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KARLA SHANGELA DA SILVA ALVES

EFEITO DO PLASMA DE BAIXA TEMPERATURA E PRESSÃO NO BIOFILME ORAL FORMADO SOBRE ESMALTE EM UM MODELO *IN SITU*

FORTALEZA
2016

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Tese apresentada ao Programa de Pós-Graduação em Odontologia da Faculdade de Farmácia, Odontologia e Enfermagem da Universidade Federal do Ceará, como requisito parcial para obtenção do título de Doutor em Odontologia. Área de concentração: Clínica Odontológica.

Orientadora: Profa. Dra. Iriana Carla Junqueira Zanin dos Santos.

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Aprovada em ___/___/___

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(Valdemir Antoneli)

RESUMO

O plasma de baixa temperatura é uma tecnologia promissora que vem sendo estudada na odontologia por suas várias propriedades, dentre elas, por sua capacidade de destruir bactérias presentes em biofilme maduro destruindo a matriz do biofilme oral. O objetivo deste estudo foi avaliar o potencial antimicrobiano do plasma de argônio de baixa temperatura em biofilmes orais formados *in situ* e verificar se o tratamento proposto danifica a estrutura do esmalte tratado. Para tanto, vinte e dois voluntários utilizaram dispositivos intraorais palatinos contendo 6 blocos de esmalte bovino que foram gotejados sacarose 10 vezes ao dia uma solução de sacarose a 10%, durante 7 dias. Ao final do período intraoral, os biofilmes formados sobre os blocos de esmalte receberam tratamento durante 5 minutos com plasma de argônio, gás argônio, clorexidina a 0,12%, ou solução salina de NaCl a 0,89%. As amostras dos biofilmes foram coletadas, pesadas, diluídas de forma seriada e plaqueadas em meio de cultivo para o crescimento de microrganismos totais, estreptococos totais, estreptococos do grupo *mutans* e lactobacilos. A fim de analisar se os tratamentos danificaram a estrutura do esmalte, os blocos foram analisados por microscopia eletrônica de varredura (MEV) e por espectroscopia Raman. O tratamento do plasma mostrou uma redução significativa na viabilidade dos microrganismos totais ($p < 0,001$), estreptococos totais ($p = 0,037$) e estreptococos do grupo *mutans* ($p = 0,004$). O gás de argônio também reduziu significativamente as contagens de estreptococos do grupo *mutans*. O tratamento com plasma não demonstrou influência sobre a viabilidade dos lactobacilos nas condições testadas ($p = 0,497$). A MEV revelou regiões lisas e homogêneas na superfície do esmalte, não sendo observadas diferenças topográficas entre os esmaltes sob biofilmes submetidos aos diferentes tratamentos. No Raman, foram identificadas quatro bandas principais presentes na fase inorgânica: 324 (V1), 582 (V2), 960 (V3) e 1045 (V4)cm^{-1} e outras 4 identificadas na fase orgânica 1448 (V5), 1465 (V6), 1653 (V7) 2943(V8) cm^{-1}, não ocorrendo diferenças estatísticas entre os espectros do Raman nos blocos de esmaltes dos diferentes tratamentos. Em conclusão, o plasma foi eficaz na redução de bactérias viáveis presentes em biofilmes orais maduros produzidas *in situ* e não alterou a superfície do esmalte sob biofilme tratado.

Palavras-chaves: Placa dentária. Gases em plasma. Agentes antimicrobianos.

ABSTRACT

The Low temperature plasma is a promising technology that has being studied in dentistry for its various properties, among them, for their ability to destroy bacteria present in mature biofilm and also destroying the polymeric matrix of oral biofilms. The aim of this study was to evaluate the antimicrobial effect of argon plasma on oral biofilms formed *in situ* and also verify if this treatment damages the structure of the treated enamel. For that, twenty-two volunteers used palatine intraoral devices containing 6 to bovine enamel slabs that were dripped sucrose 10 times a day with 10% sucrose. The intraoral period was 7 days. At the 7th day, enamel slabs were treated for 5 minutes with plasma, argon flow, 0.12% chlorhexidine, or 0.89% NaCl solution. Thus, biofilms samples were collected, weighed, serial diluted and plated in culture medium for the growth of total microorganisms, total streptococci, *mutans streptococci* and *lactobacilli*. In order to analyze whether the treatments damaged the enamel structure, the slabs were analyzed by scanning electron microscopy (SEM) and Raman spectroscopy. Low temperature plasma treatment showed a significant reduction in the viability of the total microorganisms ($p < 0.001$), total streptococcus ($p = 0.037$) and *mutans streptococci* ($p = 0.004$). Argon flow also significantly reduced the *mutans streptococci* counts. The plasma treatment did not influence on the viability of the *lactobacilli* under the conditions tested ($p = 0.497$). SEM revealed smooth and homogeneous regions on the enamel surface, unobserved topographical differences between the glazes in biofilms subjected to different treatments. In Raman spectroscopy, we identified four major bands present in the inorganic phase: 324 (V1), 582 (V2), 960 (V3) e 1045 (V4) cm^{-1} and other 4 identified in the organic layer 1448 (V5), 1465 (V6), 1653 (V7) 2943(V8) $cm\text{-}^{1}$. No statistical differences in the Raman spectra were observed for enamel of different treatments. In conclusion, the plasma was effective in reducing viable bacteria present in mature oral biofilms produced *in situ* and not alter in the enamel surface treated biofilms.

Keyword: Dental Plaque. Plasma Gases. Anti-Infective Agents.

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LISTA DE ABREVIATURAS E SIGLAS

AIR	*Argon flow*
Caae	Certificado de apresentação para apreciação ética
CAPES	Coordenação de Aperfeiçoamento de Pessoal de Nível Superior
CFU	*Colony-Forming Units*
CHX	*Chlorhexidine*
cm	Centímetros
DNA	Ácido desoxirribonucleico
F	Flúor
FM	*Future-Tech*
KV	Quilovolt
MEV	Microscópio Eletrônico de Varredura
min	Minuto
mm	Milímetro
MSA	*Mitis Salivarius Agar*
MSB	*Mitis Salivarius Bacitracina*
NaCl	Cloreto de sódio
nm	Namômentro
PLA	Plasma
SAL	*Salina solution*
SD	*Standard Deviation*
SEM	*Scanning Electron Microscopy*
SMH	*Surface Microhardness*
SMHC	*Surface Microhardness Change*
TTP	*Tissue-Tolerable Plasma*
V	Volt
µL	Microlitros
µm	Micrometro

SUMÁRIO

1 INTRODUÇÃO

A grande maioria dos microrganismos na natureza encontra-se ligada às superfícies, onde crescem na forma de biofilmes. Os biofilmes bacterianos são formados quando microrganismos unicelulares se tornam irreversivelmente aderidos a uma superfície sólida e envolvida por uma matriz de polissacarídeos extracelulares, podendo haver a formação de biofilmes a partir de uma ou de múltiplas espécies bacterianas (MAH & O`TOOLE, 2001, p. 34). Tem sido observado que bactérias inseridas nos biofilmes passam a exibir características fenotípicas distintas, as quais resultam no aumento de sua resistência aos agentes antimicrobianos de modo que essas comunidades microbianas têm grande importância em ambientes clínicos, ambientais e industriais (O´TOOLE, 2016, p. 5).

A placa dentária é um biofilme dinâmico composto por um ecossistema microbiano formado por centenas de espécies que se acumulam sobre a superfície dos dentes (FILOCHE, WONG, SISSONS, 2010, p. 10). O biofilme oral é único entre os vários tipos de biofilmes (HUANG, LI, GREGORY, 2011, p. 436) por requerer glicoproteínas salivares para anexar ao substrato. É composto por estruturas tridimensionais complexas, formado por comunidades de multi-espécies microbianas sobre o tecido oral (HE *et al*., 2015, p. 70), incorporados em uma matriz de polissacarídeos extracelulares (EPS) (HOJO *et al*., 2009, p. 983; YANG *et al.*, 2011, p. 74).

O primeiro passo na formação do biofilme oral é a ligação da película adquirida, uma fina camada de glicoproteínas de origem salivar ou bacteriana que se adere à superfície dentária. Logo em seguida, inicia-se a adesão bacteriana com as bactérias pioneiras. *Actinomyces spp, Streptococcus spp, Haemophilus spp, Capnocytophaga spp, Veillonella spp, Neisseria* são os principais gêneros de bactérias pioneiras a aderir à superfície do dente (DIGE *et.al.,* 2009, p. 70; HUANG, LI, GREGORY, 2011, p. 437). Ao longo do tempo, a diversidade bacteriana do biofilme cresce (RICKARD *et al*., 2003, p. 96), as bactérias pioneiras fornecem locais de ligações específicos às bactérias subsequentes e promovem o desenvolvimento do biofilme que aumenta gradualmente em espessura e em complexidade com a maturação da placa (HUANG, LI, GREGORY, 2011, p. 437).

