Original Article

Effect of nonthermal atmospheric plasma application at different time intervals on the dentinal shear bond strength pretreated with 2% chlorhexidine as cavity disinfectant: An *in vitro* study

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Abstract

Context: Effective use of nonthermal atmospheric plasma (NTAP) to strengthen adhesive–dentin interfacial bonding while disinfecting with chlorhexidine (CHX).

Aim: NTAP application at different time intervals on the dentinal shear bond strength (SBS) after pretreatment with 2% CHX as a cavity disinfectant.

Settings and Design: The design of the study was an *in vitro* study.

Materials and Methods: Forty permanent mandibular teeth were collected ($n = 40$) and the occlusal surfaces were flattened. For, all the specimens 37% phosphoric acid etching was done followed by pretreatment with 2% CHX as cavity disinfectant for 5 s. According to the surface treatment, divided into four groups of $n = 10$. Group I (Control): No NTAP pretreatment was done. Group II: NTAP pretreatment done for 15 s. Group III: NTAP pretreatment done for 30 s. Group IV: NTAP pretreatment done for 45 s. Later, all the specimens were treated with a bonding agent, incremental build-up of composite resin on the dentin surface was done and evaluation of SBS was done. Analyzed using One-way analysis of variance with a *post hoc* Tukey's test ($P < 0.05$).

Results: Two percent CHX pretreatment as cavity disinfectant followed by NTAP application for 30 s (Group III) exhibited greater values compared to the control group (Group I).

Conclusion: Two percent CHX pretreatment as cavity disinfectant followed by NTAP pretreatment for 30 s was found to exhibit better bond strength values compared to 15 s as well as 45 s.

Keywords: Adhesion; composite resin; nonthermal atmospheric plasma; shear bond strength

INTRODUCTION

The safety and realistic appearance of composite restorations have led to widespread clinical acceptance in dentistry. In composite restorations, the interface

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between adhesive and dentin is recognized as the weaker area.[1] Phosphoric acid is used to etch the dentin surface to eliminate the smear layer before total-etch adhesive application.[2] One of the most significant issues with the etch-rinse adhesives is the lack of durability of bond to tooth structure. Hence, the bond strength of the dentin surface is still not satisfactory.[2]

To diminish the bacteria from cavity preparations, disinfectant solutions such as 2% chlorhexidine (CHX) can be utilized.[3] CHX is a cationic bisbiguanide and is an antimicrobial agent that can inhibit the collagenolytic activity of matrix metalloproteinases (MMPs), enhancing the longevity of bond between adhesives and dentin.[4] It is capable of binding to both organic and inorganic dentin components. When CHX binds to the hydroxyapatite (dentin-inorganic component), a phosphate salt is thought to develop, increasing the surface energy of the dentin and improving the primer's ability to wet the dentin. In addition to improving dental adhesive formulation, surface modification of dentin can better provide stronger adhesive–dentin interfacial bonding.[5]

Nonthermal atmospheric plasma (NTAP) is widely used as an efficient surface modification approach to enhance the performance of a variety of natural and synthetic materials.[6] It was discovered that a brief NTAP treatment alters the nature of the exposed collagen fibers and boosts the dentin surface's hydrophilicity, allowing the adhesive to penetrate dentinal collagen fibrils more effectively and improving the bond strength at the dentin–adhesive interface. In comparison to untreated samples, adhesive–dentin interface bonding increased by approximately 60% when dentin was treated with a nonthermal plasma.[6]

NTAP pretreatment on dentin substrate appears to be a promising mode of increase in bond durability.[7,8] There appear to be quite a few studies that have assessed the effect of 2% CHX as a cavity disinfectant in combination with NTAP treatment at different time intervals. The vast majority of research studies that examined the impact of applying 2% CHX as a cavity disinfectant on the dentin substrate used bond strength tests. Hence, the aim of the current study was to assess the effect of 2% CHX pretreatment as cavity disinfectant with and without the application of NTAP at different time intervals, i.e. for 15 s, 30 s, and 45 s on the shear bond strength (SBS) of posterior bulk fill composite.

