Promotion of adhesive penetration and resin bond strength to dentin using non-thermal atmospheric pressure plasma


Non-thermal atmospheric pressure plasmas (NT-APPs) have been shown to improve the bond strength of resin composites to demineralized dentin surfaces. Based on a wet-bonding philosophy, it is believed that a rewetting procedure is necessary after treatment with NT-APP because of its air-drying effect. This study investigated the effect of ‘plasma-drying’ on the bond strength of an etch-and-rinse adhesive to dentin by comparison with the wet-bonding technique. Dentin surfaces of human third molars were acid-etched and divided into four groups according to the adhesion procedure: wet bonding, plasma-drying, plasma-drying/rewetting, and dry bonding. In plasma treatment groups, the demineralized dentin surfaces were treated with a plasma plume generated using a pencil-type low-power plasma torch. After the adhesion procedures, resin composite/dentin-bonded specimens were subjected to a micro-Raman bond-strength test. The hybrid layer formation was characterized by micro-Raman spectroscopy and scanning electron microscopy. The plasma-drying group presented significantly higher bond strength than the wet-bonding and dry-bonding groups. Micro-Raman spectral analysis indicated that plasma-drying improved the penetration and polymerization efficacy of the adhesive. Plasma-drying could be a promising method to control the moisture of demineralized dentin surfaces and improve the penetration of adhesive and the mechanical property of the adhesive/dentin interface.

Current dental-adhesive systems require the physical and chemical modifications of tooth substrates using either acids (etch-and-rinse technique) or acidic monomers (self-etch technique) (1, 2). The etch-and-rinse technique is the most common technique used by dental practitioners and provides reliable adhesion to enamel (3). However, as a result of the morphological and physiological heterogeneity of dentin, dentin adhesion with etch-and-rinse adhesive systems remains unpredictable and is dependent on the clinician’s technique (2–5). After acid etching, the demineralized dentin surface should be kept hydrated to prevent the exposed collagen network from collapsing before adequate infiltration with adhesive monomers; this clinical technique is referred to as the wet-bonding technique (6, 7). However, the extent of hydration of demineralized dentin required for optimal performance of adhesive systems is difficult to standardize in clinical practice. Therefore, adjunctive approaches, such as the use of an electric device and collagen cross-linking agents, have been investigated to improve the penetration of adhesive into the dentin surface and stabilize the hybrid layer (8–10).

Plasma, the fourth state of matter, has been adopted in modern industries for surface engineering, such as cleaning, etching, adhesion enhancement, and deposition of thin films (11). Recently, non-thermal atmospheric pressure plasma (NT-APP) has attracted considerable interest in the biomedical field (12, 13). Non-thermal atmospheric pressure plasma has a relatively low temperature and therefore, when optimally controlled, can induce changes in the surface characteristics of substrates without heat damage (14). The non-destructive property of NT-APP is particularly attractive for dental applications in which heat-sensitive substrates, such as teeth, ceramics, and resin composites, are frequently involved. RTTS et al. (15) first reported that use of a non-thermal plasma brush improved the immediate bond strength of a resin composite to dentin. Plasma treatment could increase the hydrophilicity of dentin and permit better penetration of the adhesive into the demineralized dentin surface.
(15–17). In our previous studies, a pencil-type plasma torch was developed and used to improve the resin bond strength to ceramic, enamel, and dentin (18, 19). Low-power plasma, which was generated with a pulsed power of 0.3 W to ensure that it was tolerated by the sensitive dentin, was suggested to be as effective as a high-power plasma, of 6 W, in improving the resin bond strength to dentin (20).

An NT-APP plume is usually blown out from the reactor by the flow of the working gas and thereby has an air-drying effect on the demineralized dentin surface (15, 16, 20). A plasma treatment for a few seconds can cause the dentin surface to lose moisture before application of the adhesive monomers. Based on the wet-bonding philosophy, a rewetting procedure after ‘plasma-drying’ is required to restore the moisture of dentin. However, the exact effect and application parameters of plasma-drying have not been determined when used in combination with commercially available adhesives.

