UNESP - Universidade Estadual Paulista

"Júlio de Mesquita Filho"

Faculdade de Odontologia de Araraquara

Eder Augusto Mastropietro Cavichioli

Fototerapia com luz azul combinada à aplicação de clorexidina 0,12% em um modelo ortodôntico com biofilme cariogênico

Araraquara

2020

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Dissertação apresentada à Universidade Estadual Paulista (Unesp), Faculdade de Odontologia de Araraquara, para obtenção do título de Mestre em Ciências Odontológicas, na área de Ortodontia

Orientador: Prof. Dr. Luiz Gonzaga Gandini Júnior
Coorientadora: Profa. Dra. Beatriz Helena Dias Panariello

Araraquara

2020

Cavichioli, Eder Augusto Mastropietro
Fototerapia com luz azul combinada à aplicação de clorexidina 0,12% em um modelo ortodôntico com biofilme cariogênico / Eder Augusto Mastropietro Cavichioli.-- Araraquara: [s.n.], 2020
51f.; 30 cm.

Dissertação (Mestrado em Ciências Odontológicas) – Universidade Estadual Paulista, Faculdade de Odontologia
Orientador.:Prof. Dr. Luiz Gonzaga Gandini Júnior
Coorientadora: Dra. Beatriz Helena Dias Panariello

1. Ortodontia 2. Fototerapia 3. Streptococcus mutans I.Título

Ficha catalográfica elaborada pela Bibliotecária Marley C. Chiusoli Montagnoli, CRB/5646
Universidade Estadual Paulista (Unesp), Faculdade de Odontologia, Araraquara
Serviço Técnico de Biblioteca e Documentação

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Comissão julgadora

Dissertação para obtenção do grau de Mestre em Ciências Odontológicas

Presidente e orientador: Prof. Dr. Luiz Gonzaga Gandini Júnior

2° Examinador: Profa. Dra. Fernanda Lourenção Brighenti

3° Examinador: Profa. Dra. Mirian Aiko Nakane Matsumoto

Araraquara, 03 de abril de 2020.

DADOS CURRICULARES

Eder Augusto Mastropietro Cavichioli

NASCIMENTO: 22/05/1987 – Araraquara – São Paulo.

FILIAÇÃO: Duvílio Cavichioli Júnior e Magda Regina Mastropietro Cavichioli.

2006 – 2010: Graduação em Odontologia pela Faculdade de Odontologia de Araraquara – Unesp.

2008 – 2010: Membro do Grupo PET-Odonto.

2010 – 2011: Curso de extensão em Ortodontia Preventiva e Interceptativa Extensiva pelo Grupo de Estudos Ortodônticos e Serviços – GESTOS.

2014: Especialização em Ortodontia com realizada no Grupo de Estudos Ortodônticos e Serviços – GESTOS e título obtido pela Faculdade Mozarteum de São Paulo – FAMOSP.

2016: Capacitação em Harmonização Orofacial com ênfase em Toxina Botulínica, Materiais de Preenchimento Orofacial e Fototerapia.

2017: Credenciamento no sistema Invisalign.

2018: Credenciamento no sistema Propel Orthodontics.

2018 – 2020: Cursando Mestrado em Ciências Odontológicas com concentração em Ortodontia na Faculdade de Odontologia de Araraquara – Unesp, com período no exterior como Visiting Scholar na instituição Indiana University School of Dentistry (IUSD).

AGRADECIMENTOS

Aos meus pais que sempre me deram estrutura e apoio para evoluir.

Aos meus orientadorares e coorientadora, por todo suporte necessário. Em especial, para coorientadora *Beatriz Helena Dias Panariello*, que não mediu esforços para que esse trabalho se concretizasse, sempre dando o seu melhor. Sem ela, com certeza, nada disso teria acontecido.

O presente trabalho foi realizado com o apoio da Coordenação de Aperfeiçoamento de Pessoal de Nível Superior – Brasil (CAPES) – Código de financiamento 001.

Cavichioli EAM. Fototerapia com luz azul combinada à aplicação de clorexidina 0,12% em um modelo ortodôntico com biofilme cariogênico [dissertação de mestrado]. Faculdade de Odontologia de Araraquara - Unesp; 2020.

RESUMO

Aparelhos ortodônticos fixos criam áreas de estagnação para biofilmes dentários e dificultam a limpeza dos dentes; portanto, existe o risco de desenvolver lesões incipientes de cárie durante o tratamento ortodôntico. O objetivo deste estudo é verificar se a aplicação de luz azul antes da clorexidina 0,12% no esmalte, bráquete e elástico ortodôntico reduziria ou inibiria os biofilmes maduros de *Streptococcus mutans* e seu crescimento nesses substratos 24 horas após a aplicação dos tratamentos; e se esse tratamento interfere na adesão do bráquete ao esmalte. Biofilmes de *S. mutans* UA159 foram formados por 5 dias sobre amostras compostas por esmalte bovino, bráquete ortodôntico e elástico ortodôntico. Em seguida, as amostras foram tratadas com NaCl 0,89% por 1 minuto (NaCl), luz azul por 12 minutos (BL), clorexidina 0,12% por 1 minuto (CHX) e luz azul por 12 minutos seguido da aplicação de clorexidina 0,12% por 1 minuto (BL+CHX). O acúmulo de biofilme imediatamente após os tratamentos e 24 horas após os tratamentos foram avaliados por unidades formadoras de colônias (CFU) e peso seco (DW). O pH do meio foi medido no quinto dia e sexto dia. A formação de biofilme nas amostras após os tratamentos (Imediato) e no recrescimento (Regrowth) foi avaliada visualmente por microscopia confocal de varredura a laser (CLSM). O teste de adesão (SBS) entre o bráquete e o esmalte foi feito após as amostras serem termocicladas por 500 ciclos (5°C e 55°C), tratadas e termocicladas novamente nas mesmas condições. O teste de adesão (N/mm²) foi feito com uma máquina de teste universal com uma velocidade de 1 mm/min. Após 5 dias de formação do biofilme, o tratamento com BL+CHX reduziu significativamente a viabilidade bacteriana no esmalte em comparação com o NaCl ($p = 0,004$) e BL ($p = 0,014$). Para o bráquete e o elástico, todos os tratamentos resultaram em viabilidade bacteriana semelhante ($p \geq 0,081$). No recrescimento, CHX e BL+CHX reduziram significativamente a viabilidade bacteriana no esmalte em comparação com o NaCl ($p \leq 0,015$) e BL ($p \leq 0,013$). Para o bráquete, BL+CHX reduziu significativamente a viabilidade bacteriana em comparação com NaCl ($p = 0,008$) e BL ($p = 0,009$). Para o elástico, BL+CHX eliminou o biofilme do substrato. CHX e BL+CHX reduziram significativamente a viabilidade bacteriana 24 horas após o tratamento para todos os substratos ($p \leq 0,05$). O pH do meio aumentou significativamente quando as amostras foram tratadas com CHX e BL+CHX ($p \leq 0,001$). Imagens da CLSM mostraram maior quantidade de células mortas nas amostras tratadas com BL+CHX. Não houve diferença no SBS entre os tratamentos ($p \geq 0,932$). A associação entre BL+CHX reduziu o biofilme de *S. mutans* e seu recrescimento em um modelo ortodôntico in vitro e não influenciou na resistência de adesão entre bráquete e esmalte.

Palavras chave: Ortodontia. Fototerapia. *Streptococcus mutans*.

Cavichioli EAM. Blue-light phototherapy combined with 0.12% chlorhexidine on an orthodontic cariogenic biofilm model [dissertação]. Faculdade de Odontologia de Araraquara - Unesp; 2020.