Um número estimado de 700 espécies bacterianas, incorporadas em matriz rica em polissacarídeo extracelular, foram encontradas dentro do biofilme oral (KOO, FALSETTA, KLEIN, 2013, p. 1070; NIKITKOVA, HAASE, SCANNAPIECO, 2013, p. 420). As bactérias em biofilmes mostram uma virulência mais elevada em comparação a seus homólogos planctônicos (HOJO *et al.,* 2009, p. 983), podendo ser de 10 a 1000 vezes mais resistentes às terapias antimicrobianas convencionais com agentes antibacterianos, como a clorexidina ou

os antibióticos (WELIN-NEILANDS; SVENSÄTER, 2007, p. 5635; KARYGIANNI *et al*., 2014b, p. 7330). Ocorre, no entanto, que o uso frequente desses agentes no tratamento de doenças crônicas pode resultar no desenvolvimento de resistência por algumas espécies, o que se tornou um problema de saúde pública mundial (SMITH *et al*., 2013, p. 584).

Recentemente, grandes esforços têm sido colocados na pesquisa odontológica a fim de encontrar novos métodos para eliminar microrganismos patogênicos presentes nos biofilmes orais (KARYGIANNI *et al*., 2016, p. 1) que não resultem no surgimento de cepas resistentes. Neste contexto, surgem os plasmas de baixa temperatura (PBT) para o tratamento antimicrobiano, sendo uma tecnologia alternativa de grande potencial e uma ferramenta promissora em uma variedade de aplicações biomédicas, com particular importância para combater infecções (MAI-PROCHNOWA *et al*., 2014, p. 508) por inativar bactérias (ERMOLAEVA *et al*., 2011, p. 80), fungos (FRICKE *et al*., 2012, p. 438; KLAMPFL *et al*., 2012, p. 5077), esporos (VENEZIA *et al*., 2008, p. 43), parasitas (ERMOLAEVA *et al*., 2012 p. 793) e vírus (ALSHRAIEDEH *et al*., 2013, p. 1420).

Os plasmas, que podem ser definidos como o quarto estado da matéria, são formados a partir da ionização de gases como: argônio, hélio, ozônio ou gás oxigênio. É constituído por partículas em interação permanente que incluem fótons, elétrons, íons positivos e negativos, átomos, radicais livres e moléculas excitadas e não excitadas (MOREAU; ORANGE; FEUILLOLEY, 2008, p. 611). Em geral, existem dois tipos de plasma gasoso, o plasma térmico e o não térmico. Os plasmas térmicos são obtidos a alta pressão (≥105 Pa), precisam de uma potência maior para serem formados (até 50 MW), possuem elétrons e partículas pesadas à mesma temperatura, isto é, eles estão em equilíbrio térmico entre si. Os plasmas não térmicos ou de baixa temperatura são obtidos a baixas pressões e potências sendo caracterizados por uma temperatura muito elevada dos elétrons mais do que a do gás (temperatura macroscópica), enquanto os íons e os átomos neutros são obtidos a uma temperatura muito menor (normalmente temperatura ambiente) e consequentemente não apresentam um equilíbrio termodinâmico local (MAI-PROCHNOWA *et al*., 2014, p. 508; SCHOLTZ *et al*., 2015, p. 58). Um plasma é às vezes chamado de "quente" se ele está quase totalmente ionizado ou "frio" se apenas uma pequena fração (por exemplo, 1%) das moléculas do gás estão ionizadas (MOREAU; ORANGE; FEUILLOLEY, 2008, p. 612).

As vantagens do PBT sobre outros agentes antimicrobianos são que ele pode ser usado para um tratamento localizado, fornecendo uma resposta bactericida instantânea (segundos de aplicação), o que torna a probabilidade do desenvolvimento de resistência bacteriana ao PBT baixa, e a terapia tem demonstrado efeitos colaterais mínimos. Além disso, a temperatura do PBT é compatível com os tecidos das células mamárias, o que incentiva a sua utilização *in*

vivo (FLUHR *et al*., 2012, p. 130, PARTECKE *et al*., 2012, p. 1). Espécies reativas são geradas transitoriamente pelo PBT através de diversas vias de colisões e dissociações. Estas espécies reativas oxidantes têm fortes efeitos sobre as estruturas externas das células, seja um revestimento de esporos ou de uma membrana celular. As gorduras insaturadas na bicamada lipídica das membranas celulares são susceptíveis ao ataque por radicais de hidroxila, comprometendo a função da membrana e as proteínas em membranas. Os revestimentos dos esporos são susceptíveis ao dano oxidativo por ataque químico pelas espécies reativas. Uma vez que a membrana celular e os revestimentos dos esporos foram parcialmente degradados, as espécies reativas podem danificar o material genético e as moléculas dentro do microrganismo, levando a sua destruição (HOFFMANN; BERGANZA; ZHANG, 2013, p. 21; MAI-PROCHNOWA *et al*., 2014, p. 508; SCHOLTZ *et al*., 2015, p. 53).

Adicionalmente, os tratamentos com PBTs para várias aplicações biomédicas e médicas, incluindo cicatrização de feridas, não mostraram efeitos adversos e forneceram provas da segurança da tecnologia (FLUHR *et al*., 2012, p. 130). Pesquisas têm demonstrado que o tratamento com o plasma a baixa temperatura (PBT) pode matar bactérias orais (YAMAZAKI *et al*., 2011, p. 390; GORYNIA *et al*., 2013, p. 1; BLUMHAGEN *et al*., 2014, p. 89) e inibir completamente a formação de um biofilme rico em matriz (DUARTE *et al.*, 2011, p. 1).

Vários autores têm estudado as possíveis utilidades do PBT no campo da odontologia: no clareamento dentário (LEE *et al*., 2010, p. 240; SUN *et al.,* 2010, p. 1892; JIE *et al.,* 2010, p. 3143; PARK *et al*., 2011, p. 170; SEOUL *et al.,* 2013, p. 265), na esterilização de instrumental (RUPF *et al.,* 2012, p. 126; KOBAN *et al*., 2011, p. 956; SU-JIN *et al*., 2013, p. 2; IDLIBI *et al.,* 2013, p. 369) e na adesão das restaurações a dentina (RITTS *et al.,* 2010, p. 510, HIRATA *et al.,* 2016, p.215). Além disso, diversos autores testaram a ação antimicrobiana do plasma em biofilmes *in vitro* bacterianos de *S. mutans* (GOORE *et al*., 2006, p. 1317), *B. cereus* e *G. Stearothermophilus* (MORRIS *et al*., 2009, p. 55)*, L. acidophilus* e *S. mutans* (BO *et al*., 2011, p. 48), *P. Gingivalis* (MAHASNEH *et al*., 2011, p. 191), *S. mutans* (SUN *et al.,* 2011, p. 143702) e em canais radiculares (JIANG *et al*., 2009, p. 479; LU *et al*., 2009, p. 668; SHAUDINN *et al*., 2013, p. 1).

Assim, a tecnologia do PBT tem recebido crescente atenção como uma possível terapia para o tratamento e/ou prevenção de biofilmes. Muitos estudos sugerem a terapia com PBT um importante agente antibacteriano, mas relatam terapias utilizando uma variedade de diferentes dispositivos geradores de plasma com diferentes tipos de gases e tempo de tratamentos dos biofilmes diferentes, o que torna um desafio mostrar a reprodutibilidade dos resultados. Em contraste com seu efeito antimicrobiano, ainda não se tem conhecimento de

como o PBT afeta a estrutura do esmalte. Além disso, é necessário determinar parâmetros seguros para a utilização do PBT nos tecidos dentais antes que o mesmo seja recomendado para o uso clínico. Até esta data, tem havido algumas investigações em relação ao tratamento de bactérias orais com PBT (BIN *et al*., 2006, p. 1317; SLADEK *et al*., 2007 p. 318; YAMAZAKI *et al*., 2011, p. 384; GORYNIA *et al*., 2013, p. 1; BLUMHAGEN *et al*., 2014, p. 84; SLADEK *et al*., 2014), mas não há estudos com biofilmes formados *in situ*, de modo a investigar a terapia sob condições mais próximas àquelas encontradas na cavidade bucal dos seres humanos.

