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Effect of Argon Plasma on Root Dentin after Use of 6% NaOCl

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The aim of this study was to evaluate the effect of argon plasma on dentin surface after use of 6% NaOCl. Sixty bovine incisors had their crowns removed, the roots split, and the segments planed. One hundred twenty segments of the cervical third were used. The samples were divided in two groups (n=60): Control group: immersed in 6% NaOCl, washed, dried and then immersed in 17% EDTA, washed and dried and Argon group: after treatment described for the Control group, non-thermal argon plasma was applied for 30 s. Ten samples were evaluated by scanning electron microscopy in each group. Other ten samples were analyzed by Fourier transform infrared spectroscopy (FTIR). Thirty samples were analyzed with a goniometer to measure the contact angle between the dentin surfaces and solutions, to determine the surface free energy. The last ten samples were used to evaluate the wettability of AH Plus sealer. Data were statistically analyzed using Kruskal Wallis and Mann-Whitney tests ($p<0.05$). The results of this study showed that argon plasma did not modify the surface topography. FTIR analysis showed chemical modifications after plasma treatment. Argon plasma increased the surface free energy of dentin and AH Plus wettability. In conclusion, argon plasma treatment modified chemically the dentin surface. This treatment increased the surface free energy and wettability of an epoxy resin root canal sealer, favoring its bonding to dentin surfaces.

Key Words: sodium hypochlorite, plasma gases, surface properties, topography, wettability.

Introduction

Effective cleaning and shaping of the root canal, and creating a coronal and apical seal are essential goals for successful endodontic treatment and prognosis (1,2). Among the auxiliary chemical substances used in endodontics during chemical-mechanical preparation, sodium hypochlorite, in different concentrations, has been most commonly used. This endodontic irrigant is able to destroy a broad spectrum of microbes and to dissolve organic tissue; however, it is known that sodium hypochlorite solutions have cytotoxic effects and modify the organic components of dentin, especially collagen. Consequently, sodium hypochlorite may affect the mechanical properties of dentin by degradation of the organic dentin components (3,4). Furthermore, due to the oxidative power of NaOCl, which leaves an oxygen-rich layer on the dentin surface, its use may affect the wettability and penetration of the resin sealer into dentin as well as its polymerization (4,5).

Plasma treatment is an effective and clean technology, since the bulk properties of the materials may remain unaltered or well maintained after the treatment (6). Non-thermal gas plasmas are partially ionized gases that contain highly reactive particles, including electronically excited atoms, molecules, ionic and free radical species, while the gas phase remains near room temperature. Depending on the plasma chemistry or gas composition, these highly reactive plasma species can react with, clean and etch surface materials, bond to various substrates, or combine to form a thin layer of plasma coating, and consequently modify the surface characteristics (7). Previous studies have demonstrated that non-thermal plasma was effective and efficient in root canal disinfection (8,9), bonding to composite restorative materials, cleaning of instruments (10) and for tooth whitening (11,12). Argon plasma was indicated mainly to improve the adhesion process of resin-based materials to dentin (13).

This study was designed to evaluate the effect of argon plasma on the dentin surface after the use of 6% NaOCl. The null hypotheses tested were that argon plasma treatment produces no topographic (i) or chemical (ii) changes on dentin surfaces previously treated with 6% NaOCl; this treatment had no influence in the surface free energy (iii), as well as in the wettability (iv) of the AH Plus epoxy resin root canal sealer.

Material and Methods

Sample preparation

Sixty bovine incisor teeth with straight root canals, mature root apices and similar anatomical characteristics were selected for this study. The crowns were removed at the dentinoenamel junction and the roots were split longitudinally (mesiodistally) with a rotary diamond disk (KG Sorensen, Barueri, SP, Brazil) at low speed. The cervical third was selected and used in the present study.

The samples were flattened with abrasive paper (211Q and P220, 3M Brazil, Sumaré, SP, Brazil). One hundred and twenty smooth and flat surfaces with a standardized smear layer were obtained. After this, the samples were divided into 2 groups (n=60):

Control group: the samples were immersed in 6% NaOCl for 30 min simulating the irrigation procedure used during the chemomechanical preparation, washed with 10 mL of distilled water and dried with absorbent paper. The samples were then immersed in EDTA for 3 min, washed with distilled water and dried with nitrogen gas.