ABSTRACT

Fixed orthodontic appliances create areas of stagnation for dental biofilms and make it difficult to clean the teeth; therefore, there is a risk of developing incipient lesions of caries during the orthodontic treatment. The objective of this study is to verify if the application of blue light prior to 0.12% chlorhexidine (CHX) on enamel, orthodontic brackets and elastics would reduce or inhibit mature *Streptococcus mutans* biofilms and their regrowth on these substrates 24 h after the application of the treatment; and if this treatment would interfere with bracket adhesion to the enamel. Biofilms of S. mutans UA159 were formed for 5-days over samples composed by a bovine enamel, an orthodontic bracket and an orthodontic elastic. Then, the samples were treated with 0.89% NaCl for 1 minute (NaCl), blue light for 12 minutes (BL), 0.12% chlorhexidine for 1 minute (CHX) and BL for 12 min + 0.12% CHX for 1 min (BL+CHX). Biofilm accumulation immediately after treatments and 24-h after treatments (regrowth) were evaluated by colonies forming units (CFU) and dry weight (DW). The pH of the spent media was measured on the 5th and 6th day. Biofilm formation on the samples after the treatments and on the regrowth was visually evaluated by confocal laser scanning microscopy (CLSM). Shear bond strength (SBS) between bracket and enamel was evaluated after specimens were thermocycled for 500 cycles (5° and 55 °C), treated and thermocycled again in the same conditions. Shear forces (N/mm^2) were applied to the specimens with a universal testing machine at a crosshead speed of 1 mm/min. After 5 days of biofilm formation BL+CHX significantly reduced the bacterial viability on Enamel compared to NaCl ($p=0.004$) and BL ($p=0.014$). For Bracket and Elastic, all the treatments resulted in similar bacterial viability ($p\geq0.081$). In the regrowth, CHX and BL+CHX significantly reduced the bacterial viability in the Enamel compared to the NaCl ($p\leq0.015$) and BL ($p\leq0.013$). For Bracket, BL+CHX significantly reduced the bacterial viability compared to NaCl ($p=0.008$) and BL ($p=0.009$). For the Elastic, BL+CHX eliminated the biofilms from the substrate. CHX and BL+CHX significantly reduced the bacterial viability 24 h after treatment for all substrates ($p\leq0.05$). The media pH significantly increased when samples were treated with CHX and BL+CHX ($p\leq0.001$). CLSM images showed greater amount of dead cells in the samples treated with BL+CHX. There was no difference on the SBS between the treatments ($p\geq0.932$). The association between BL and CHX reduced S. mutans biofilm and its regrowth on an in vitro orthodontic model and did not influence on the bonding strength between bracket and enamel.

Keywords: Orthodontics. Phototherapy. *Streptococcus mutans*.

SUMÁRIO

1 INTRODUÇÃO

Várias doenças em humanos são causadas por biofilmes, incluindo aquelas que ocorrem na boca. Entre elas, a cárie continua sendo uma das doenças orais mais presentes que comprometem a saúde e o bem-estar de crianças e adultos[1]. Esta doença resulta de interações complexas entre microrganismos orais específicos, fatores do hospedeiro e dieta, que promovem o estabelecimento de biofilme cariogênico na superfície dos dentes[2,3]. Os aparelhos ortodônticos criam áreas de estagnação para o biofilme dental e dificultam a limpeza dos dentes. As superfícies irregulares dos bráquetes ortodônticos limitam o mecanismo natural de autolimpeza feitos pela musculatura oral e saliva[4]. A diversidade de dispositivos utilizados em aparelhos ortodônticos pode promover alterações específicas no ambiente bucal, como pH ácido, aumento da adesão de microrganimos e desenvolvimento de biofilme[5,6]. O aumento da proliferação de bactérias facultativas, incluindo o *Streptococcus mutans*, leva a uma diminuição do pH que inclina o equilíbrio desmineralização-remineralização em direção à perda mineral que, por sua vez, pode levar ao desenvolvimento de lesões de mancha branca e, eventualmente, a cárie com cavitação[7,8].

O risco de desenvolver lesões cariosas incipientes durante o tratamento ortodôntico não deve ser subestimado pelos ortodontistas[9]. Com base em registros fotográficos antes e após o tratamento ortodôntico, um estudo mostrou uma alta incidência de lesões de mancha branca em pacientes após o tratamento ortodôntico (72,9%), e a incidência de lesões cavitadas nessa população foi de 2,3%[9]. Sexo, idade e higiene bucal no início do tratamento não foram associados ao desenvolvimento da lesão, mas uma associação significativa foi evidenciada com o aumento da duração do tratamento[9]. O problema do desenvolvimento de lesões de mancha branca é um desafio alarmante e merece atenção significativa de pacientes e profissionais, o que deve resultar em maior ênfase na prevenção eficaz da cárie[9]. Os métodos preventivos de cárie incluem instruções sobre higiene bucal e hábitos alimentares, bem como a prescrição de dentifrícios com flúor (≥ 1000 ppm) e enxaguatórios bucais[10]. No entanto, é necessário implementar um programa preventivo de higiene bucal para pacientes ortodônticos, pois a higiene bucal é mais difícil de manter quando há bráquetes, fios e outros acessórios[11,12]. Nesse contexto, o controle efetivo do biofilme dental por métodos mecânicos sofre algumas limitações em pacientes com aparelhos

ortodônticos fixos[13,14]. Assim, deve ser considerado o importante papel dos agentes químicos utilizados para melhorar a saúde bucal dos pacientes em tratamento ortodôntico[15].

A clorexidina (CHX) tem sido amplamente utilizada como agente tópico na descontaminação de cáries e mucosa bucal devido seus excelentes resultados contra muitas bactérias orais[16], sendo considerada padrão ouro no controle químico dessas bactérias[17]. No entanto, a eficácia da CHX é reduzida contra bactérias organizadas em biofilmes[18,19]. Isso pode ocorrer devido a interações iônicas entre a matriz de exopolissacarídeos (EPS) carregada negativamente, que compreende a maior parte do volume do biofilme e as moléculas de CHX carregadas positivamente[18]. À medida que a CHX catiônica interage com a matriz de EPS aniônica, a carga da matriz torna-se neutra, reduzindo as forças de repulsão entre as partes carregadas e permitindo maiores aproximações entre as cadeias poliméricas, o que leva a redução do volume ocupado pelo biofilme[18]. Uma matriz mais compacta pode inibir a difusão de solutos no biofilme, incluindo a própria CHX[18].

A terapia antimicrobiana fotodinâmica tem sido indicada como uma alternativa às terapias convencionais para o tratamento de doenças bucais de origem microbiana. Essa terapia é baseada no uso de fotosensibilizadores que iniciam uma resposta fotoquímica quando expostos à luz de um comprimento de onda específico. Em um estudo que investigou os efeitos antimicrobianos das luzes azul (400-440 nm) e vermelha (570-690 nm) em combinação com fotossensibilizadores curcumina e azul de toluidina em *S. mutans*, observou que as luzes em combinação com os fotossensibilizadores promoveram a inativação das células planctônicas e *S. mutas* em tempos de iluminação muito curtos[20]. No entanto, a principal limitação da terapia fotodinâmica antimicrobiana é o desafio do fotossensibilizador de penetrar na profundidade do biofilme[20]. Estudos que avaliam a eficácia da terapia fotodinâmica antimicrobiana em *S. mutans* sugerem que a terapia é eficaz e promove a erradicação dos microrganismos em sua forma planctônica[21]; no entanto, a erradicação de microrganismos organizados em biofilmes ainda não foi observada. Provavelmente, isso se deve ao efeito protetor da matriz de EPS, que dificulta a ação do agente fotossensibilizador. Assim, a fototerapia com luz azul sem a presença de um fotossensibilizador parece ser uma alternativa promissora à terapia fotodinâmica antimicrobiana para superar esse problema[22]. A fototerapia sem fotossensibilizadores exógenos demonstrou que *S. mutans* perderam a capacidade de se reorganizar em

biofilme após exposição à luz azul (68-680 J/cm^2; 400 a 500 nm)[23]. Além disso, foi demonstrado que a aplicação de luz azul (72 J/cm^2; 400-440 nm) duas vezes ao dia impedia o desenvolvimento a matriz de EPS nos biofilmes de *S. mutans*[22]. Assim, pode-se hipotetizar que a associação da luz azul, que reduz significativamente a matriz de EPS do biofilme de *S. mutans*, possa facilitar a penetração da CHX.

Uma força de adesão confiável entre bráquete e dente é essencial para o sucesso do tratamento ortodôntico[24]. Reynolds[25] descobriu que uma força de adesão entre 5,9 e 7,8 Mpa é adequada para manter a adesão do bráquete ao dente. A queda dos bráquetes ortodônticos pode frustar o clínico, afetar significativamente a eficiência do tratamento e ter um impacto financeiro no tratamento[26] e, portanto, devemos fazer o possível para evitá-lo. Os efeitos das aplicações de CHX na adesão dos bráquetes aos dentes foi avaliado anteriormente[27,28], mostrando que a CHX não interfere na adesão. Por outro lado, foi demonstrado que a aplicação de Laser de Diodo (445 nm) por 15 segundos na base do bráquete reduziu significativamente os valores de SBS[29]. No entando, até onde sabemos, nenhum estudo avaliou os efeitos da luz azul combinada com clorexidina no SBS dos bráquetes ortodônticos.

