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ROBERTO HANIERY PONTE ALVES

EFEITOS DO PLASMA DE BAIXA TEMPERATURA SOBRE OS SUBSTRATOS DE ESMALTE E DENTINA

FORTALEZA
2015

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Dissertação apresentada ao Programa de Pós-Graduação em Odontologia da Universidade Federal do Ceará, como requisito do exame de defesa de Mestrado em Odontologia.

Área de concentração: Clínica Odontológica

Orientadora: Profª Drª. Iriana Carla Junqueira Zanin dos Santos

Coorientadora: Profª Drª. Lidiany Karla Azevedo Rodrigues

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“Ninguém pode voltar atrás e fazer um novo começo, mas qualquer um pode recomeçar e fazer um novo fim”.

Chico Xavier

RESUMO

O plasma de baixa temperatura (PBT) tem tido seu uso investigado na destruição dos biofilmes orais, no entanto,seu efeito sobre os substratos dentários ainda não está completamente esclarecido. O objetivo deste trabalho foi avaliar o efeito do PBT na dureza superficial, no ângulo de contato, na morfologia das superfícies e composição química do esmalte e dentina bovinos. Para tanto, foram analisados a dureza de superfície Knoop (DS), as alterações nos ângulos de contato (AC), a morfologia de superfície por Microscopia Eletrônica de Varredura (MEV) e sua composição química através da Espectofotometria Raman (ER). Inicialmente, foram confeccionados blocos de esmalte e dentina com dimensões de 4x4x2 mm preparados a partir de dentes bovinos. Todos os blocos foram polidos e divididos em três grupos (n=6): Plasma de Argônio, Gás de Argônio e Controle. A MS foi avaliada antes e depois de cada tratamento, realizando-se cinco penetrações em cada espécime (50g/5s para esmalte e 25g/5s para dentina). A determinação do AC foi realizada dispersando uma gota de água sobre a superfície tratada e observando o ângulo de contato obtido em 1 min e 1 hora, utilizando fotografias digitais de alta resolução e o programa Image J. As alterações físicas na morfologia do esmalte e dentina submetidos ao PBT foram observadas a partir da MEV. Adicionalmente, a proporção relativa do conteúdo orgânico e mineral do esmalte e dentina após os tratamentos foram analisados por ER. Os dados foram avaliados por análise de variância (ANOVA) – 1 via para MS e 2 vias (tratamento e tempo) para AC, após Tukey teste ($\alpha < 0,05$). Os resultados da MS demostraram não haver diferença significativa no esmalte ($p = 0,087$) após os tratamentos. No entanto, a MS da dentina diminuiu após o tratamento de plasma de argônio ($p = 0,005$). Os resultados indicaram que os valores de ângulo de contato da água diminuíram após 1 minuto da aplicação de plasma de argônio no esmalte ($p < 0,05$) e após 1 minuto e 60 minutos da aplicação de plasma de argônio na dentina ($p < 0,05$). Os resultados da ER demonstraram que os tratamentos com o plasma de argônio e gás de argônio apresentaram menores intensidades nas bandas da matriz orgânica para o esmalte e para a dentina. Em adição, a MEV demonstrou que, além de remover a smear layer externa aos túbulos dentinários, o tratamento com PBT promoveu rachaduras na superfície do esmalte e da dentina. Em conclusão, o PBT diminuiu a dureza superficial da dentina e melhorou potencialmente o molhamento da superfície do esmalte e dentina. Alterações morfológicas superficiais e diminuição da matrix orgânica foram observadas para ambas as estruturas dentais, depois dos tratamentos com plasma de argônio e gás de argônio.

Palavras-chave: Gases de plasma; Teste de dureza; Espectroscopia Raman; Microscopia eletrônica de varredura.

ABSTRACT

Low-temperature plasma (LTP) is a promising technology with the potential benefit of destroying the matrix of the oral biofilm and also produce antimicrobial effect on the bacterial cell. However, the effect of LTP on dental structures is not clear. The objective of this study was to evaluate the effect of LTP on enamel and dentin substrates. Therefore, we analyzed Knoop microhardness to calculate the percentage of surface hardness (SH), changes in contact angles (CA), the surface topography by Scanning Electron Microscopy (SEM) and also the chemical composition by Raman spectrometry (RS). Initially, 4x4x2 mm enamel and dentin blocks were prepared from bovine incisors. All blocks were polished and divided into three groups (n = 6); argon plasma, argon flow rate and control. The SH was assessed before and after each treatment, performing five penetrations in each specimen (50g / 25g and 5s enamel / dentin 5s). Contact angles changes were performed by dispersing water drop on the treated surfaces and observing the contact angle obtained after 1 min and 1 hour. For that, high-resolution digital photos and the Image J program was used. Physical changes in the structure of enamel and dentin submitted to LTP were observed from the SEM. Additionally, the relative proportion of the organic and mineral content of the enamel and dentin after treatment were analyzed by RS. Data were evaluated by analysis of variance (ANOVA) - 2-way (treatment and time) and Tukey test ($\alpha < 0.05$). For the SH the results showed no significant difference in the enamel ($p=0.087$), however, the SH of dentin decreased after treatment with argon plasma ($p=0.005$). The results indicated that water contact angle values in enamel decreased after 1min of the application of argon plasma ($p<0.05$). In the dentin the contact angle decreased after 1 and 60 min of the application of argon plasma ($p<0.05$). The RS results showed than argon plasma and argon gas presented lower organic matrix bands, similar results were observated for both enamel and dentin. The SEM showed that in addition to removing the external smear layer to the dentinal tubules, treatment with LTP promoted cracks in the enamel and the dentin surfaces. In conclusion, the use of argon plasma decreased the superficial hardness in dentin and, potentially increased the wetting in booth substract. Superficial morphological change and decreased of the organic matrix were observed in booth estructures after treatment with argon plasma and argon flow rate.

Keywords: Plasma gases; Hardness test; Raman spectroscopy; Scanning electron microscopy.

SUMÁRIO

1 INTRODUÇÃO GERAL

O plasma é considerado o quarto estado da matéria, apresentando uma grande quantidade de espécies altamente reativas, como íons, elétrons, radicais livres e nêutrons excitados eletronicamente. Existem plasmas que podem chegar a uma temperatura média de 10.000 ^{0}C (plasmas térmicos), enquanto os plasmas de baixa temperatura chegam a uma temperatura média de 40 ^{0}C (YASUDA *et al*., 2005; TENDERO *et al*., 2006). No caso dos equipamentos de baixa temperatura, os plasmas podem ser gerados a partir de diversos gases, como o hélio (SHASHURIN *et al.,* 2008; JIANG *et al*., 2009), o argônio (ZHANG *et al.,* 2012; ERMOLAEVA *et al.,* 2012), o nitrogênio (ARDHAOUI *et al*., 2013), misturas oxigenadas (HELLER *et al*., 2012) e ar ambiente (KOLB *et al.,* 2008).