Ar group: after the treatment described for the control group, non-thermal argon plasma was applied.

All samples were immersed in the same irrigant (30 mL) at the same time. They were also removed from the solution concomitantly (18,20,22). Thus, it was possible to standardize the duration of the immersion with utmost precision.

Plasma Treatment

A glass reactor was used for non-thermal plasma treatments. This reactor consists of a 5 cm diameter and 30 cm long tube, evacuated by a mechanical pump down to pressures under 2 Pa. Gas was allowed to fill the reactor up to a pressure of 10 Pa. Non-thermal plasma was generated in the glass cylinder under vacuum by the action of the induced magnetic field from the current passing through an electrical coil surrounding the cylinder. Surfaces were then treated using argon gas (White Martins, Rio de Janeiro, RJ, Brazil) at 60 W for 30 s. At the end of the process, RF (radio frequency) was turned off before the samples were exposed to air.

Topographical Analysis

For this purpose, the samples were prepared and analyzed by a scanning electron microscope (JSM 6460 LV; JEOL, Tokyo, Japan). For each group, ten samples were used and three microphotographs were obtained at 1000x magnification, totaling 30 microphotographs per group. The images were evaluated qualitatively with regards to the presence or absence of topographical changes.

Chemical Analysis

In order to analyze the effect of the argon plasma treatment on dentin, ten dentin surfaces of each group were evaluated using Fourier transform infrared spectroscopy (FTIR). Infrared analysis was performed using a Nicolet 6700 spectrometer (Thermo Scientific, Waltham, MA, USA) in the ATR mode and all spectra were acquired using an absorbance mode in the 650-4000 cm^{-1} range.

Contact Angle Analysis

Surface Free Energy

Ramé-hart goniometer (Ramé-hart Instrument Company, Netcong, NJ, USA) was used to measure the contact angle between the surfaces and the following solutions: water (polar), ethyleneglycol (polar) and diiodomethane (apolar). For each group, thirty samples were evaluated, ten in contact with each solution. In each drop, forty measurements were made, with 1 s interval between each measurement. Based on the data obtained for the three solutions above, the Ramé-hart software was able to measure the surface free energy, in addition to the polar and dispersed components of the different groups.

Endodontic Sealers Wettability

The Ramé-hart goniometer was used to measure the contact angle between the surfaces and the resin-based sealer AH Plus (Dentsply, Petrópolis, RJ, Brazil).

The sealer was manipulated according to the manufacturer's specifications. A drop of sealer (0.1 mL) was deposited on the dentin surfaces with a 0.5 mL BD ultrafine syringe (Becton Dickinson, Franklin Lakes, NJ, USA).

For each group, ten samples were evaluated. The sealer dynamic wettability was followed up and computed for 1 min (sixty measurements with 1 s interval between each measurement). Final contact angle values were evaluated as well as the dynamics of the sealer on dentin surface.

The following formula was used to evaluate the sealer dynamic wettability (SDW):

$$SDW (\%) = \frac{\text{(initial angle - final angle)}}{\text{initial angle}} \times 100$$

Contact angle data were computed with the Origin - Pro 70 software program, and analyzed statistically by the Kolmogorov-Smirnov, Kruskal Wallis and Mann-Whitney tests ($p<0.05$).

Results

Figure 1 shows representative photomicrographs of the different groups. It may be observed that argon plasma treatment produced no changes on the surface when compared with the control group.

Figure 2 shows FTIR spectra of the different groups. Spectra were normalized using the phosphate peak (1025 cm^{-1}). Chemical modifications were observed after plasma treatment in the organic and inorganic components of dentin. Alterations were observed in the 870 cm^{-1} peaks, which correspond to carbonate and phosphate peak (1025 cm^{-1}). Organic alterations were observed in the 1630 cm^{-1} peak corresponding to the stretching vibration of C=O bond (vC=O) from amide I; the 1580 cm^{-1} peak corresponding to dehydrated amide I; and the band between 1500 and 1600 cm^{-1}, which corresponds to the stretching vibration of C-N bond (νC-N) from amide II. The 1230 cm^{-1} peak corresponds to amide III and the 1170 cm^{-1} peak corresponds to dehydrated amide III.