A prática clínica requer uma alternativa fácil, rápida e eficaz para reduzir o biofilme de *S. mutans* em pacientes em tratamento ortodôntico com aparelhos fixos. A aplicação de luz azul antes da aplicação de clorexidina 0,12% na redução do biofilme de *S. mutans* nos dentes, bráquetes ortodônticos e elásticos ainda não foi estudada. Portanto, o objetivo deste estudo é avaliar o acúmulo de biofilme de *S. mutans* no esmalte, bráquete e elástico para verificar se a aplicação de luz azul antes da clorexidina 0,12% reduz ou inibie a formação do biofilme e seu recrescimento nesses substratos após 24 horas. Além disso, objetivamos analisar se a aplicação desses tratamentos interferiria na adesão do bráquete ao esmalte. Os resultados deste estudo podem ajudar os ortodontistas a prevenir lesões de cárie em seus pacientes, principalmente naqueles com maior risco de desenvolvimento de cárie. Além disso, os resultados obtidos com a aplicação da luz azul associada à clorexidina 0,12% nos bráquetes e elásticos podem representar uma alternativa promissora, prática e rápida de ser realizada no consultório odontológico para redução de *S. mutans* nos pacientes durante o tratamento ortodôntico.

2 PROPOSIÇÃO

1. Verificar se a aplicação de luz azul (fototerapia) anteriormente à clorexidina 0,12% é eficaz na descontaminação de bráquete, elástico e esmalte.
2. Avaliar se os tratamentos aplicados não interferem na adesão do bráquete ao esmalte.

3 PUBLICAÇÕES

3.1 Publicação 1*

Association between two therapies on *Streptococcus mutans* biofilm and its regrowth on an *in vitro* orthodontic model.

Eder A. M. Cavichioli[1], Beatriz H. D. Panariello[2], Sabrina Feitosa Sochacki[3], Luiz Gonzaga Gandini Junior[1], Simone Duarte*[2]

[1] Department of Orthodontics and Pediatric Dentistry, School of Dentistry at Araraquara, UNESP São Paulo State University, Araraquara, São Paulo, Brazil

[2] Department of Cariology, Operative Dentistry and Dental Public Health, Indiana University School of Dentistry, Indianapolis, Indiana, USA

[3] Department of Biomedical Sciences and Comprehensive Care, Indiana University School of Dentistry, Indianapolis, Indiana, USA

*Corresponding Author
Simone Duarte
Department of Cariology, Operative Dentistry and Dental Public Health
Indiana University School of Dentistry
1121 W. Michigan Street, DS 406
Indianapolis, IN, 46202, USA
Tel: 317-274-5437
E-mail: siduarte@iu.edu

* O artigo segue as normas da *American Journal of Orthodontics and Dentofacial Orthopedics*, para o qual submetemos para publicação.

ABSTRACT

Fixed orthodontic appliances create areas of stagnation for dental biofilms and make it difficult to clean the teeth; therefore, there is a risk of developing incipient caries lesions during the orthodontic treatment. The objective of this study is to determine if the combination of two different therapies, phototherapy by blue light and the antimicrobial 0.12% chlorhexidine (CHX) on enamel, orthodontic brackets and elastics would reduce or inhibit mature *Streptococcus mutans* biofilms and their regrowth on these substrates 24 h after the application of the treatment. Biofilms of *S. mutans* UA159 were formed for 5-days over samples composed by a bovine enamel, an orthodontic bracket and an orthodontic elastic. Then, the specimens were treated with 0.89% NaCl for 1 min (NaCl), blue light for 12 min (72 J/cm^2) (BL), 0.12% chlorhexidine for 1 min (CHX) and BL for 12 min followed by 0.12% CHX for 1 min (BL+CHX). Biofilm were evaluated by colonies forming units (CFU) and dry weight (DW) immediately after treatments and 24-h after treatments (regrowth). The pH of the spent media was measured on the 5th and 6th day. Biofilm formation on the samples after the treatments and on the regrowth was visually evaluated by confocal laser scanning microscopy (CLSM). After 5 days of biofilm formation BL+CHX significantly reduced the bacterial viability on enamel compared to NaCl ($p=0.004$) and BL ($p=0.014$). For bracket and elastic, all the treatments resulted in similar bacterial viability ($p\geq0.081$). In the regrowth, CHX and BL+CHX significantly reduced the bacterial viability in the enamel compared to the NaCl ($p\leq0.015$) and BL ($p\leq0.013$). For bracket, BL+CHX significantly reduced the bacterial viability compared to NaCl ($p=0.008$) and BL ($p=0.009$). For the elastic, BL+CHX eliminated the biofilms from the substrate. CHX and BL+CHX significantly reduced the bacterial viability 24 h after treatment for all substrates ($p\leq0.05$). The media pH significantly increased when samples were treated with CHX and BL+CHX ($p\leq0.001$). CLSM images visually showed an abundant quantity of red cells in the samples treated with BL+CHX. The association between BL and CHX reduced *S. mutans* biofilm and its regrowth on an *in vitro* orthodontic model.

Keywords: Biofilms; Orthodontics; *Streptococcus mutans;* enamel; Orthodontic brackets; Orthodontic elastics;

1. INTRODUCTION

Several diseases in humans are caused by biofilms, including those that occur in the mouth. Amongst them, dental caries continue to be one of the most present and costly biofilm-dependent oral diseases that compromise the health and well-being of children and adults[1]. This disease results from complex interactions between specific oral microorganisms, host factors and diet, which promote the establishment of cariogenic biofilms on the surface of teeth[2,3]. Fixed orthodontic appliances create areas of stagnation for the dental biofilm and make it difficult to clean the teeth. The irregular surfaces of orthodontic brackets limit the natural self-cleaning mechanism of oral musculature and saliva[4]. The diversity of devices used in orthodontic appliances can promote specific alterations in the oral environment, such as acid pH, increased adhesion of microorganisms and development of biofilm[5,6]. Increased proliferation of facultative bacterial populations, including *Streptococcus mutans*, leads to a decrease in pH that tilts the demineralization-remineralization equilibrium toward mineral loss which, in turn, can lead to the development of white spot lesions and, eventually, to cavitation and caries that extends to the dentine[7,8].

The risk of developing incipient carious lesions during orthodontic treatment should not be underestimated by orthodontists[9]. Based on photographic records before and after orthodontic treatment, a study showed a high incidence of white spot lesions in patients after orthodontic treatment (72.9%), and the incidence of cavitated lesions in this population was 2.3 %[9]. Sex, age and oral hygiene at the start of treatment were not associated with lesion development, but a significant association was evidenced with increased treatment duration[9]. The problem of developing white spot lesions is an alarming challenge and deserves significant attention from patients and professionals, which should result in greater emphasis on effective caries prevention[9]. Caries preventive methods include instructions on oral hygiene and eating habits, as well as the prescription of fluoride dentifrices (≥ 1000 ppm) and mouthwashes[10]. However, it is necessary to implement a preventive oral hygiene program for orthodontic patients, since oral hygiene is more difficult to maintain when brackets, wires and other accessories are present[11,12]. In this context, the effective control of dental biofilm by mechanical methods suffers some limitations in patients with fixed orthodontic appliances[13,14]. Thus, the important role of chemical agents used to improve the oral health of patients undergoing orthodontic treatment should be considered[15].

Chlorhexidine (CHX) has been widely used as a topical agent in the decontamination of dental cavities and buccal mucosa due to its excellent results against many oral bacteria[16], being considered gold standard in the chemical control of these bacteria[17]. However, the efficacy of CHX is reduced against bacteria organized in biofilms[18,19]. This may occur due to ionic interactions between the negatively charged exopolysaccharide (EPS) matrix, which comprises the bulk of the biofilm volume, and the positively charged CHX molecules[18]. As the cationic CHX interacts with the anionic EPS matrix, the matrix charge becomes neutral, reducing the repulsion forces between the charged parts and allowing greater approximations between the polymer chains, which lead to the reduction of the volume occupied by the biofilm[18]. A more compact matrix may inhibit the diffusion of solutes in the biofilm, including CHX itself[18].

Photodynamic antimicrobial chemotherapy (PACT) has been indicated as an alternative to conventional therapies for treating oral diseases dependent on microorganisms. This therapy is based on the use of photosensitizers that initiate a photochemical response when exposed to light of an specific wavelength. In a study investigating the antimicrobial effects of blue (400-440 nm) and red (570-690 nm) lights in combination with the curcumin and blue toluidine photosensitizers on *S. mutans*, lights in combination with photosensitizers promoted the inactivation of planktonic cells of *S. mutans* in very short times of illumination[20]. However, the major limitation of PACT is the challenge for the photosensitizer to penetrate the depth of the biofilm[20]. Studies that evaluate the effectiveness of PACT in *S. mutans* indicate that the therapy is effective and promotes eradication of the microorganisms in their planktonic form[21]; however, the eradication of microorganisms organized in biofilms has not yet been observed. This is probably due to the protective effect of the biofilm matrix, which hinders the action of the photosensitizing agent. Thus, blue-light phototherapy without the presence of a photosensitizer appears to be a promising alternative to antimicrobial photodynamic therapy to overcome this problem[22]. Phototherapy without exogenous photosensitizers demonstrated that *S. mutans* lost the ability to reorganize into biofilm after exposure to blue light (68-680 J/cm^2; 400 to 500 nm)[23]. In addition, it was demonstrated that the application of blue light (72 J/cm^2; 400-440 nm) twice daily reduced the amount of insoluble polysaccharides in the EPS matrix in *S. mutans* biofilms[22]. Thus, it can be hypothesized that the association of two therapies, (i) blue light and (ii) CHX can be beneficial. Essentially, application of blue light, which

significantly reduces EPS from the *S. mutans* biofilm matrix, may facilitate the penetration of CHX within the biofilm, since CHX, that is cationic, interacts with the negative charge of the matrix of EPS and diffusion is difficult through biofilm[18].