The aim of this study was to investigate the effect of plasma-drying on the bond strength of an etch-and-rinse adhesive to dentin compared with that of the contemporary wet-bonding technique. The null hypothesis tested was that plasma-drying in adhesion procedures would not influence the microtensile bond strength (MTBS) of resin composite to dentin. Formation of the hybrid layer at the adhesive/dentin interface was characterized by micro-Raman spectroscopy and scanning electron microscopy analyses.

**Material and methods**

**Dentin specimen preparation**

Sound, caries-free human third molars were collected according to protocols approved by the Institutional Review Board of Seoul National University Dental Hospital (IRB No. ERI12003) and were stored in a solution of 0.5% chloramine-T at 4°C until required for use. The teeth were cut using a low-speed diamond saw (Isomet; Buehler, Lake Bluff, IL, USA) at the occlusal enamel to expose the dentin surfaces. The exposed dentin surface was polished with 600-grit silicon carbide abrasive papers under water cooling. The teeth were randomly assigned to four groups according to the adhesion procedure: a wet-bonding group (positive control), a plasma-drying group, a plasma-drying/rewetting group, and a dry-bonding group (negative control).

**Adhesion procedure and plasma treatment**

The dentin surface was demineralized for 15 s with 35% phosphoric acid (Scotchbond Etchant; 3M ESPE, St Paul, MN, USA) and then rinsed thoroughly with water. In the wet-bonding group, the demineralized dentin surface was blot-dried with a moistened Kimwipes tissue (Kimberly-Clark, Roswell, GA, USA) before applying a two-step etch-and-rinse adhesive (Adper Single Bond 2; 3M ESPE). Adper Single Bond 2 adhesive contains 2,2-bis[4-(2-hydroxy-3-methacryloxypropoxy) phenyl]-propane (BisGMA), hydroxyethyl methacrylate (HEMA), dimethacrylates, ethanol, water, photoinitiators, and silica nanofillers. In the plasma-drying group, the demineralized dentin surface was exposed to a plasma plume for 20 s at a distance of 5 mm from the nozzle. The plasma plume was generated at a pulsed power of 0.3 W using the pencil-type plasma torch (20). Helium as working gas was delivered at a flow rate of 2 l min⁻¹. The plasma-dried dentin surface was immediately coated with the adhesive. In the plasma-drying/rewetting group, the demineralized dentin surface was treated with the plasma torch in the same manner as for the plasma-drying group, but the plasma-dried dentin surface was rewetted with a moistened Kimwipes tissue before applying the adhesive. In the dry-bonding group, the demineralized dentin surface was dried with oil/water-free compressed air for 20 s before the adhesive was applied. In all test groups, the applied adhesive was gently dried with oil/water-free compressed air to evaporate solvents and light-cured for 10 s using a light-emitting diode curing unit (Elipar FreeLight 2; 3M ESPE). A resin composite (Filtek Z-250; 3M ESPE) was incrementally built on the dentin surface and each increment was light-cured for 20 s. The bonded specimens were stored in a humid chamber at 37°C for 24 h before being tested.

**Microtensile bond strength test**

The bonded specimens were trimmed into an hourglass shape and then sectioned into slabs with a cross-sectional bonded area of approximately 0.8 mm² × 0.8 mm². Finally, 24 specimens were obtained from each group for a MTBS test (n = 24). The specimens were attached to a testing jig and loaded at a crosshead speed of 0.5 mm min⁻¹ in a universal testing machine (LF Plus; Lloyd Instruments, Fareham, UK) until failure. The dimension of the adhesive interface was measured for each specimen using a digital caliper to determine the MTBS value.

