International Journal of Multiphysics
Volume 18, No. 4, 2024
ISSN: 1750-9548

Investigating the Effect of Cold Atmospheric Plasma Treatment on the Tooth Enamel Acid Resistance (In-Vitro Study)

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Abstract

Introduction: Caries is the main cause of toothache. Cold atmospheric plasma can clean and disinfect the decay cavity without cutting. The purpose of the present research is Investigating the effect of cold atmospheric plasma treatment on the tooth enamel acid resistance (in-vitro study).

Methods & Materials: In this experimental-laboratory study, 42 samples with dimensions of 5 x 5 mm were prepared from the enamel of extracted human incisors. The samples were randomly divided into two groups. One of the groups was treated with cold plasma for 30 seconds (CAP group) and the other group was not treated at this stage (NO CAP group). Next, the samples of both groups were etched with 37% phosphoric acid for 30 seconds and then washed for 30 seconds. Then the samples were cut in half by ISOMET 2000 Precision Saw low speed cutting machine. The cut section of the samples was examined by scanning electron microscope and the depth of demineralization was measured from the surface to the deepest point of the enamel.

Results: The average depth of demineralization in the group prepared with cold plasma was 11.37 microns and the average depth of demineralization in the group prepared without cold plasma was 7.65 microns. The Mann-Whitney U test was used to determine the significance of the difference between the average depth of demineralization of the groups,. The average depth of demineralization in the group prepared with cold plasma (CAP) was significantly higher than the group without preparation with cold plasma (NO CAP). ($P<0.05$)

Conclusion: In the conditions of this study, preparation with cold atmospheric plasma reduces the resistance of tooth enamel to acid and increases the depth of demineralization and improves acid etching technique.
Keywords: Plasma, electron microscope, demineralization, enamel

Introduction

The mouth is a microbial habitat with more than 700 species that live in harmony with the human body. However, periodontal disease and caries are two common diseases in dentistry. Caries is the main cause of toothache and can occur both in the crown and the root of the tooth. Dental caries is defined as local destruction of tooth tissue by acids produced by bacteria (1,2). This acid causes the local pH values to decrease below a critical value and as a result the tooth tissues are demineralized (3). If the release of calcium, phosphate, and carbonate continues outside the tooth, a cavity will eventually form (4). This destruction can happen in their enamel, cementum and underlying dentin. Caries starts with small areas of demineralization in the enamel and can be stopped in the early stages or reversed through absorption of calcium, phosphate, and fluoride, but it is often not self-limiting and without proper treatment, demineralization can progress through the dentin to the pulp. slow and eventually cause tooth loss (5). To treat a damaged tooth, dentists recommend removing tooth decay and filling cavities with suitable materials (6). Caries management with restorative treatment, despite the limitations and the possibility of needing to repeat the restorative treatment, is still the preferred treatment method in many countries(7). However, since years ago in some regions such as Scandinavia, restorative treatments mostly have been replaced by caries prevention methods (8). Currently, polymer composites are widely used as dental restorative materials due to their superior properties such as biocompatibility, aesthetics, antibacterial properties, and non-toxicity compared to old filling materials. These materials have shown good physical, mechanical, and thermal properties (9). In recent research, various approaches have been used, including reducing shrinkage stress, increasing fracture toughness, creating antibacterial and self-healing capabilities to increase the lifespan of dental composites and reduce treatment failure.

Acid etching of enamel is used as a mechanism to increase the adhesion of resin restorative materials. The goals of acid etching are to chemically clean the enamel, improve wettability and increase the surface area available for polymer contact, and create micropores in which polymer tags can form (10).

Plasma is the fourth state of matter, which was discovered by British physicist Sir William Crookes in 1879, but the name plasma was used by American chemist Irving Langmuir in 1929. As the most common state of matter, it covers more than 99% of the visible world. Plasma is a collection of isolated particles. When electrons are separated from atoms and molecules, those particles change state and become plasma. Plasmas are inherently energetic because electrons use constant energy. If the energy is lost, the electrons reconnect and the plasma particles turn into gas once again. Unlike ordinary matter, plasma can exist over a wide range of temperatures without changing state. Popular plasmas include auroras, lightning, neon signs, and fluorescent lights. Outside the container, the plasma looks like a gas and the particles have no definite shape. But unlike gas, magnetic and electric fields can control plasma and transform it into useful and malleable structures (11).