2 PROPOSIÇÃO

Avaliar o potencial antimicrobiano do plasma de argônio de baixa temperatura em biofilmes orais formados *in situ* e os seus possíveis efeitos na morfologia e composição química do esmalte bovino sob o biofilme irradiado.

3 CAPÍTULO

Esta tese 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 dissertação de mestrado e tese de doutorado e permite a inserção de artigos científicos de autoria e coautoria do candidato. Por se tratar de pesquisa envolvendo seres humanos, ou parte deles, o projeto de pesquisa deste trabalho foi submetido à apreciação do Comitê de Ética em Pesquisa da Faculdade de Medicina da Universidade Federal do Ceará via Plataforma Brasil, tendo sido aprovado sob Caae - 40975514.0.0000.5054 (ANEXO A). Assim sendo, essa tese de doutorado é composta de um capitulo que contém um artigo que será submetido para publicação no periódico “Caries Research” (ANEXO B).

3.1 Capítulo 1

Effect of tissue-tolerable plasma on oral biofilms formed *in situ*. Karla Shangela da Silva Alves, Roberto Haniery Pontes Alves, Ramille Araújo Lima, Maria Silmara Alves de Santana, Lidiany K. A.Rodrigues, Simone Duarte, Iriana C. J. Zanin.

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Short Title: Plasma treatment on *in situ* biofilms

Keywords: Dental plaque, Oral biofilms, plasma gases, *in situ* study.

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ABSTRACT

The Tissue-Tolerable Plasma - TTP is a promising technology that has being studied in dentistry due to not only its ability to kill bacteria present in mature biofilm and but also to destroy the polymeric matrix of oral biofilms. The aim of this study was to evaluate the antimicrobial effect of argon TTP on oral biofilms formed *in situ* and also verify if this treatment damages the structure of enamel under treated biofilm. For that, twenty-two volunteers used palatine intraoral devices containing bovine enamel slabs that were treated 10 times per day with a 10% sucrose. After a 7 day intraoral period, the biofilm formed over the enamel slabs were treated for 5 minutes, as follows: TTP, argon flow, 0.12% chlorhexidine, or 0.89% NaCl solution. Thus, biofilms samples were collected, diluted in decimal series and plated in culture medium for the growth of total microorganisms, total streptococci, *mutans streptococci* and *lactobacilli*. In order to analyze whether the treatments damaged the enamel structure, the slabs were analyzed by scanning electron microscopy (SEM) and Raman spectroscopy. Tissue tolerable plasma treatment showed a significant reduction in the viability of the total microorganisms ($p < 0.001$), total streptococcus ($p = 0.037$) and *mutans streptococci* ($p = 0.004$). Argon flow also significantly reduced the *mutans streptococci* counts. The plasma treatment did not influence on the viability of the lactobacilli under the conditions tested ($p = 0.497$). SEM revealed smooth and homogeneous regions on the surface of the treated enamel, unobserved topographical differences between the enamel subjected to different treatments. In Raman spectroscopy, we were identified four major bands present in the inorganic phase: 324 (V1), 582 (V2), 960 (V3) e 1045 (V4) cm^{-1} and other 4 identified in the organic layer1448 (V5), 1465 (V6), 1653 (V7) 2943(V8) cm^{-1}. No statistical differences in the Raman spectra were observed for enamel of different treatments. In conclusion, the plasma was effective in reducing viable bacteria present in mature oral biofilms produced *in situ* and it did not modify the morphology and composition the enamel.

INTRODUCTION

Biofilms models may help us to accurately predict, in a controlled and simplified way, a clinical outcome which can lead us to preventive actions for diseases [Featherstone, 1996]. One example of biofilm model is the *in situ* biofilm, which may be formed on different substrates that are introduced inside the oral cavity into oral devices. Several models, which enable the development of non-disturbed oral biofilm, are found in literature. The first report was done by Ahrens et al. [1976] and they developed a model based on acrylic splints on which enamel slides were positioned. Since then, this system has been widely used with a variety of substrates including glass [Arweiler et al., 2004], bovine enamel [Jentsch et al., 2002], bovine dentin [Zaura-Arite et al., 2001], human enamel [Teixeira et al., 2012] and dentin [Lima et al., 2009] and hydroxyapatite discs [Sreenivasan et al., 2009].

Antimicrobial agents are used due its ability to avoid dental biofilm formation, but biofilm species may exhibit several resistance mechanisms against them [Mah&O'Toole 2001]. In addition, the age and the structure of a biofilm may also restrict the penetration of the antimicrobial agent and deeper cells in the biofilm could not be affected [Zanin et al., 2005]. Disruption of the oral microflora and the difficulty of maintaining therapeutic concentrations of antimicrobials in the oral cavity are also problems associated with its usage [Zaura-Arite et al., 2001]. Thus an antimicrobial adjuvant would be a valuable alternative in treating oral infections.

Regarding these alternative adjuvants, Tissue-Tolerable Plasma - TTP, that represents the fourth state of matter after solid, liquid, and gaseous [Morfill et al., 2009], emerges as an alternative once it is a technology that inhibits biofilm formation with the potential benefit of destroying oral biofilm matrix [Duarte et al., 2011]. Plasma is a partially ionized gas generated by an electrical discharge, which creates a highly reactive environment with ions, electrons, excited atoms and molecules, vacuum ultraviolet and ultraviolet irradiation, free radicals and chemically reactive particles [Koban et al., 2010; Zamperini et al., 2010]. The production of stable plasma at atmospheric pressure has attracted attention for treating living cells and tissues without thermal damage [Brun et al., 2012]. It is also site specific, targeting only the infected area [Goree et al., 2006], and it seems to preserve the material's bulk properties [Zamperini et al., 2010]. In addition, plasma is usually produced by low-toxicity gases and elaborates its activity by producing a mixture of products that decay within a few seconds after the treatment process.

Therefore, this approach has been suggested as environmentally friendly with no harmful residues [Alkawareek et al., 2012].

TTP has recently been extensively studied by researchers as a possible therapy in dentistry. Researchers have mostly investigated the antimicrobial effects produced by plasma as a means to remove dental biofilms and eradicate oral pathogens. It has been shown that antimicrobial effects the TTP are carried out by induction reactive, photons and reactive particles, ions, and molecules [Hasse et al., 2015]. The efficacy of TTP against an extended spectrum of fungi and bacteria, including *S. mutans*, has been shown in several *in vitro* studies [Rupf et al., 2010; Matthes et al., 2013; Bender and Kramer, 2014; Preissner et al., 2015], but no studies report results in multispecies biofilms. Furthermore, it has been applied effectively in antimicrobial therapy in case reports and clinical studies in animals and humans, especially in otorhinolaryngology and dermatology [Isbary et al., 2013; Klebes et al., 2015]. Strikingly, its efficacy seems to be irrespective of microbial resistance patterns [Haertel et al., 2014; Daeschlein et al., 2015] and no negative side effects to viable tissues have been described, despite slight warming and temporary redness [Lademann et al., 2009; Fluhr et al., 2012]. In this way, TTP has been suggested for different applications in dentistry [Kim et al., 2014].

Therefore, plasma is emerging as a physical treatment with microbicidal potential on bacteria, parasites, fungi, spores, and viruses [Sladek, 2005; Fridman et al., 2007; Kolb et al., 2008; Von Woedtke et al., 2008; Dobrynin et al, 2009; Rupf et al., 2010]. Also, TTP may allow its application inside the mouth because of it is compatible with the mammalian tissues, which encourages their use *in vivo* [Fluhr et al., 2012; Partecke et al., 2012; Delben, 2016]. The first hypothesis to be tested in this study is that TTP have antimicrobial effect on microorganisms present in biofilms formed *in situ*. Furthermore, the hypothesis that plasma treatment will damage the enamel structure will be tested.

MATERIALS AND METHODS

Ethical Aspects

This study was approved by the Institutional Ethical Committee and all volunteers gave informed consent (Sisnep Caae - 40975514.0.0000.5054). .

Experimental design

For this *in situ* experiment, a single-blind split mouth design was used in two intra-oral phases of 7 days (7d) each, in which 22 volunteers wore palatal devices containing six bovine enamel slabs, positioned in pair of three. At the end of each intraoral phase, the device was randomly split and each half (containing three enamel slabs) was allocated into one of the following treatments: Plasma (PLA); Argon flow (AIR); Chlorhexidine 0.12% (CHX) or Salina solution 0.89% (SAL) as described in Fig 1. In this way, at the end of the two clinical phases, all volunteers were submitted to the four different treatments (Fig.2).

