Effects of a helium cold atmospheric plasma on bonding to artificial caries-affected dentin

Xuan QI[1]*, Xiao-Ming ZHU[2]*, Xiaoqiang LIU[1], Jing LI[3], Lu-Xiang ZHAO[4], He-Ping LI[3] and Jianguo TAN[1]

[1] Department of Prosthodontics, Peking University School and Hospital of Stomatology & National Center of Stomatology & National Clinical Research Center for Oral Diseases & National Engineering Laboratory for Digital and Material Technology of Stomatology, Beijing, 100081, China
[2] Second Clinical Division, Peking University School and Hospital of Stomatology & National Center of Stomatology & National Clinical Research Center for Oral Diseases & National Engineering Laboratory for Digital and Material Technology of Stomatology, Beijing, 100101, China
[3] Department of Engineering Physics, Tsinghua University, Beijing, 100084, China
[4] Department of Mechanical Engineering, Tsinghua University, Beijing, 100084, China
Corresponding authors, Jianguo TAN; E-mail: kqtanjg@bjmu.edu.cn, He-Ping LI; E-mail: liheping@tsinghua.edu.cn

This study investigated the effects of a helium cold atmospheric plasma (CAP) on the bonding performance and surface modification of the caries-affected dentin (CAD). Artificial CAD was created by pH-cycling. The microtensile bond of CAD were examined before and after CAP treatments at 24 h and after 2-year aging. The effects of surface modification were studied with contact-angle measurement, scanning electron microscopy and X-ray photoemission spectroscopy. Thirty-second CAP treatment increased the immediate bond strength of CAD to a level that was statistically the same as sound dentin, and slowed the aging process of the bonding as well. The CAP treatment induced modified CAD surface with increased wettability, cleaner appearance, and increased percentage of the mineral-associated elements and oxygen. This research demonstrated that the helium CAP jet treatments of 30 s and 45 s improved the bond strength of the artificial CAD, and was considerably effective in its surface modification.

Keywords: Cold plasma, Dental caries, Dental bonding, Tensile strength, Composite resin

INTRODUCTION

Caries is considered as the most common reason for tooth defect. With a deep understanding to caries and the development of adhesive techniques, the contemporary caries management is becoming more conservative and preventive. According to the minimally invasive concept, caries excavation should be stopped immediately when leathery/firm affected dentine is reached, so that the remineralizable caries affected dentin (CAD) is preserved[1]. However, the bond strength of resin to CAD has been reported to be significantly lower than that to sound dentin (SD)[2]. This is due to some features of CAD, including thicker and 'collagen' smear layer[3], the lower mineral content of inter-tubular dentin, acid-resistant mineral crystals clogging in dentinal tubules[4], and more activated collagenolytic enzymes[5]. As CAD becomes an increasingly common substrate in practice, its bonding performance requires further research and development.

Many methods aiming at different characteristics of CAD have been tried to improve its bonding performance, but the efficiency is still limited or controversy. The most known chemo-mechanical caries removal system uses a mixture of sodium hypochlorite and a variety of amino acids. This method has high technical sensitivity and only softened carious dentine can be removed[6]. Other chemical cross-linkers, such as glutaraldehyde and grape seed extract, have been reported to further strengthen the partly demineralized CAD and achieve bonding values comparable with those of sound dentin. But their use has been limited due to the instability of the cross-links and cytotoxicity[7]. In regard to tubular occlusions, the use of strong acid cannot dissolute the intratubular minerals, but causes high demineralization, leaving exposed and non-reinforced fibrils under the hybrid layer[8]. Chlorhexidine, a matrix metalloproteinase inhibitor, can preserve bond strength of sound dentin, however, it is less effective in CAD because the demineralized dentin is hard for chlorhexidine to bind to[9]. Physical treatment is another alternative method to remove caries, as it leaves no residues after treatment and can improve the rigidity of the collagen[10]. Conventional physical crosslinking methods include the use of ultraviolet, lasers, *etc*. The use of ultraviolet-A (UVA) to improve the mechanical properties of carious affected dentin has been reported[11]. However, to be clinically relevant, the bond-strength of carious affected dentin is still lower than that of the sound dentin[12]. Different lasers have been applied to remove caries and the smear layer of CAD. However, for these purposes, they required relatively high-energy densities, which had undesirable side effects on the dental tissues making the long-term effect uncertain[13].

Recently, cold atmospheric plasma (CAP), as a clean and safe treatment method, has attracted wide

---

attention from researchers. Its abundant chemically reactive species with high electron energies qualify it to have multiple functions in surface modification, including plasma overcoat, plasma surface gradient and surface etching[14]. It has been reported that the CAP treatment can successfully improve the bond strength of sound dentin[15-19]. The efficacy is thought to be related to the improvements in several critical properties for bonding. This suggests that CAP has the potential to act on multiple weakness points of bonding in CAD: the abundant reactive oxygen species (ROS) and reactive nitrogen species (RNS) in CAPs can deactivate oral pathogens[20,21]; its highly reactive radicals can clean the smear layer and improve surface wettability, thereby improves the dentin permeability[17,22]; it may also strengthen the weak hybrid layer of CAD by increasing dentin collagen stiffness[18] and enhancing polymerization of resin[23]. Among all the possible mechanisms, this research focuses on the surface modification of the CAD induced by CAPs, as the physical and chemical properties of the surface have the most direct influence on the bonding performance. Based on this, this study aims to evaluate the effects of the CAP treatment on the bonding to CAD by measuring the immediate bond strength and analyzing the physicochemical properties of the modified surface (surface wettability, surface morphology, and chemical composition). The null hypothesis was that the CAP had no effects on bonding to CAD.