Based on the relative temperatures of electrons, ions, and neutral particles, plasmas are classified as "thermal" or "nonthermal." Thermal plasma has electrons and heavy particles at the same temperature, i.e. they are in thermal equilibrium with each other. Non-thermal plasmas, on the other hand, have ions and neutrals at much lower temperatures (sometimes room temperature), while electrons are much hotter. In recent years, cold atmospheric plasma sources (less than 40°C at the point of application) have been introduced, allowing the extension of plasma therapy to living tissues .

There are different methods for producing plasma, including dielectric barrier discharge, atmospheric pressure plasma jet, plasma needle and plasma pencil. Gases that can be used to produce CAP are: helium, argon, nitrogen, heliox (combination of helium and oxygen) and air. Because of its ability to inactivate microorganisms, cause cell detachment and death in cancer cells, researchers have been interested in finding applications of CAP in dentistry and oncology (12).

Plasma applications are known for sterilization of medical equipment, packaging in the food industry, implants, wound healing and blood coagulation. Applications of CAP in dentistry include: tooth decay removal, sterilization, biofilm removal, root canal disinfection and bleaching (13).

Necrotic, infected, and demineralized tissue must be removed before filling cavities. This work can be done using ozone therapy, mechanical grinding or laser techniques (14-18). Unfortunately, these methods can be destructive as they may remove excess healthy tissue to ensure no decay remains (12).

Cold atmospheric plasma can clean and disinfect the decay cavity without cutting. This technology is able to destroy Streptococcus mutans bacteria (19,20). Cold plasma is considered as an alternative method to overcome the limitations of conventional biofilm removal methods. Due to its improved chemistry, cold plasma contains many active plasma species, including various charged particles and reactive oxygen species, which are effective in inactivating bacteria (21). This method can provide a less invasive way to remove dental caries before tooth filling, since it works at room temperature and does not cause any pain, destruction or pathological changes in healthy tissue (12, 22). Cold plasma does not cause hyperemia, swelling or wound due to lack of thermal damage (23). The advantages of this method include safety and proper efficiency, less time, low processing temperature, and use in living and non-living tissues (24). Plasma can also change the surface properties of enamel and dentin (25, 26) and improve conventional restorative methods and adhesion between teeth and restorative materials (27).

With the advancement of dental materials and clinical techniques, composites have become the most widely used direct restorative materials to meet the aesthetic needs of patients for the repair of tooth decay, tooth crown fracture, tooth wear, and congenital defects (28). In this method, the adhesion of the restorative material to the tooth is very important. Adhesion depends on the wetting and spreading of the adhesive material on the entire surface (30). Polymerization shrinkage creates stress at the restoration-tooth interface, resulting in debonding of the restoration when the shrinkage stress exceeds the bond strength. This, in turn, leads to a number of potential clinical problems such as postoperative hypersensitivity, secondary caries, and pulp inflammation as a result of infiltration of saliva, bacteria, and other irritants through the debonded space (28). Despite numerous researches, various forms of fracture or secondary caries have had an adverse effect on the lifetime of dental composite restorations and lead to 1-3% of the annual failure rate of dental composite restorations (31).

The use of methods that create greater bond strength has a significant effect on reducing the possibility of debonding of the restoration. Many common methods include the use of phosphoric acid for cleaning, increasing surface roughness and improving wettability on the surface of enamel and dentin (32). In some studies, it has been stated that cold plasma can increase the strength of the composite bond to dentin and enamel as an auxiliary method for the phosphoric acid method (25, 33). Therefore, in this study, the effect of cold atmospheric plasma on the acid resistance of tooth enamel was investigated.

Methods and Materials

This case-control in-vitro research was approved by the ethics committee of Aja University of Medical Sciences (Ethics ID: IR.AJAUMS.REC.1402.119). In order to measure the sample volume, G*Power software was used. Considering type one error (α=0.05), effect size (Effect size=0.8) and study power of 80% (β-1=0.80), the required sample size was calculated as 21 samples in each group. A number of extracted maxillary central teeth with intact crowns were selected for study. The enamel of the labial surface of the teeth was thoroughly cleaned with a rubber cap and pumice stone to remove any remaining blood and tissue, and then washed and dried. The crowns of the teeth were checked by an operator for being healthy and free of cracks and decay using an optical microscope, and problematic teeth were excluded from the study. (Image 3-1) Then the enamel of the labial surface was cut into 5x5mm enamel pieces by a diamond disc. 42 pieces of enamel were prepared and randomly divided into two groups (CAP and NO CAP). The samples were kept in distilled water during the study.

