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Nonthermal Atmospheric Plasmas in Dental Restoration

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Abstract

It is well known that the service life of contemporary composite restoration is unsatisfactory, and longevity of dentin bonding is one of the major culprits. Bonding is essentially a hybridization process in which dental substrate and adhesive resin interact with each other through an exchange process. Thus, the longevity of dentin bonding can only be improved with enhanced qualities in substrate, adhesive resin, and their interaction within the hybridization zone. This review aims to collect and summarize recent advances in utilizing nonthermal atmospheric plasmas (NTAPs)—a novel technology that delivers highly reactive species in a gaseous medium at or below physiologic temperature—to improve the durability of dentin bonding by addressing these 3 issues simultaneously. Overall, NTAP has demonstrated efficacies in improving a number of critical properties for dentin bonding, including deactivation of oral pathogens, modification of surface chemistry/properties, resin polymerization, improvement in adhesive-dentin interactions, and establishment of auxiliary bonding mechanism. While a few preliminary studies have indicated the benefit of NTAP to bond strength and stability, additional researches are warranted to employ knowledge acquired so far and to evaluate these properties in a systematic way.

Keywords: dentin, adhesives, dental bonding, surface properties, composite resins, polymerization

Existing Problems in Dental Adhesion

In today's dental practice, tooth restoration with composite material is an increasingly common procedure, especially when aesthetic considerations are of priority. However, the longevity of composite restoration has long been criticized as being inferior to its amalgam counterpart due to unsatisfactory bonding performance of dental adhesives (De Munck et al. 2005; Bernardo et al. 2007). From a physicochemical point of view, dental bonding is weak because it is a process in which dental substrate and resin—2 highly heterogeneous entities with drastically different properties—are joined together via hybridization. As such, the overall quality of bonding can be improved only when the qualities of 3 major contributing factors are accounted for: dental substrate, resin, and their interaction/hybridization.

It has been long recognized that oral bacteria may accelerate bond degradation of the tooth-restoration interface (De Munck et al. 2005). Thus, one important aspect of dental substrate quality involves the deactivation of adherent oral bacteria on teeth following minimally invasive cavity preparation (Shafiei and Memarpour 2012). Another aspect of substrate quality is surface energy (i.e., wettability), which dictates how well adhesives spread and penetrate into dental substrates (Pashley et al. 1992). Regarding resin, its quality is directly related to polymerization. Suboptimal polymerization is common in current dentin bonding procedures due to the incompatibility between hydrophobic photoinitiator and hydrophilic component of adhesive (Wang and Spencer 2005; Wang et al. 2006), the latter of which is ubiquitously existent in almost all dental adhesives to facilitate resin infiltration into water-filled demineralized dentin collagen matrix resulting from acid etching. Compromised polymerization not only leads to an unstable resin phase but also leaves demineralized dentin collagen exposed to the degenerative oral environment, leading to an unstable collagen phase as well (Pashley et al. 2011; Van Meerbeek et al. 2011). Thus, resin polymerization is quintessential to the resin-collagen microinterlocking mechanism, whose stability underlies dentin bonding durability (Nakabayashi et al. 1982). Finally, resin-substrate interaction is influenced by factors such as intactness of smear layer and extent of tubule opening, and it can be augmented by any auxiliary interaction between collagen and resin phases to complement the aforementioned mechanical interlocking. With these understandings, the current review focuses on nonthermal atmospheric plasma (NTAP), a novel technology that has the potential to simultaneously address qualities of dental substrate, resin, and their interaction and enhance the longevity of dental bonding as a result. A brief summary of the NTAP applications pertaining to dental bonding is shown in the Table.

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Table. Brief Summary of the NTAP Carrier Gas, Flow Rate, Treatment Time, and Effects Pertaining to Dental Bonding.

NTAP Carrier Gas	Power	Time	Observations Related to Bonding	Reference
He at 2,000 sccm with O_2 (0.5%) feeding	Pulse frequency (20 kHz) and voltage (6 kV)	120 s	*B. cereus*, methicillin-resistant *S. aureus, E. coli*, and *P. aeruginosa* killed	Alkawareek et al. 2014
He	150 to 340 mW	60 s	*S. mutans* killed in dental model cavity	Bedem 2005
Ar at 3,000 sccm with optional O_2 feeding at 50 or 1,500 sccm	3 W	20 s	Top layer of *L. acidophilus* and *S. mutans* biofilm deactivated; O_2 addition no effect on deactivation effectiveness	Blumhagen et al. 2014
He at 1,000 to 2,000 sccm with optional O_2 (2%) feeding	Pulse frequency (10 kHz), width (1.6 ns), and voltage (8 kV)	120 to 480 s	Significantly decreased colony-forming unit of *E. faecalis*; ultrastructural changes observed after treatment for 120 s	Cao et al. 2011
Ar at 3,000 sccm	5 W	40 s	Enhanced degree of monomer conversion	Chen, Zhang, et al. 2012
He at 4,500 sccm with optional O_2 (1% to 10%) feeding	24 W	Up to 180 s	He/O_2 (2.5%) reached the maximum sterilization efficiency of *E. faecalis* on filter paper	Chen, Huang, et al. 2012
Ar at 2,000 sccm	5 to 10 W	5 to 45 s	Decreased water contact angle on enamel, dentin, and cured composite	Chen et al. 2013
Ar at 2,000 sccm	5 to 15 W	60 to 480 s	HEMA grafting with simultaneous NTAP and HEMA treatment of demineralized dentin collagen	Chen et al. 2014
He at 50 sccm with TEGDMA feeding	Pulse frequency (15 kHz) and voltage (2 kV)	N/A	TEGDMA deposition on dental ceramics.	Cho et al. 2011
Ar at 3,000 sccm	2 to 3 W	30 s	Immediate μTBS of Single Bond Plus to dentin increased by NTAP	Dong et al. 2013
Ar at 3,000 sccm	2 to 3 W	30 s	No HEMA grafting with sequential NTAP and HEMA treatment of demineralized dentin collagen	Dong et al. 2014
Ar at 3,000 sccm	2 to 3 W	30 s	Higher μTBS of OptiBond All-in-One self-etch adhesive to dentin after 24 h and 2 mo of water storage	Dong et al. 2015
Airflow at 29 sccm	~135 W	10 to 30 s twice daily for 5 d	*A. naeslundii, C. albicans, S. gordonii, S. mutans, S. oralis*, and *S. sanguinis* inhibited on agar plates	Duarte et al. 2011
He at 50 sccm	Vrms (1.13 to 1.98 kV), Irms (7.07 to 14.14 mA), and 15 kHz	60 s	Improved surface hydrophilicity on dental ceramics	Han et al. 2012
He at 2,000 sccm	Conventional: 2.4 kV, 2.5 mA, and 8.0 kHz Pulsed: ~2 kV, 0.4 kHz, and 5 voltage peaks (500 ns) at 12.5-ms intervals	30 s	Reduced water contact angle on dentin; increased μTBS of Scotchbond Multi-Purpose Plus to dentin immediately and after 5,000 cycles of thermocycling	Han et al. 2014
Ar at 5,000 sccm	8 W	30 s	Reduced water contact angle on dentin; increased immediate μTBS to dentin for Scotchbond Universal but not Clearfil SE; no increased μTBS for either adhesive after 1-y storage	Hirata et al. 2015
Ar at 1,500 sccm with optional O_2 feeding at 250 sccm	15 W	Up to 300 s	Amount of O_2 addition and type of supporting medium influenced the killing of *E. coli* and *M. luteus*	Huang et al. 2007
Ar at 5,000 sccm	8 W	180 s	Significantly higher *E. faecalis* reduction than 0.1% chlorhexidine irrigation and a comparable one with 0.6% sodium hypochlorite	Jablonowski et al. 2013
Ar at 5,000 sccm with optional O_2 (0.2% to 1%) feeding	8 W	30 to 120 s	Water contact angle of dentin reduced	Koban et al. 2011
He at 3,525 sccm with optional H_2O_2/H_2O (30%) feeding	Pulse frequency (2.4 GHz), width (5 μs), and power (250 W); mean power, 2 W	N/A	Decreased water contact angle on enamel and dentin	Lehmann et al. 2013