**Micro-Raman spectroscopy**

Micro-Raman spectral analysis was performed to evaluate the penetration of the adhesive monomers, especially BisGMA, into the demineralized dentin, as well as to evaluate the polymerization efficacy of the adhesive at the adhesive/dentin interface. A Renishaw InVia Raman microscope (Renishaw, Wotton-under-Edge, UK) was used with a 785 nm diode laser at 125 mW and focused through a 100 × Leica lens (Leica Microsystems, Wetzlar, Germany) to a beam diameter of approximately 1 μm. Raman spectra were acquired across the adhesive/dentin interface at 0.5 μm intervals from the dentin to the adhesive layer. Penetration of the adhesive monomers was compared by measuring the penetration of BisGMA into the demineralized dentin and the relative content of BisGMA in the adhesive, which were determined based on the ratio of band intensities of 1613 cm⁻¹ (phenyl C-O-C of BisGMA) and 1667 cm⁻¹ (amide I of collagen) to the band intensity of 1640 cm⁻¹ (aliphatic C=C)/1609 cm⁻¹ (aromatic C=C) was also determined to compare the polymerization efficacy of the adhesive between the test groups. Band ratios were calculated using the spectral subtraction technique (21, 22).

**Scanning electron microscopy**

The micromorphological characteristics of the adhesive/dentin interface were observed using a scanning electron microscope (Leica, Cambridge, UK) at 1500× and 5000× magnification. The micromorphological characteristics of the adhesive/dentin interface were observed using a scanning electron microscope (Leica, Cambridge, UK) at 1500× and 5000× magnification.
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microscope (JSM-840A; JEOL, Tokyo, Japan). Selected specimens from each group were immersed in 6N hydrogen chloride (HCl) for 3 s to dissolve the mineral component of the dentin. This was followed by immersion in 5% sodium hypochlorite (NaOCl) for 4 min to remove the remaining organic components. After washing twice with water, the prepared specimens were desiccated overnight in a vacuum jar and sputter-coated with gold.

Statistical analysis
Using the statistical software spss 12.0 (IBM, Armonk, NY, USA), one-way ANOVA was performed to compare the MTBS data. The Tukey’s HSD test was used for post hoc pairwise comparisons at a significance level of $\alpha = 0.05$. In addition, the MTBS data were also analyzed using the Weibull statistics to obtain the Weibull modulus ($m$) and characteristic strength ($\sigma_0$).

Results
One-way ANOVA indicated significant differences in the MTBS between the groups (Table 1, $P < 0.001$). The plasma-drying group presented the highest mean MTBS value of 61.2 ± 14.3 MPa, followed by the plasma-drying/rewetting group (58.6 ± 14.6 MPa) and the wet-bonding group (52.2 ± 16.8 MPa). The bond strength of the plasma-drying group was significantly higher than that of the wet-bonding group ($P < 0.05$). The dry-bonding group presented the lowest mean MTBS value, of 28.9 ± 9.5 MPa ($P < 0.05$). According to the Weibull statistics, the plasma-drying group possessed a higher Weibull modulus ($m = 4.93$) and characteristic strength ($\sigma_0 = 66.8$ MPa) than the plasma-drying/rewetting ($m = 4.50$; $\sigma_0 = 64.3$ MPa) and wet-bonding ($m = 3.45$; $\sigma_0 = 58.1$ MPa) groups (Table 1). The dry-bonding group possessed the lowest Weibull modulus ($m = 3.39$) and characteristic strength ($\sigma_0 = 32.3$ MPa).

Representative micro-Raman mapping spectra acquired across the adhesive/dentin interface for a specimen selected from the wet-bonding group is shown in Fig. 1. The intensities of the Raman bands associated with the adhesive monomer (1113, 1454, and 1609 cm$^{-1}$) decreased gradually from the adhesive layer to the dentin. Figure 2 shows the band ratios of 1113 cm$^{-1}$/1667 cm$^{-1}$, 1113 cm$^{-1}$/1454 cm$^{-1}$, and 1640 cm$^{-1}$/1609 cm$^{-1}$ as a function of the position across the hybrid layer, from the bottom part to the upper part of the hybrid layer. The band ratio of 1113 cm$^{-1}$/1667 cm$^{-1}$, which indicated BisGMA penetration, gradually decreased when approaching the bottom part of the hybrid layer in all the test groups (Fig. 2A). However, in the upper part of the hybrid layer, the plasma-drying group possessed a higher 1113 cm$^{-1}$/1667 cm$^{-1}$ ratio than obtained for the wet-bonding and plasma-drying/rewetting groups. The band ratio of 1113 cm$^{-1}$/1454 cm$^{-1}$ indicated the relative content of BisGMA in the penetrating adhesive (Fig. 2B). The corresponding ratios in the plasma-drying and dry-bonding groups were similar ratios to 1113 cm$^{-1}$/1454 cm$^{-1}$, except at the bottom part of the hybrid layer. The ratio of the plasma-drying group was apparently greater than those of the wet-bonding and plasma-drying/rewetting groups across the hybrid layer. The band ratio of 1640 cm$^{-1}$/1609 cm$^{-1}$ was inversely proportional to the degree of conversion of the adhesive (Fig. 2C). The dry-bonding group presented the lowest ratio, which indicated the highest polymerization.