MATERIALS AND METHODS

The teeth used in this study were extracted non-carious, unerupted human third molars, which were obtained with individuals' informed consent under a protocol approved by Peking University Institutional Review Board (PKUSSIRB-201522043a). The teeth were stored in saline containing 0.02% sodium azide at 4°C and used within one month after collection.

Specimen preparation
To prepare the artificial CAD, the occlusal-third of the crown was removed horizontally to expose the dentin surface using a water-cooled low-speed diamond saw (Isomet 1000, Buehler, Lake Bluff, IL, USA). Two layers of acid-resistant nail varnish (Revlon, New York, NY, USA) was applied on the entire surface of each specimen except for the dentin surface. Then, the specimens underwent a pH-cycling procedure consisting of 8 h in demineralizing solution and 16 h in remineralizing solution. The demineralizing solution contained 2.2 mM $CaCl_2$, 2.2 mM NaH_2PO_4, and 50 mM acetic acid adjusted to pH 4.5 using NaOH. The remineralizing solution contained 1.5 mM $CaCl_2$, 0.9 mM NaH_2PO_4 and 150 mM KCl adjusted to pH 7.0 using NaOH[24,25]. Specimens were rinsed thoroughly with deionized water (Direct-Q UV, Millipore, Molsheim, France) every time when changing the solution. This procedure lasted for 14 days at 37°C without agitation (Fig. 1).

The molars that did not undergo pH-cycling were collected for the sound dentin group, which were cut horizontally at occlusal-third of the crown to expose the dentin surface before use.

Dentin surfaces of all the specimens were wet-ground with a 600-grit SiC sandpaper for 1 min to creat uniform smear layers before CAP treatments.

Plasma jet treatments
In our study, the CAP Med-I, which is developed by Tsinghua University, was applied to generate a helium CAP jet for the treatment of the dentin surfaces. The CAP Med-I is an atmospheric-pressure dielectric-barrier-discharge plasma jet generation system. As shown in Fig. 2a, it consists of the high-voltage alternating-current (HVAC) power supply module, the helium flow rate control unit, the driving frequency and voltage output modulation knobs, and the co-axial type plasma jet generator. A detailed description on CAP Med-I can be referred to a former study[26]. The CAP jet was generated with the high-purity helium at the flow rate of 8.1 slpm as the plasma forming gas under the discharge voltage of 2.8 kV and the frequency of 17 kHz. The distance between the nozzle exit of the plasma generator and the dentin surface was kept at 1.0 cm, as shown in Fig. 2b. With careful control of the power input, the measured gas temperature at 1.0 cm downstream of the plasma torch nozzle exit was approximately 38±2°C, which was
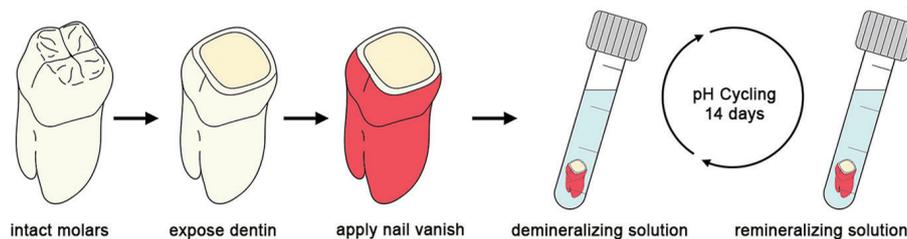

Fig. 1   Schematic representation of the production procedures for artificial CAD in the study.
The crowns of intact human third molars were reduced occlusally to expose the dentin, and entire surface except for the dentin surface were covered by nail varnish. Then, the specimens underwent a pH-cycling procedure with 8 h in demineralizing solution and 16 h in remineralizing solution, and lasted for 14 days at 37°C.
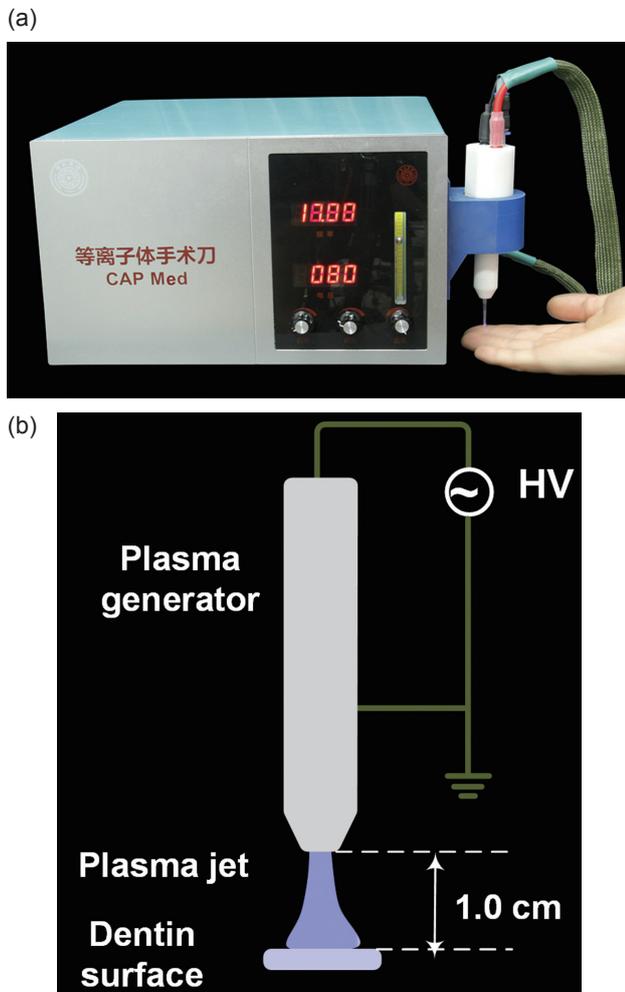
(a)

(b)

Fig. 2 Picture and schematic of experimental set up for CAP treatments.
(a) picture of the CAP machine for the treatment of dentin. (b) schematic of experimental set up for CAP treatments.

within a safe temperature range for the pulp treatment theoretically.