Figure 1 – Description of the treatments in which the enamel slabs were allocated.

Groups	Treatment
PLA	Plasma plume scanning enamel surface during 5 min
AIR	Argon gas flow scanning enamel surface during 5 min
CHX	A drop of 50 µL on each slab during 5 min
SAL	A drop of 50 µL on each slab during 5 min

Tissue-Tolerable Plasma

The Tissue-Tolerable Plasma TTP source that was utilized in this study was developed by the Leibniz Institute for Plasma Science and Technology (Neoplas Tools – KINPen, Greifswald, Germany) and consists of a hand-held unit (length = 170 mm, diameter = 20 mm, weight = 170g) connected to a high-frequency power supply (frequency 1.82MHz, 2–6 kV peak-to-peak, 8 W system power) for the generation of a plasma jet at atmospheric pressure. The hand-held 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 (slm) 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 10mm [Delben, 2016]. The gas flow/TTP plume was targeted to all surface of enamel slabs, and a surface scanning were performed manually by a single operator.

Specimen preparation

Bovine teeth were used to perform this *in situ* study. The teeth were stored in 0.01% (v/v) thymol solution at 4°C for 30 days until be used [Cury et al., 2000]. Enamel slabs with 4 x 4 x 2 mm were obtained using a water-cooled diamond saw and a cutting machine (IsoMet Low Speed Saw; Buehler, Lake Bluff, IL, USA). The adjustment of the enamel to obtain flat plates was done with the aid of a low-speed polishing machine and 320 grit paper (Carbimet Paper Discs; Bhuheler-met® II, USA), under water-cooling. Afterwards, the specimens were polished using three different silicon carbide waterproof papers (320, 600, and 1,200-grit) as well as polishing cloths with 1µm diamond paste (Buehler®, Illinois, USA).

Microhardness analysis

Surface Microhardness (SMH) was performed using a Knoop indenter with a 50g load for 5s in a Future-Tech FM Microhardness Tester coupled to the FM-ARS software (Future-Tech Corp., Tokyo, Japan). Initial SMH measurements were made in the center of the enamel surface making one row of five indentations. For selection purposes, enamel slabs with SMH values ranging from 272- 440 Knoop hardness number were selected in a total of two-hundred and sixty-four slabs that were used in this experiment [Meredith et al., 1996]. After the intra-oral phase and treatments, SMH measurements were repeated spaced at 100 µm on the side right of the five baseline indentations. Also, the percentage of surface microhardness change (%SMC) was calculated (%SMC = SMH after treatment – SMH baseline x 100/ SMH baseline).

In situ palatal devices

After baseline SMH, the slabs were autoclaved (121 ºC, 15 min) [Yamamoto et al., 2005] and stored in 100% humidity until being inserted into the palatal appliances. For each subject, two acrylic palatal devices were prepared, in which two cavities (18 x 6 x 3 mm) were made on the left and right sides; and three slabs were attached with wax in each cavity. In order to allow biofilm accumulation, and to protect it from mechanical disturbance, a plastic mesh was positioned on the acrylic resin, leaving a 1 mm space from the slab surface [Hara et al, 2003].

In situ study Population

Twenty-two healthy volunteers (16 women and 6 men), aged from 19 to 34 years, able to comply with the experimental protocol, were selected to participate in this study. All

participants received oral and written instructions about the experimental design. The inclusion criteria were normal salivary flow rate, normal buffering capacity of saliva and *S. mutans* colony-forming units ($CFUmg^{-1}$) in biofilms of at least 10^5 after 36 h of oral hygiene suspension. Exclusion criteria included active caries lesions, use of antibiotics within the past 3 months prior to the study, and the use of fixed or removable orthodontic devices.

In situ biofilm formation

During the lead-in period (7d) and throughout the clinical 2 phases (7d each), the volunteers brushed their teeth with a fluoridated dentifrice [Sorriso Super Refrescante – a calcium carbonate based dentifrice, 1,450 µg fluoride (F) g^{-1}, as monofluorophosphate [MFP, Colgate-Palmolive, São Paulo, SP, Brazil]. Also, the volunteers received oral and written instructions to wear the appliances at all times, including at night. They were allowed to remove the appliances only during meals, when consuming acid drinks, and when performing oral hygiene. When removed, the devices were kept moist in plastic boxes to keep the bacterial biofilm viable [Cury et al., 2000]. The cariogenic challenge was provided by the volunteers who dripped a 10% sucrose solution onto all the enamel slabs, 10 times a day, according to a predetermined schedule (at 08:00, 09:30, 11:00, 12:30, 14:00, 15:30, 17:00, 18:30, 20:00, and 21: 30 h) [Duggal et al., 2001]. Before replacing the palatal appliance in the mouth, a 5-min waiting time was standardized to allow diffusion of the sucrose into the dental biofilm. Brushing with the dentifrice was performed three times a day, after mealtimes when the volunteers habitually carried out their oral hygiene procedures. The appliances were brushed extra-orally, except for the slab area, to avoid disturbing the biofilm. All volunteers consumed fluoridated water (0.70 mg F 1^{-1}), and no restriction was made with regard to the volunteers' dietary habits.

Treatment of in situ biofilms

Microbiological analyses of the oral biofilm were performed on the 7th day of the experiment immediately after performing the treatments. The distribution of treatments on the palatal device in each intra-oral phase was determined randomly by raffle. All volunteers attended to laboratory in fasting and one drop of 10% sucrose was added to each slabs before the device was removed from mouth. Thirty minutes after, the plastic meshes of the devices were removed with a scalpel blade (#15C), the biofilm formed *in situ* were exposed, and the treatments with PLA, AIR, CHX or SAL were performed. Biofilms were then scraped carefully and weighed using microcentrifuge tubes, to which 1000 µl 0.89% NaCl solution was added. To disperse the biofilms, the samples were

sonicated at an output of 7W (Sonifier450D, São Bernado do Campo,SP, Brazil) using 3 pulses of 15 s with interval of 15 s of rest on ice. Afterwards, the suspension was serially diluted (1:10, 1:100, 1:1.000, 1:10.000, 1:100.000, and 1:1.000.000) in 0.89% NaCl solution. In order to assess microorganism viability, samples were plated in triplicate on the following culture media: mitis salivarius agar (MSA), containing 15% sucrose, to determine total streptococci; MSA agar plus 0.2 units of bacitracin ml^{-1} to determine *mutans streptococci* [Gold et al., 1973], rogosa agar supplemented with 0.13% glacial acetic acid to assess the number of lactobacilli [Rogosa et al., 1951] and blood agar to determine total microorganisms. The plates were incubated for 48 h at 37°C in a partial atmosphere of 5% CO_2. Representative colonies of *mutans streptococci*, total streptococci, *lactobacilli,* and total microorganisms were counted using a colony counter, and the results were expressed as colony-forming units (CFU) mg^{-1} of biofilm.

Scanning Electron Microscopy (SEM)

For SEM, samples from bovine enamel slabs rinsed with distilled water, were vacuum dried for 24h and then mounted on a SEM stub (aluminum discs), fixed with double sided adhesive carbon tape, coded and mounted onto aluminium stubs with Acheson silver DAG (Agar Scientific, U.K.) and then coated with a 15 nm thick layer of gold, using a Polaron SEM coating unit. The specimens were then examined using an SEM (Inspect™ S50, Jeol, Tokyo, Japan), operating at 20 KV and working distance 10mm. Images were taken at 2 levels of magnification (×5000 and ×10000) in order to assess for changes in surface structure and captured using an specific software (EDS Software for SEM, Oxford instruments). The images were analyzed visually.

Raman Spectroscopy

The Raman spectra were acquired on a Raman spectrometer (Xplora HORIBA, Kyoto, Japan). For excitation of the samples it was used a HeNe laser operating at 632.8 nm wavelength. The enamel slab sample was placed onto a substrate, the laser beam was focused on the sample surface using a microscope (OLYMPUS, Japan) with lens of 10x and numerical aperture 0.75 forming a spot approximately 4 μm over the surface of the sample, and three points were chosen for Raman measurement with an exposure time of 30 s. The first point was located in the central region of the dental slab, and the others were located to the right and to the left of this first point. The distance between the points was 1 mm. The Raman system was calibrated with a silicon semiconductor using the Raman peak at 521 cm^{-1}. All spectra were taken and collected in the region from 200 to

3600 cm^{-1} and allowed a characterization of both mineral content (hydroxyapatite) and organic (essentially collagen) constituents. 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).