Table 1
Microtensile bond strength (MTBS, in MPa) and Weibull parameters (Weibull modulus, $m$; characteristic strength in MPa, $\sigma_0$) of the resin composite to dentin for different adhesion procedures, with and without treatment with a non-thermal atmospheric pressure plasma (NT-APP).

<table>
<thead>
<tr>
<th>Group</th>
<th>MTBS (SD)$^A$</th>
<th>$m$ (90% CI)$^B$</th>
<th>$\sigma_0$ (90% CI)$^C$</th>
<th>Correlation coefficient $r$</th>
</tr>
</thead>
<tbody>
<tr>
<td>Wet bonding</td>
<td>52.2 (16.8)$^b$</td>
<td>3.45 (3.26–3.65)</td>
<td>58.1 (24.6–79.8)</td>
<td>0.988</td>
</tr>
<tr>
<td>Plasma-drying</td>
<td>61.2 (14.3)$^a$</td>
<td>4.93 (4.53–5.33)</td>
<td>66.8 (36.5–83.4)</td>
<td>0.976</td>
</tr>
<tr>
<td>Plasma-drying/rewetting</td>
<td>58.6 (14.6)$^ab$</td>
<td>4.50 (4.25–4.75)</td>
<td>64.3 (33.2–82.0)</td>
<td>0.989</td>
</tr>
<tr>
<td>Dry bonding</td>
<td>28.9 (9.5)$^c$</td>
<td>3.39 (3.17–3.60)</td>
<td>32.2 (13.4–44.5)</td>
<td>0.985</td>
</tr>
</tbody>
</table>

$^A$Mean values with the same superscript letter are not significantly different, based on the Tukey’s HSD post-hoc test ($\alpha = 0.05$).

$^B$The higher the Weibull modulus, the more reliable the adhesion procedure.

$^C$The higher the characteristic strength, the higher the adhesion effectiveness.
efficacy. The plasma-drying group possessed a lower $1640 \, \text{cm}^{-1}/1609 \, \text{cm}^{-1}$ ratio than those of the wet-bonding and plasma-drying/rewetting groups. Representative scanning electron microscopy images of the adhesive/dentin interface are shown in Fig. 3. The wet-bonding, plasma-drying, and plasma-drying/rewetting groups presented intimate contact between the adhesive layer and hybrid layer with well-developed resin tags, whereas the dry-bonding group presented distinct gaps in the interfacial region. The plasma-drying group presented a more uniform and homogeneous adhesive/dentin interface than the wet-bonding and plasma-drying/rewetting groups. Furthermore, in the plasma-drying group, lateral branches of resin tags, which projected into dentinal canaliculi, were abundant and clearly observed throughout the entire length of the resin tags.

**Discussion**

Non-thermal atmospheric pressure plasmas include a large amount of charged species, radicals, and energetic photons, which can enhance the surface energy of a substrate, making it perform better in molecular interactions (11). Treatment of demineralized dentin surfaces with NT-APP is a promising approach to improve the penetration of adhesive and resin bond strength to dentin (15–17, 20). Conversely, a rewetting procedure is necessary after plasma treatment, in accordance with the wet-bonding philosophy (15, 16, 20). In the present study, the bond strength for the plasma-drying group was significantly greater than that of the wet-bonding group and it was double that of the dry-bonding group (Table 1). The structural integrity of the collagen network may be maintained despite the loss of water by the plasma-drying, allowing adequate penetration of adhesive monomers. A possible mechanism for the effect of plasma-drying is that many energetic and chemically reactive species in plasma lead to structural changes of the exposed collagen fibers by breaking intrafibrillar bonds, such as hydrogen bonds, thereby preventing collapse of the collagen network, even under dry conditions (15, 23).