(continued)

Table. (continued)

NTAP Carrier Gas	Power	Time	Observations Related to Bonding	Reference
He with O_2 feeding	Pulse frequency (10 kHz), width (1600 ns), voltage (8 kV)	300 s	*P. gingivalis* killed; no pathologic changes in rabbit oral mucosa	Liu et al. 2011
He at 1,000 sccm	5 to 15 W	Up to 900 s	*S. aureus, B. subtilis, P. aeruginosa*, and *E. coli* destroyed in a minute; mesenchymal stem cells and rat skin not affected	Lunov et al. 2015
He at 5,000 sccm	Pulse frequency (5 kHz), width (500 ns), and voltage (8 kV)	300 to 660 s	Significant dose-related inactivation of *P. gingivalis*	Mahasneh et al. 2011
He at 2,000 sccm	N/A	5 to 35 s	*S. mutans* biofilms destroyed with no morphologic modifications in pig gingiva	Molnar et al. 2013
Ar at 2,500 sccm	5 W	Up to 300 s	Immediate μTBS of Single Bond Plus to peripheral dentin increased by 30-s NTAP treatment	Ritts et al. 2010
He/O_2/N_2 at 2,000/1,200/1,500 sccm	2.5 W	0.3 to 0.9 s/mm^2	*L. casei, S. mutans, C. albicans*, and *E. coli* killed on agar plates and dentin slices	Rupf et al. 2010
Ar at 5,000 sccm with O_2 feeding	8 W	5 to 20 s	Water contact angle reduced on Y-TZP and Ti surfaces	Silva et al. 2011
He at 2,000 sccm	150 mW	Up to 60 s	Top layer of *S. mutans* biofilms inhibited	Sladek et al. 2007
Ar at 5,000 sccm	8 W	10 s	Water contact angle reduced on Y-TZP surfaces	Valverde et al. 2013
Ar at 500 to 3,500 sccm	5 to 15 W	Up to 300 s	*S. mutans* and *L. acidophilus* seeded on various tooth models deactivated	Yang et al. 2011
Ar at 1,000 to 3,500 sccm	10 to 20 W	300 s	*E. coli* and *M. luteus* seeded in porous solid medium, liquid medium, and colloid medium deactivated	Yu et al. 2007
Ar at 3,000 sccm	2 to 3 W	30 s	Adhesive penetration into demineralized dentin collagen improved	Zhang et al. 2014
He at 2,000 sccm with O_2 (1%) feeding	Pulse frequency (8 kHz), width (1600 ns), and voltage (8 kV)	720 s	Simulated root canals sterilized	Zhou et al. 2010

N/A, not available; NTAP, nonthermal atmospheric plasma; TEGDMA, triethyleneglycol dimethacrylate; μTBS, microtensile bond strength.

Nonthermal Atmospheric Plasma

Plasma is the fourth state of matter and constitutes >99% of the universe (Tendero et al. 2006). It is essentially an ionized gas generated by an electromagnetic field. For more information on the classification, source, and general application of plasma, see the excellent reviews by Tendero et al. (2006), Fridman et al. (2008), and Bárdos and Baránková (2010). From the perspective of dental applications, the generating conditions of plasma, particularly temperature and pressure, bear direct implications to the feasibility of its clinical use. Plasma that is nonthermal (i.e., its gas phase is at room temperature, as opposed to thousands of degrees) and atmospheric (i.e., it is produced at atmospheric pressure, as opposed to vacuum) is the desired type. NTAP can be created and manipulated into various physical shapes and compact sizes that can be handheld and conveniently operated by dental practitioners in clinics. Figure 1a shows a schematic illustration of the apparatus for NTAP brush production, and Figure 1b shows its appearance (Chen et al. 2013). Like all plasmas, NTAP is a mixture of highly reactive particles, including electronically excited atoms/molecules, ionic and free radical species, and ultraviolet (UV) photons. Depending on the plasma chemistry and gas composition, these highly reactive plasma species react with, clean and etch surface materials, bond to various substrates, or combine to form a thin layer of plasma coating, consequently altering the surface characteristics for multiple applications (Fig. 1c; Shohet 1993; Yasuda 2005).