Clinical practice requires an easy, quick and effective alternative to reduce *S. mutans* biofilm in patients undergoing orthodontic treatment with fixed appliances. The application of blue light prior to the application of 0.12% chlorhexidine on *S. mutans* biofilms on teeth, orthodontic brackets and elastics have not been studied yet. Therefore, the objective of this study is to evaluate the accumulation of *S. mutans* biofilms on enamel, orthodontic brackets and elastics to verify whether the application of blue light prior to chlorhexidine 0.12% reduces or inhibits biofilms and the 24 h regrowth of *S. mutans* on these substrates. Moreover, we aimed to analyze if the application of these treatments would interfere with bracket adhesion to the enamel. The results of this study may help orthodontists to control caries lesions in their patients, especially on those who are at higher risk for caries development. In addition, the results obtained with the application of blue light associated with chlorhexidine 0.12% on brackets and elastics may represent a promising alternative, practical and quick to be carried out in the dental office for the reduction of cariogenic biofilm in patients during treatment orthodontic.

2. MATERIALS AND METHODS

2.1 Light Source

A commercially available, non-coherent blue light (LumaCare Model L-122, Medical Group, Newport Beach,CA) was used in this study with a wavelength of 420 nm, spot size of 113.1 mm^2, and fixed output of 95.5 mW/cm^2.

2.2 Preparation of the specimens for microbiological tests

The bovine enamel chips used in this study were acquired from the Indiana University (IU) Oral Health Research Institute (OHRI). All chips were prepared using a standardized jig and received a final dimension of 5 x 5 x 5 millimeters. The enamel chips were polished using a sequence of 1200, 2400, 4000 grit polishing carbide paper under water cooling followed by a diamond polishing suspension. After the enamel was treated with 35% phosphoric acid conditioner gel for 30 sec (Potenza Attaco, PHS do Brasil, Joinville, Santa Catarina, Brazil, lot 20092018-2077), rinsed with water for 30 sec and dryed for 5 sec. A small layer of primer (Transbond XT Light Cure Adhesive

Primer, 3M Unitek, Monrovia, CA, USA, lot N982379) was applied for 20 sec using a micro brush, dried for 5 sec and photopolymerized with a 1600 LED mW/cm² polymerization lamp (Smart Lite Max L.E.D. Curing Light, DENTSPLY Caulk, Milford, Detroit, USA) for 10 sec[24]. The intensity of the light was checked (~1750 mW/cm²) with a radiometer (Cure Rite, Curing Light Meter, Dentsply Caulk, Division of Dentsply International Inc., Milford, DE, USA). A small amount of the orthodontic adhesive (Transbond XT Plus, 3M Unitek, Monrovia, CA, USA, lot 1827400502) was applied to the base of the bracket (Kirium, slot 0.022", Abzil 3M, São José do Rio Preto, SP, BR). The bracket was seated on the previously prepared enamel. The excess of resin was removed with a dental explorer. The 1600 LED mW/cm² polymerization lamp was placed at a distance of 2-3 mm above the bracket by mesial and this region was polymerized for 5 sec. This step was repeated for the distal. The enamel chip-bracket substrates were then sterilized in ultrapure water using an autoclave prior to the inoculation with *S. mutans* biofilm. The orthodontic elastics (Alastik Easy-To-Tie Obscure, 3M Unitek, Monrovia, CA) were exposed to UV light for 30 min for decontamination and were attached to the brackets using a sterile dental explorer. Sixty bracket-enamel-elastic substrates were prepared in order to conduct a duplicate study with three separate occasions (n=6) for the treatment and n=6 for the regrowth evaluation. The remaining specimens were utilized for confocal imaging analysis.

2.3 Bacterial Strains, Bacterial Growth, and Biofilm Formation

S. mutans UA159 (ATCC 700610) was obtained from single colonies isolated on blood-agar plates, placed in tryptone yeast-extract broth containing 1% glucose, and incubated for 18 h at 37°C under microaerophilic conditions (5% of CO_2). Biofilms of *S. mutans* UA159 were formed on the enamel, bracket and elastic substrates. The samples were placed in a 24-well culture plate, suspended in 1 mL of medium containing 1% sucrose, and stored at 37°C and 5% CO_2 for a total of 5 days. Fresh medium was replaced once daily. After 5 days, the treatments were performed and part of the samples were processed for CFU and dry-weight quantification, and another part was stored at 37°C under microaerophilic conditions (5% of CO_2) for additional 24-h for the regrowth evaluation. The design of the study is depicted in figure 1.

2.4 Experimental Groups

After 5 days biofilm formation, samples were exposed to 0.89% saline solution (NaCl) for 1 min, chlorhexidine (CHX) for 1 min, non-coherent blue light (BL) – 72 J/cm² of energy density (approximately 12 min) and non-coherent blue light – 72 J/cm² plus chlorhexidine for 1 min (BL+CHX). During the non-coherent blue light exposures, the sample was removed from the media and placed in a new 24-well plate to prevent any overexposure of the other samples to the BL. For all exposures, the light source tip was set at a standard distance of 0.5 cm away from the sample.

2.5 Biofilm Analysis

2.5.1 TREATMENT Group

After the treatment, the samples were separated into enamel, bracket and elastic substrates using a sterile orthodontic debonding plier (Dentronix, Cuyahoga Falls, Ohio) and a dental explorer. Elastics and brackets where placed in 5 mL of sterile 0.89% NaCl solution each. Adherent cells of the bovine enamel chip were harvested by gently scraping the enamel surfaces with a sterile spatula. Harvested cells were then placed in 3 mL of sterile saline solution. The spatula was then rinsed twice with 1 mL of sterile saline and the bovine enamel chip was rinsed once with 1 mL of sterile saline solution, resulting in a total of 5 mL of sterile saline solution. Biofilms were sonicated using three 10-second pulses at an output of 7 W (Fisher Scientific, Sonic Dismembrator model 100, USA). The resulting homogenized suspension was then used for dry weight (DW) and colony forming unit (CFU) analysis.

2.5.2 REGROWTH Group

After the treatment, the samples from the Regrowth group were incubated in fresh TSB+YE+1% sucrose media at 37°C under microaerophilic conditions (5% of CO_2) for additional 24-h for the evaluation of the efficacy of the treatments on biofilm regrowth one day after application. Samples were then separated into enamel, bracket and elastic substrates using a sterile orthodontic debonding plier and a dental explorer and evaluated for CFU and DW, as the TREATMENT group.

2.6 Dry Weight (DW)

Three (3) mL of cold 100% proof ethanol were added to 1 mL of biofilm and the suspension was stored at negative 20°C for at least 18 h. The resulting precipitate was

centrifuged at 10,000 rpm for 10 min at 4°C. The pellet was washed with 1 mL of cold ethanol to precipitate the soluble polysaccharides and centrifuged at 12,000 rpm for 10 min. The resulting supernatant was discarded and the samples were let dry in a desiccator for 7 days and then weighed. All samples were weighed in the pre-weighed centrifuge tubes in which they were dried. The final weights collected were compared to initial centrifuge tube weights resulting in the final biofilm weight.

2.7 Colony Forming Units (CFU)

An aliquot of 0.1 mL of the homogenized suspension was serially diluted (1:10, 1:100, 1:1000, 1:10,000, 1:100,000) and plated onto blood agar and incubated for 48 h (5% CO_2, 37°C). Later, the number of CFU/mL of recovered *S. mutans* were counted and recorded.

2.8 Confocal scanning laser microscope (CSLM)

Confocal scanning laser microscopy was used to visually analyze the live and dead bacterial cells found on the biofilm surface. Samples were stained using a LIVE/DEAD® BacLight™ Bacterial Viability Kit (L13152; Molecular Probes, Inc.) and incubated at room temperature in the dark for 15 min to allow penetration of the fluorophores inside the bacterial cells. Confocal 3D tile scans were acquired with an Olympus FV1000 MPE confocal/2-photon system (Olympus, Center Valley, PA) available at the ICBM Imaging facility, Indianapolis, IN, using Olympus UPlanSApo 4x/0.16 objective lens, 800x800 pixels frame size, and 4s/pixel scanning rate. Images were collected using a sequential illumination scanning mode set up for two channels: 488nm excitation/500-545nm emission, and 559nm excitation/570-670nm emission. Tile scans were stitched into large mosaics using Olympus Fluoview FV10-ASW software (version 04.02.02.09) and 3D rendering images were generated using Imaris software (Bitplane, Concord, MA).