O plasma de baixa temperatura (PBT) é uma tecnologia inovadora que está sendo extensivamente estudada devido à sua aplicabilidade clínica, biológica em biomaterias. Alguns tratamentos com o PBT têm amplo sucesso nas engenharias de superfície no processamento de materiais no estado sólido, especialmente na limpeza e condicionamento, proporcionando melhorias no desenvolvimento de biomaterias (SHOHET, 1991; SHI *et al.,* 2002), na cicatrização de feridas (ERMOLAEVA *et al.,* 2012), na dermatologia (HEINLIN *et al*., 2011), na esterilização de materias cirúrgicos (SU-JIN *et al.,* 2013; AHMAD NOUR *et al.,* 2013) na descontaminação bacteriana (HELLER *et al*., 2012), na adesão de materias adesivos e restauradores (CHEN *et al*., 2013; TEIXEIRA *et al.,* 2015), apresentando bons resultados, mesmo com poucos tempo de aplicação.

A rede química resultante envolve mais de 200 reações, com produtos incluindo O_3, NO, NO_2, etc. As principais moléculas reativas produzidas pelo PBT são geradas por diversas reações transitórias, vias de colisões e dissociação de elétrons do plasma com o oxigênio atmosférico. Supõe-se que os radicais de oxigênio e à base de nitrogênio são os contribuintes mais significativos para os efeitos de esterilização do plasma. Essas espécies reativas de oxigênio têm fortes efeitos sobre as estruturas externas das células, seja sobre o revestimento de endosporos ou sobre a membrana celular, resultando na oxidação e degradação das proteínas (LEVINE, 2011). A presença de O_2 no plasma de ar conduz uma geração de O_3, que interfere com a respiração celular proporcionando forte efeito bactericida (MORRIS, 2009).

O tratamento com o PBT, utilizando o gás argônio, pode ser utilizado na Odontologia na destruição da matriz do biofilme, expondo as células bacterianas, em conjunto com o efeito antimicrobiano (YANG *et al*., 2011; DUARTE *et al*., 2011). O PBT poderia ser usado em

várias situações de cuidados orais, tanto na remoção mecânica do biofilme quanto na prevenção da cárie dentária e em adição aos tratamentos dentários oferecidos aos pacientes na prática odontológica geral (JIANG *et al.,* 2009; PAN *et al*., 2010; SUNG *et al*., 2013).

Entre as vantagens do PBT, observa-se que este pode ser usado para um tratamento localizado antibacteriano ou antisséptico em superfícies vivas e inanimadas (GURSOY *et al*., 2013), fornecendo uma resposta bactericida instantânea, com segundos ou poucos minutos de aplicação (JIANG *et al*., 2009), diminuindo a probabilidade do desenvolvimento de resistência bacteriana e servindo como uma alternativa para a prevenção das infecções. Além disso, a terapia PBT tem demonstrado efeitos colaterais mínimos compatíveis com células de mamíferos e sem muita oscilação térmica, incentivando a sua utilização *in vivo* (FLUHR *et al.*, 2012; PARTECKE *et al.,* 2012; LAROSSI *et al*., 2015).

Além do efeito antimicrobiano, o PBT pode alterar as características das superfícies poliméricas, fibras de colágeno e superfícies dentinárias, uma vez que aumenta a energia de superfície do substrato, aumentando a hidrofilia das superfícies tratadas (D'AGOSTINO *et al.*, 2005; CHEN *et al*., 2013; DONG *et al*., 2013; YASUDA, 2005; TEIXEIRA *et al*., 2015). O PBT parece alterar a estrutura química das fibras de colágeno aumentando a hidrofilicidade da superfície da dentina, permitindo uma melhor penetração do adesivo e aumentando em cerca de 60% a força de união na interface adesivo-dentina (RITTS *et al.,* 2010). Assim, o Plasma de Baixa Temperatura (PTB) tem sido estudado com diversos propósitos na Odontologia. No entanto, o efeito desse tratamento na estrutura do esmalte e dentina ainda não está esclarecido. Dessa forma, o objetivo deste trabalho foi avaliar o efeito do PBT na dureza superficial, no ângulo de contato, na morfologia das superfícies e composição química do esmalte e dentina bovinos.

2.1 OBJETIVOS GERAIS

Avaliar as propriedades dos substratos de esmalte e dentina bovina após exposição ao tratamento com plasma de argônio de baixa temperatura. Inicialmente, as alterações na microdureza e na morfologia de superfície bem como na molhabilidade dos substratos serão avaliadas. Adicionalmente a composição química do esmalte e da dentina tratada com plasma será analisada.

3 CAPITULO

Esta Dissertação de Mestrado está baseada no artigo 46 do Regimento Interno do Programa de Pós-graduação em Odontologia da Universidade Federal do Ceará, que regulamenta o formato alternativo para Dissertações de Mestrado e Teses de Doutorado, e permite a inserção de artigos científicos de autoria ou coautoria do candidato. Dessa forma, esta tese é composta por um capítulo contendo artigo a ser submetido para publicação em revista científica, conforme descrito a seguir:

Artigo

"Effecs of low temperature plasma on bovine enamel and dentin substrates"

Roberto Haniery Ponte Alves, Sônia Luque Peralta, Lidiany Karla Azevedo Rodrigues, Simone Duarte, Iriana Carla Junqueira Zanin.

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Title: “Effecs of low temperature plasma on bovine enamel and dentin substrates”

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ABSTRACT

The aim of this study was to evaluate the effect of low-temperature plasma (LTP) on the dental enamel and dentine. Surface hardness Knoop (SH), contact angle (CA), Scannig Electron Microscopy (SEM) and Raman spectrophotometry (RS) were used. Thus, enamel and dentin slabs were prepared from bovine teeth divided in three groups (n=6); argon plasma, flow control and negative control. The SH was assessed before and after in each treatment. The wettability was evaluated by measuring the contact angle of a drop of water on the substrates 1 and 60 minutes after treatment. The relative proportions of mineral and organic content present in the enamel and dentin were analyzed by RS. Morphologic changes were analyzed using SEM. Data were evaluated by ANOVA- one way for SH and two ways (treatment and time) for CA followed by a Tukey test ($p<0.05$). Surface microhardness results showed no significant difference in the enamel ($p=0.087$). However, SH of dentin decreased after treatment with argon plasma ($p=0.05$). Contact angle values decreased in enamel and dentin after 1min ($p<0.05$) and remain lower in dentin after 60 min of argon plasma treatment ($p<0.05$). Raman spectrophotometry demonstrated that argon plasma and flow control presented lower organic matrix bands both to enamel and dentin. Scanning electron microscopy showed that LTP treatment only removes the external smear layer but also produced cracks in the enamel and the dentin surfaces. In conclusion, the use of argon plasma decreased the superficial hardness in dentin, and potentially increased the wetting in booth substracts. Morphological changes and decreased in organic matrix were observed after treatment with argon plasma and argon gas.

Keywords: Plasma gases; Hardness test; Raman spectroscopy; Scanning electron microscopy.

3.1 INTRODUCTION

Plasma, considered “the 4th state” of matter, is a partially ionized gas with ions, electrons, and uncharged particles such as atoms, molecules, and radicals. There are two types of plasma: thermal and non-thermal also called Low Temperature Plasma (LTP). Thermal plasma has electrons and heavy particles (neutrals and ions) at higth temperature. LTP is a non-thermal, since the gas phase may be controlled at an ambient temperature[1].