Deactivation of Oral Pathogens

As a gaseous medium, NTAP has the capability to penetrate irregular cavities/fissures and kill bacteria. Plasma treatment of tooth cavities or dental surfaces allows us to avoid contamination, actively fight bacterial infections, offer additional cleaning to the decayed matters in the tooth cavities, and prepare/engineer the dentin and adhesive surface/interface for strong and durable bonding to composite restorative materials with a cohesive treatment process.

A broad spectrum of bacteria can be deactivated/destructed by NTAP, including Gram-negative *Pseudomonas aeruginosa* and *Escherichia coli* and Gram-positive *Staphylococcus aureus, Bacillus subtilis*, and *Micrococcus luteus* (Huang et al. 2007; Yu et al. 2007; Zhou et al. 2010; Alkawareek et al. 2014; Lunov et al. 2015). It was found that NTAP's antibacterial activity was not due to UV emission (Lunov et al. 2015) but rather to reactive oxygen species, which led to oxidative damages to cell membrane, DNA, and proteinaceous enzymes

(Alkawareek et al. 2014). Thus, the antibacterial efficacy of NTAP was tunable by adjusting oxygen feeding to the plasma-generating devices (Huang et al. 2007; Yu et al. 2007; Zhou et al. 2010). When it comes to oral bacteria, twice daily NTAP treatment for 10 to 30 s each time could eradicate several dental microorganism biofilms, including *Actinomyces naeslundii, Candida albicans, Streptococcus gordonii, Streptococcus mutans, Streptococcus oralis*, and *Streptococcus sanguinis*, likely due to disruption of the polysaccharides in the biofilm matrix (Duarte et al. 2011). Periodontal pathogen *Porphyromonas gingivalis* and endodontic pathogen *Enterococcus faecalis* were inhibited by NTAP alone or in conjugation with conventional antibacterial approaches (Zhou et al. 2010; Cao et al. 2011; Liu et al. 2011; Mahasneh et al. 2011; Chen, Huang, et al. 2012; Jablonowski et al. 2013). Moreover, NTAP treatment did not change the viability of mesenchymal cells, nor did it induce morphologic alteration to mucosa and periodontal tissues, thus alleviating such concerns as adverse effect to healthy tissues when NTAP is used in oral applications (Liu et al. 2011; Molnar et al. 2013; Lunov et al. 2015). While pulpal cells are crucial to tooth restoration as well, their response to NTAP treatment has not been evaluated yet.

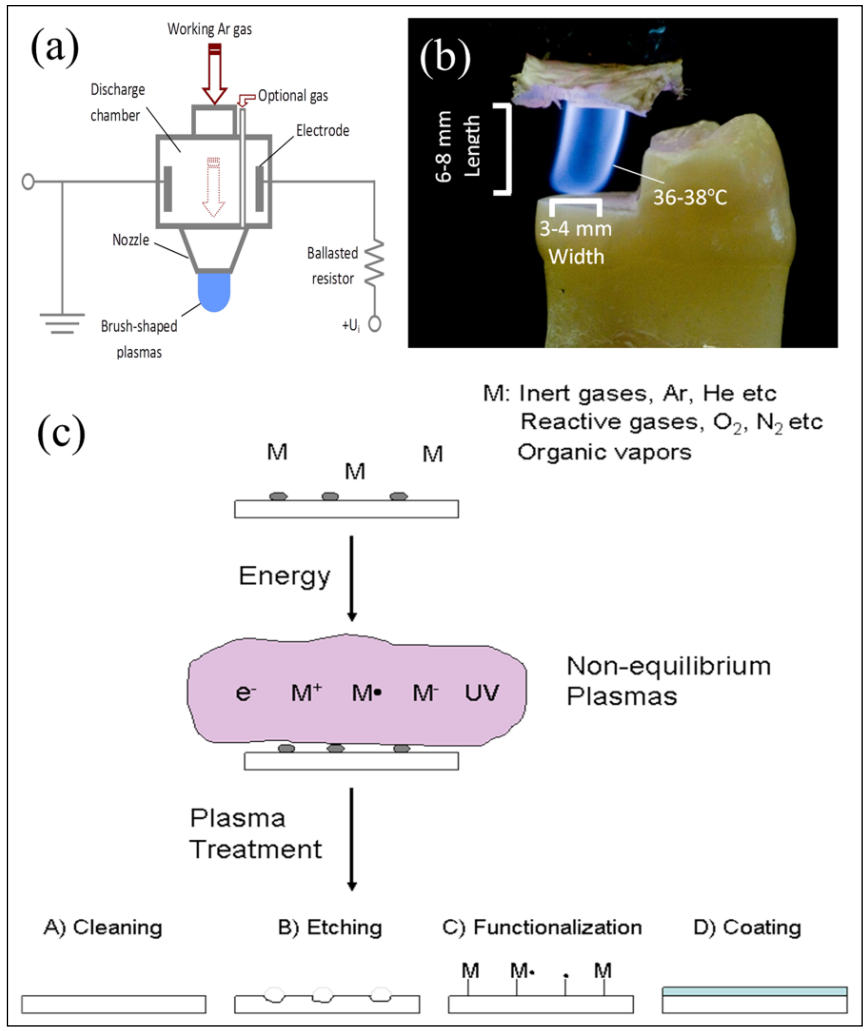

Figure 1. Schematic diagram (**a**) and photograph (**b**) of nonthermal atmospheric plasma brush setup, with schematic illustration (**c**) of plasma treatment effects on a substrate surface.