Cold plasma application

CAP group samples were removed from distilled water and then dried. Next, the surface of the enamel samples were exposed to cold plasma (kv 16) for 30 seconds. In this study, a DBD probe (Dento Panel, Fanavaran Sepid Jamegan, Tehran, Iran) was used to produce plasma. With the help of a glass lamp and neon gas, the DBD probe enables the transfer of plasma on the glass surface, by ionizing air molecules, it creates many active species.

Acid application

The samples of both groups (CAP and NO CAP) were removed from distilled water and dried. Then, the surface of the enamel samples was exposed to 37% phosphoric acid gel (Exetch, Parlaco, Tehran, Iran) for 30 seconds. After applying acid, the samples were washed for 30 seconds and dried for 20 seconds and then placed in distilled water.(34)

Cutting samples

The samples of both groups were cut from the middle by a low-speed cutting machine (ISOMET 2000 Precision Saw, Buehler, Lake Bluff, USA) and a double-sided diamond disc with a thickness of 0.4 mm (ISOCUT, Buehler, Lake Bluff, USA) under continuous water flow.(35)

Examining the samples with SEM

The samples were briefly dried and fixed on the base, then coated with gold by a coating machine (Coater Q150T, Quorum technology, England). The cut sections of the samples were examined by a scanning electron microscope (Quanta 450, FEI, USA) with an accelerating voltage of 15 kV and a magnification of 2000 times. The depth of demineralization was measured from the surface to the deepest point of the enamel according to the index provided by the microscope.(Fig1)

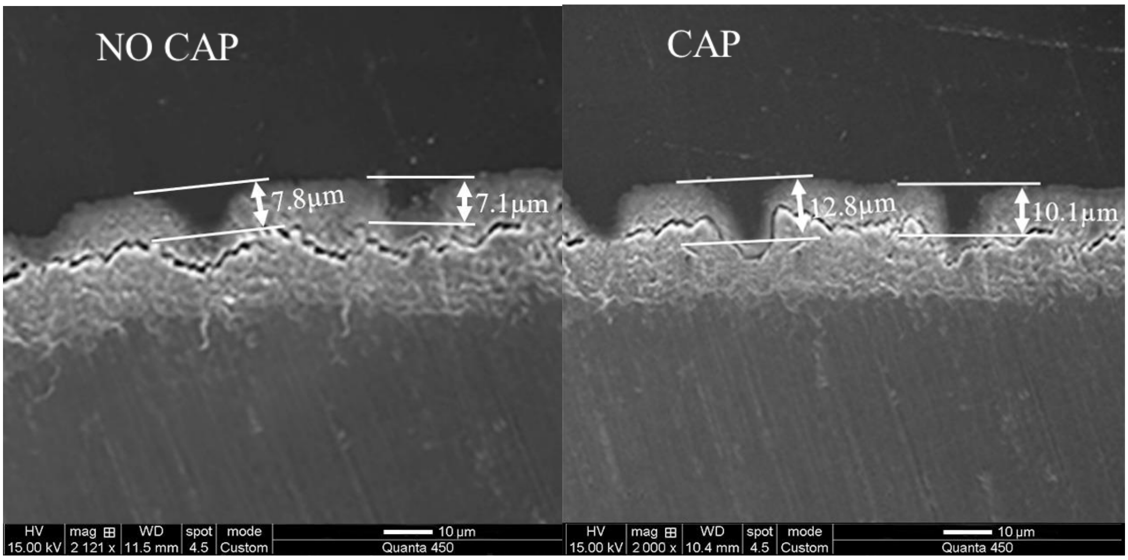

Fig1. Sample images recorded by SEM of the samples of two groups

SPSS version 26 software (IBM, New York, USA) was used for data analysis. The evaluation of the study data is shown in the descriptive section using mean, standard deviation and tables and graphs. In the analytical part, in order to check the significance of the difference in the average depth of demineralization of the group prepared with cold plasma and without cold plasma, and due to the non-normal distribution of the data, Mann-Whitney U analysis was used to check the significance of the difference between the two groups.