Different studies showed that Low Temperature Plasma (LTP) represent a technology that can improve interactions between materials and biological systems[2,3]. During the last decades, the interest in using alternative therapies for prevention and treatment of dental caries has increased. Many treatments have been employed to alter the substrate to improve the surfaces properties such as adhesive ability, wettability, surface cleaning and permeability[4,5,6,7].

In addition to the surface modification, several studies demonstrate other biological effect of LTP. Data in the literature studied the effectiveness of LTP against oral bacteria, including *S. mutans* [8]. Many studied indicated that LTP could be a promising technique in various dental clinical applications such as bacterial disinfection and early caries prevention[9,10,11]. Other studies in dentistry have investigated eradicate oral pathogens[12] and disinfect root canals[13]. Previous studies indicate that LTPs were highly effective in oral bacterial disinfection[14], interfacial bonding improvement of composite restorations[14,3]. Thus, LTPs are a promising therapy to prevent and control oral diseases. In addition, LTP can be used for localized treatment providing an instantaneous bactericidal response[12,15] which makes the likelihood of the development of bacterial resistance low and with minimal side effects[16]. Moreover, the temperature of the LTPs is compatible with mammalian tissue, which encourages their use *in vivo*[17,18].

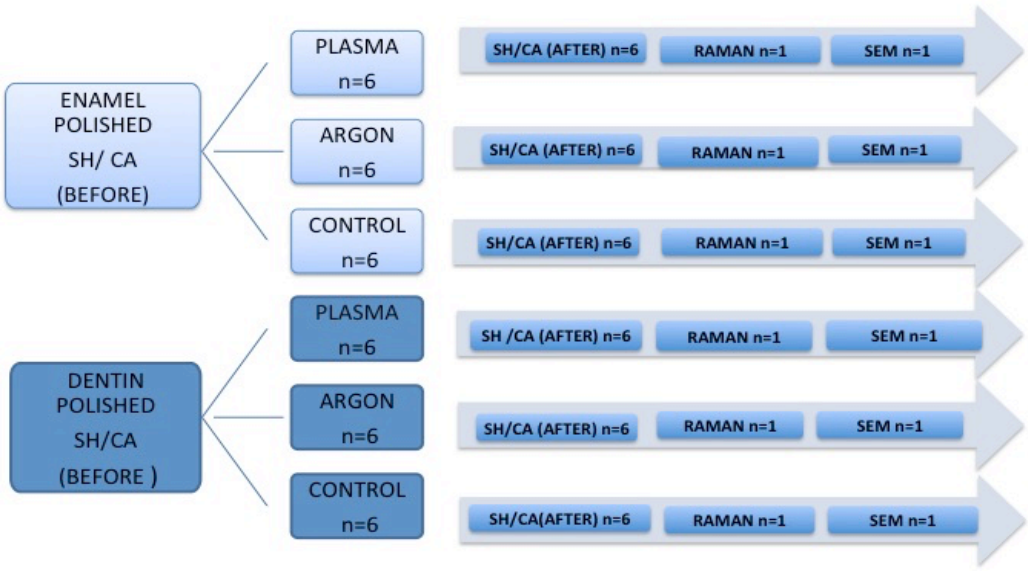
The effects of plasma treatment on enamel and dentin structure have not been completely understood yet. Both substances have different complex and inhomogeneous structures, which contain organic and inorganic components and need more investigations with respect to their responses on plasma treatment. Enamel which is derived from ectoderm, is a very brittle tissue composed of mostly hydroxyapatite mineral (~96%), water (~3%), and trace of organic matrix (~1%), the dentin is derived from the mesoderm, is a flexible, mineralized tissue composed of 70% (weight%) inorganic material, 20% organic material, and 10% fluid[19]. The differences in these properties between enamel and dentin as well as

within the dentin pose challenges for dental treatment. Therefore, the purpose of this study was to evaluate the effect of low-temperature plasma (LTP) on the dental enamel and dentine through the techniques of surface hardness (SH), contact angle (CA), Electron Microscopy (SEM) and Raman spectrophotometry (RS).

3.2 MATERIALS AND METHODS

Experimental Design

For the in vitro experiment, enamel and dentin polished slabs were randon selected and divided in three groups (n=6); argon plasma, flow control, and negative control (0.04 cm^2). The SH was assessed before and after each treatment. The wettability was twice evaluated during 1minute with a 60minute interval by measuring the contact angle of a water drop on the substratum. In order to assess the chemical and physical changes promoted by the treatments, the specimens of each group were analyzed by Raman spectroscopy followed by SEM.



Low Temperature Plasma (LTP): The LTP used in this study (KinPen 09, INP Greifswald, Germany) (Foest et al., 2005) consists of a hand-held unit (170 mm length, 20 mm diameter, weighing 170 g) connected to a high-frequency power supply (frequency 1.1MHz, 2–6 kV peak-to-peak, 10 W system power) for the generation of a plasma jet at atmospheric pressure. The handheld unit has a pin-type electrode (1 mm diameter) surrounded by a 1.6 mm quartz capillary. An operating gas consisting of Argon at a flow rate of 5 standard liters per minute was used. The plasma plume emerging at the exit nozzle is about 1.5 mm in diameter and extends into the surrounding air for a distance of up to 10 mm[20]. Time of argon plasma or flow control treatment was 1 minute.

*Sample preparation***:** For the present study, 18 bovine incisors stored in a 0.01% (v/v) thymol solution at 4ºC for thirty days were used[21,22]. Slabs with 4x4x2 mm were cut from the center portion of crowns using water-cooled diamond disks (Extec Corp., Enfield, CT, USA) and a cutting machine (IsoMet Low Speed Saw, Buehler, Lake Bluff, IL, USA). The necessary adjustments in the slabs were perfomed using a low-speed water-cooled polishing machine with 320 grit paper (Carbimet Paper Discs). Eighteen slabs of both enamel and dentin were made. Afterwards, the specimens were polished using three different silicon carbide waterproof papers (320, 600 and 1200 grit) as well as polishing cloths with 1 μm diamond paste (Buehler, Lake Bluff, IL, USA). The samples were cleaned in an ultrasonic bath for 15 min. In order to avoid dehydration, the specimens were stored in 100% humidity until being used.

Microhardness evaluation: Microhardness measurements were performed in a hardness tester (Shimadzu HMV-2000 Shimadzu Corporation, Kyoto, Japan) using a Knoop diamond under a 50 g load for 5 s to select enamel slabs with similar hardness (314 to 387) and a Knoop diamond under a 25 g load for 5 s to select dentin slabs with similar hardness (53 to 65). After initial selection of slabs, another microhardness measurement was performed after argon plasma or flow control treatments in the center area of the slab. For that, immediately after treatments 5 penetrations (100 μm distant between them) were performed. The percentage of surface hardness loss (%SHL) was obtained by subtraction of initial surface hardness (SHi) and final surface hardness (SHf) values as follows: [%SHL = (SHi – SHf) × 100 / SHi].[22]

Surface contact angle measurements: The wettability of the surfaces to water was determined with static contact angle measurements. The evaluation of the contact angle was performed using 3 µl of distilled and deionized water droplet on each surface of enamel and dentin. For all slabs, contact angles were measured before any treatment (t=0), 1 minute after (t=1 min) and 60 minutes after (t=60 min) for negative control, flow control and argon plasma groups. In each point, images were captured using a digital camera (Nikon D3000, Nikon Inc, Chiyoda-ku, Tokyo, Japan) fixed with objective lens (Medical Nikkor 120mm). The contact angle (θ) measurements were obtained using the computer software Image J 1.46r (National Institutes of Health, Bethesda, MD, USA) and the statistical analysis was performed using the average of angles obtained from the six pictures in eacth group[24].