With regard to cariogenic bacteria—which underlie secondary caries formation and therefore directly affect dental adhesion durability—their destruction by NTAP has been confirmed through various tooth-mimicking substrates (Blumhagen et al. 2014), cavity-simulating models (Bedem 2005), as well as dental substrates, including dentin and enamel (Rupf et al. 2010; Molnar et al. 2013). Generally, NTAP treatment for 5 to 300 s led to total destruction of bacteria species evaluated regardless of carrier gas. For instance, 2 bacteria species closely related to dental caries, *S. mutans* and *Lactobacillus acidophilus*, were cultured on filter paper, glass slides, and polytetrafluoroethylene films, which represent fissures, smooth surfaces of teeth, and resin, respectively, and then subject to NTAP treatment (Yang et al. 2011). Bacteria death was evident, as seen in the morphologic changes with various treatment times (Fig. 2). Bacteria with larger size (*L. acidophilus*) were more resistant to NTAP, and DNA/protein UV absorbance intensity suggested that cell content leakage occurred immediately after plasma exposure. The limitations of NTAP with regard to inhibiting biofilm were discussed in a few studies (Sladek et al. 2007; Blumhagen et al. 2014), which showed incomplete removal or recovery of *S. mutans* biofilms after NTAP treatment. It was believed that biofilm thickness might have prevented plasma from exerting a bactericidal effect to bacteria at the bottom and/or that cell debris from killed bacteria on the surface blocked further penetration of plasma. Clearly, additional work is warranted to optimize NTAP's use in dental surface sterilization.

Dental Surface Modification

Considering the high hydrophilicity of contemporary dental adhesives, a hydrophilic enamel/dentin surface is expected to facilitate resin-tooth hybridization and consequently enhance bonding performance. Additionally, a hydrophilic adhesive resin surface has the potential to retard bonding degradation by reducing *S. mutans* adhesion (Brambilla et al. 2014) and thus reducing the risk of secondary caries. However, it should also be kept in mind that high hydrophilicity brings about the risk of

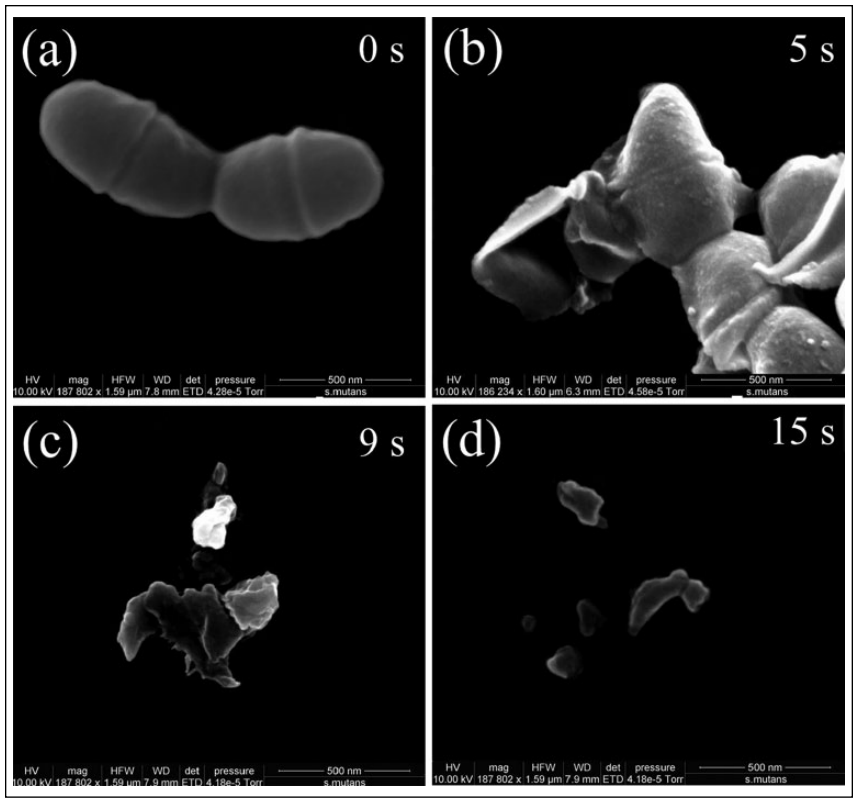

Figure 2. Scanning electron microscopic images of *S. mutans* cells of (**a**) untreated control, plasma treated for 5 s (**b**), 9 s (**c**) and 15 s (**d**). Plasma conditions were 2000 sccm argon flow rate and 10 W DC power input. Reprinted with permission from Yang et al. (2011).

adhesive-phase separation due to preferential penetration of its hydrophilic components. Moreover, increased hydrophilicity of dentin/enamel may result in a higher degradation rate by facilitating penetration of water and hydrolytic enzymes at the interface, which may lead to a rapid and dramatic decrease in bond strength in the long term.

To gauge the hydrophilicity of dental surfaces, contact angle experiments have been performed, where lower water contact angles designate higher hydrophilicity. With argon or helium as carrier gas, with or without additional O_2/H_2O_2 gas feeding, NTAP has demonstrated the ability to reduce water and ethanol contact angles on a variety of dental substrates, including zirconia, titanium, bovine and human dentin/enamel, as well as cured dental composites (Koban et al. 2011; Silva et al. 2011; Chen et al. 2013; Lehmann et al. 2013; Valverde et al. 2013; Han et al. 2014; Hirata et al. 2015). NTAP's alteration to surface hydrophilicity is believed to involve 2 mechanisms: etching/ablation and modification. Regarding the etching/ablation process, energy-dispersive x-ray (EDX) and x-ray photoelectron spectroscopy (XPS) analyses demonstrated that NTAP-treated dentin and enamel exhibited more prominent mineral signals (Ca and P), and NTAP-treated composite exhibited more prominent filler signals (Si, Zr, Y, and F), which indicated that NTAP changed the surface chemical composition by selectively etching away susceptible protein (as opposed to mineral) in tooth and polymer (as opposed to filler) in composite. As a result, more hydrophilic mineral and filler were exposed, leading to enhanced surface hydrophilicity. Similarly, weaker carbon signals were spotted on NTAP-treated zirconia and titanium surfaces, attributed to the removal of organic contaminants (Silva et al. 2011; Valverde et al. 2013). Due to this etching effect, NTAP treatment significantly increased surface roughness of bovine dentin, but the difference in bovine enamel was not statistically significant possibly due to low protein content (Lehmann et al. 2013). Roughness change in NTAP-treated human dentin was reported to be insignificant, but it was likely a result of nonpolished dentin specimens (Koban et al. 2011). In addition, NTAP's etching effect was found to cause changes to the secondary structure of demineralized dentin collagen, as evidenced by shifts of Fourier transform infrared spectroscopy (FTIR) bands, including amide A, amide II, and amide III (Chen et al. 2014). It implies that excessive NTAP treatment may damage dentin collagen and thus decrease bond strength (discussed below). Regarding the modification process, EDX/XPS studies affirmed significant increase in oxygen content on NTAP-treated dental surfaces, reflecting the important role of reactive oxygen species in introducing polar, oxygen-containing groups to the substrates (Koban et al. 2011; Silva et al. 2011; Lehmann et al. 2013; Valverde et al. 2013; Chen et al. 2014). XPS narrow scans suggested that NTAP treatment induced substantial increase in C-O-, O=C-O-, and CO_3^{2-} signals on enamel (Lehmann et al. 2013), and FTIR analysis revealed an increase in the amount of carbonyl groups (1,760 cm^{-1}) on demineralized dentin collagen (Ritts et al. 2010).