2.9 Statistical Analysis

Normal distribution of data was verified by the Shapiro–Wilk test and homogeneity of variance was checked by the Levene test ($\alpha = 0.05$). The quantitative data of Log_{10} (CFU/mL) and dry weight were statistically analyzed by two-way analysis of variance (ANOVA) considering the different substrates (enamel, bracket and elastic) and the different treatments (NaCl, BL, CHX, BL+CHX). For multiple comparisons Tukey post-

hoc test was applied (α= 0.05). Comparisons between treatment and regrowth regarding the pH, Log_{10} (CFU/mL), DW and SBS were analyzed by one-way ANOVA with Tukey post-hoc test (α= 0.05). Analyses were done in the software SPSS (IBM® SPSS® Statistics, version 26, Chicago, IL).

3. RESULTS

3.1 Colony forming units (CFU)

For treatment after 5 days of biofilm formation (Figure 2), for the Log_{10} (CFU/mL), two-way ANOVA analysis showed factors “substrates” (p=0.009) and “treatments” (p≤0.001) significantly influenced the results. Enamel substrate results are significantly different from bracket (p=0.032) and elastic (p=0.013) results, enamel being more susceptible for biofilm accumulation than bracket and elastic, which showed similar susceptibility for biofilm accumulation (p=0.934). When comparing the same substrates treated with different substances, it was observed that, for enamel, BL+CHX significantly reduced the bacterial viability compared to negative control NaCl (p=0.004) and to BL (p=0.014), and approached CHX values (p=0.087). In contrast, CHX values approached NaCl (p=0.967) and BL (p=0.999). For bracket, all treatments presented similar bacterial viability (p≥0.823), and the same was observed for elastic (p≥0.081).

Concerning regrowth (Figure 3), when samples where incubated and evaluated 24 h after being treated, for Log10 (CFU/mL), two-way ANOVA analysis showed factors “substrates” (p=0.001) and “treatments” (p≤0.001) significantly interfered in the results. Enamel substrate results are significantly different from elastic (p=0.001) and similar to bracket (p=0.055), enamel and bracket being more susceptible for biofilm accumulation then the other substrates. Comparison between the same substrates treated with different substances showed that, for Enamel, CHX and BL+CHX significantly reduced bacterial viability compared to negative control NaCl (p=0.015 and p≤0.001, respectively) and to BL (p=0.013 and p≤0.001, respectively). NaCl and BL values are similar (p=1.000). For Bracket, BL+CHX significantly reduced the bacterial viability compared to NaCl (p=0.008) and BL (p=0.009) and approached CHX results (p=0.989). However, CHX results approached NaCl (p=0.107) and BL (p=0.110). For elastic, BL+CHX eliminated biofilms from the substrate (values equal to

zero), while CHX significantly reduced bacterial viability compared to NaCl (p=0.015) and BL (p=0.013), but did not eliminate biofilms like BL+CHX did.

Comparison between treatment after 5 days of biofilm formation and regrowth 24 h after treatment (6 days) showed that, for all substrates, CHX and BL+CHX significantly reduced the bacterial viability 24 h after treatment (Figures 4, 5 and 6). This trend was not observed for NaCl and BL (p≥0.05). For enamel (Figure 4), when comparing treatment with CHX after 5 days of biofilm formation and regrowth 24 h after treatment the reduction in viability was significant with p≤0.001, as well as when comparing treatment with BL+CHX and results of this treatment after 24 h (p≤0.001). For bracket (Figure 5), treatment with CHX and results of this treatment after 24 h showed significant reduction with p=0.045, for BL+CHX reduction was even more significant, p=0.003. Finally, for elastic (figure 6), comparison between treatment and regrowth with CHX showed significant reduction with p≤0.001, and for BL+CHX bacterial viability was reduced to zero, showing that this treatment eliminated bacteria from elastic 24 h after being applied.

3.2 pH

The pH of the spent media was measured 5 days after biofilm formation and 24 h after application of treatments (Figure 7). Comparison of the pH among treatments after 5 days of biofilm formation and regrowth 24 h after treatments (6 days) showed CHX and BL+CHX significantly increased pH of the media (p≤0.001).

3.3 Dry weight (DW)

Regarding the treatment after 5 days of biofilm formation, for dry weight (Figure 8), two-way ANOVA analysis showed factors “substrates” (p=0.043) and “treatments” (p=0.878) did not influence on the results, which means dry weight is similar between all substrates and all treatments (p≥0.05). For the regrowth (Figure 9), two-way ANOVA analysis showed factors “substrates” (p≤0.001) significantly influenced in results, however, factor “treatments” (p=0.022) did not. Bracket showed significantly less biofilm biomass (dry weight) than enamel and elastic (p≤0.001), while enamel and elastic have similar biofilm biomasses (p=0.288). Treatments are not significantly different between each other concerning the same substrates (p≥0.05).

Comparison among treatments after 5 days of biofilm formation and regrowth 24 h after treatment (6 days) showed that, for bracket (Figure 10), CHX significantly

reduced biofilm biomass 24 h after treatment (p=0.010). For the remaining substrates, there was no significant differences in dry weight between treatment regrowth (Figure 11 and 12).

3.4 Confocal scanning laser microscope (CSLM)

Biofilm accumulation on samples immediately after treatments and 24-h after treatments (regrowth) was visually analyzed by CLSM (Figure 13). Live cells are stained green and dead cells are stained red. Based on a visual analysis, CHX and, specially, BL+CHX, have larger amounts of dead cells present compared to NaCl and BL. These results corroborate with the CFU data.

4. DISCUSSION

Fixed orthodontic appliances create a big challenge for good oral hygiene and provide larger surface area for biofilm adherence and development. The irregular shapes of the appliances also limit self-cleansing capacity of saliva, lips, tongue, and cheeks. These obstacles can lead to increased risk of incipient caries on dental surfaces not usually susceptible to carious occurrence[25]. Consequently, for patients with high risk of caries, there is a risk for the formation of white spots due to enamel demineralization[25]. Because of that, dentists should incorporate caries risk assessment into initial evaluations of orthodontic patients, and risk-specific prevention and management protocols can help to eliminate or minimize this clinical problem[25]. In view of that, oral biofilm control alternatives are needed to complement oral hygiene of high-risk orthodontic patients[15].

Results obtained with the combination between BL and CHX (BL+CHX) either immediately or 24 h after its application showed more efficacy in *reducing S. mutans* viability for most cases, being more efficient than only CHX in case of REGROWTH in elastic, in which it was observed that BL+CHX reduced the viable colony counts to zero. Chlorhexidine is a broad-spectrum antiseptic that acts on gram-positive and gram-negative bacteria and yeast. It has great affinity to the cellular walls of microorganisms, salivary mucopolysaccharides and hydroxyapatite[26]. Its molecule bind to the negatively charged bacterial cell surface, altering and disrupting the integrity of the cell membrane, causing bacterial death[27]. Chlorhexidine was selected as the

test substance because it is the best-characterized and most effective chemical antiplaque agent[17,28] and the concentration selected (0.12%) corresponds to that used clinically for substitutive plaque control[28].

Association between CHX and BL had immediate effects on reduction of *S. mutans* viability on enamel and late effects on reduction of *S. mutans* on enamel, bracket and elastic, observed in the REGROWTH test. This result corroborates with the confocal images. This might have happened due to the disturbance of exopolysaccharides matrix caused by BL, which might have facilitated penetration of CHX into the biofilm. Previous study demonstrated application of BL for approximately 12 min reduced insoluble extracellular polysaccharides levels on matrix of *S. mutans* biofilms[22]. In addition, it was observed that BL negatively affected re-organization of *S. mutans* biofilm[23]. In contrast, CHX has an immediate bactericidal effect, followed by prolonged bacteriostatic action resulting from its adsorption into biofilm-coated enamel surface[28]. The disorganization of *S. mutans* biofilm promoted by BL added to CHX immediate and prolonged antiseptic effects might explain the good results obtained with the combination of BL+CHX. Therefore, we believe that one effect complemented another.

The pH outcomes confirmed our CFU results. Both CHX and BL + CHX were effective in increasing pH of the medium when we compared the immediate TREATMENT group and the REGROWTH group. For both treatments, there was an increase in pH 24 h after their application. Since *S. mutans* metabolizes medium sugars, which makes the medium acidic, the fact that pH has increased indicates there was a reduction of microorganisms in the media. Therefore, increasing pH of the oral environment and dental biofilm may cooperate in changing the microflora toward less acidogenic and/or aciduric strains, contributing to the reductions of the occurrence of caries[25]. In contrast, as already expected, application of NaCl or BL to mature *S. mutans* biofilm did not reduce the bacterial viability immediately after the treatment nor 24 h after treatments. This result is in accordance to the acid pH observed in regrowth after NaCl and BL treatments, indicating there was a large amount of bacteria metabolizing the sucrose in the media. The low-pH milieu at the tooth-biofilm interface stimulates enamel demineralization[29]. It was previously demonstrated that BL itself does not have an antimicrobial effect when compared to chlorhexidine; conversely, its effects are mainly related to reduction of extracellular polysaccharide levels[22,30].