Raman Spectroscopy: Raman spectroscopy is a vibrational spectroscopy technique and qualitative analysis used to assess light scattered from biological molecules and ions, so that the wavelength difference between scattered and incident light corresponds to molecular vibration, leading to frequency shift bands (labeled in cm^{-1}) in the Raman spectrum[25]. Raman spectroscopy has been widely used to investigate the molecular structure of biological tissues and has many benefits: no special treatment is required, analysis can be performed at room temperature, the technique has good reproducibility, and the presence of a molecule to which a spectrum is assigned can be appraised by calculating the spectrum peak[26]. The relative amounts of organic and mineral material in enamel and dentin were obtained for argon plasma, flow control and negative control treatments. Spectra of the specimens were obtained using an FT-Raman Spectrometer (RFS 100/S – Bruker Inc., Karlsruhe, Germany) withone Ge diode detector cooled by liquid N_2. In order to excite the spectra, the focused λ = 1064.1 nm line of an air cooled Nd:YAG laser source was used. The maximum laser power incidence on the sample surface was about 500 mW and the spectrum resolution was 2 cm^{-1}. The specimens were positioned in the sample-holder located in the sample compartment and the IR354 lens collected radiation scattered at 180°. The FT-Raman spectra were obtained using 150 scans in three different points of the exposed slab area. The first point was located in the central region of the dental slab, and the others were located to the right or to the left of this first point. The distance between the points was 1 mm. The explored frequency ranged from 20 to 4000 cm^{-1} and allowed a characterization of both mineral content (hydroxyapatite) and organic (essentially collagen) constituents. In order to normalize measurements and allow their comparison we used the band surface parameter, which corresponds to the area under the

curve for the "n" analyzed band[27]. The bands at 962 (v1), 1070 (v2) and 2943 (v3) cm^{-1} were analyzed, in the selected slabs, after treatments. These bands correspond to phosphate, carbonate and organic matrix[28], respectively. The curve was identified for each band using an OriginPro 8.6 32 bit software system (Operating System:7, Copyright©2012 by OriginLab Corporation, Northampton:MA. USA).

Scanning electron microscopy (SEM): The slabs were fixed on stubs with carbon tape or adhesive containing powdered graphite (Ceil, São Paulo, SP, Brazil) and sputter-coated with gold in an ion coater (Denton Desk II, Denton Vacuum LLC, Moorestown, NJ, USA). Particle size and morphology were examined under vacuum with a SEM (Inspect™ S50, Jeol, Tokyo, Japan). The images were obtained using an specific software (EDS Software for SEM**,** Oxford instruments) and 3 different magnifications were used (500X, 1000X and 5000X)[29]. The images were analysed visually.

Statistical analysis: Surface microhardness values (Knoop hardness number) and contact angle were evaluated for equal variances and normal distributions of erros by Shapiro-Wilk test. Therefore, the data were analysed by a one-way ANOVA for microhardness and two-way ANOVA test (time and treatment) for contact angle, followed by a Tukey test. The significance level was set at 5%. The software SigmaStat for Windows, (version 3.5 2007, Chicago, Illions, USA) was used.

3.3 RESULTS

Figure 1 shows the difference between the initial and final microhardness in control, argon flow rate and argon plasma group shows in percentage. The mean values of initial surface (SH) and post surface (SH1) hardness were similar in enamel (p=0,087) (Fig 1A) among the three groups. The microhardness measurements decreased in dentin compared argon plasma and argon flow rate treatments demonstrated statistical significant differences between argon plasma and control (p= 0.005) (Fig 1B).

The mean values and the standard deviations of enamel contact angle are expressed in Figure 2A. Two-way analysis of variance (treatment type and time) showed a significant effect for the factor time (p=0.025), no significant effect for the treatment type (p=0.092) and no interaction between them (p=0.059). Argon gas treatment showed similar results at the

different times tested ($p>0.005$). Argon plasma treatment showed statistically lower mean than control after 1 min and 60 min ($p<0.05$). When the type of treatment was compared, statistical differences were fond after 60 min ($p<0.05$).

The water contact angle results for dentin are shown in Figure 2B. Two-way analysis of variance (treatment type and time) showed no statistical difference for the factor time ($p=0.087$). Statistical significant difference was observed for the treatment type ($p<0.001$) and for the interaction between them ($p=0.042$). Argon flow rate results demonstrated similar contact angle at the different tested times. When the two treatments were compared at the different times, statistically significant differences are found after 1 min and 60 min ($p<0.05$). For the argon plasma treatment, the contact angle results for control group was statistically higher than after plasma treatment 1min and 60 min ($p<0.05$).

Selected Raman bands related to phosphate, carbonate and organic matrix and their positions are shown in Figures 3-4. The bands observated are; at v_1=962, v_2=1070 and v_3=2943 cm^{-1}. Both treatments with argon flow rate and argon plasma showed a decrease in organic matrix bands for the enamel and dentin substrate.

The surface morphology of enamel and dentin submitted to different treatments are presented in Figure 5. Representative SEM images of control, argon flow rate and argon plasma treatments visually demonstrate that both treatments remove smear layer and also promote cracks on enamel and dentin surfaces, with more evident alterations in dentin surfaces.

3.4 DISCUSSION

The gas utilized in this experiment was the argônio, a commonly used operating gas in NTP (Norma Temperature and Pressure) technology because of its relatively low cost and high sputtering yield[30]. The alterations in microhardness can be related to the loss or gain of minerals (demineralization and remineralization) of the dental structure. The results obtained in the present study demonstrated that the enamel microhardness was not affected by argon flow rate and argon plasma treatment. On the other hand, the results demonstrated that argon plasma decrease significantilly the microhardness of dentin. These results might be an indication, that LTP is able to etch organic substrate[31] and to remove organic sheaths of the surface, being a promosing technique in various dental clinical applications. Further studies have to be conducted to determine whether the modifications on dentin microhardness are

clinically relevante once recent researches demonstrated that the bleaching process, regardless of whether or not it was light activated or different concentration, led to relevant changes in the chemical composition of enamel and dentin in different concentration[32,33].