Microtensile bond strength (μTBS) test
Due to limited previous literatures and reported effect sizes, the pre-experiments with 1 tooth (16 beams from each teeth) for each group were conducted. The effect size was calculated by One-Way Analysis of Variance F-Tests in Power Analysis and Sample Size software (PASS, version 15, NCSS, LLC, Kaysville, UT, USA) using the results of pre-experiments. The estimated sample size turned out to be smaller than the actual one, indicating that the actual sample size was statistically adequate. Two teeth were included and the sample size increased to 32 (16 beams×2 teeth) in the final test. *Post hoc* sample size calculation was conducted to ensure proper statistical analyses and reproducibility.

Twenty teeth were randomly divided into five experimental groups according to whether they were treated with pH-cycling and the period of CAP treatments: (i) sound dentin without pH-cycling or CAP treatments (SD group, positive control); (ii) artificial CAD without CAP treatments (CAD group, negative control); (iii) artificial CAD treated with CAP for 15, 30, 45 s (CAD CAP-15s, CAD CAP-30s and CAD CAP-45s groups).

The two-step self-etching adhesive system (Clearfil SE Bond, Kuraray Noritake Dental, Tokyo, Japan) was applied to all groups according to the manufacture's instructions. The resin composite (Clearfil AP-X, Kuraray Noritake Dental, Osaka, Japan) was then applied on the top of the adhesive three or four times, and light-cured for 20 s after each application. After stored in distilled water at 37°C for 24 h, the prepared teeth were sectioned perpendicular to the bonded surfaces using the water-cooled diamond saw to produce a series of 0.7×0.7×8 mm beams. Sixteen beams in the center of each tooth were collected and further divided into two subgroups according to the μTBS testing time: one subgroup was immediately submitted to the μTBS test (*n*=32), the other subgroup was stored for 2 years in 0.5% chloramine solution at 37°C (*n*=32). The μTBS test was performed using a micro tensile tester (EZ-L- 1kN, Shimadzu, Kyoto, Japan) at a 0.5 mm/min strain rate until failure (Autograph DCS-5000, Shimadzu).

Surface contact angle measurement
The wettability of the surfaces to water was determined by static contact angle measurements with a contact angle goniometer (SL200, USA Kino Industry, Norcross, GA, USA) using tangential line method. The sample size was calculated following the same process with μTBS test. Ten teeth were randomly divided into five experimental groups in the same way as the μTBS. One disc of 2 mm thick dentin without pulp exposure was obtained from each tooth by an extra cut under the dentin surface horizontally. Right after plasma treatments, 0.5 µL deionized water droplet were applied on the surface with an automatic piston syringe at three different locations for each sample (*n*=6).

Scanning electron microscopy (SEM)
Discs of SD or CAD were prepared and grouped in the same way as the contact angle measurement. After dehydrating and drying[17], the specimens were sputter-coated (Model 681, Gatan, Pleasanton, CA, USA) with Au-Pd alloy. Then the samples were observed in a field emission SEM (Helios Nanolab 600i, FEI, Hillsboro, OR, USA) using the TLD (through the lens detector) at 10 kV accelerating voltage.

X-ray photoemission spectroscopy (XPS) measurement
The un-treated SD and CAD (control groups) and 30-s CAP-treated surfaces were recruited for surface chemical analysis by XPS (PHI Quantera SXM, ULVAC-PHI, Kanagawa, Japan). Survey spectra were acquired at 280 eV pass energy with a step size of 1.0 eV, and basic elements of tooth, including carbon, nitrogen, oxygen, phosphorus, calcium, were measured.

Statistical analysis
The data of µTBS test and surface contact angle measurement were analyzed using statistical software SPSS (IBM SPSS Statistics, Version 25.0, IBM, Armonk, NY, USA). The statistical unit was beams for µTBS, and discs for surface contact angle. Group analysis was performed by one-way analysis of variance (ANOVA) for comparison of means or by Welch test when Levene test for homogeneity of variances failed. Statistical significance was preset at α=0.05.

RESULTS

µTBS test
Table 1 shows the statistical comparison of the µTBS values of all the groups at 24 h and after aging storage for 2 years. The immediate µTBS strength of CAD was significantly lower than that of SD ($p<0.05$). After CAP treatments, the µTBS values increased significantly compared with the CAD group despite the treatment duration ($p<0.05$). The group treated for 30 s had the highest µTBS (49.27±2.26 MPa), and was statistically the same with the SD group (49.87±1.64 MPa). After 2-year aging, µTBS values of all the groups droppped significantly ($p<0.05$). But the group treated for 30 s (31.13±2.16 MPa) and 45 s (32.93±1.15 MPa) still had higher values compared with non-treated CAD (20.41±3.54 MPa), and are close to that of SD (35.56±2.42 MPa) ($p<0.05$).

Surface contact angle measurement
Figure 3 shows the water contact angle values of all the group. The contact angle of the CAD group (63.0±5.5°) was higher than that of the SD group (57.8±3.9°), which indicated that it was more hydrophobic. But the difference was not statistically significant. After treated with CAP for 15 s (59.6±3.1°), the contact angles decreased, but was still statistically the same as the untreated CAD group. With the increase of treatment time, contact angles continued to decrease, meaning that the hydrophilicity increases. Group CAD CAP-

30s (48.7±5.2°) and CAD CAP-45s (48.0±5.1°) have the lowest contact angle, and the differences with that of the CAD group was significant; no statistical differences are found within the two groups, which means that the hydrophilicity is stable at a certain level (Fig. 3).