Regarding substrates, enamel showed higher CFU counts then brackets and elastic immediately after TREATMENT. We hypothesize this might have happened because glucans present in biofilm's extracellular matrix enhances local adhesion strength of *S. mutants* on apatitic surfaces[31], such as enamel. Extracellular polysaccharides (EPS) are the key elements of matrix in cariogenic biofilms and are known as critical virulence factors related with dental caries[32,33]. Furthermore, EPS assembly on surfaces increases local accumulation and of microorganisms on teeth and might be responsible for mechanical properties of cariogenic biofilms, for example, adhesive strength and cohesiveness[33].

In the present study we observed dry weight of biofilms formed on enamel, bracket and elastic after NaCl, BL, CHX and BL+CHX immediately after treatments are similar. Dry weight comprehends bacteria and extracellular matrix components. Since extracellular matrix components were not quantified in the present study, it is possible that treatments that reduced CFU counting (CHX and BL+CHX) did not reduce extracellular matrix components, such as polysaccharides, extracellular DNA or proteins[33], but further studies are necessary to evaluate the impact of extracellular matrix components on the biofilm dry weight. In contrast, there was a significant decrease on dry weight of biofilm formed on bracket in the REGROWTH. We hypothesize metal surface of the bracket tested in this study tends to accumulate fewer microorganisms. Stainless steels vary in surface properties like chemistry, topography, roughness and surface energy, which might determine the effect of surface chemistry on bacterial adhesion[34].

5.CONCLUSION

Considering orthodontic treatment is a critical phase for some patients with difficulty in controlling oral hygiene and the good immediate and prolonged results obtained in this study, BL+CHX may be considered an alternative therapy to traditional methods in order to reduce the risk of white spots around the brackets due to site demineralization. However, evidences derived from clinical observations are needed to confirm our findings. In conclusion, association between blue light and chlorhexidine reduced *S. mutans* biofilm and its regrowth on an in vitro orthodontic model**.**

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FIGURES

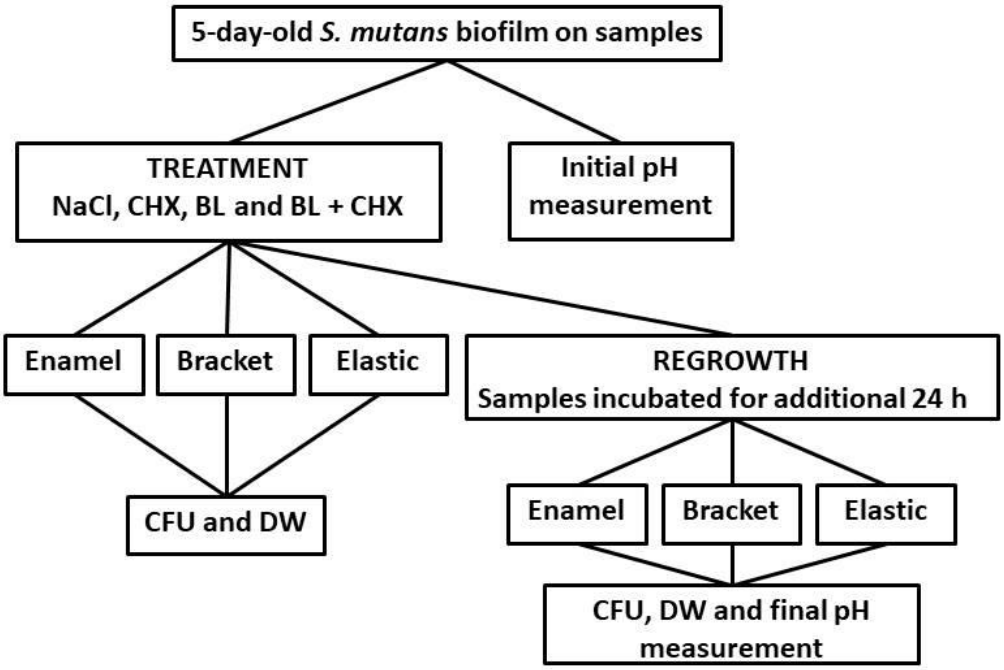

Figure 1. Flowchart of the study design.

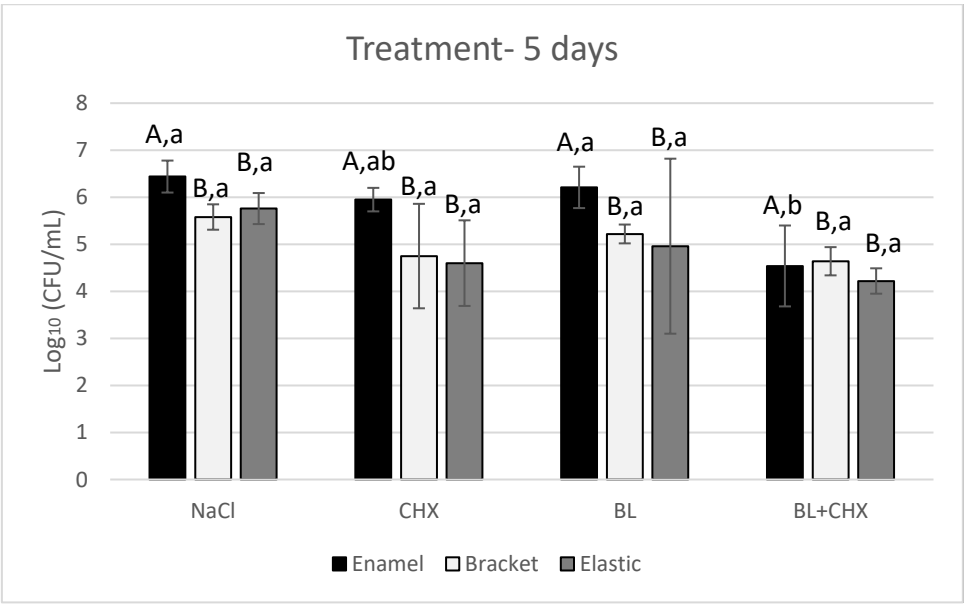

Figure 2. Log_{10} (CFU/mL) mean ± standard deviation for the 4 treatments (NaCl, CHX, BL and BL+CHX) and three substrates (enamel, bracket and elastic) 5 days after biofilm formation (Treatment- 5 days). Uppercase letters depict differences between the substrates, while lowercase letters depict the differences between the treatments in the same substrate. Two-way ANOVA with Tukey post-hoc test was applied ($p \leq 0.05$). Different letters represent statistical difference. *value equal to zero

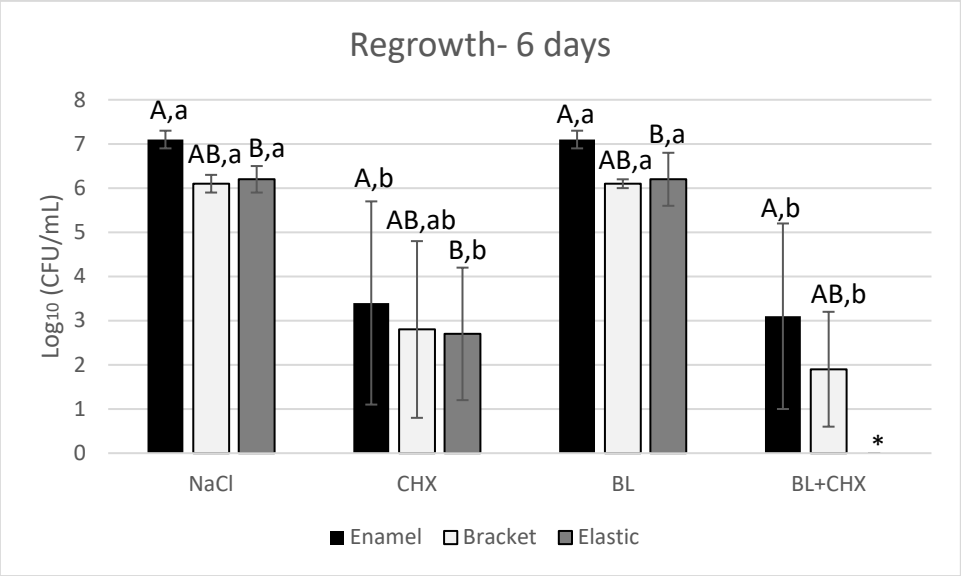

Figure 3. Log_{10} (CFU/mL) mean ± standard deviation for the 4 treatments (NaCl, CHX, BL and BL+CHX) and three substrates (enamel, bracket and elastic) 24 h after application the of the treatments (Regrowth- 6 days). Uppercase letters depict differences between the substrates, while lowercase letters depict the differences between the treatments in the same substrate. Two-way ANOVA with Tukey post-hoc test was applied ($p \leq 0.05$). Different letters represent statistical difference. *value equal to zero