Statistical analysis

The sample size was calculated by using data from an initial study, assuming on a medium effect size of 0.5, significance level 5%, and statistical power 95%, it was found that the study required 16 participants. Twenty- two participants were screened to ensure that at least 16 subjects would successfully complete the study. Each participant wore an upper removable appliance containing six enamel slabs. The study consisted of two phases and each phase lasted for 7-days. Therefore, a total number of 264 enamel slabs were required for the whole study.

The normality distribution of the data was checked using the Kolmogorov-Smirnov statistical test. The mean and the standard deviation (SD) of the numbers of surviving microorganisms for each treatment were calculated. Colony-forming units were transformed into log 10 colony-forming units in order to reduce variance heterogeneity. To determine the differences between test and control values, the unpaired one-way analysis of variance followed by a Holm-Sidak test or non-parametric Kruskal-Wallis followed by a Dunn's Multiple Comparison Test were used when appropriate. Paired- t test was used to compare baseline and after intraoral phase SMH. Differences among the experimental periods in relation to %SMC were tested by Kruskal-Wallis One Way Analysis of Variance on Ranks. For Raman in order to normalize measurements and allow their comparison, the band height parameter was used. The mean of the peaks height were compared using test one-way analysis of variance (ANOVA). Significance level was set at 5% ($p < 0.05$) using the software SigmaPlot 11.0 (La Jolla, CA, USA).

RESULTS

Microhardness analysis

The results for enamel surface microhardness before and after treatment and also the percentage of microhardness change can be found in table 1. The comparison of different treatments showed no statistical significant difference among them ($p= 0.36$).

Microbiological viability

All volunteers showed normal patterns in the salivar tests with mean values of 1.56 ml/min salivary flow rate, normal buffering capacity (pH= 5.13) and 7.37 x 10^6 CFU ml^{-1} of *mutans streptococci* in biofilms formed after 36 h of oral hygiene suspension. The effect of plasma treatment on viability of *in situ* biofilms can be found on Figure 3. Plasma treatment showed a significant reduction for total microorganism ($p<0.001$), total streptococci ($p= 0.037$), and *mutans streptococci* ($p= 0.004$) counts. *Mutans streptococci* counts were also reduced by argon gas treatment. Thus, plasma treatment did not demonstrate influence on lactobacilli viability ($p= 0.497$). For the other tested microorganisms there were no statistical difference among salina solution, argon gas and CHX treatments. The percentage of viable *mutans streptococci* related to total streptococci was 7.86% in plasma treatment, 10.23% in argon treatment, 15.34% in salina group and 8.10% in chlorhexidine treatment.

Scanning Electron Microscopy (SEM)

SEM analysis revealed smooth and homogenous surface topography of enamel at both x5000 (Figure 4: 1A, 2A, 3A and 4A) and x10000 whereas in region enamel the demineralized showed irregularities and stepped lamination appearance (Figure 4: 1B, 2B, 3B, and 4B) magnification. No difference was observed among the surfaces of different treatment groups.

Raman Spectroscopy

The average Raman spectra for the four treatment groups in the region of 200-3600 cm^{-1} are shown in Fig. 5. All spectra were normalized based on the 960 cm^{-1} phosphate peak, which was the most intense peak. The region spanning from 240 to 1.045 cm^{-1} was characteristic of phosphate and carbonate groups and representative of the mineral phase of enamel and the region spanning from 1448 to 3080 cm^{-1} was characteristic of C-H bond stretching and amide bending vibrational modes from proteins in the organic matrix of the enamel.

The band in the range of v1 (240-330 cm^{-1}) represents the carbonate vibration, bands at v2 (582 cm^{-1}), v3 (960 cm^{-1}), v4 (1045 cm^{-1}), represent the phosphate vibrations in hydroxyapatite, and bands v5 (1448 cm^{-1}), v7 (1653 cm^{-1}) amide bending vibrational modes from proteins in the organic matrix of the enamel and v6 (1465 cm^{-1}), v8 (2943 cm^{-1}) representing C-H.

In Fig. 06, Raman-spectra from enamel slabs with treatment plasma (in black), argon flow (in red), 0.89% NaCl saline solution (in blue) and chlorhexidine (in green) are shown with similar vibrational modes. Although in the peaks intensity of the SAL group was higher than the other groups, no statistically significant differences were detected in the peak intensity observed for all bands in all tested groups (p= 0.123).

DISCUSSION

The main finding of the present study is that Tissue-Tolerable Plasma was effective against mature oral biofilm formed *in situ*, being more effective in reduce bacterial viability than chlorhexidine, a well-known antimicrobial substance. Additionally, this is the first *in situ* study evaluating the antimicrobial effectiveness of Tissue-Tolerable Plasma treatment on oral biofilms. The oral biofilm containing pathogenic bacteria communities is one of the major factor associated with dental caries [Guo et al., 2015]. Also, microorganisms growing in biofilms may be up to 1000 times more resistant to antimicrobial agents than their planktonic counterparts [Costerton et al., 1999; de Melo et al., 2013]. In this way, the interest in new strategies to effectively inactivate pathogenic bacteria in oral biofilms has emerged in the scientific community and the use of the Tissue-Tolerable Plasmas are one of these new therapies because of its effectiveness against oral microorganisms [Gorynia et al., 2013; Blumhagen et al., 2014; Sladek et al., 2014].

This study compared the antimicrobial effect of several treatments on oral biofilms. CHX solution is a golden standard to inactivate or prevent dental plaque formation [Matthews, 2011] when compared to other chemical agents used in dentistry [Filoche et al., 2005]. However, a 0.1% CHX solution seems to be inefficient against mature oral biofilms [Vitkov et al., 2005]. The main advantage of using CHX is its wide antimicrobial spectrum, acting on both Gram-positive and Gram-negative microorganisms and its prolonged and continuous effect even in the presence of blood and other body fluids [Rosenthal et al., 2004]. However, the prolonged use of CHX can cause mucous peeling, stains on the teeth, alterations in the sense of taste, compromising of the wounds healing and reduction of fibroblast adhesion to radicular surfaces [Zheng and Wang, 2011]. Thus a potential antimicrobial adjuvant alternative to CHX with less side-effect would be of great value in dentistry. In our study, plasma treatment showed better results than CHX in reducing total microorganism, total streptococci and *mutans streptococci*, similarly found

by the Koban et al. [2010] *in vitro* using TTP treatment against oral biofilms formed on titanium discs.

In our study, complex *in situ* multispecies biofilms were significantly affected by TTP after 5 min treatment. Although we did not investigate the mechanism of action of TTP on bacterial species, there are many possible mechanisms that may explain its bactericidal activity, such as electron bombardment of cell membranes, charge accumulation, chemical reactions such as oxidation, destruction of nucleic acids by ultraviolet radiation, and ablation of the cell membrane [Laroussi, 2005]. In all cases, the site of action for the plasma deactivating mechanisms is either at the cell membrane or the DNA. Argon plasma acts via a complicated mechanism that includes a synergetic action of reactive species of different kinds, including ionized argon gas molecules [Shimizu et al., 2008]. TTP reactive particles produce a general mechanical effect on the surface of living organisms, which has been called etching [Lerouge et al., 2000a; Moisan et al., 2001; Moreau et al., 2008].

Etching is due to the reaction of highly reactive gas radicals with organic materials, generating products that disrupt membrane surface. It may cause perforations in the membranes of microorganisms, which, in turn, remove the obstacle to secondary reactive species that might be formed in the medium. The effectiveness of etching depends on plasma composition [Lerouge et al., 2000b]. Park et al. [2014], related that it is possible that several components work together to produce a synergistic effect rather than a single component contributing to the sterilization. TTPs may produce a large amount of reactive oxygen species (ROS) when it passes through air, and in particular, high levels of OH are generated when plasma reacts with water or tissue fluid. It is well known that OH effectively kills bacteria. Furthermore, the half-life of plasma-generated ROS is very short, and hence, its retention in the oral cavity is short and less likely to induce harmful effects on tissues.