It is worth mentioning that the majority of NTAP devices reported in dental studies have been based on oxygen-containing feeding gases, such as ambient air, O_2, H_2O_2, and H_2O (Fig. 1a). A few studies included vaporized triethyleneglycol dimethacrylate, a relatively hydrophobic monomer (compared with hydroxyethylmethacrylate [HEMA]) in the feeding gas, and verified triethyleneglycol dimethacrylate deposition on dental ceramic surfaces (Cho et al. 2011; Han et al. 2012). In addition, adjustment of feeding gas to a fluoride-containing compound such as SF_6 resulted in plasma affording hydrophobic properties to surfaces (Barni et al. 2005). So far, such hydrophobic rendering of tooth surfaces has not been reported yet, but it has the intriguing potential to address the overhydrophilicity of current dental adhesives (Tay and Pashley 2003) by compatibilizing tooth surface with more hydrophobic bonding systems. It may enhance the penetration of hydrophobic

components (e.g., bisphenol A and glycidyl methacrylate [BisGMA]) into dentin, resulting in stronger bonding and better sealing (Sherriff 2005).

Resin Polymerization

It has been long documented that plasma can induce polymerization by direct energy transfer (i.e., bombarding reactive species, including radicals, ions, and metastable species, to monomer) and indirect energy transfer (e.g., absorption of UV-visible emission by monomer; Epaillard et al. 1989; Gong et al. 1998; Çökeliler et al. 2007). NTAP is believed to enhance polymerization via the same mechanisms.

Using FTIR, Chen et al. (Chen, Zhang, et al. 2012) compared the degree of conversion (DC) of model self-etch adhesives initiated by traditional camphorquinone/amine photoinitiators and NTAP. It was found that at the same light or NTAP exposure time (40 s), the DC of the NTAP-initiated groups was consistently greater than that of the photoinitiated groups with the same adhesive composition (Fig. 3). Moreover, the DC of photoinitiated adhesives suffered from increased water content, whereas that of NTAP-initiated adhesives did not. From the perspective of radical generation, these phenomena reflect the long-discovered incompatibility between camphorquinone/amine photoinitiation and acidity (and, thus, water content; Tay et al. 2003)—an issue that is nonexistent in NTAP initiation, as NTAP provides a steady supply of radicals generated in the discharge chamber (Fig. 1a). Clearly, NTAP initiation holds advantage over photoinitiation in terms of monomer conversion in the areas where water or dentinal fluids exist. In another study, model total-etch adhesives were applied to acid-etched dentin surface with 30 s of NTAP treatment and then light cured (Zhang et al. 2014). Spatially resolved DC obtained by Raman mapping demonstrated that the NTAP-treated specimens exhibited more complete monomer conversion, especially at the resin-collagen interface (>90%), indicating that the energy transferred from plasma remained active, which led to additional initiation and higher DC. It is worth mentioning that besides enhanced monomer conversion, plasma-induced polymerization is unique in the initiation mechanism, which features diradical production following plasma bombardment and hemolytic scission of vinyl species (Yasuda 1985). As a result, adhesive surface is believed to contain a large quantity of radicals after polymerization, which remain active to enhance the curing of the subsequent composite placement at the adhesive-composite interface.