The results of the micro-Raman spectroscopy analysis indicated that the plasma-drying group presents a higher degree of BisGMA penetration than the wet-bonding and plasma-drying/rewetting groups, although the differences between the groups in the penetration of BisGMA decreased when approaching to the bottom part of the hybrid layer (Fig. 2A). In addition, the relative content of BisGMA in the infiltrating adhesive was greater in the plasma-drying group than in the wet bonding and plasma-drying/rewetting groups (Fig. 2B). Although the effect of the plasma treatment on the penetration of adhesive was more prominent in the upper part of the hybrid layer, the plasma treatment generally improved the penetration of BisGMA into the demineralized dentin. The band ratios of $1113 \, \text{cm}^{-1}/1667 \, \text{cm}^{-1}$ (amide I of collagen): the degree of BisGMA penetration into the demineralized dentin; (B) $1113 \, \text{cm}^{-1}/1454 \, \text{cm}^{-1}$ (CH$_2$ of all monomers): the relative content of BisGMA in the infiltrating adhesive; (C) $1640 \, \text{cm}^{-1}$ (aliphatic C=C)/$1609 \, \text{cm}^{-1}$ (aromatic C=C): the relative polymerization efficacy of the adhesive.

The plasma-drying group possessed a lower $1640 \, \text{cm}^{-1}/1609 \, \text{cm}^{-1}$ ratio than those of the wet-bonding and plasma-drying/rewetting groups. Representative scanning electron microscopy images of the adhesive/dentin interface are shown in Fig. 3. The wet-bonding, plasma-drying, and plasma-drying/rewetting groups presented intimate contact between the adhesive layer and hybrid layer with well-developed resin tags, whereas the dry-bonding group presented distinct gaps in the interfacial region. The plasma-drying group presented a more uniform and homogeneous adhesive/dentin interface than the wet-bonding and plasma-drying/rewetting groups. Furthermore, in the
group were comparable with that of the dry-bonding group in the upper part of the hybrid layer. In contrast to the dry-bonding group, the plasma-drying group presented a uniform adhesive/dentin interface with good integrity (Fig. 3C,D). Therefore, the highest bond strength for the plasma-drying group can be explained by the high BisGMA content in the hybrid layer. BisGMA has better mechanical properties than monomethacrylates, such as HEMA, but, because of its hydrophobicity and large molecular size does not adequately infiltrate the wet dentin surface (24). The plasma-drying would displace excess water from the interfibrillar space without collapse of the collagen network, resulting in improved BisGMA penetration and bond strength.

A previous study, using an argon plasma brush (17), showed that plasma treatment improved the penetration of adhesive into the demineralized dentin surface and the polymerization efficacy of a model adhesive. However, in contrast to the results of the current study, the improvement in the penetration of adhesive was ascribed mainly to hydrophilic HEMA. The different results may be attributed to the differing composition of adhesives and the differing characteristics of the plasmas. The previous study (17) used a model adhesive with a higher concentration of HEMA (a mass ratio of BisGMA to HEMA of 0.43) than the currently used adhesive (a mass ratio of BisGMA to HEMA of approximately 1.5). In addition, argon plasma makes a polymer surface more hydrophilic than does helium plasma (25, 26). When compared with helium plasma, argon plasma generates larger amounts of reactive species, such as hydroxyl groups, and is more effective at inducing chain scission, etching, and cleaning through ion bombardment (25–27). The improved hydrophilicity of the dentin surface, in conjunction with the more hydrophilic adhesive, may markedly enhance HEMA penetration. However, the high contents of HEMA in the adhesive/dentin interface adversely influence mechanical properties and make the interface prone to hydrolytic degradation (28). Enhancing the infiltration of hydrophobic monomers, such as BisGMA, is desired for achieving high bond strength and durable adhesion to dentin, and thus the plasma treatment demonstrated in the present study seems promising.