MATERIALS AND METHODS

Ethical clearance was obtained from the Ethical Committee (Pr.252/IEC/SIBAR/2018). Forty freshly extracted human permanent mandibular molar teeth (n = 40) which were free of caries, fracture lines, cracks, and any previous restorations were selected. The teeth were stored in 0.2% thymol and any tissue tags were removed with a scaler. Using a diamond disk and slow-speed handpiece with water coolant, the occlusal surface was flattened by eliminating the cusps, grooves, and ridges thus reducing 1–2 mm of occlusal tooth structure. With the use of silicon carbide abrasive papers of 600 grit, the exposed dentin surfaces were polished under water cooling. All the specimens were placed in ultrasonic cleanser to remove any of the foreign particles left over the dentinal surface.

Surface treatment

An acid-etch system (Scotchbond Multi-Purpose Plus; 3M ESPE, USA) was used, according to the manufacturer's instructions. All the prepared dentinal surfaces (n = 40) were acid-etched with 37% phosphoric acid gel for 15 s (Uni etch BAC 32%, Bisco, USA), and rinsed for 15 s. Then, the specimens were re-wetted with 2% CHX for 5 s, employing a new micro brush until the appearance of sleekness. The excess solution was removed with absorbent paper after 5 s.

Plasma treatment

NTAP was applied on the rewetted surfaces of specimens in experimental groups [Figure 1]. Plasma treatment was conducted at the Department of Chemistry, Indian Institute of Space Science and Technology, Thiruvananthapuram, Kerala, India. The NTAP treatment was carried out in a glass reactor. This reactor has a tube that is 5 cm in diameter and 30 cm long. A mechanical pump is used to evacuate the reactor at pressures <2 Pa. Up to a pressure of 10 Pa, gas was permitted to flood the reactor. NTAP was produced within the glass cylinder under vacuum due to the induced magnetic field brought about by the current running through an electrical coil around the cylinder.[9,10] Helium-argon gas was used to treat surfaces at 60 W for 15, 30, or 45 s. Before exposing the specimens to air, the radio frequency was turned off at the end of the process.[9,10]

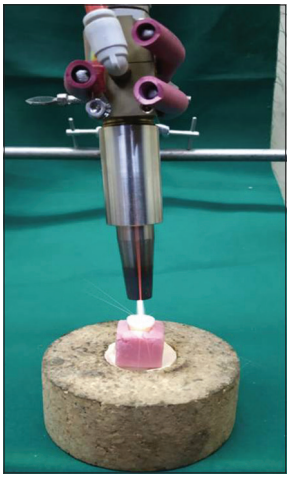


Figure 1: Nonthermal atmospheric plasma application on the rewetted surface of a specimen

After rewetting with 2% CHX as cavity disinfectant, depending on the duration of plasma application, the specimens were allocated into four groups, each with a specimen size of ten ($n = 10$).

Group I (Control Group): No plasma application was performed in this group.

Group II (NTAP 15 s): The rewetted surfaces were treated with NTAP for 15 s, 5 mm away from the nozzle.

Group III (NTAP 30 s): The rewetted surfaces were treated with NTAP for 30 s, 5 mm away from the nozzle.

Group IV (NTAP 45 s): The rewetted surfaces were treated with NTAP for 45 s, 5 mm away from the nozzle.

An acid-etch adhesive (Adper Single Bond 2, 3M ESPE, USA) was then applied to the dentinal surfaces of the specimens in each of the four groups and the specimens were polymerized using a light-emitting diode curing unit (Mediplus, India) with an output power of 550 mW/cm^2 for 10 s. For all the specimens, composite placement was completed following the bonding process. A plastic matrix measuring 2 mm by 2 mm was prepared and placed on the occlusal surfaces. The composite (Spectrum, Dentsply, USA) was placed gradually, with each increment polymerizing for 30 s. For 24 h, the specimens were kept in distilled water. The Universal Testing Machine (UTM) from Instron Hydraulics was used to assess the SBS. Every specimen was positioned in the UTM such that the plunger's leading edge was aimed at the interface between the composite resin and the occlusal surface at a speed of 1 mm/min.

Statistical analysis

The SPSS 22.0 (Statistical Package for Social Science, IBM Corporation Ltd., Armonk, New York, USA) was used for the statistical analysis. Mean and standard deviation were estimated for all four groups. The mean values were compared using one-way analysis of variance (ANOVA) followed by *post hoc* Tukey's test with the level of significance set at $P < 0.05$.