Dentin surface SEM examination
Figure 4 shows the typical SEM micrographs of all the groups. The surface of CAD (b) appeared to have a
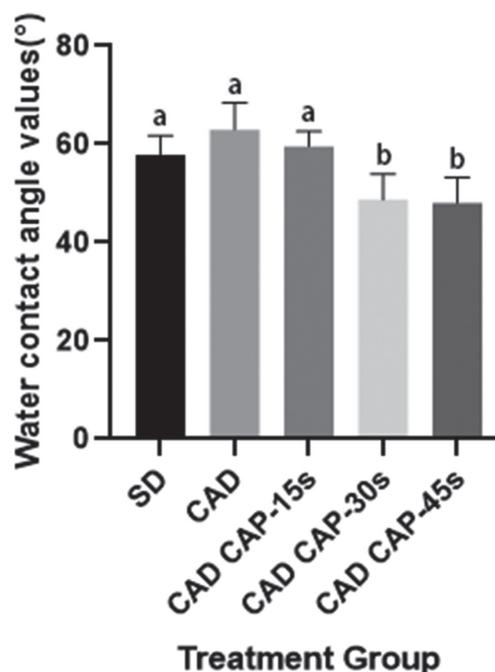

Fig. 3 Water contact angle values of SD and non-treated CAD, and CAD treated with CAP for different durations.
SD: sound dentin, CAD: caries affected dentin, CAP: cold atmospheric plasma. Identical letters indicate no significant difference for each value ($p>0.05$).

Table 1 µTBS data (in MPa) of the resin composite to the dentin surfaces measured at 24 h after CAP treatments and after 2-year water storage

Group	µTBS (Sd)	
	At 24 h	After 2 years
SD	49.87 (1.64)[Aa]	35.56 (2.42)[Ba]
CAD	35.62 (5.02)[Ab]	20.41 (3.54)[Bd]
CAD CAP-15s	37.17 (4.29)[Ab]	23.07 (4.64)[Bd]
CAD CAP-30s	49.27 (2.26)[Aa]	31.13 (2.16)[Bc]
CAD CAP-45s	48.83 (1.59)[Aa]	32.93 (1.15)[Bb]

SD: sound dentin, CAD: caries affected dentin, CAP: cold atmospheric plasma. Sd: standard deviation. Values are given in the form of mean (Sd, *n*=32). Different uppercase letters within a row indicate significant differences between the testing times, and different lowercase letters within a column indicate significant differences among the different groups ($p<0.05$).
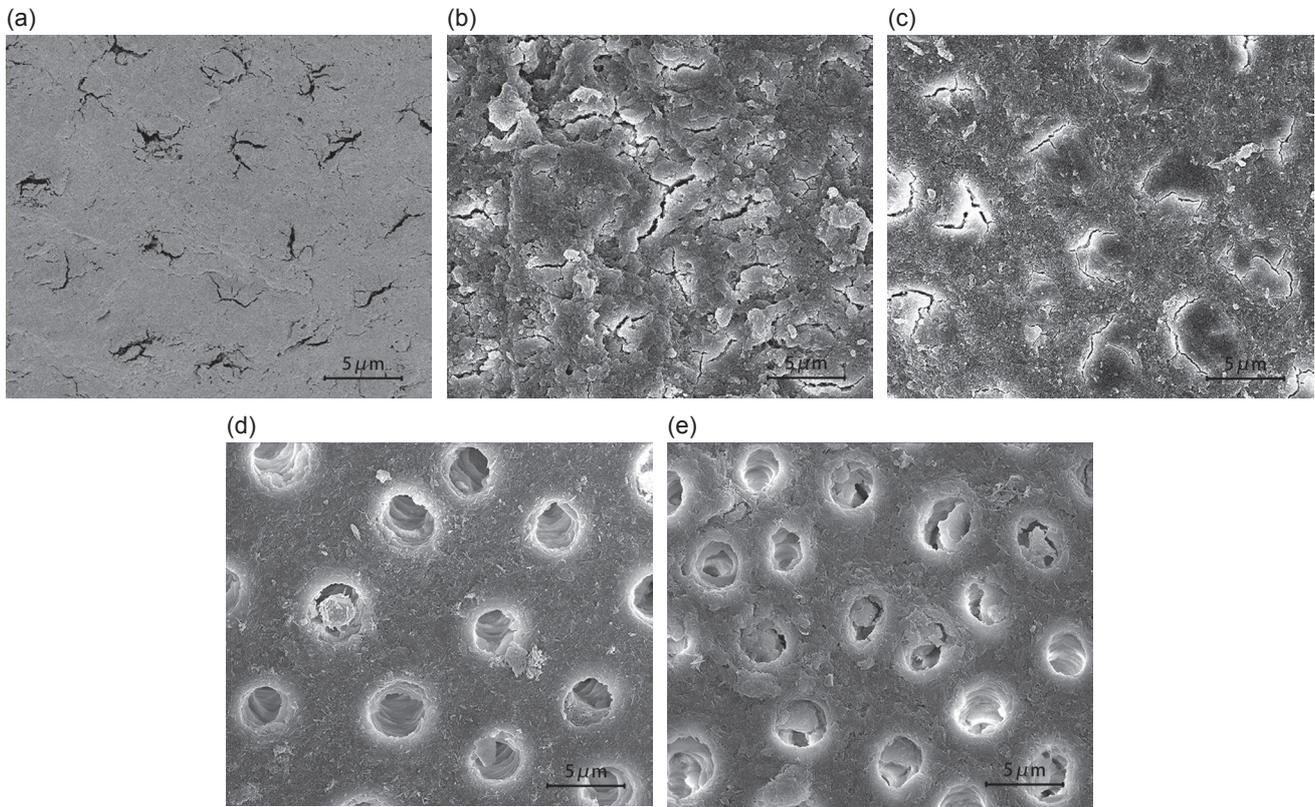
Fig. 4 Typical SEM micrographs of SD, non-treated CAD and CAD treated with CAP for different durations. (a) SD, (b) CAD, (c) CAD CAP-15s, (d) CAD CAP-30s, (e) CAD CAP-45s.
SD: sound dentin, CAD: caries affected dentin, CAP: cold atmospheric plasma. The surface of CAD (b) appeared to have thicker and fluffier smear layer compared with SD (a), and many of the dentin tubules were clogged. No changes were found in CAD after treated with plasma for 15 s (c). When the treatment time extended to 30 s and 45 s, the surfaces became cleaner and the dentin tubules covered by the smear plugs were opened, exposing the deeper mineral crystal occlusions inside some tubules (d, e).