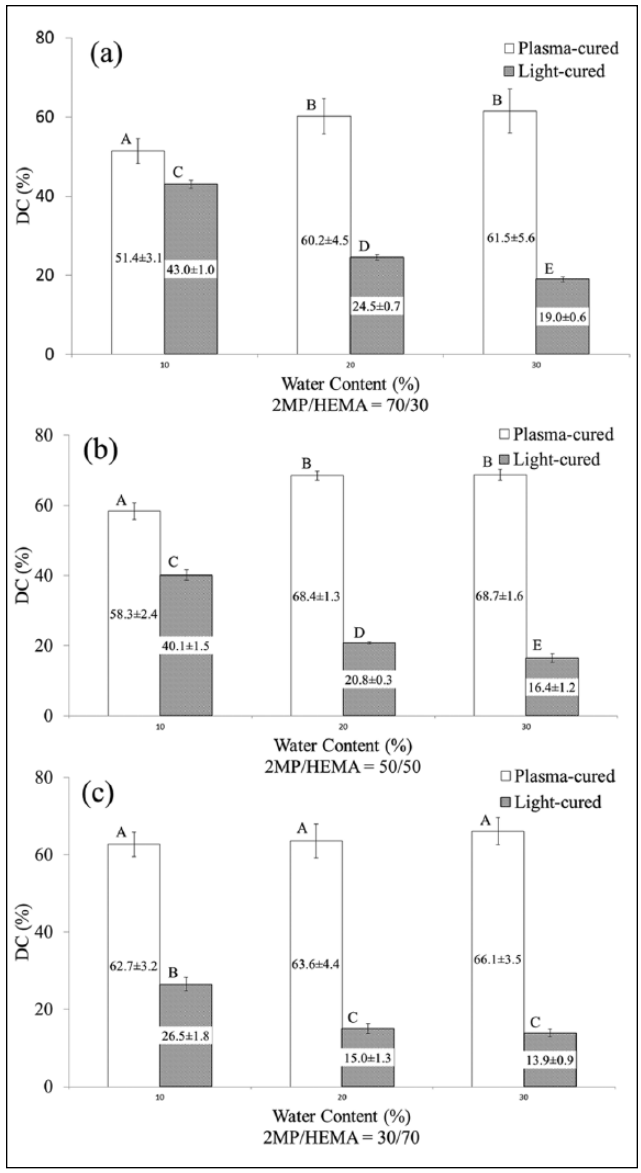

Figure 3. Percent monomer degree of conversion (DC) values of the model adhesives having different monomer mass ratios and different water contents (wt%). The adhesives were either plasma-cured or light-cured for 40 s. In each figure, means with different letters are significantly different ($P < 0.05$). Reprinted with permission from Chen et al. (2012).

Resin-Substrate Hybridization

Hybridization of adhesive resin with enamel/dentin is essentially an exchange process, in which minerals removed from acid etching are replaced by adhesive monomers, which then become interlocked in the created porosities upon polymerization (Van Meerbeek et al. 2006). Due to the intrinsically simpler chemical and structural composition of enamel, bonding to enamel has been found far less susceptible to failure than bonding to dentin (Cardoso et al. 2011). Thus, the majority of published work regarding NTAP's effect on resin-substrate hybridization actually involves bonding to dentin exclusively. The prerequisites of an excellent dentin-adhesive hybridization include an optimal adhesive penetration and a stable dentin-adhesive physicochemical interaction. Ultimately, high-quality hybridization is manifested as robust, durable bond strength. This section discusses NTAP's influence on dentin-adhesive hybridization from these 3 perspectives.

Resin Penetration

For total-etch adhesives, application of resin (in the form of either a separate priming agent or a "one bottle" containing both priming and bonding agents) follows tooth conditioning

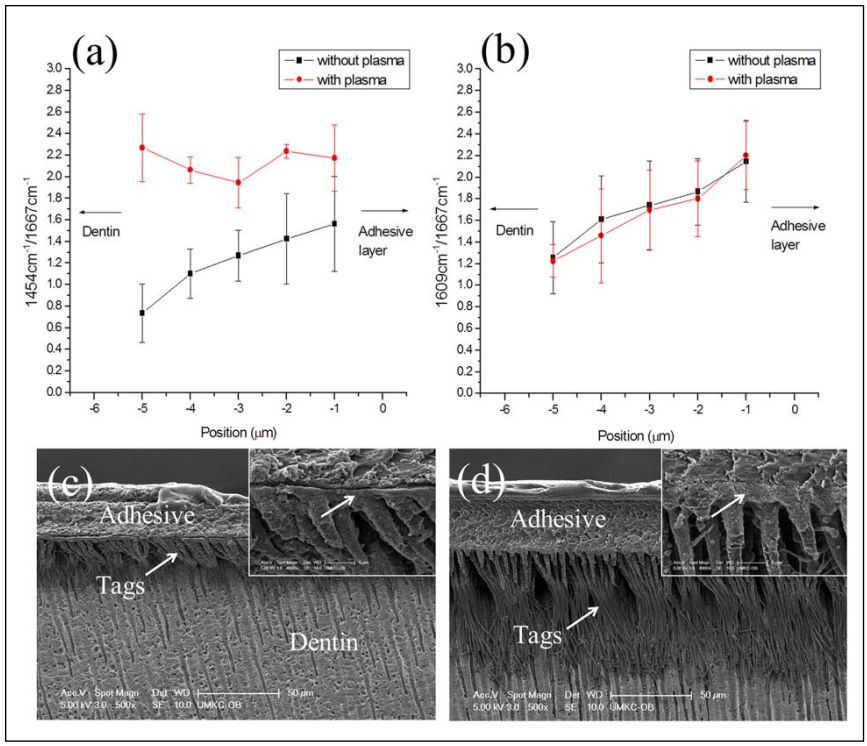

Figure 4. Micro-Raman band ratios of (**a**) 1,454 cm^{-1}/1,667 cm^{-1} and (**b**) 1,609 cm^{-1}/1,667 cm^{-1} as a function of position in the adhesive/dentin interface region for the specimens without and with plasma treatment. Representative scanning electron microscope micrographs of the adhesive/dentin interface for the specimens (**c**) without and (**d**) with plasma treatment. Arrows in the inserted images indicate the hybrid layer. Adapted from Zhang et al. (2014).

with acid (Pashley et al. 2011). The acid etching removes smear debris/layer and opens dentin tubules; therefore, resin penetration for total-etch adhesives primarily involves infiltration of resin into demineralized dentin collagen matrix and open tubules, leading to the formation of adhesive/dentin hybrid layer and resin tags. Using Raman mapping, Zhang et al. (2014) investigated a model BisGMA/HEMA adhesive's penetration into demineralized dentin surface following NTAP treatment for 30 s. The band ratio of 1,454 cm^{-1}/1,667 cm^{-1}, which represents the overall amount of adhesive with respect to collagen, significantly increased after NTAP treatment (Fig. 4a), indicating that NTAP treatment induced a greater adhesive penetration into dentin collagen. However, the band ratio of 1,609 cm^{-1}/1,667 cm^{-1}, which represents the amount of BisGMA with respect to collagen, had no significant change (Fig. 4b), suggesting the overall increase in resin infiltration was not due to the hydrophobic component BisGMA but rather the hydrophilic component HEMA. Scanning electron microscope images (Fig. 4c, d) also showed that resin tags were longer and more tortuous with lateral projections (Han et al. 2014; Zhang et al. 2014), reflecting an enhanced penetration of resin into dentin tubules and tubule branches thanks to more hydrophilic tubule walls as a result of NTAP treatment. The results were consistent with the notion (mentioned in the Dental Surface Modification section) that NTAP could increase the hydrophilicity/wettability of dentin collagen or substrates, which caused the preferential infiltration of hydrophilic components. Although the degradation of collagen is decreased due to increased penetration, such a differential penetration between hydrophilic and hydrophobic components of an adhesive may bring about the risk of phase separation particularly at the bottom of hybrid layer.