Contact angle evaluation has been widely used to measure surface wettability of different materials. Specifically, water contact angle is considered to be useful indication of the surface tension and to reflect the wetting of the substrate by water[4,34]. The results indicated that contact angle values decreased considerably after 1min argon plasma treatment for both enamel and dentin. For enamel, argon plasma treatment showed significant lower contact angle after 1min. For dentin, this effect lasted for the 60 minutes of the experiment. Sumarizing, the argon plasma treatment was able to significantty increase of surface wetting with water which effect on dentin more pronounced than on enamel. The reduction of the amount of carbon compounds on the surfaces which are transformed by chemical reactions is assumed to be the main reason for better wetting. A strong fluid penetration into the surface was also seen for dentin. These results are similar to those found in other studies on the same substrate[14,35,36] the X-ray Photoelectron Spectroscopy (XPS) analysis showed that after 30 seconds of plasma treatment, the atomic percent of carbon (C) decreased in the substrates, suggesting that the C-containing materials were removed by the treatment[14]. It is also known that the plasmas are able to etch the surface and remove organic matter via breaking of C-C and C-H bonds. Polymeric matrix on the surface can be degraded into loosely-bonded oligomers, low-molecular-weight oxidized material, or even volatile species by plasmas[37].

Based on the concept that the intensity of the phosphate, carbonate and organic matrix bands are related to the amount of each component in the analyzed substrate, it seems reasonable to assume that the bands intensity reduction (organic matrix) could be that LTP is able to etch the surface and remove organic matter via breaking of C-C and C-H bonds, because this bonds are the most chemically energizable in the molecule[38].

The SEM results of enamel indicated a decreased of the smear layer after application of the argon plasma and argon gas, and morphological change type cracks were visible after plasma application. These results are different to those found by[34] using a different type of plasma. This could be explained because those authors used a helium gas flow of 3.525 standard liters per minute and a system power of 2W while in our study a argon plasma with a higher flow rate (5 standard liters per minute) and power source (10W) was used. For the dentin, similar SEM results were observed, with a decrease in smear layer and morphological change as slots, which could be explained because some intertubular areas are visible on

dentin. Dentinal tubules are filled with smear plugs and the intertubular areas appear slightly roughened. No collagen fibers are visible on this surface after argon plasma treatment and the orifices of dentine tubules appear irregularly, some of these results are similar to those founded by[34].

3.5 CONCLUSION

The use of argon plasma indicated decreased the superficial hardness of dentin and increased the surface energy, wettability in both tested subtracts. It was demonstrated that water contact angle values decreased after 1 min plasma treatment in dentin. Morphological changes and decrease in the organic matrix were observed in enamel and dentin after argon plasma and argon gas treatments. Summarising, the argon plasma is able to modify tooth surfaces under tested conditions and showed who may offer possibility to integrate the treatment as an additional step to optimize the adhesion of dental materials to enamel and dentin.

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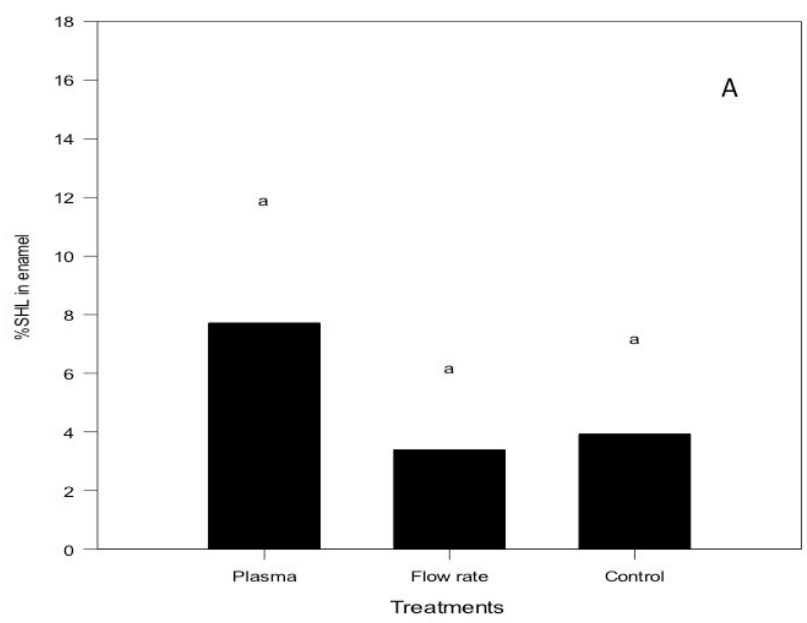


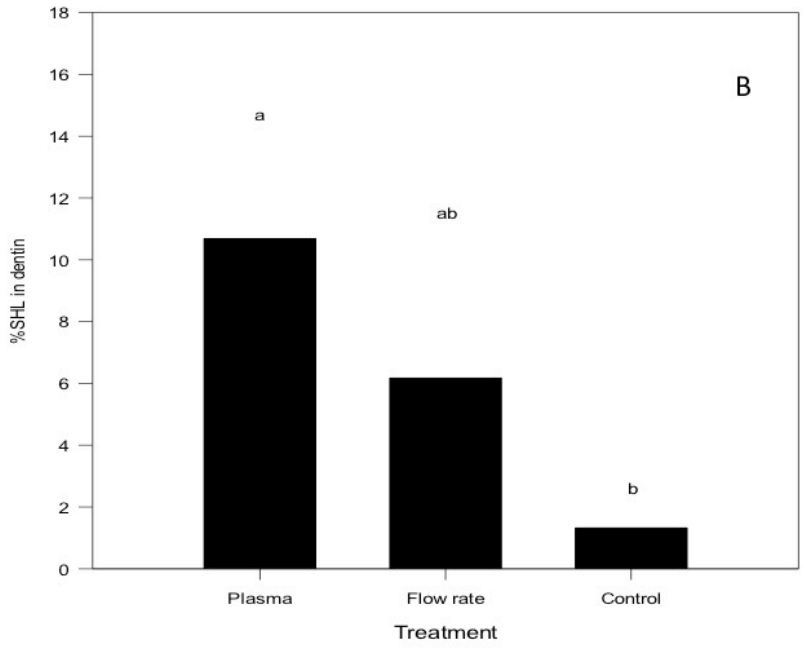
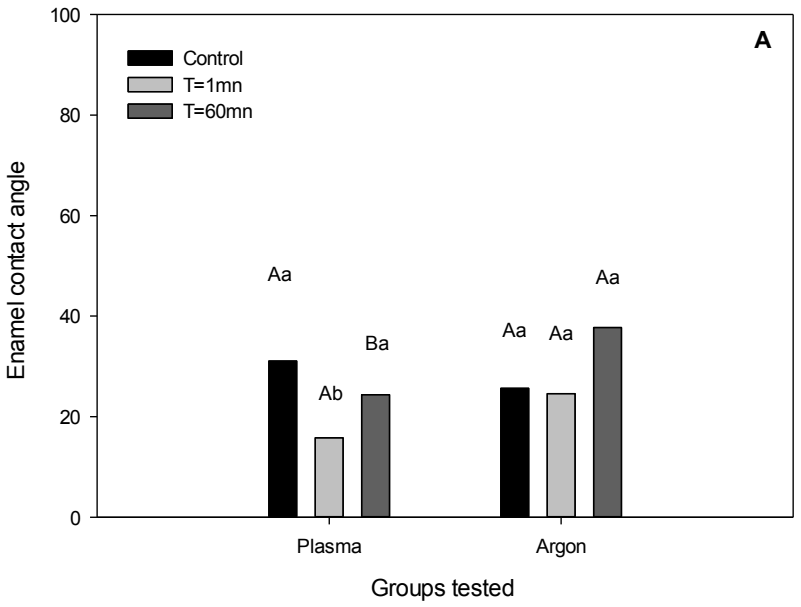


Figure 1. Microhardness (A) Enamel polished and (B) Dentin polished submitted to different treatments. Data represent mean values (n = 6) of the percentage of enamel surface hardness loss (%SHL) according to the treatments and the bars show standard deviations. Data followed by different letters differ statistically ($p < 0.05$). Low case letters show the statistical difference.