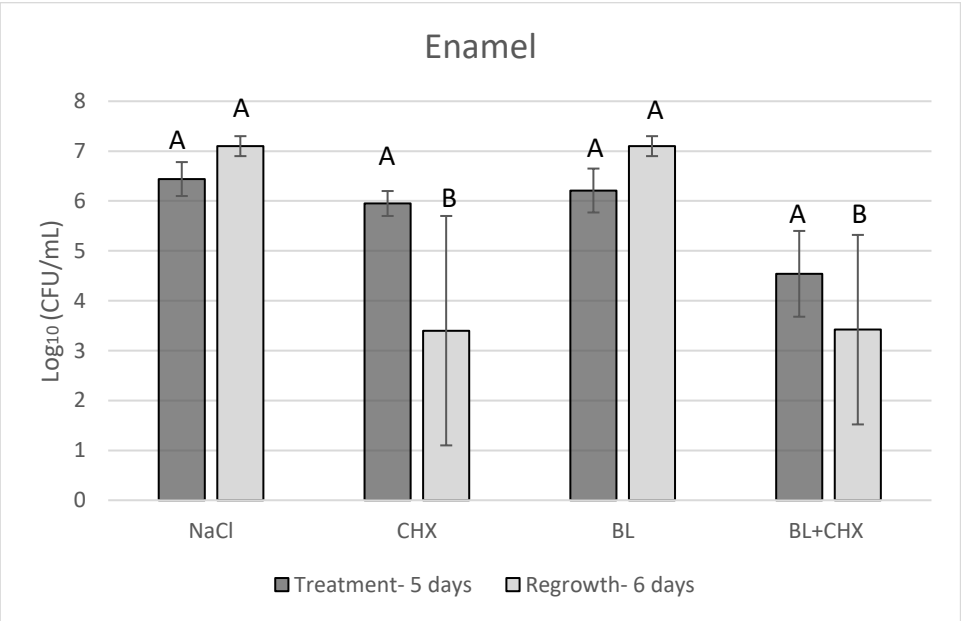

Figure 4. Mean ± standard deviation of Log10(CFU/mL) comparing the same treatment 5 days after biofilm formation and 24 h after the treatment (regrowth- 6 days) on Enamel. Data was analyzed by one-way ANOVA with Tukey post-hoc test ($p \leq 0.05$). Different letters represent statistical difference.

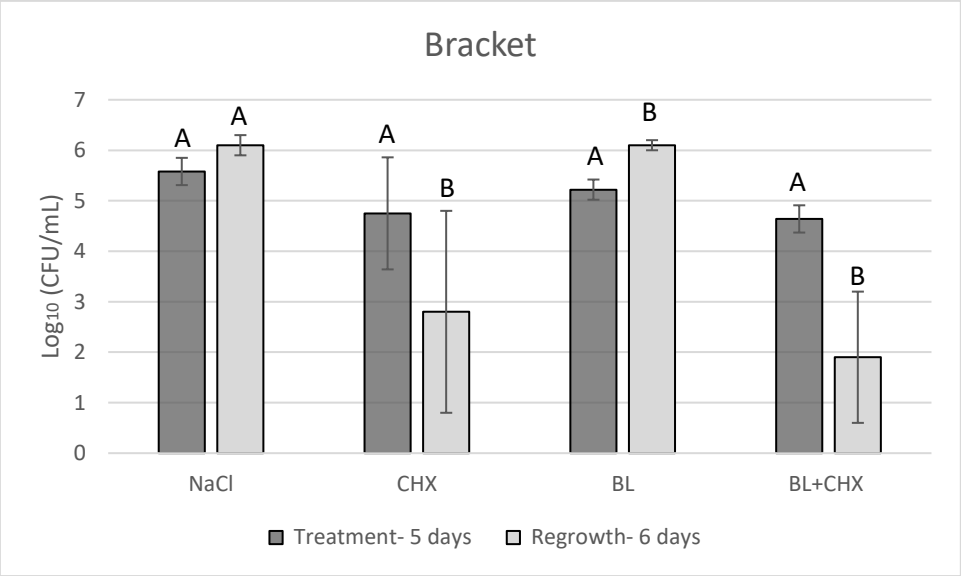

Figure 5. Mean ± standard deviation of Log10(CFU/mL) comparing the same treatment 5 days after biofilm formation and 24 h after the treatment (regrowth- 6 days) on Bracket. Data was analyzed by one-way ANOVA with Tukey post-hoc test (p≤0.05). Different letters represent statistical difference.

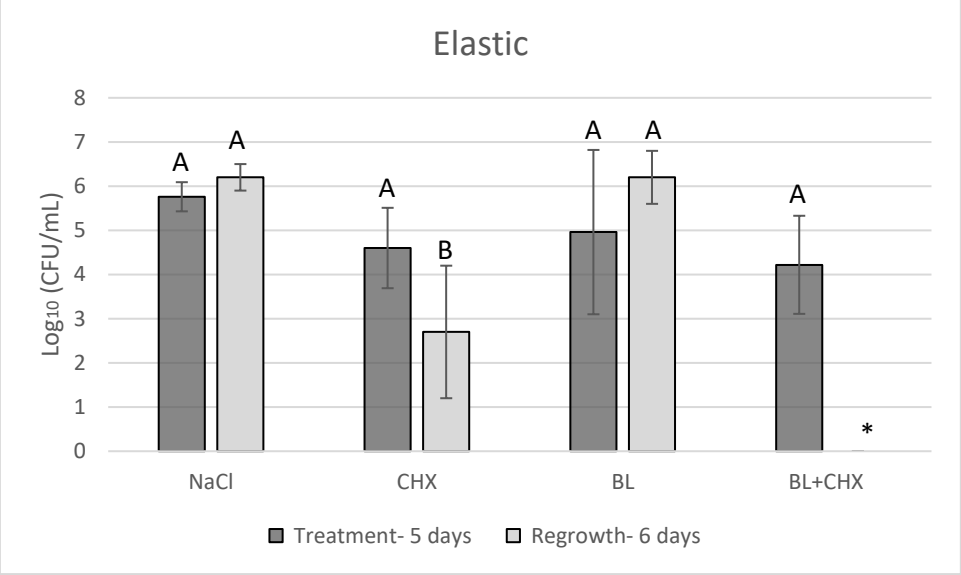

Figure 6. Mean ± standard deviation of Log10(CFU/mL) comparing the same treatment 5 days after biofilm formation and 24 h after the treatment (regrowth- 6 days) on Elastic. Data was analyzed by one-way ANOVA with Tukey post-hoc test (p≤0.05). Different letters represent statistical difference. *value equal to zero

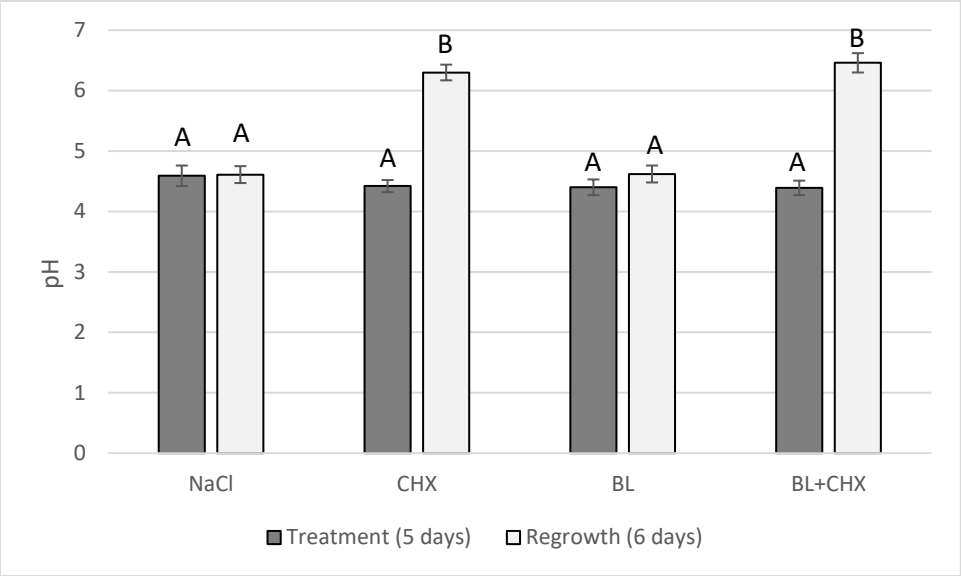

Figure 7. Mean ± standard deviation of pH comparing the same treatment 5 days after biofilm formation and 24 h after the treatment (regrowth- 6 days). Data was analyzed by one-way ANOVA with Tukey post-hoc test (p≤0.05). Different letters represent statistical difference.