Figure 3 shows representative images of contact angle between dentin surface and solutions (water, ethyleneglycol and diiodomethane) and AH Plus sealer. Table 1 shows the polar and dispersed components, and surface free energy of the different groups. Statistical analyses showed that plasma treatments increased the surface free energy and favored the dispersed component.

Regarding sealer wettability, argon plasma treatment reduced the final contact angle values and improved significantly the dynamic wettability of AH Plus sealer.

Discussion

Previous studies have reported the positive effect of plasma treatment on the bonding properties of different dental substrates (6,7,13,14). Additionally, recent studies

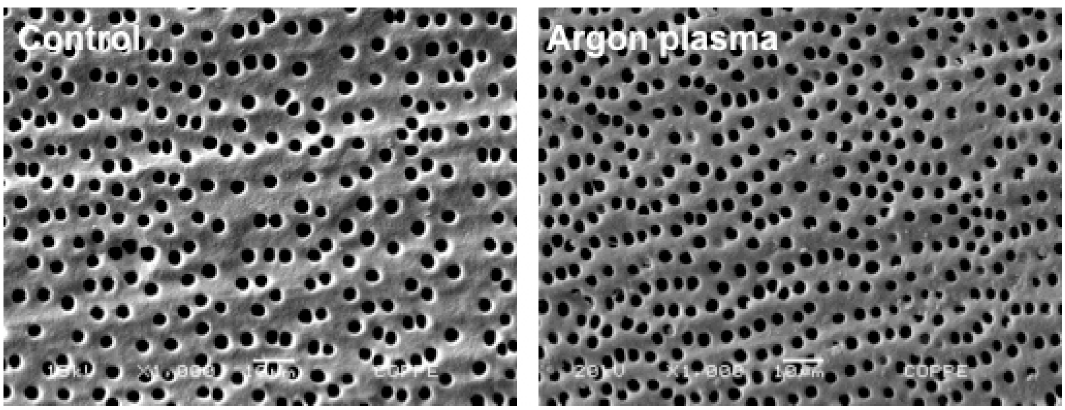

Figure 1. Representative photomicrographs (×1000) of control and argon plasma groups.