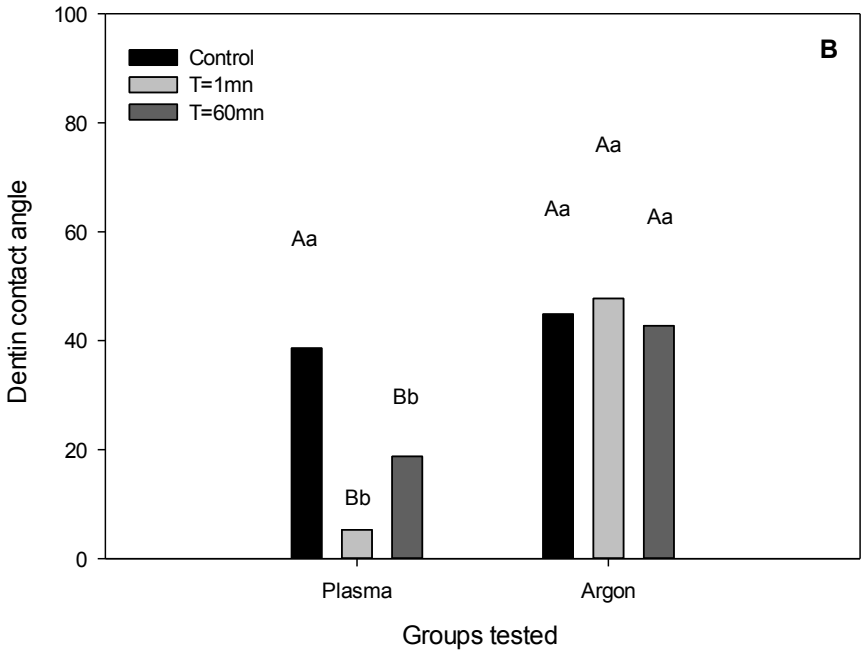


Figure 2. Contact angle (A) Enamel polished and (B) Dentin polished submitted to different treatments during 1min and 60min. Data represent mean values (n = 6) and standard deviations. Data followed by different letters differ statistically ($p < 0.05$). Capital letters compare treatments; Low case letters compare different times.

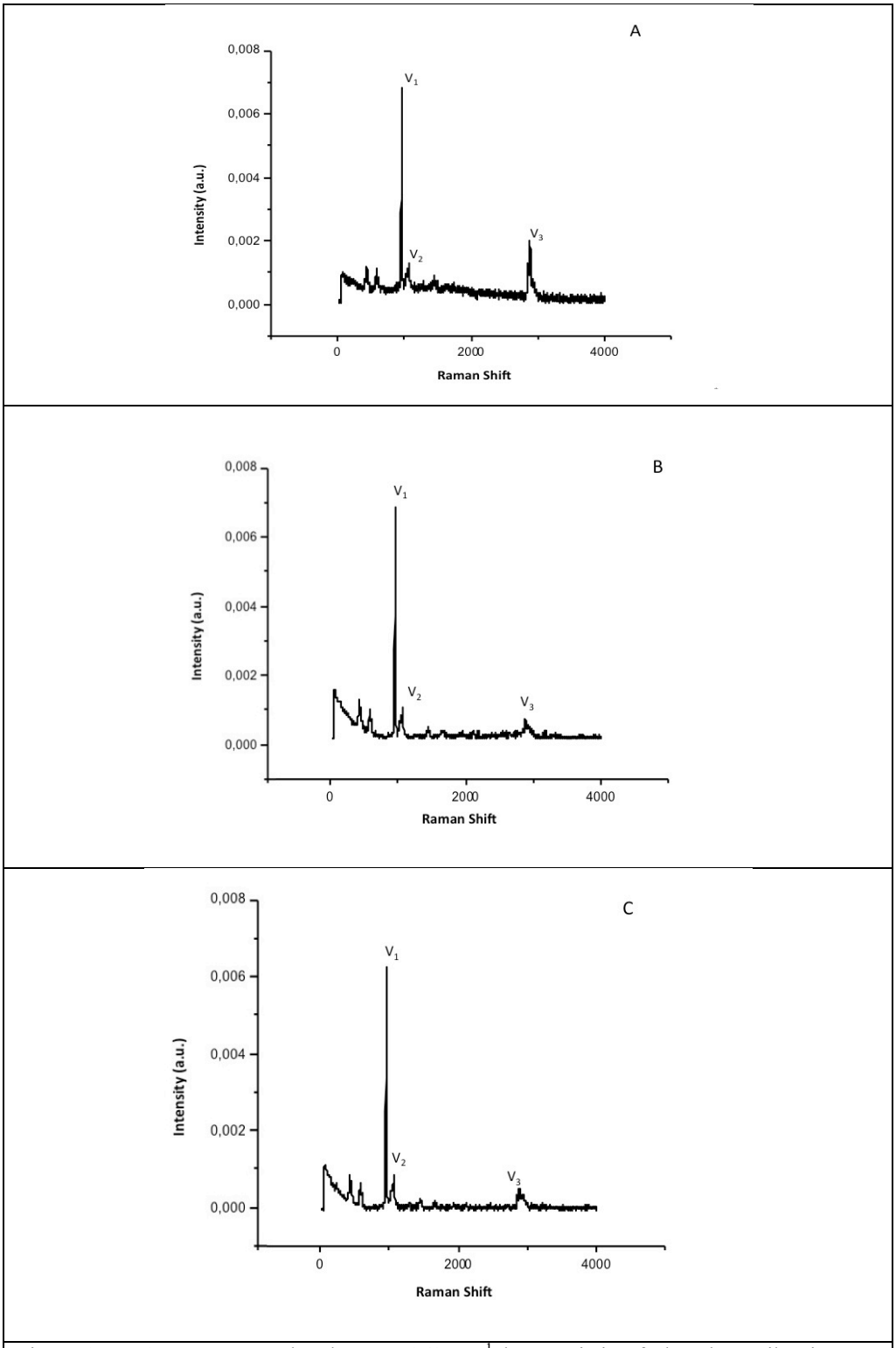


Figure 3. ENAMEL Raman band at v_1= 962 cm^{-1}characteristic of phosphate vibration v_2= 1070 cm^{-1} characteristic of carbonate vibration and v_3= 2943 cm^{-1} characteristic of organic matrix vibration. A, B and C. (A) Control; (B) Argon flow rate and (C) Argon plasma treatment.