For self-etch adhesives, demineralization of dentin surface, dissolution of smear layer, and resin infiltration into collagen matrix (and possibly tubules) occur concomitantly (Van Meerbeek et al. 2011). Consequently, self-etch adhesives' penetration into dentin is a more complex process influenced by additional factors, such as smear layer permeability. Using scanning electron microscope analysis, Dong et al. (2015) found that 30 s of NTAP treatment caused partial opening of dentin tubules on polished dentin surface, which significantly increased the permeability of smear layer as measured by water contact angle. In corroboration, they saw longer resin tags and thicker hybrid layer when OptiBond All-in-One self-etch adhesive (Kerr, Romulus, MI, USA) was used to bond to NTAP-treated dentin.

Resin Grafting to Collagen

As mentioned earlier, current dental adhesives afford adhesive-dentin bonding solely by a mechanical mechanism—that is, microinterlocks between resin polymer and collagen fibrils (Nakabayashi et al. 1982). The fact bears 2 implications. First, hydrophilic resin monomers such as HEMA are indispensable for a good bonding due to the hydrophilicity of collagen matrix. Second, introducing additional interactions, such as chemical bonds between collagen and HEMA, could result in better adhesive-dentin bonding. On top of that, chemical bonds between HEMA and collagen could also address the reported leakage of HEMA from resin matrix (Spencer and Wang 2002).

Dong et al. (2014) subjected demineralized dentin collagen to sequential treatment of NTAP (30 s) and HEMA (2 min) and then removed physically adsorbed HEMA by acetone rinsing. The resultant dentin collagen showed no characteristic FTIR bands attributed to HEMA, including ester C=O stretching at 1,720 to 1,730 cm^{-1}; therefore, they concluded that no HEMA-collagen chemical bond was furnished by NTAP treatment. In contrast, Chen et al. (2014) subjected demineralized dentin collagen to simultaneous NTAP and HEMA/water mixture treatment for different times (1 to 8 min) and investigated the FTIR spectral change following water rinsing. They observed prominent bands attributed to HEMA, including ester C=O stretching (Fig. 5a, b). Since NTAP treatment induced HEMA polymerization, they fabricated demineralized dentin collagen

with light-cured HEMA to see if the emerged HEMA bands were due to polymerization-induced HEMA-collagen entanglement. The results (Fig. 5c) ruled out such a possibility and confirmed that simultaneous NTAP and HEMA treatment did chemically graft HEMA molecules onto dentin collagen fibrils. An optimal combination of NTAP power and treatment time seemed to exist, as the amount of HEMA grafted was not monotonically correlated to either. Regardless of NTAP parameters, TEM analysis revealed no obvious morphologic changes to dentin collagen's characteristic banding structure, which suggested that HEMA grafting by NTAP was not at the expense of collagen integrity and therefore should not pose a threat to bond strength. The results from these publications could be understood from 2 perspectives. First, establishment of HEMA-collagen chemical bonds most likely required active sites existent on both materials. It occurred only when dentin collagen and HEMA were bombarded by plasma at the same time, as reported by Chen et al. (2014). Second, NTAP treatment dehydrated dentin surface, which resulted in a collapsed dentin collagen matrix. Since pure HEMA would not infiltrate into collapsed dentin collagen (Pashley et al. 2007), no HEMA grafting could take place, as seen by Dong et al. (2014).

Bond Strength

With regard to total-etch adhesives, Ritts et al. (2010) evaluated the immediate bonding performance of a commercial 2-step total-etch adhesive with various NTAP treatment times. The NTAP treatment (30 to 300 s) was applied after acid etching and before rewetting and adhesive application. It was found that 30 s of treatment increased the microtensile bond strength (μTBS) and modulus in a location-dependent manner but that treatment >30 s did not, possibly a ramification of plasma's etching effect that excessively destructed collagen fibrils in longer times. In a follow-up experiment using the same adhesive and NTAP protocol, comparison of NTAP-treated and non-NTAP-treated dentin bonding was made on the same teeth to suppress noises due to interspecimen discrepancy. With a fixed treatment time of 30 s, significantly higher μTBS was recorded on NTAP-treated dentin, and fractographic analysis revealed that NTAP-treated specimens exhibited less frequent interfacial and mixed failures (Dong et al. 2013). In a recent study, Han et al. (2014) used a 3-step total-etch adhesive for bonding to dentin. The NTAP treatment (30 s) followed acid etching and preceded rewetting and primer application. Significantly higher immediate μTBS (66.2 ± 16.0 MPa for pulsed plasma, 76.9 ± 20.2 MPa for conventional plasma) was reported as compared with control (45.9 ± 14.5 MPa). Moreover, the μTBS of NTAP-treated specimens did not decrease after 5,000 cycles of thermocycling (77.9 ± 8.8 MPa for pulsed plasma, 78.1 ± 12.6 MPa for conventional plasma). While the μTBS of the control group did not significantly decrease either (40.4 ± 11.5 MPa), it was significantly lower than that of NTAP-treated groups.