Table 2 Surface chemical compositions (in atomic percentage of each element, %) of SD, non-treated CAD and CAD treated with CAP for 30 s

	SD	CAD	CAD CAP-30s
C1s (%)	29.17	52.17	45.40
N1s (%)	4.87	11.14	6.74
O1s (%)	47.29	30.04	36.64
P2p (%)	7.48	2.59	4.37
Ca2p (%)	11.19	4.06	6.84

SD: sound dentin, CAD: caries affected dentin, CAP: cold atmospheric plasma

thicker and looser smear layer compared with that of SD (a), and many of the dentin tubules were covered. No changes were found in CAD after treated with plasma for 15 s (c). When the treatment time was extended to 30 s and 45 s, the surface of CAD became cleaner, and the dentin tubules covered by the smear layer plugs were open, exposing the deeper mineral crystal occlusions inside some tubules (d, e).

XPS examination
Figure 5 provides the XPS wide-scan spectra of the sound dentin (a), untreated CAD (b), and CAD treated with CAP for 30 s (c). In sound dentin, the most dominant peaks were O1s peak (530.1 eV) and Ca2p1 peak (346.1 eV). In CAD, both peaks dropped significantly, and C1s peak (284.0 eV) increased and became one of the foremost peaks. After the CAP treatment, O1s peak and Ca2p1 peak increased back to the dominant peaks, and the C1s
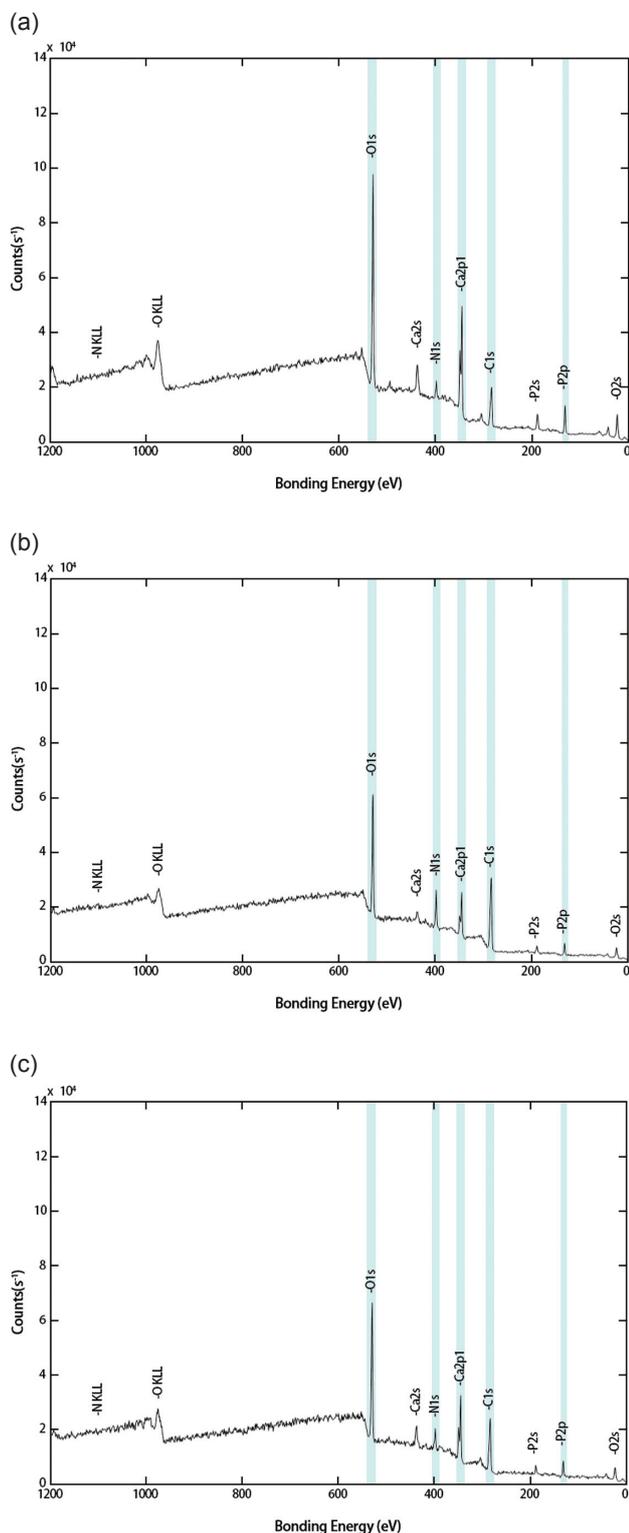
Fig. 5 XPS of SD, non-treated CAD and CAD treated with CAP for 30 s. (a) SD, (b) CAD, (c) CAD CAP-30s. SD: sound dentin, CAD: caries affected dentin, CAP: cold atmospheric plasma.

peak dropped.