Results

In the conditions of this study, the average depth of demineralization in the group prepared with cold plasma (11.37 microns) was higher than the average depth of demineralization in the group prepared without cold plasma (7.65 microns).(Table 1)

Table 1. Descriptive indices of the depth of demineralization of the two studied groups

Preparation	Min (µm)	Max (µm)	Avg (µm)
CAP	8.7	13.7	11,37
NO CAP	6.6	8.8	7.65

Examining the samples with the Shapiro Wilk test showed non-normal distribution of the data; Therefore, in order to determine the significance of the difference between the average demineralization depth of the groups, the non-parametric Mann-Whitney U test was used with a significance limit of less than 0.05. Standard deviation, median and IQR were calculated separately for each group as shown in Table 2.

Table 2. Comparison of Average and median depth of demineralization of preparation group with cold plasma and without cold plasma

Groups	CAP	NO CAP
Avg.	11.37	7.65
Standard deviation	1.43	0.58
Median	11.4	7.6
IQR	2.4	0.9
P-Value	<0.001	

According to the information obtained in this study, the average depth of demineralization in the group prepared with cold plasma (CAP) was significantly higher than the group without preparation with cold plasma (NO CAP). (P-Value < 0.001)

Discussion

With the advancement of dental materials and clinical techniques, composites have become the most widely used direct restorative materials to meet the aesthetic needs of patients for the repair of tooth decay, crown fracture, tooth wear, and congenital defects. Along with the advantages of composites, we should also pay attention to the disadvantages caused by the weak properties of the materials and the quality of the methods of use. Disadvantages such as shrinkage stress caused by polymerization, technical sensitivity of materials and sensitivity to humidity during use, which potentially increases the risk of debonding and causes a number of clinical problems such as sensitivity after treatment, secondary decay, pulp inflammation due to saliva and bacterial penetration. to the space between the restorative material and the dental structure (28). These problems can increase treatment costs and dissatisfaction of the dentist and the patient. In recent studies, attempts have been made to reduce shrinkage stress, increase fracture toughness, create antibacterial and self-healing capabilities to improve the properties of dental composites and reduce treatment failure. Also, increasing the quality and strength of the bond between the restorative material and the dental structure is very important to reduce the possibility of debonding of the restorative and to reduce the mentioned problems, and in recent years, it has attracted the attention of researchers and manufacturers of dental materials and equipment.

Phosphoric acid etching is commonly used in clinical and laboratory research on the enamel surface to provide better bond strength. The acid etching technique can dissolve in the organic components of the enamel, create irregularity or microporous structure on the surface of the enamel and help to form a mechanical bond. Meanwhile, after etching with phosphoric acid, the free energy of the enamel surface increases, which causes the wetting of the bond on the enamel surface(36).

Plasma, the fourth state of matter, can rapidly change the surface energy without affecting the bulk properties of the material. Plasma produces radical species and energetic photons that are capable of modifying certain surfaces, increasing surface energy and hydrophilicity due to the removal of hydrocarbon groups and creation of hydroxyl groups. Active species with the action of ions can bind to atoms or other molecules in the air such as oxygen and change the surface energy (37).

The present study was conducted with the aim of investigating the effect of cold plasma on tooth enamel acid resistance by examining the depth of demineralization as one of the most important factors in the acid etching system.

In this study, 42 samples including 21 samples from each group were prepared and after preparation, their etching depth was measured and compared.

In the conditions of this study, the average depth of demineralization in the group prepared with cold plasma was 11.37 microns and the average depth of demineralization in the group prepared without cold plasma was 7.65 microns. As a result, the first and second hypotheses were rejected and the third hypothesis was confirmed.

According to the information obtained in this study, the average depth of demineralization in the group prepared with cold plasma was significantly higher than the group without preparation with cold plasma. (P-Value < 0.05)

In the study of Lehman et al. in 2013, which was conducted with the aim of investigating the changes in the surface properties of dental structures due to cold atmospheric plasma, cold plasma increased the surface roughness of dentin samples. Cold plasma did not have such an effect on etched dentine and polished enamel samples and etched enamel (25).