With regard to self-etch adhesives, Dong et al. (2015) investigated the effect of 30 s of NTAP treatment on the μTBS of a 1-step adhesive, Optibond All-in-One (Kerr), both

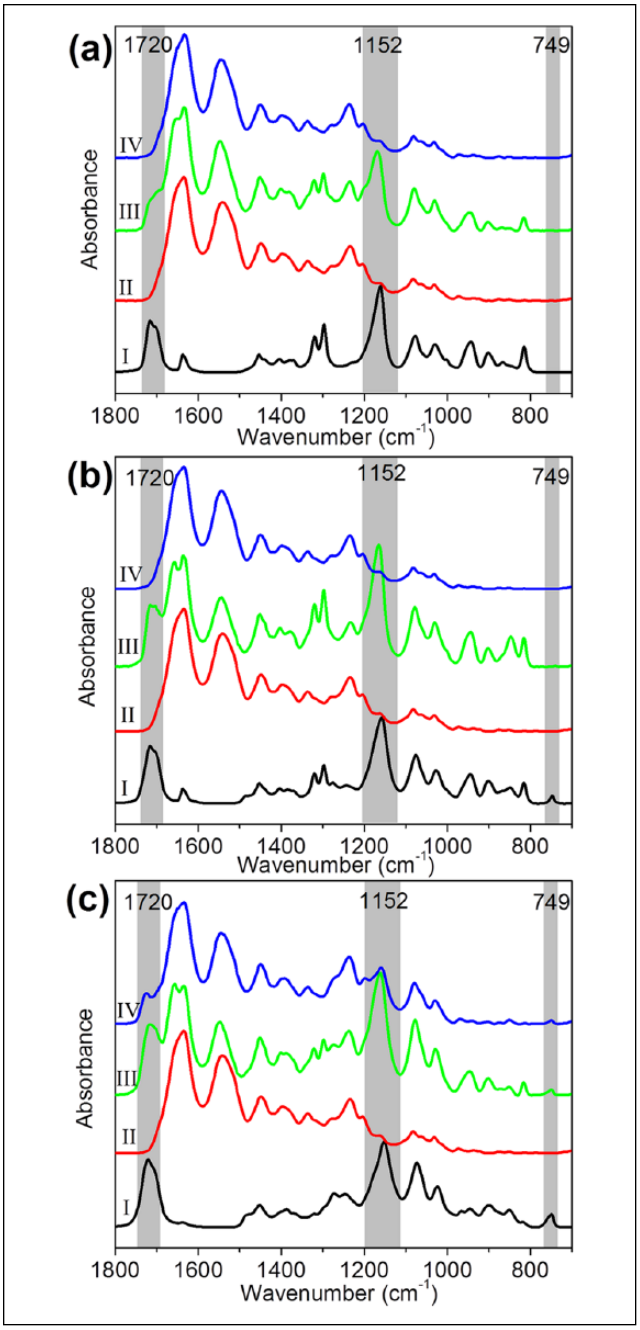

Figure 5. FT-IR spectral comparison of (**a**) uncured, (**b**) light-cured, and (**c**) plasma-exposed groups (input power of 15W, treatment time of 4 min). The spectra from bottom to top in each figure represent (I) HEMA (II) non-HEMA-treated dentin collagen film (III) HEMA-treated collagen film before water rinse, and (IV) HEMA-treated collagen film after water rinse. Reprinted with permission from Chen et al. (2014).

immediately and after 2 mo of storage. The NTAP treatment followed smear-layer creation on dentin and preceded rewetting and adhesive application. It was found that the immediate μTBS of NTAP-treated specimens (69.7 ± 11.5 MPa) was 22.1% higher than control (57.1 ± 17.5 MPa). After 2 mo of storage in phosphate-buffered saline buffer (pH = 7.4), the superiority of NTAP-treated specimens was more prominent,

as the μTBS of treated specimens (63.6 ± 14.4 MPa) was 30.1% higher than control (48.9 ± 14.6 MPa). It was claimed that the partially opened dentin tubules observed after NTAP treatment facilitated the adhesive's penetration into the smear layer, which resulted in thicker hybrid layer and, consequently, better bonding performance. In a separate study, Hirata et al. (2015) tested a 1-step self-etch adhesive, Scotchbond Universal (3M ESPE, St. Paul, MN, USA), and a 2-step self-etch adhesive, Clearfil SE Bond (Kuraray, Japan). The NTAP treatment (30 s) followed dentin smear-layer creation, but no rewetting was mentioned before adhesive or primer application. Significantly higher immediate μTBS was reported for Scotchbond Universal but not Clearfil SE Bond, whereas after 1-y water storage, there was no difference between NTAP-treated and control groups regardless of adhesive used. It is not clear whether the discrepancy in after-storage bond strength between these 2 studies was due to different adhesives used, different storage time, or rewetting.

Conclusions and Perspective

By delivering reactive species, including ions, radicals, and UV photons, NTAPs have exhibited various biological and chemical effects critical to dental bonding. Oral pathogens, including cariogenic bacteria, can be killed within a clinically relevant time frame, but further studies are warranted with respect to the plasma penetration and removal of established, thick biofilms. Significant increase in surface hydrophilicity/wettability is furnished on dental surfaces, including enamel, dentin, and resin/composite, reflecting plasma's capability to etch/ablate and introduce functional groups to surfaces by bombardment of reactive species. Plasma-induced polymerization has been confirmed, with monomer conversion superior to traditional light curing. Enhanced penetration of adhesives has been seen on NTAP-treated dental surfaces resulting in better hybrid layer and longer resin tags. NTAP has also been found to induce chemical grafting of HEMA to dentin collagen, signifying additional bonding mechanism between adhesive and collagen besides pure mechanical interlocking.

Compared with the number of fundamental studies above, investigations have been somewhat lacking with respect to NTAP's overall effect when incorporated into actual bonding procedures. Reports so far have indicated that NTAP treatment enhanced bond strength both immediately and after aging on a case-by-case basis. However, more vigorous and clinically relevant storage shall be performed to gauge NTAP's long-term influence to bonding. Additionally, the current design of bonding experiments has not properly reflected NTAP's antibacterial activity. Bacteria-infectious substrates, such as carious teeth and endodontic preparations, should be included in the future, as opposed to noncarious teeth only. Critically, it is troublesome that little effort has been directed toward the establishment of a guideline by which NTAP can be rationally integrated into bonding procedures. More systematic NTAP studies on dental restoration under clinically relevant settings are needed in the future.

Author Contributions

Y. Liu, contributed to conception, design, data acquisition, and interpretation, drafted and critically revised the manuscript; Q. Liu, contributed to design, data acquisition, and interpretation, drafted the manuscript; Q.S. Yu, contributed to conception and design, critically revised the manuscript; Y. Wang, contributed to conception, design, data acquisition, analysis, and interpretation, critically revised the manuscript. All authors gave final approval and agree to be accountable for all aspects of the work.

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