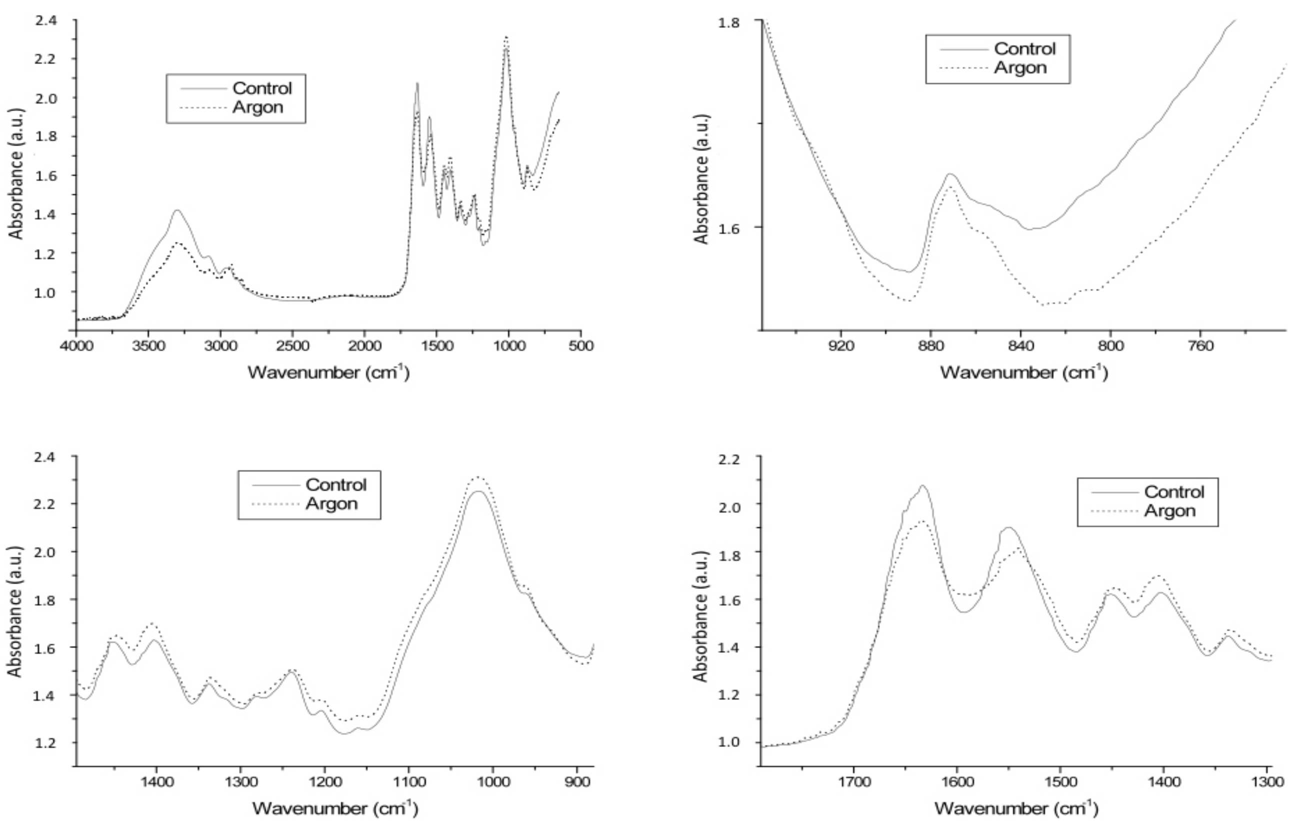

Figure 2. FTIR spectra of the control and argon groups.

have proposed the use of 6% NaOCl for irrigation (15-17). In the present study, the effect of argon plasma treatment on the dentin surface after the use of 6% NaOCl was evaluated topographically and chemically, as well as their influences on the surface free energy and resin-based sealer wettability.

With regards to the topographical analysis, the first tested null hypothesis was accepted. After argon plasma treatment, the dentin surface showed a similar morphology as that of control group (6). Pretreatment with 6% NaOCl had no effect in the surface changes.

Evaluating the FTIR spectra, the tested second null hypothesis was rejected. After plasma treatment, chemical alterations were observed in the organic and inorganic compounds of dentin. As regards organic compounds, plasma treatment reduced the amide I and II bands, sensitive markers for the collagen component of dentin. Relative to the inorganic compound, the carbonate band (870 cm^{-1}) was increased after plasma treatment (18,19). This occurs because dentin surfaces can be etched away by the plasma (7). Argon showed to have a significant chemical effect on the surface modification of the dentin substrate without topographical destructive effects (6).

Adhesion processes are mainly influenced by the relative surface free energy (wetting ability) of the solid surface (20). The surface free energy is a measure of the surface reactivity or adhesiveness to its environment. This phenomenon occurs as a result of interatomic attraction. It is termed contact angle and it has an inverse relationship with surface free energy (wettability), i.e., the lower the contact angle, the greater the surface free energy and, hence, the greater the adhesion (20,21). In the present study, the third and forth null hypotheses were rejected. Plasma treatment increased the surface free energy of dentin favoring mainly the dispersed component. This component is associated with the surface hydrophobicity (22).

Regarding resin-based sealer wettability, FTIR results associated with the surface free energy analysis could explain the favorable results. Due to the higher surface free energy associated with the improvement of dispersed component, it favors the reduction of contact angle values and dynamic wettability of AH Plus, a sealer with hydrophobic characteristics (5,23). In the present study, the plasma treatment reduced the organic compounds of dentin, favoring the wettability of hydrophobic materials, like AH Plus (8). In the present study, argon plasma showed positive effects on bonding to dentin surfaces after use of sodium hypochlorite. Though it is a promising technology, due to its novelty, it has high cost.

In conclusion, the present study showed that argon plasma treatment modified chemically the surface and had no effect in the topography. This treatment increased the surface free energy and wettability of resin-based sealer, favoring the bonding to dentin surfaces. Further studies with regards to the bond strength are necessary to confirm its effect on the adhesion to root canal.