Quantitative atomic percent concentrations for N1s peak (398.8 eV), P2p peak (132.2 eV) and the three substrates mentioned above are shown in Table 2. Compared with SD, CAD before treatment had a lower atomic percent of elements associated with mineral (Ca2p: 11.19% in SD, 4.06% in CAD; P2p: 7.48% in SD, 2.59 in CAD), and higher associated with organic debris (C1s: 29.17% in SD, 52.17% in CAD; N1s: 4.87% in SD, 11.14% in CAD). The CAP treatment reversed those trends, that is to say, the mineral-associated elements increased (Ca2p 6.84%; P2p 4.37%) and organic-associated elements decreased (C1s 45.4%; N1s 6.74%).

DISCUSSION

The purpose of this study was to evaluate the effects of the helium CAP jet on the immediate bonding strength of CAD, and to explore its mechanisms from surface modification perspective. A significant increase was observed in the immediate and aging µTBS values of CAD after the helium CAP jet treatment, especially in the group treated for 30 s, which showed cleaner surfaces with better wettability and higher concentrations of O, Ca and P. So the null hypothesis that CAP had no effects on bonding to CAD has to be rejected.

The present study uses pH-cycling to obtain artificial CAD samples for µTBS and surface modification examinations. Most studies about CAD use natural samples, which are prepared by removing natural caries-infected lesions *in vitro*[27]. However, the variation of natural CAD is too great to be used as a standardized substrate for the laboratory research[26]. And the arc form of the cavities makes it difficult to prepare a smooth surface for the bonding experiment. Therefore, in recent years, more and more studies choose artificial CAD samples[28,29]. Although it is difficult to completely stimulate the chemical and histologic characteristics of natural lesions (*e.g.*, the host matrix metalloproteinase activated by bacterial acids)[30], the important advantage of artificial CAD is that they provide a standardized substrate. Among them, pH cycle is considered to resemble a natural affected caries dentin layer after caries removal, especially in terms of µTBS and mineral density[24,25].

Surface wettability has a great influence on the dentin-resin bonding. The changes of CAD before and after the helium CAP jet treatment are evaluated in this study. With the high hydrophilicity of contemporary dental adhesives, the hydrophilic surface of lower water contact angle is conducive to resin-tooth hybridization and better bonding performance. However, if the surface is super hydrophilic, different adhesive-phases will separate and the resin polymerization will be affected, leading to a poor adhesive durability[31]. The contact angles of CAD drop significantly after 30 s CAP treatment, and no further decrease is observed when the exposure time is extended to 45 s. That is to say, the hydrophilicity increases within an appropriate range, facilitating the penetration of resin adhesives to dentin

surface, and consequently, the higher µTBS values are gained in these two groups.

Many studies have confirmed the capability of CAP treatment to reduce the water contact angles of dentin[16,32,33], and the two mechanisms involved are surface etching/ablation and modification. In this study, the etching effect of CAP on the smear layer of CAD can be confirmed by SEM and XPS examinations. The surface of CAD before treatment (Fig. 4b) appears to have a thicker and fluffier smear layer compared with that of SD (Fig. 4a), this is a coincidence with the reports of Taniguchi *et al.*[3]. No noticeable changes of CAD are observed after 15-s CAP treatment, but after being treated for 30 s and 45 s (Figs. 4d, e), the surfaces become cleaner and the dentin tubules covered by the smear plugs are opened, exposing the deeper mineral crystal occlusions inside some tubules. As is shown in XPS wide-scan spectra (Fig. 5) and quantitative atomic percent concentrations (Table 2), CAD before treatment possesses higher C and N signals and lower Ca and P signals compared with those of SD, suggesting a protein-rich smear layer on CAD surfaces. Those trends are reversed after treated with CAP for 30 s with an increase of P (from 2.59% to 4.37%) and Ca (from 4.06% to 6.84%) signals, and a decrease of C (from 52.17% to 45.40%) and N (from 11.14% to 6.74%) signals, indicating that the helium CAP jet etches away the protein-rich smear layer and exposes hydrophilic mineral, and thus, leads to enhanced surface hydrophilicity.

By etching the smear layer, the CAP treatment exposes the dentin surface with higher mineral contents. This, on the one hand, can enhance the surface hydrophilicity; while on the other hand, can improve the quality of the hybrid layer and improve the long-term bonding performance. After 2-year aging, µTBS values of all the groups drop significantly. But the groups treated for 30 s (31.13±2.16 MPa) and 45 s (32.93±1.15 MPa) still have higher values compared with that of the non-treated CAD (20.41±3.54 MPa), and are close to that of SD (35.56±2.42 MPa) ($p<0.05$). The developed durability of bonding suggests that CAP can improve the quality of resin-CAD hybrid layer. The composition of smear layer is related to the characteristics of the tissue cut away. Compared with sound dentin, caries-infected dentin is partially demineralized with destroyed structures of dentin collagen and contains more water. Therefore, the CAD smear layer is thicker, richer in water and organic components, and is hard to be removed by traditional etch-and-rinse adhesives[34]. Self-etching adhesives are applied directly on the smear layer of dentin surface, that is to say, the smear layer becomes a part of the hybrid layer, whose thickness and quality will affect the adhesive/dentin bonding strength. By partly removing the smear layer, the CAP treatment may induce a better hybrid layer and improve the long-term bonding performance.