A lower band ratio of 1640 cm\(^{-1}\)/1609 cm\(^{-1}\) indicated higher polymerization efficacy in the interfacial region (Fig. 2C). The dry-bonding group showed a lower 1640 cm\(^{-1}\)/1609 cm\(^{-1}\) ratio throughout the examined region than the other groups, but this did not imply that a high-quality hybrid layer was formed. In the dry-bonding group, the adhesive could simply cover the collapsed collagen structure instead of infiltrating. The low 1640 cm\(^{-1}\)/1609 cm\(^{-1}\) ratio for the dry-bonding group can be explained by the low amount of residual water, as well as by poor hybridization between the adhesive and the collapsed collagen matrix (29–31). On the other hand, it is noteworthy that the polymerization efficacy for the plasma-drying group was comparable with that of the dry-bonding group. Excess residual water causes phase separation between the hydrophobic and hydrophilic components and compromises the polymerization efficacy of the adhesive monomers (29, 32). Plasma treatment effectively removed residual water from the dentin surface without causing collapse of the demineralized collagen matrix. In addition, energetic and chemically reactive species included in the plasma can induce polymerization of the adhesive monomers and enhance interaction between the dentin substrate and the infiltrating adhesive.

Scanning electron microscopy observations demonstrated that the plasma-drying group had abundant and well-developed lateral branches of resin tags, which
anastomosed with adjacent dentinal tubules (Fig. 3C, D). Hydrophobic monomers, such as BisGMA, do not adequately infiltrate the demineralized dentin where there is excess residual water. However, complete removal and/or replacement of water in the interfibrillar space is practically unattainable (33, 34). Plasma-drying may effectively remove residual water from the interfibrillar space, allowing effective penetration of the adhesive monomers. In addition to the high BisGMA content in the hybrid layer, well-developed resin tags could contribute to improving the bond strength for the plasma-drying group.

The Weibull statistics was used for interpreting bond-strength data in a viewpoint of fracture analysis, which can be explained with crack distribution. The MTBS data fitted the Weibull distribution well, as shown by correlation coefficient values of > 0.9 for all test groups (Table 1). Most studies on the strength of brittle materials, such as ceramics and resin composites, have used means and SD, which, however, do not provide information about unpredictable brittle failure as a result of the presence of critical flaws (35–37). The overall performances of adhesives bonded to dentin can be better evaluated by predicting the likelihood of failure at specific stress levels. The Weibull parameters provide insights into the reliability (Weibull modulus m) and the probability of failure for a given stress level (35–39). A higher m indicated a narrow spread of data and a high reliability of the characteristic strength (σ0), which represents the stress responsible for 63.2% of the sample failures. An adhesion procedure with a high Weibull modulus is considered to be reliable and less technique-sensitive. The plasma-treatment groups (plasma-drying and plasma-drying/rewetting) showed higher Weibull moduli m than the wet-bonding group. Plasma-drying improved the reliability of dentin adhesion by making adhesion procedures less dependent on the moisture content of demineralized dentin. When adhesion procedures are clinically performed, plasma treatment will expand the window of opportunity in which optimal intertubular and intratubular dentin infiltration are simultaneously achieved and reduce the technique sensitivity of etch-and-rinse adhesive systems.

In conclusion, plasma treatment after acid etching enhanced the penetration of adhesive, especially hydrophobic BisGMA, and as a result improved the bond strength of the resin composite to dentin. Moreover, omitting the rewetting procedure after plasma treatment enhanced the effect of plasma treatment in improving the dentin adhesion. Plasma-drying will be a reliable method to control the moisture of demineralized dentin surfaces in adhesion procedures. The effect of plasma treatment on dentin adhesion should be further investigated with different adhesive systems. In addition, future studies on plasma treatment need to evaluate the long-term durability of dentin adhesion and clinical performance.

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Conflicts of interest – The authors declare no conflicts of interest.

References
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