually mixed. The use of capsule type resin cement could reduce those voids and provide higher bond strength.

The effects of plasma treatment also depend on the plasma chemistry and the energy given to the plasma. Helium gas and a mixture of helium with 0.3% oxygen were used in this study. However, there was no significant difference in SBS between these two systems (helium or helium/oxygen) (Fig 1), whether plasma-only or with subsequent re-wetting. Neither the short time of plasma treatment nor the low quantity of O species in the helium/oxygen groups seemed to affect the polymerization of the self-adhesive resin cement.

Many studies have found that 30 seconds of plasma treatment of dentin surfaces is effective in enhancing the bond strength of dental adhesives.[15,19,21] Prolonged treatment times may damage the dentin surface and decrease interfacial bonding strength.[15] However, the conditions of this in vitro study did not take into account the effects of pulpal pressure in in vivo conditions. The permeability of dentin may have resulted in oversaturation of the dentin surface with water. In addition, future studies, with more homogeneous self-adhesive resin cement, with fewer voids, on the long-term durability of adhesion after plasma treatment must be investigated.

Conclusions

Within the limitations of this study, the use of helium/oxygen plasma jet followed by re-wetting enhances the bonding effectiveness of the RelyX™ U200 self-adhesive resin cement applied to coronal dentin. These results can be attributed to the cleaning effect and surface wettability of plasma, enhancing the penetration of self-adhesive resin cement and the formation of resin tags, both of which improve bond quality.

Conflict of interest statement

The authors declare no conflicts of interest in this study.

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