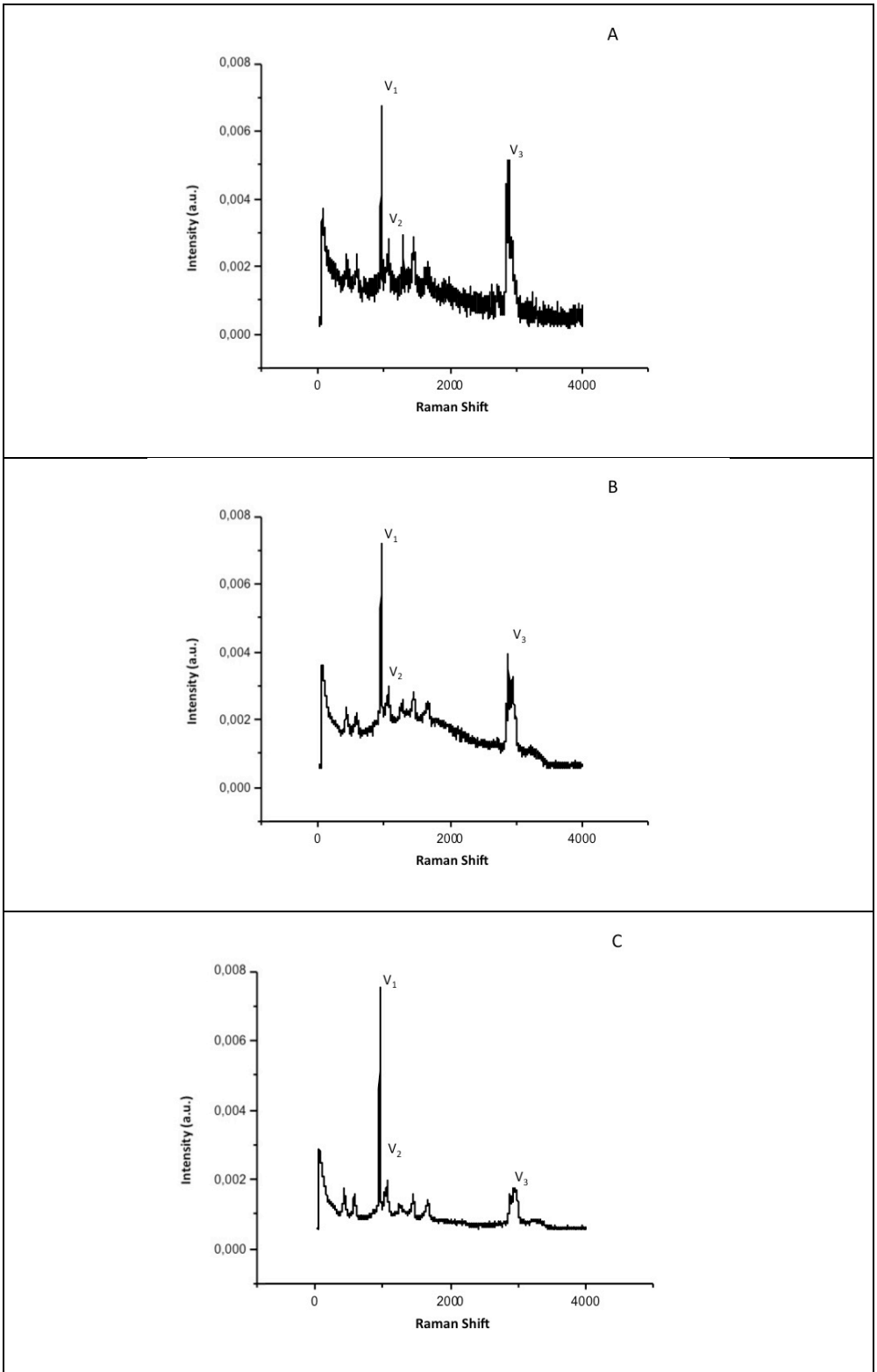
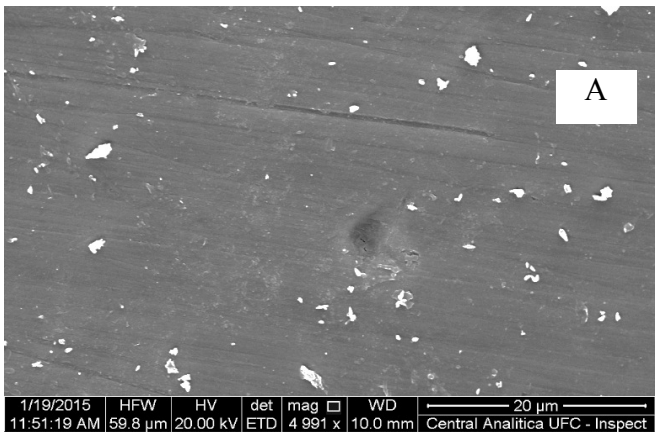
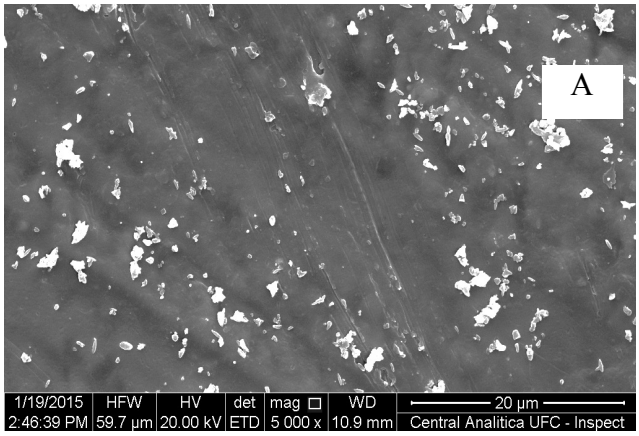
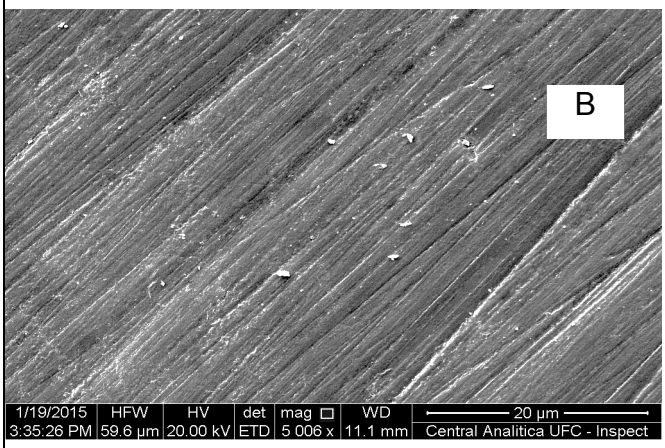
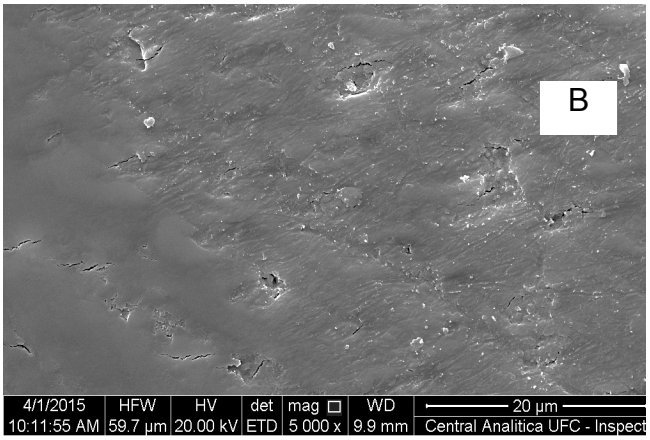