The TTP treatment was more effective to reduce total microorganisms than chlorhexidine treatment. Ermolaeva et al. [2011] suggested that, in general, TTP argon plasma is more effective against Gram-negative than Gram-positive bacteria because of the differences in cell-wall structure. In our study, we did not test plasma treatment in gram-negative bacteria disconnected of other microorganism, but our oral biofilms contain both gram-negative and positive bacteria and the treatment was effective in reduce them. So, further experiments are needed to evaluate the effectiveness of plasma treatment on gram-negative bacteria.

As observed to total microorganisms, the counts of total streptococci after TTP treatment was inferior than observed to chlorhexidine. The degree of inhibition is probably related to the thickness of the biofilm once thicker biofilm, such as those formed in the presence of sucrose [Roberts et al. 2002], would present a greater challenge for the penetration of plasma free radicals [Sladek and Stoffels, 2005; Vleugels et al. 2005]. This could also inhibit the antibacterial activity of the TTP, killing cells only at the surface of biofilms and inducing a sublethal response to cells attached to the substrate [Sladek and Stoffels 2005]. Also, the partial antimicrobial effect achieved in our study may be explained by characteristics of different bacteria present in biofilms that turn them more resistant to antimicrobial treatments. In this way, Gorynia et al., [2013] tested argon plasma in *S. sanguinis* biofilms and found that TTP was not effective on this bacteria which may be attributed to its fast metabolic ratio and also to its ability of attaches to surfaces 10 to 100 times stronger *than S. mutans, S. mitis* and *S. salivarius*, generating more stable biofilms [Rosan et al., 1982].

Despite the majority of the studies test TTP treatment against planktonic cultures, Goree et al. [2006] investigated the use of a plasma needle in *mutans streptococci* colonies and observed that TTP was able to kill bacteria after 10 seconds of treatment. They concluded the plasma could provide an attractive alternative treatment for dental clinic. Yang et al. [2011] and Blumhagen et al. [2014] used the TTP argon plasma against *mutans streptococci* and *Lactobacillus acidophilus* and observed that TTP was effective in reducing the number of *S. mutans* after 11-15 sec and that results were dependent of the bacterial supporting media. Similar results were also observed on monospecies biofilms with different types of plasma source such as helium and oxigen gas [Sladek et al., 2004; Yamazaki, 2011]. Also, the percentage of viable cariogenic *mutans streptococci* related to total streptococci was almost half in TTP treatment compared to negative control.

In our study there was not observed statistically significant reductions on viability of *Lactobacilli sp* among the tested treatments. Yang et al., [2011] found that longer plasma treatment was required for deactivating *L. acidophilus* than used to kill *S. mutans*. The sterilization mechanisms of TTP indicate that bacterial sizes and structure would affect the plasma effectiveness and efficiency in bacterial deactivation. The bigger cell size of *L. acidophilus* (~ 1 × 3 μm in diameter) would receive less free radicals per unit of cell making than harder to kill than the smaller *S. mutans* (~ 1 μm in diameter). In order to achieve the same intensity of injection plasma for a single cell, under the same plasma conditions,

several minutes of exposure time were needed to kill *L. acdophilus*, while only tens of seconds of exposure time was needed for *S. mutans*.

In the present study, enamel demineralization was observed after 7 days of plaque accumulation at high frequency of exposure to sugar (Table 1). It was observed a reduction in superficial enamel microhardness (SMH) due to the cariogenic challenger to which enamel blocks were submitted. The SMH is considered a very sensitive method of evaluating early caries lesion [Zero, 1995]. Tenuta et al. (2003) suggest that it takes around 3-4 days for dental plaque to manifest its cariogenicity on dental substrate but this period could be shorter for bovine enamel [Featherstone and Mellberg, 1981]., Thus, the mineral loss found in our study is similar to those founded for human enamel by other authors [Park et al., 2004]. Also, the treatment with argon plasma, argon flow, 0.12 % chlorhexidine, and 0.89% NaCl solution had no effect on SMC%, suggesting that the plasma treatment do not interfere in enamel hardness under tested conditions. These results indicated that plasma treatment did not induce damage in the enamel surface despite enamels slabs have suffered cariogenic challenge. Chen et al. [2013] also did not observed surface modification of several dental substrates submitted to by Tissue-Tolerable Plasma treatment.

The raman spectroscopy allows a molecular analysis of mineralized dental tissues. The information is provided as curves representing the intensity of the signal according to the frequency and mathematical analyses may be performed in order to provide comparative and quantitative analysis [Tramini et al., 2000]. To our knowledge, this is the first study that has characterized and compared the molecular structure of the enamel submitted to the plasma treatment. The reading of the slab in three different points allows us to compare statistically all the treatments, however, no significant difference in mean intensity of the peaks.

In conclusion, our results demonstrate that the argon TTP under tested conditions was effective in reducing the viability of *mutans streptococci*, total streptococci and total microorganism, without damage the surface of enamel.

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Tables

Table1: Surface microhardness analysis of enamel slabs according to the treatment (mean ± SD).

Treatment	SMH		%SMC
	Baseline	After intraoral phase	
Plasma	324.03 ± 31.82a	210.98 ± 90.53b	-34.99 ± 21.92A
Argon flow	312.87 ± 26.90 a	230.81 ± 51.37 b	- 26.32 ± 09.77A
NaCL 0,89%	318,35 ± 30,61 a	193.11 ± 87.99 b	-39.06 ± 21.79A
Chlorexidine	315,88 ± 28.46 a	212.45 ± 70.03 b	-32.49 ± 14.78 A

Different letters indicate statistically significant differences ($p < 0.05$). Capital letters compare the columns; low case letters compare the lines. SMH = surface microhardness; SMC = surface microhardness change.

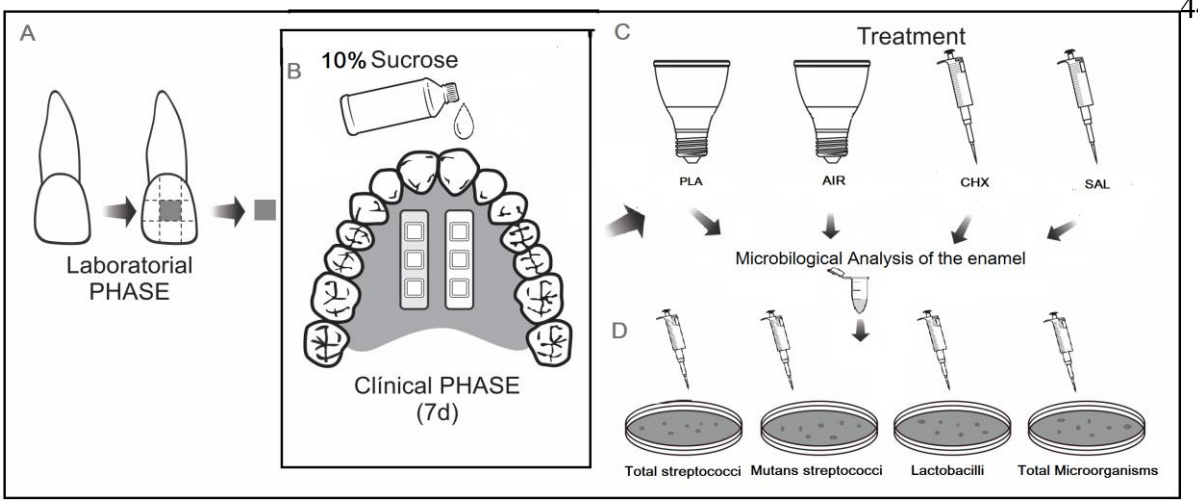

Figure 2 – Experimental Design. In (A) enamel slabs from bovine teeth stored in 0.01% thymol. (B) Clinical phase to provide a cariogenic challenge. (C) Each slab following treatments for 5 min: PLA, AIR, CHX and SAL. (D) Biofilm samples plated in triplicate in MSA, MSB Agar, Rogosa Agar and in Blood Agar.