RESULTS

Table 1 shows the mean values, standard deviation, and overall significance of all four groups evaluated by one-way ANOVA ($P < 0.05$). Table 2 represents the pairwise comparison of the groups done by *post hoc* Tukey's test ($P < 0.05$). Graph 1 represents the box plot diagram showing the upper, and lower bound values and median of all the groups. The inference from Graph 1 suggests that there was a statistically significant difference among the groups ($P < 0.001$). The plasma-treated groups exhibited a statistically significant increase in SBS values as compared with the untreated control group. When compared for pair-wise analysis, a statistically significant difference ($P < 0.001$) exists between Group I and Group III. Greater SBS values were observed with Group III in comparison with Group I, Group II and Group IV. A decrease in SBS values was observed in Group I in comparison with Group II, Group III, and Group IV whereas Group II and Group IV showed similar SBS values.

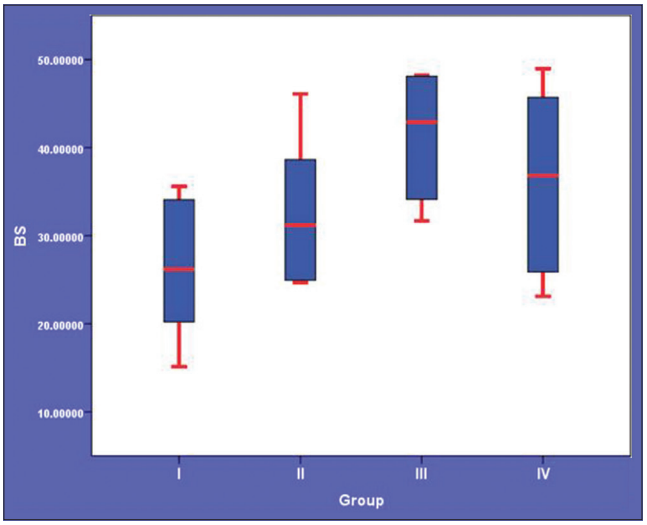
Table 1: Mean values, standard deviation, overall significance of all the four groups evaluated by one-way analysis of variance ($P<0.05$)

Group	*n*	Minimum	Maximum	Mean	SD	*P*
I	10	15.12	32.00	24.97	6.44	<0.001*
II	10	24.94	46.10	33.97	6.96	
III	10	36.90	48.21	43.83	4.64	
IV	10	23.12	45.71	35.82	7.52	

*Statistically significant. SD: Standard deviation

Table 2: Pair-wise comparison of the groups done by *post hoc* Tukey's test ($P<0.05$)

Comparison between the groups	*P*
Group I versus Group II	0.01
Group I versus Group III	<0.001
Group I versus Group IV	0.002
Group II versus Group III	0.009
Group II versus Group IV	0.92
Group III versus Group IV	0.042



Graph 1: Box plot diagram showing the upper, lower bound values and median of all the groups and there are no outliers

DISCUSSION

To establish a strong bond between composite resin and intact dentine, dentine bonding is a common step in modern dental restorative procedures. Inadequate penetration of adhesive monomers into the full depth of the demineralized layer, on the other hand, could result in leakage and a marginal gap in this area, exposing the collagen fibrils to the oral environment and hastening the hydrolytic degradation of the hybrid layer.[11] Another

factor that contributes to the decreased longevity of the restorations is the breakdown of exposed collagen by collagenolytic host-derived enzymes including MMPs.[12]

A common antibacterial agent with a wide range of effectiveness against oral bacteria is 2% CHX.[13] In addition to being utilized as a disinfectant, CHX, a potent MMP inhibitor, was demonstrated to significantly diminish dentinal collagenolytic activity.[14] Gomes *et al*.[15] reported that the dentine degrading activity might be reduced by the application of 2% CHX to the dentin surface after the application of phosphoric acid, to the exposed denuded collagen layer. Because of the characteristics of CHX, like ready binding to phosphate groups, strong positive (+ve) ionic charge and strong affinity to tooth surfaces and finally increasing the surface-free energy, its application after etching would increase the wettability of the primers, thereby improving the adhesion.[16]

Moreover, 2% CHX reduces or avoids the autodegradation of exposed collagen fibrils inside an incompletely formed hybrid layer, enhancing the long-term durability of the hybrid layer and bond strength.[14] Specific host-derived proteinase activity seems to be prevented even after a short-time application of CHX in contact with the demineralized dentin.[17,18]

Therefore, it would be ideal to use CHX for only a short amount of time as part of the effort to improve the bonding procedure. In a study conducted by Kang *et al*.[19] to preserve dentin bond strength, a quick application of 2% CHX for 5 s following 37% phosphoric acid etching may be sufficient.