In the present study, the use of cold plasma along with etching with phosphoric acid increased the surface roughness compared to etching with phosphoric acid in enamel samples. The results related to the group of ivory samples (unetched) in the study of Lehman et al. can be considered consistent with the present study. On the other hand, at first glance, the results related to etched ivory, polished enamel, and etched enamel groups in Lehmann et al.'s study seem inconsistent with the present study. The reason for this difference can be attributed to the following:

Etched ivory group and etched enamel group: difference in the order of preparation methods in two studies (in the present study, cold plasma was applied first and then phosphoric acid, but in Lehmann's study, phosphoric acid was applied first and then cold plasma)

Polished enamel group: In this group, only cold plasma preparation was done and no phosphoric acid preparation was done.

In the study of Ritz et al., which was conducted in 2010 with the aim of investigating the therapeutic effects of non-thermal plasma on dentin surfaces used for composite restoration, the results showed that the bond strength of composite restoration to peripheral etched dentin after 30 seconds of plasma treatment has increased significantly (64%). Also, compared to the control group, more fractures were of the cohesive type. However, the bond strength to the etched inner dentin showed no improvement after plasma preparation (38).

The increase in bond strength in this study, which was the result of changing the surface properties of dentin due to the use of plasma, can be caused by the increase in surface roughness and is in line with the present study. The lack of increase in bond strength in the inner dentin group can be caused by high amounts of water and low mineral content in deep dentin.

The study by Strazi et al. in 2021 investigated how cold plasma affects the characteristics of dentin surface on bovine dentin samples. Argon gas without plasma production did not cause a significant difference in dentin surface characteristics compared to the control group, but preparation with cold plasma significantly It significantly decreased the contact angle values and increased the total free energy of dentin surface interaction. There was no significant difference in surface roughness, morphology and chemical composition in the use of argon gas, cold plasma, and different oxygen concentrations (39).

The results obtained in the present study show that the average depth of demineralization is 11.37 microns in the group prepared with cold plasma, which is significantly higher than the average depth of demineralization of the group without preparation with cold plasma (7.65 microns) and the increase in roughness It shows the surface in preparation with cold plasma. The reason for this difference can be attributed to the use of bovine ivory samples in the astrazi study and human enamel samples in the present study, as well as the difference in the surface preparation method between the two studies (in the present study, all samples were treated with 37% phosphoric acid in the second step while in the study of astrazi, only preparation was done with argon gas and cold plasma).

In Hirata et al.'s study in 2015, which aimed to determine the effect of atmospheric plasma on the microtensile bond strength of dentin with two self-etch adhesive systems after one year of storage in water, the bond strength increased for a tested adhesive. This increase in bond strength can be due to the change in the surface properties of dentin and the increase in the depth of demineralization and is in line with our study.(40)

In the study by Hirata et al. in 2016, which was conducted with the aim of investigating the effect of atmospheric plasma on the strength of the micro-tensile bond of dentine with two systems of adhesive etching and washing, after one week and one year of storage in water, for the groups that plasma after H acid was used in the one-week evaluation, a significant increase in bond strength was observed, and the percentage of cohesive failures also increased. On the other hand, for the groups where plasma was used before acid etching, there was no difference in bond strength and fracture patterns compared to the control groups (without plasma application) (41).

The lack of change in bond strength and the percentage of fracture patterns for the groups where plasma was used before acid etching in the above study can indicate the lack of effect of plasma on the surface properties of dentine, which is inconsistent with the results of the present study, and the reason for this difference can be attributed to two factors:

1) Using ivory samples in Hirata's study and enamel samples in the present study

2) Evaluation of the results of the study with different methods (in Hirata's study, the strength of the bond was examined, which factors other than surface characteristics can also be involved, while in the present study, the depth of demineralization was examined by scanning electron microscope)

Conclusion

The present study showed that under the conditions of this study, preparation with cold atmospheric plasma reduces the resistance of tooth enamel to acid and increases the depth of demineralization by phosphoric acid and improves the performance of acid etching.

Conflicts of interest

The authors declare that there are no known conflicts of interest.

Acknowledgement

The research team would like to thank and appreciate Aja university faculty of dentistry, Shahid Beheshti Faculty of Dentistry Research Center, and Tehran University Faculty of Metallurgy.

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