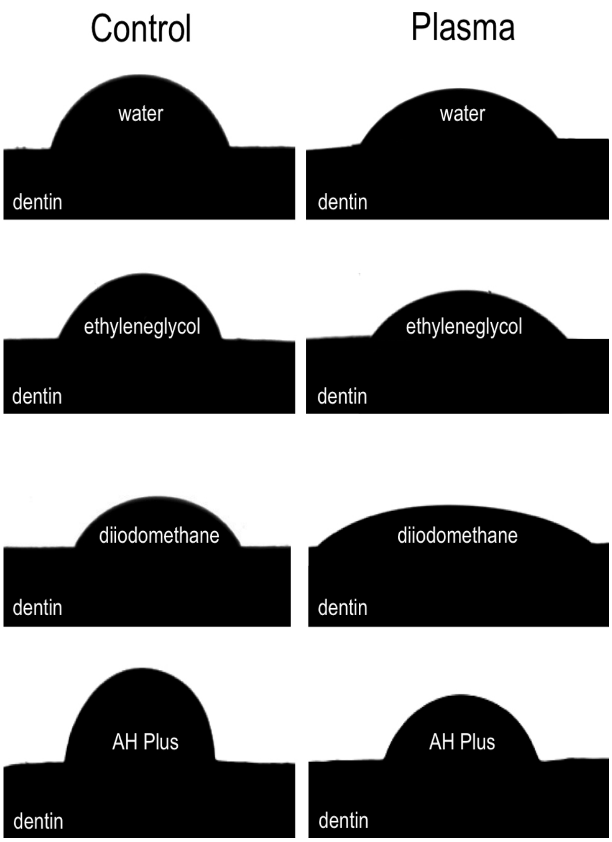

Figure 3. Representative images of contact angle between dentin surface and solutions/sealer.

Table 1. Mean and standard deviation surface free energy, components of the different groups and values of contact angle and sealer dynamic wettability (SDW)

Group	Polar component	Dispersed component	Surface free energy	AH Plus Contact angle	AH Plus SDW
	mJ/m^2	mJ/m^2	mJ/m^2	°	(%)
Control	24.01±3.8a	32.65±2.9b	56.66±3.4b	74.86±14.9b	1.8±1.9b
Argon plasma	25.25±1.5a	36.76±1.5a	62.01±0.9a	61.24±3.4a	4.9±0.9a

Resumo

O objetivo do presente estudo foi avaliar o efeito do plasma de argônio na superfície dentinária após o uso de NaOCl 6%. As coroas de 60 incisivos bovinos foram removidas, as raízes clivadas e os segmentos planificados. Cento e vinte segmentos referentes ao terço cervical foram utilizados. As amostras foram divididas em dois grupos (n=60): Grupo Controle: imersos em NaOCl 6%, lavados, secos, imersos em EDTA 17%, lavados e secos e Grupo Argônio: após o tratamento descrito no grupo controle, foi aplicado plasma de argônio não térmico por 30 s. Em cada grupo, 10 amostras foram avaliadas por microscopia eletrônica de varredura. Outras dez amostras foram analisadas por espectroscopia no infravermelho por transformada de Fourier (FTIR). Trinta amostras foram analisadas com um goniômetro para medir o ângulo de contato entre a superfície dentinária e as soluções e determinar a energia livre de superfície. As últimas dez amostras foram utilizadas para avaliar a molhabilidade do cimento AH Plus. Os dados foram analisados estatisticamente usando os testes de Kruskal Wallis e Mann-Whitney (p<0,05). Os resultados do estudo mostraram que o plasma de argônio não modificou a topografia de superfície. A análise por FTIR mostrou modificações químicas após o tratamento de plasma. O plasma de argônio aumentou a energia livre da superfície dentinária e a molhabilidade do cimento AH Plus. Conclusão, o tratamento com plasma de argônio modificou quimicamente a superfície dentinária. Este tratamento aumentou a energia livre de superfície e a molhabilidade de um cimento endodôntico à base de resina epóxi, favorecendo as características adesivas da superfície dentinária.

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