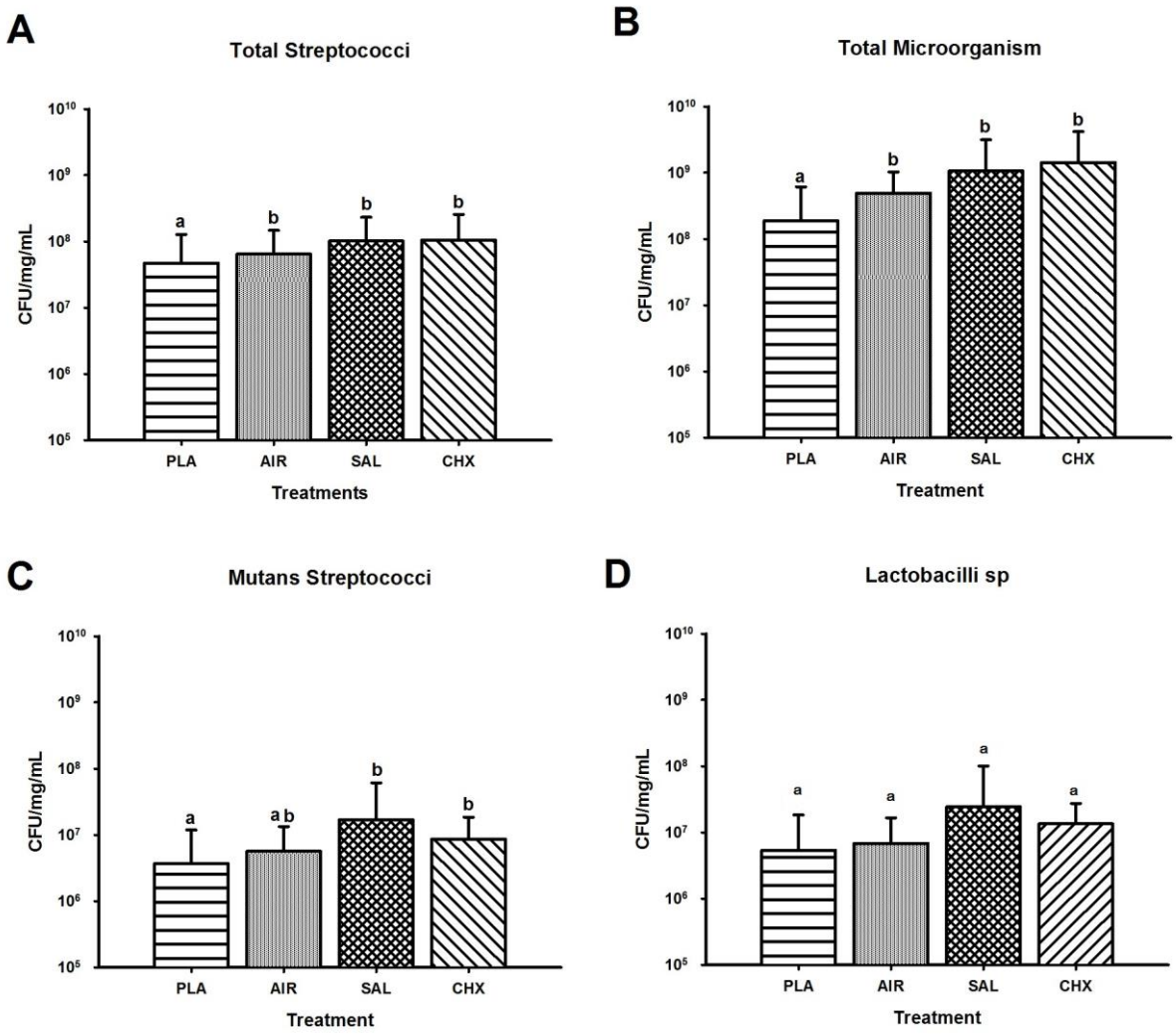

Figure 3 – Effects of the treatments PLA, AIR, SAL and CHX on viability of (A) total streptococci, (B) total microorganisms, (C) *mutans streptococci* and (D) *lactobacilli sp.* Data represent the mean values and error bars represent standard deviations. Data followed by different letters differ statistically ($p < 0.05$).

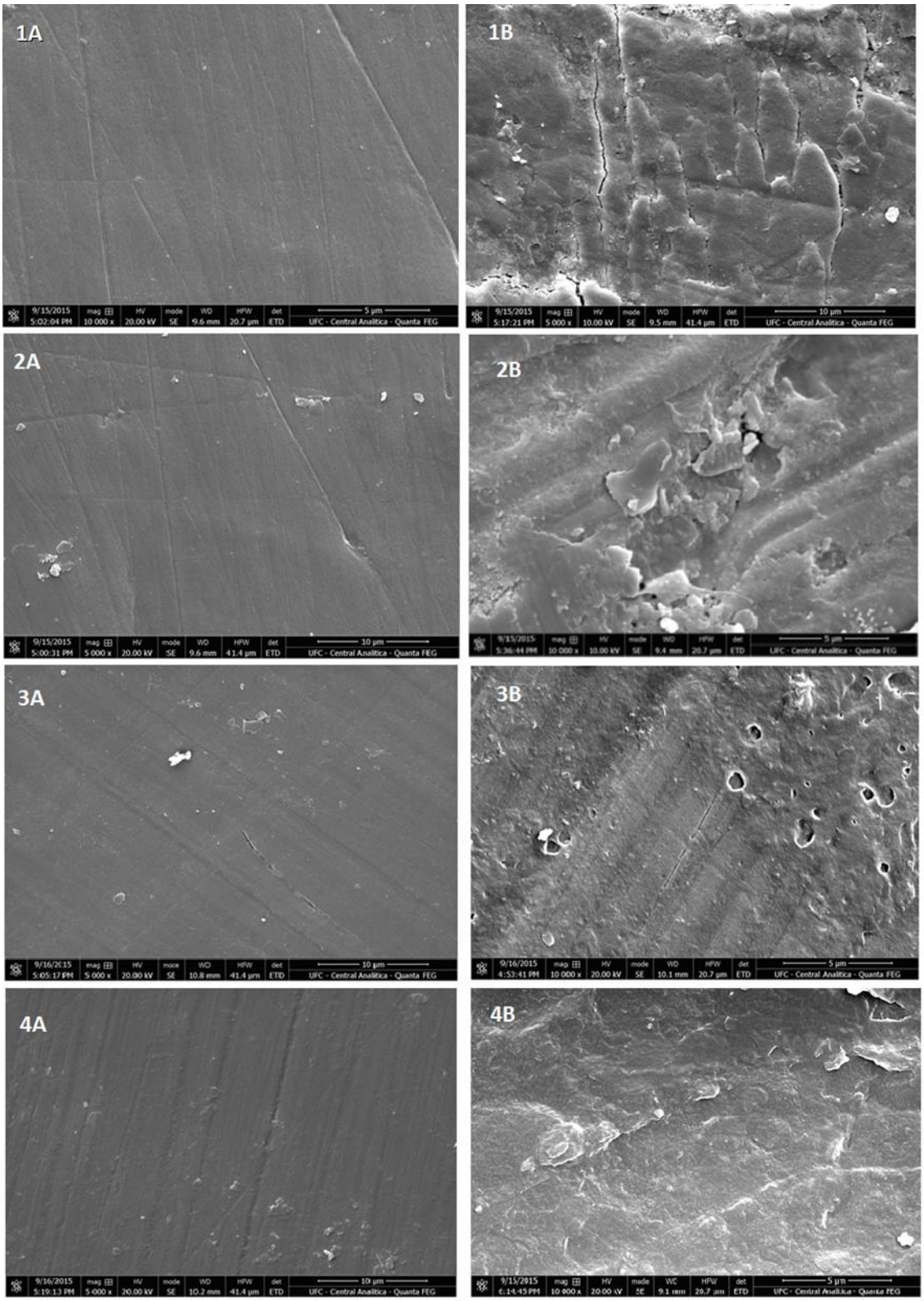

Figure 4 – SEM analysis respectively to PLA, AIR, CHX, SAL treatments at x5000 (1A, 2A, 3A and 4A) and x10000 (1B, 2B, 3B, and 4B) region desmineralised the enamel.