The structure, morphology, and chemical composition of dentin surfaces can vary, which may have an impact on how well dentin bonding systems adhere to dentin surfaces over time. Lately, efforts have been made to develop dentin surface modifications, such as chemical or electrical approaches, altering the dentin surface that would facilitate easier penetration of bonding agents.[11] The desired type of plasma is atmospheric (it is produced at atmospheric pressure as opposed to vacuum) and is nonthermal (i.e. its gas phase is at room temperature as opposed to thousands of degrees).[20] It was suggested in dentistry to promote coronal adhesion, adhesion between fiber posts and resin cements, and root canal disinfection. There were several different gases used, including oxygen, helium, and argon.[21]

By applying plasma to the etched dentin surface with a NTAP brush, total-etch adhesive/dentin bonding is much improved. Plasma therapy was used to modify the exposed collagen structure and enhance the hydrophilicity of the dentin surface.[22] One potential method for improving the surface bonding properties of various materials is surface treatment with plasma, which enhances surface bonding characteristics and uses a cohesive treatment technique to prepare the dentin and adhesive interface for strong and durable bonding to composite restorative materials.[23] Nonthermal plasma treatment has been shown in numerous investigations to strengthen the bonding of restorative composites to dentin.[8,9,22,24]

The NTAP (15, 30, 45 s) was applied after acid etching, after rewetting with 2% CHX and before adhesive application.[20] Argon plasma therapy outperformed the control group. When argon plasma treatment is done for 30 s, it produces the highest values of bond strength, while the treatments for 15 and 45 s produce the lowest values of bond strength. The bond strength of adhesive systems to dentin was found to be increased following the application of argon plasma treatment for 30 s, but extended exposure times may result in lower bond strength values.[21] The SBS was shown to increase after 30 s of treatment but not after >30 s, probably as a result of the etching impact of plasma, which excessively destructed collagen fibrils at longer times. NTAP typically modifies or etches the surface. New chemical structures are typically created during the initial activities. Chemical surface structures are removed through etching. According to research done by previous studies, applying argon plasma to dentin before restoration improved the adhesive properties of dentin.[6] It was suggested that the partially opened dentin tubules that were seen following NTAP treatment made it easier for the adhesive to penetrate the smear layer, creating a thicker hybrid layer, and, as a result, greater bonding performance was seen.[20]

The possible reason for the decreased SBS values when NTAP was treated for 45 s compared to 30 s is, that when argon plasma of 45 s was applied, it can be seen that all peaks increased in relation to the phosphate peak. This rise in all peaks could be related to a decline in dentin phosphate concentration as the phosphate peak was the one utilized to normalize all spectra. Thus, the adverse outcomes of the 45-s results could be attributed to extended plasma exposure, which could begin to degrade dentin's inorganic structure by the reduction of phosphate species to carboxylic groups.[21]

The results of this investigation showed that dentin that had previously been exposed to 2% CHX showed improved bond strength of dentin after being treated with argon plasma for 30 s. Some recent investigations have shown the chemical alterations brought about by plasma treatment and their influence on adhesive properties have been demonstrated.[7] According to statistical data obtained, argon plasma therapy appears to be a potential approach for improving the adhesion of composite to dentin.[6] Consequently, larger research should be done to learn more about how argon plasma affects the organic dentin component, analyze the various application times and their impact on adhesive qualities, and create a standardized regimen for argon plasma treatment in patients.

CONCLUSION

NTAP enhanced the adhesive penetration and increased the dentin and adhesive interfacial bonding. 2% CHX pretreatment as cavity disinfectant followed by NTAP treatment for 30 s has shown higher SBS of dentin in comparison to the control group and was found to exhibit improved bond strength values compared to 15 s as well as 45 s.

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Conflicts of interest

There are no conflicts of interest.

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