In the present research, significant changes are observed after 30-s CAP jet treatment, which were also reported in some previous studies[18]. But the morphologic changes of the dentin surfaces after plasma treatments varied among different studies. Some studies claimed that no morphologic changes were tested after CAP treatment[33]. These divergent results are related to the differences of both dentin surfaces and CAPs. On the one hand, the characteristics of dentin surfaces can affect their sensitivity to the CAP treatment. Chen *et al.* found no changes in SEM images of SD surfaces before and after 30-s plasma treatment[33]. However, Zhu *et al.* applied CAP to demineralized dentin for only 10 s and observed partial collagen collapse[18], suggesting a negative correlation between the mineral level of dentin surface and its reaction sensitivity to CAP. The XPS test in this study shows that the elements associated with mineral are lower in CAD compared with that of the SD (Table 2, Ca2p: 11.19% in SD, 4.06% in CAD; P2p: 7.48% in SD, 2.59 in CAD). And the loss of minerals can significantly reduce the mean elastic modulus and nanohardness of CAD[35,36]. What's more, the CAD surface (Fig. 4b) is looser than that of SD (Fig. 4a) under SEM test. Those differences in chemical composition, hardness and morphology make the CAP more effective on CAD than it is on SD. On the other hand, the different treating effects might be attributed to the different kinds of CAPs: the CAPs used by Chen *et al.*[33] were generated by direct-current glow discharges with argon as the plasma forming gas, while Zhu *et al.*[18] treated the dentins with the helium CAPs produced by the radio-frequency atmospheric pressure glow discharges. Compared to the helium CAPs generated by the dielectric barrier discharges employed in our study, the chemically reactive species may be somewhat different from those of the CAPs used by Chen *et al.*[33].

Besides the etching effect, the CAP treatment also induces a chemical modification on the CAD surface. The O content in CAD increases from 30.04% to 36.64% after the helium CAP jet treatment of 30 s (Table 2). This is coincidence with previous studies[19,33]. The chain scission and oxidation of the long carbon chain substance by the plasma treatment are thought to be the major reasons for the increase of oxygen[37]. Since the plasma consists of abundant chemically reactive species with high electron energies, the oxygen in ambient air can be excited by the plasmas to form abundant highly reactive oxygen species, and then, reacts with the surfaces. On the one hand, the high energy species in plasmas could cut the long hydrophobic carbon chain, and graft oxygen-containing functional groups to induce oxidation. On the other hand, these species could break the hydrogen bond between different peptide chains, consequently, loose structure and expose hydrophilic amino acid inside, resulting in a chemically modified surface[14].

Previous studies found that the CAPs were effective in improving the dentin collagen stiffness, enhancing polymerization of resin, and inhibiting matrix metalloproteinase[18,23]. Although those are all based on sound dentin, it is suggested that the CAP has potential in enhancing the aging bonding strength of CAD. We will continue the relative studies in future.

In summary, the foregoing results show that, by etching and chemical modification, the helium CAP jet

　　　　　　　　　　　　　　Dent Mater J 2022; 41(1): 101–109

treatment is effective in improving the immediate bond strength of artificial CAD using the self-etching systems, and slows the aging process of the bonding. A higher-hydrophilic and cleaner surface could be easily achieved by the CAP treatment.

CONCLUSION

Within the limitations of this study, we conclude that the helium CAP jet treatments for 30 s and 45 s is effective in improving the immediate bond strength of the artificial CAD, and slows the aging process of the bonding. CAD is frequently encountered in present clinical practice with minimum invasive philosophy, and the CAP treatment is a technique with bright prospects in enhancing the bonding performance of this challenging substrate. A higher-hydrophilic and cleaner surface could be easily achieved by the CAP treatment.

ACKNOWLEDGMENTS

REFERENCES

1) Chen Z, Chen RT. Dental caries revisited. J Oral Sci Res 2020; 36: 1-6.
2) Isolan CP, Sarkis-Onofre R, Lima GS, Moraes RR. Bonding to sound and caries-affected dentin. A systematic review and meta-analysis. J Adhes Dent 2018; 20: 7-18.
3) Taniguchi G, Nakajima M, Hosaka K, Iwamoto N, Ikeda M, Foxton RM, et al. Improving the effect of NaOCl pretreatment on bonding to caries-affected dentin using self-etch adhesives. J Dent 2009; 37: 769-775.
4) Jr GWM, Marshall SJ, Kinney JH, Balooch M. The dentin substrate: structure and properties related to bonding. J Dent 1997; 25: 441-458.
5) Vidal CM, Tjaderhane L, Scaffa PM, Tersariol IL, Pashley D, Nader HB, et al. Abundance of MMPs and cysteine cathepsins in caries-affected dentin. J Dent Res 2014; 93: 269-274.
6) Ericson D, Zimmerman M, Raber H, Ouml, Trick B, Bornstein R, et al. Clinical evaluation of efficacy and safety of a new method for chemo-mechanical removal of caries. A multi-centre study. Caries Res 1999; 33: 171-177.
7) Macedo GV, Yamauchi M, Bedran-Russo AK. Effects of chemical cross-linkers on caries-affected dentin bonding. J Dent Res 2009; 88: 1096-1100.
8) Yoshiyama M, Urayama A, Kimochi T, Matsuo T, Pashley DH. Comparison of conventional vs self-etching adhesive bonds to caries-affected dentin. Oper Dent 2000; 25: 163-169.
9) Giacomini MC, Scaffa P, Chaves LP, Vidal C, Machado TN, Honorio HM, et al. Role of proteolytic enzyme inhibitors on carious and eroded dentin associated with a universal bonding system. Oper Dent 2017; 42: E188-E196.
10) Wollensak G, Iomdina E. Long-term biomechanical properties of rabbit cornea after photodynamic collagen crosslinking. Acta Ophthalmol 2009; 87: 48-51.
11) Ahn Y, Lamy R, Darling CL, Stewart JM, Pinzon LM. Photochemical crosslinking of caries-affected dentin combined with total- or self-etch systems. Am J Transl Res 2018; 10: 2990-2995.
12) Wang Y, Spencer P, Walker MP. Chemical profile of