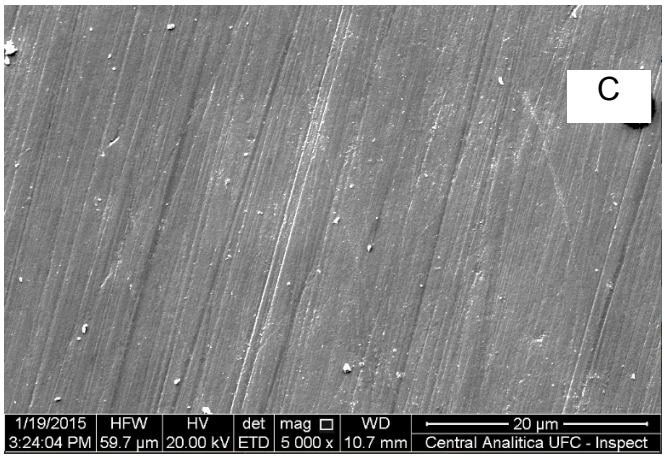
Figure 4. DENTIN Raman band at v_1=962 cm^{-1} characteristic of phosphate vibration, v_2=1070 cm^{-1} characteristic of carbonate vibration and v_3=2943 cm^{-1} characteristic of organic matrix vibration. A, B and C. (A) Control; (B) Argon flow rate and (C) Argon plasma treatment.











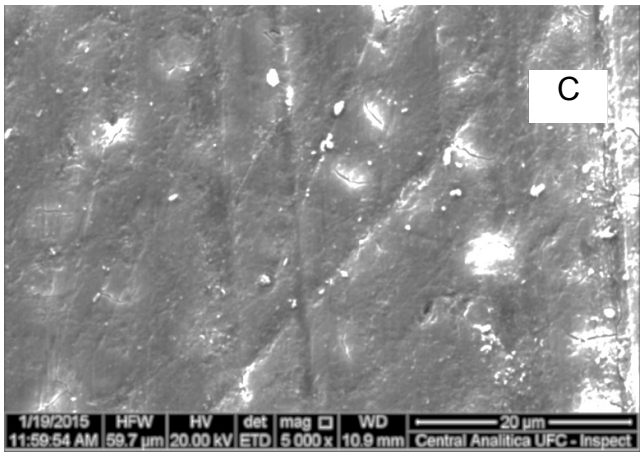


Figure 5a. Representative scanning electron micrograph images of the surfasse morfology after treatments (5000X). Enamel polished (A) Control; (B) Argon flow rate; (C) Argon plasma.

Figure 5b. Representative scanning electron micrograph images of the surfasse morfology after treatments (5000X). Dentin polished (A) Control; (B) Argon flow rate; (C) Argon plasma.

4. CONCLUSÃO GERAL

A utilização de plasma de argônio diminuiu a dureza superficial de dentina e aumentou a molhabilidade da superfície do esmalte e dentina. Mudanças na morfologia das superfícies tratadas e diminuição na matriz orgânica foram observadas no esmalte e dentina depois dos tratamentos com plasma de argônio e gás de argônio. Resumindo, o plasma de argônio foi capaz de modificar as superfícies dos dentes nas condições testadas proporcionando aumento da energia superfial.

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Anexo

Physics of Plasmas (PoP)

Manuscript Length for PoP Letters and Brief Communications

Manuscripts for Letters in Physics of Plasmas, as well as for Brief Communications, should not exceed 3500 words (approximately four printed journal pages). Abstract, title, author list, references and acknowledgments are all excluded from the 3500-word limit. Figures, tables, and equations, however, are included and must be accounted for by calculating a word count equivalent to the space they occupy. Circumvention of the length limitation by dividing a long article into smaller parts is contrary to the purpose of this journal.

Please use these guidelines for estimating length of PoP Letters and Brief Communications

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Tables: 6.5 words per line, plus 13 words for single-column tables. 13 words per line, plus 26 words for double-column tables.

Equations: 16 words per row for single-column equations. 32 words per row for double-column equations.

If the total number of words (text + figures + tables + equations) is 3500 or less, the length is acceptable.

Acceptable file formats

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intelligible to a broad audience. The manuscript must be in good scientific AmericanEnglish. All pages need to be numbered consecutively.

The **manuscript should be arranged** in the following order: title, author(s), affiliation(s), abstract, text, acknowledgments, appendixes, and references. Figures, with figure captions, may be embedded within the manuscript to assist the reviewers. In addition, please submit separate figure source files.

Series publications should be submitted in sequential order (Part I or I, Part II or II, etc.) and properly identified in the references. For series publication of closely related papers, the descriptor "Part I," or simply "I," will not be included in the title of an article unless Part II has been submitted for publication in the journal.

The **abstract** should serve as an index (including all subjects, major and minor, about which new information is given) and as a summary (presenting the conclusions and all results of general interest in the article). It should be one paragraph with approximately 250 words. The abstract should not contain displayed mathematical equations, footnotes, references, graphics, or tabular material.

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Equations should be punctuated and aligned to bring out their structure and numbered on the right. Mathematical operation signs indicating continuity of the expression should be placed at the left of the second and succeeding lines. Use × rather than a centered dot, except for scalar products of vectors. The solidus (/) should be used instead of built-up fractions in running text, and in display wherever clarity would not be jeopardized. Use "exp" for complicated exponents.

Notation must be legible, clear, compact, and consistent with standard usage.

Footnote to the title should be set as a "Note" above the byline footnotes. For footnotes to the bylines, use a), b), c), etc. Avoid textual footnotes by inserting the information in the text. Footnotes within tables should be designated by lowercase roman letter superscripts and given at the end of the table.

References should be set as a double-spaced list at the end of the text. The names, including initials, of all authors in each reference should be given (in the text, the use of "et al." is permissible). For footnotes to title and bylines, use a), b), c), etc. Avoid lengthy textual footnotes by inserting the information in the text. Footnotes within tables should be designated by lowercase roman letter superscripts and given at the end of the table. All references to books and journal articles, listed at the end of the paper, are to appear in only one of these three formats:

By number, in order of first appearance, presenting the names of the authors, the journal name, volume, first page number only, and year, as in:19L. M. Pecora, T. L. Carroll, G. A. Johnson, D.J. Mar, and J. F. Heagy, *Chaos* **7**, 520 (1997). This paper will be listed as the 19th in the list of references and cited as 19 or Ref. 19.

In **alphabetical order** according to the first author's last name, including, in addition to the name, the title of the paper cited, journal name, volume, first and last page, and year, as in: Pecora, L. M., Carroll, T. L., Johnson, G. A., Mar, D. J., and Heagy, J. F., "Fundamentals of synchronization in chaotic systems, concepts, and applications," *Chaos* **7**, 520–543 (1997). This paper will be cited as "Pecora et al. (1997)." If there are several papers by the same author(s) and the same year, they should be distinguished by letters, as in (1997a).

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