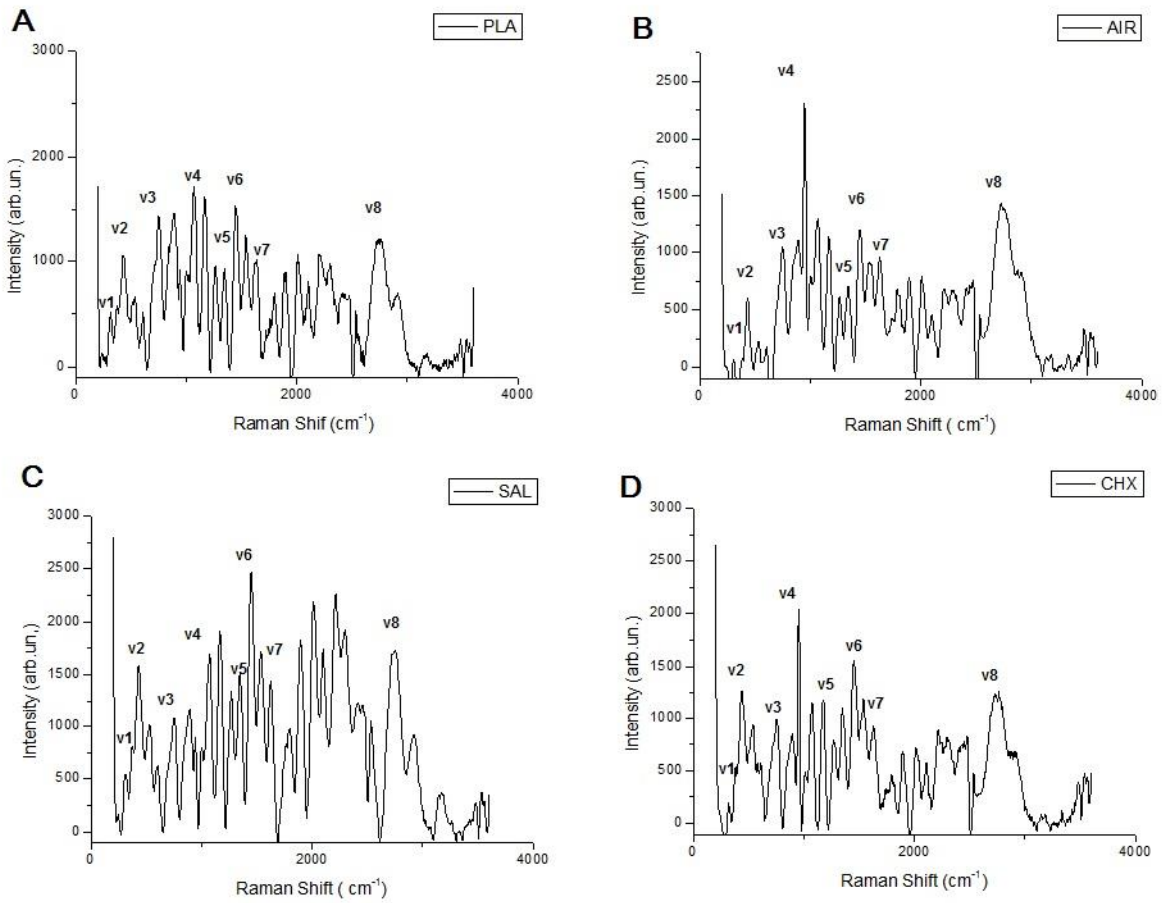

Figure 5: Raman band at v1=324 cm-^{1} characteristic of carbonate vibration, v2=582 cm-^{1}, v3=960 cm^{-1}, v4= 1045 cm^{-1} characteristic of phosphate vibration, v5= 1448 cm^{-1}, v6= 1465 cm^{-1}, v7= 1653 cm-^{1} and v8 = 2943 cm-^{1} phase organic. In (A) PLA; (B) AIR; (C) SAL; (D) CHX treatments.

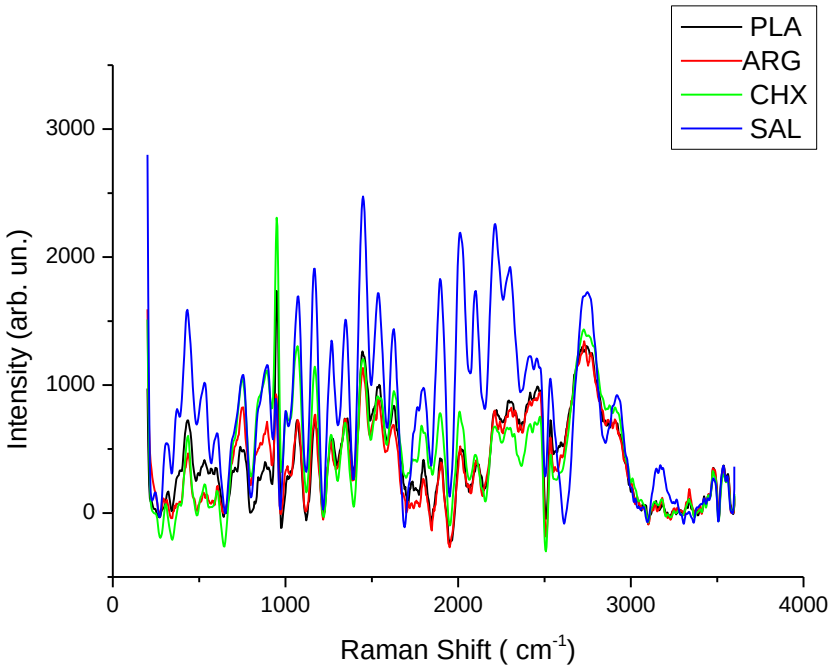

Figure 6 – Raman spectra of treatments plotted together. Treatments PLA (in black), AIR (in red), SAL (in blue) and CHX (in green).

4 CONCLUSÃO

Nas condições testadas o Plasma de Baixa Temperatura foi eficaz na redução da viabilidade de *Streptococcus mutans*, estreptococos totais e microrganismos totais, sem alterar a estrutura do esmalte sob biofilme irradiado.

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ANEXO A – APROVAÇÃO DO COMITÊ DE ÉTICA

UNIVERSIDADE FEDERAL DO CEARÁ/ PROPESQ

PARECER CONSUBSTANCIADO DO CEP

DADOS DO PROJETO DE PESQUISA

Título da Pesquisa: Atividade do plasma de baixa temperatura em biofilmes orais formados in situ
Pesquisador: Karla Shangela da Silva Alves
Área Temática:
Versão: 2
CAAE: 40975514.0.0000.5054
Instituição Proponente: UNIVERSIDADE FEDERAL DO CEARA
Patrocinador Principal: Financiamento Próprio

DADOS DO PARECER

Número do Parecer: 1.000.400
Data da Relatoria: 26/03/2015

Apresentação do Projeto:
Projeto de doutorado de Karla Shangela da Silva Alves sobre a utilizacao do plasma de baixa temperatura (PBT)na terapia anti-placa e anti-carie, ja que esta tecnologia provoca destruicao da matriz extracelular de bacterias e, estudos preliminares demonstraram a sua eficacia sobre biofilme oral maduro. Serao selecionados vinte voluntarios (estudantes do curso de Odontologia da FFOE-UFC) que receberao um dispositivo palatino intra-oral fabricado com metal, esmalte e tela para retencao de bacterias e formacao de biofilme. Apos 7(sete) dias, estes dispositivos serao removidos e tratados, em laboratorio, com terapia PBT. Cada amostra tera uma area irradiada e uma outra nao, esta ultima, considerado o controle. Apos o tratamento, os especimes serao analisados quanto a viabilidade bacteriana, propriedades bioquimicas da matriz do biofilme, determinacao das possiveis alteracoes da superficie do esmalte e caracterizacao molecular da populacao microbiana dos biofilmes tratados pela tecnica do PCR-DGGE. A Analise de Variancia e o teste Tukey serao utilizados tendo o nivel de significancia estabelecido em 5%.

Objetivo da Pesquisa:
Objetivo Primário:
Elucidar e aperfeiçoar uma abordagem única para um agente anti-placa, usando os efeitos sinérgicos do PBT de (i) destruir a matriz extracelular do biofilme maduro e (ii) a ação antimicrobiana, entretanto sem prejudicar o esmalte.

Endereço: Rua Cel. Nunes de Melo, 1127
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ANEXO B – NORMAS DA REVISTA A SER SUBMENTDIDO

KARGER

Caries Research

Contents: all years

Guidelines for Authors
www.karger.com/cre_guidelines

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Aims and Scope

'Caries Research' is an international journal, the aim of which is to promote research in dental caries and related fields through publication of original research and critical evaluation of research findings. The journal will publish papers on the aetiology, pathogenesis, prevention and clinical control or management of dental caries. Papers on health outcomes related to dental caries are also of interest, as are papers on other disorders of dental hard tissues, such as dental erosion. Aspects of caries beyond the stage where the pulp ceases to be vital are outside the scope of the journal. The journal reviews papers dealing with natural products and other bacterial inhibitors against specific criteria, details of which are available from the Editor.

Submission

Manuscripts written in English should be submitted online:

Online Manuscript Submission

Should you experience problems with your submission, please contact:

Prof. David Beighton
(Editor-in-Chef, Caries Research)
Department of Microbiology
The Henry Wellcome Laboratories for Microbiology and Salivary Research