adhesive/caries-affected dentin interfaces using Raman microspectroscopy. J Biomed Mater Res A 2010; 81A: 279-286.
13) Trevelin LT, Silva BTFD, Freitas PMd, Matos AB. Influence of Er:YAG laser pulse duration on the long-term stability of organic matrix and resin-dentin interface. Lasers Med Sci 2019; 34: 1391-1399.
14) Li HP, Zhang XF, Zhu XM, Zheng M, Liu SF, Qi X, et al. Translational plasma stomatology: Applications of cold atmospheric plasmas in dentistry and their extension. High Voltage 2017; 2: 188-199.
15) Ritts AC, Li H, Yu QS, Xu CQ, Yao XM, Hong L, et al. 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.
16) Hirata R, Teixeira H, Ayres A, Machado L, Coelho P, Thompson V, et al. Long-term adhesion study of self-etching systems to plasma-treated dentin. J Adhes Dent 2015; 17: 227-233.
17) Dong X, Li H, Chen M, Wang Y, Yu Q. Plasma treatment of dentin surfaces for improving self-etching adhesive/dentin interface bonding. Clin Plasma Med 2015; 3: 10-16.
18) Zhu XM, Zhou JF, Guo H, Zhang XF, Liu XQ, Li HP, et al. Effects of a modified cold atmospheric plasma jet treatment on resin-dentin bonding. Dent Mater J 2018; 37: 798-804.
19) Zhu XM, Guo H, Zhou JF, Zhang XF, Chen J, Li J, et al. Influences of the cold atmospheric plasma jet treatment on the properties of the demineralized dentin surfaces. Plasma Sci Technol 2018; 20: 044010.
20) Mai-Prochnow A, Murphy A, McLean K, Kong M, Ostrikov K. Atmospheric pressure plasmas: Infection control and bacterial responses. Int J Antimicrob Agents 2014; 43: 508-517.
21) Gilmore B, Flynn P, O'Brien S, Hickok N, Freeman T, Bourke P. Cold plasmas for biofilm control: opportunities and challenges. Trends Biotechnol 2018; 36: 627-638.
22) Zhang Y, Yu Q, Wang Y. Non-thermal atmospheric plasmas in dental restoration: Improved resin adhesive penetration. J Dent 2014; 42: 1033-1042.
23) Chen M, Zhang Y, Yao X, Li H, Yu Q, Wang Y. Effect of a non-thermal, atmospheric-pressure, plasma brush on conversion of model self-etch adhesive formulations compared to conventional photo-polymerization. Dent Mater 2012; 28: 1232-1239.
24) Marquezan M, Correa FN, Sanabe ME, Rodrigues Filho LE, Hebling J, Guedes-Pinto AC, et al. Artificial methods of dentine caries induction: A hardness and morphological comparative study. Arch Oral Biol 2009; 54: 1111-1117.
25) Joves GJ, Inoue G, Nakashima S, Sadr A, Nikaido T, Tagami J. Mineral density, morphology and bond strength of natural versus artificial caries-affected dentin. Dent Mater J 2013; 32: 138-143.
26) Li D, Li G, Li J, Liu ZQ, Li HP. Promotion of wound healing of genetic diabetic mice treated by a cold atmospheric plasma jet. IEEE Transactions on Plasma Science 2019; 47: 4848-4860.
27) Meraji N, Nekoofar MH, Yazdi KA, Sharifian MR, Fakhari N, Camilleri J. Bonding to caries affected dentine. Dent Mater 2018; 34: e236-e245.
28) Nicoloso G, Antoniazzi B, Lenzi T, Soares F, Rocha R. The bonding performance of a universal adhesive to artificially-created caries-affected dentin. J Adhes Dent 2017; 19: 317-321.
29) Jiang M, Mei M, Wong M, Chu C, Lo E. Influence of silver diamine fluoride treatment on the microtensile bond strength of glass ionomer cement to sound and carious dentin. Oper Dent 2020; 45: E271-E279.
30) Matos AB, Wu CD, Li W, Bedran-Russo AK. Gelatinolytic activity and adhesion studies of artificial caries-affected

dentin do not simulate natural caries. J Adhes Dent 2020; 22: 415-420.

31) Liu Y, Liu Q, Yu QS, Wang Y. Nonthermal atmospheric plasmas in dental restoration. J Dent Res 2016; 95: 496-505.

32) Stasic J, Selaković N, Puač N, Miletić M, Malović G, Petrović Z, *et al*. Effects of non-thermal atmospheric plasma treatment on dentin wetting and surface free energy for application of universal adhesives. Clin Oral Investig 2019; 23: 1383-1396.

33) Chen M, Zhang Y, Sky Driver M, Caruso A, Yu Q, Wang Y. Surface modification of several dental substrates by non-thermal, atmospheric plasma brush. Dent Mater 2013; 29: 871-880.

34) Spencer P, Wang Y, Walker MP, Swafford JR. Molecular structure of acid-etched dentin smear layers —In situ study.

J Dent Res 2001; 80: 1802-1807.

35) Ito S, Saito T, Tay FR, Carvalho RM, Yoshiyama M, Pashley DH. Water content and apparent stiffness of non-caries versus caries-affected human dentin. J Biomed Mater Res B Appl Biomater 2005; 72: 109-116.

36) Ito S, Hashimoto M, Wadgaonkar B, Svizero N, Carvalho R, Yiu C, *et al*. Effects of resin hydrophilicity on water sorption and changes in modulus of elasticity. Biomaterials 2005; 26: 6449-6459.

37) Sun S, Sun J, Yao L, Qiu Y. Wettability and sizing property improvement of raw cotton yarns treated with He/O 2 atmospheric pressure plasma jet. Appl Surf Sci 2011; 257: 2377-2382.