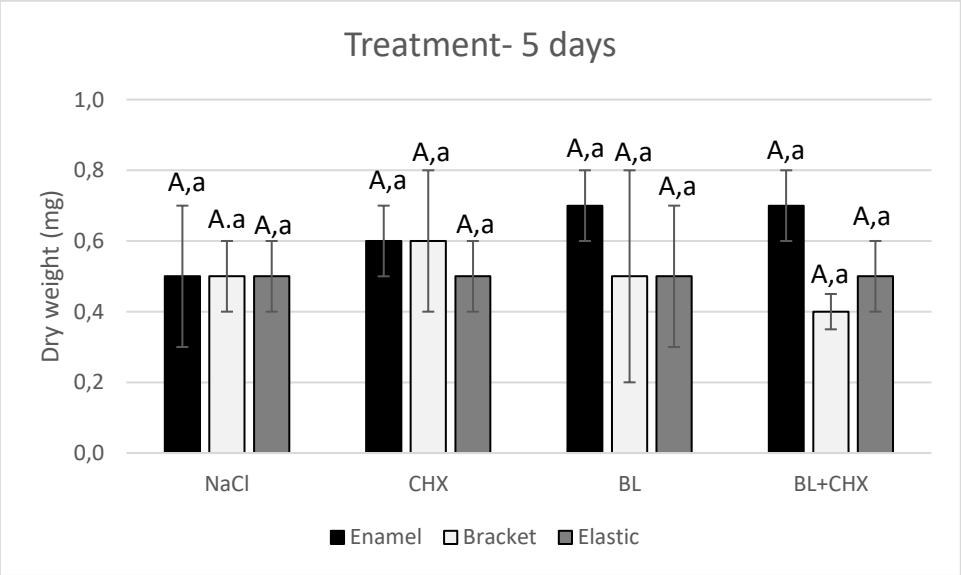

Figure 8. Dry weight mean ± standard deviation for the 4 treatments (NaCl, CHX, BL and BL+CHX) and three substrates (enamel, bracket and elastic) 5 days after biofilm formation (Treatment- 5 days). Uppercase letters depict differences between the substrates, while lowercase letters depict the differences between the treatments in the same substrate. Two-way ANOVA with Tukey post-hoc test was applied (p≤0.05). Different letters represent statistical difference.

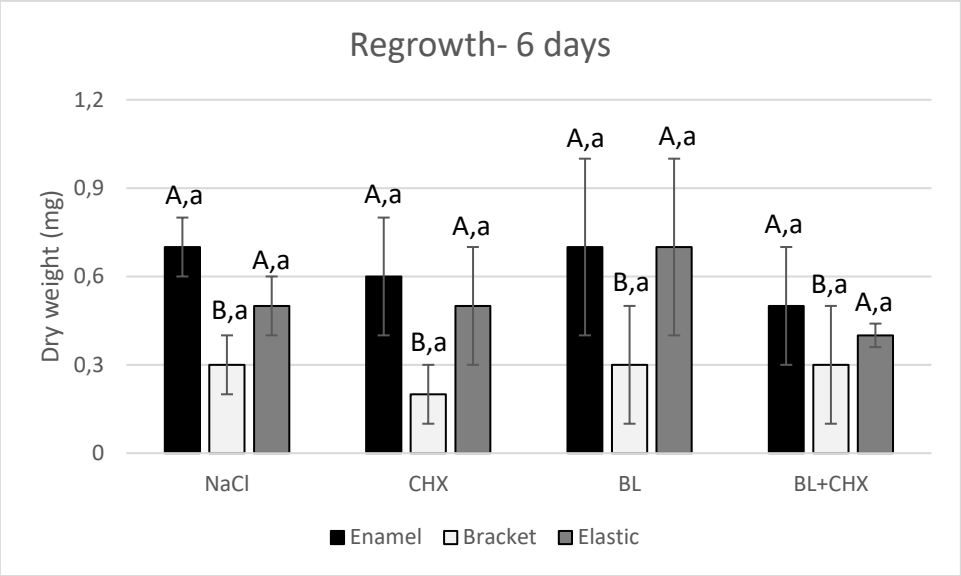

Figure 9. Dry weight mean ± standard deviation for the 4 treatments (NaCl, CHX, BL and BL+CHX) and three substrates (enamel, bracket and elastic) 24 h after the application of the treatments (Regrowth- 6 days). Uppercase letters depict differences between the substrates, while lowercase letters depict the differences between the treatments in the same substrate. Two-way ANOVA with Tukey post-hoc test was applied (p≤0.05). Different letters represent statistical difference.

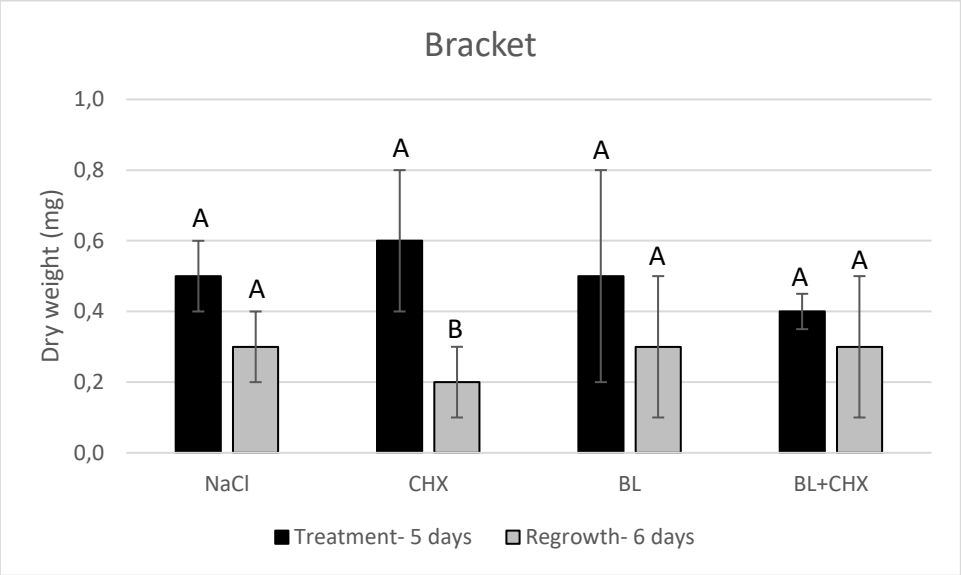

Figure 10. Mean ± standard deviation of dry weight comparing the same treatment 5 days after biofilm formation and 24 h after the treatment (regrowth- 6 days) on Bracket. Data was analyzed by one-way ANOVA with Tukey post-hoc test (p≤0.05). Different letters represent statistical difference.

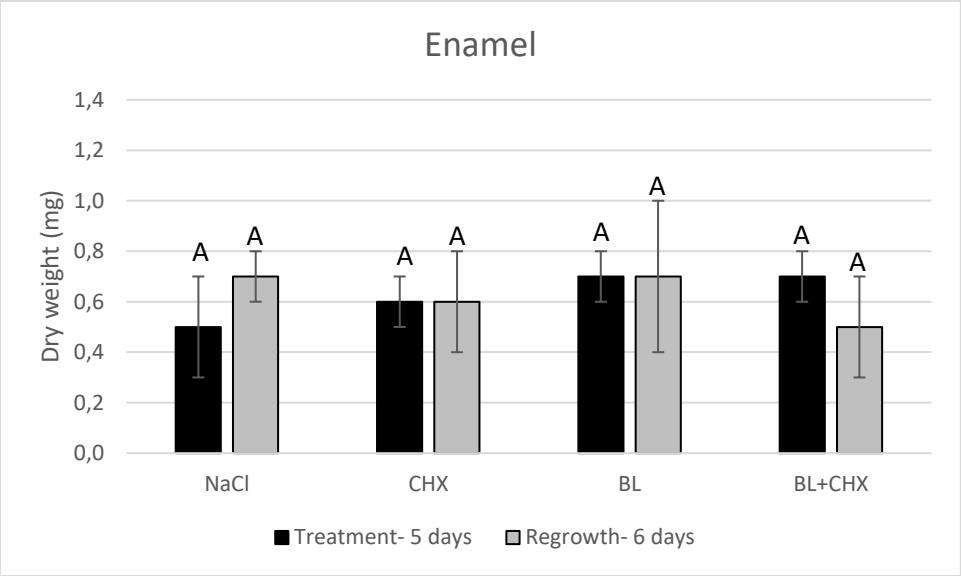

Figure 11. Mean ± standard deviation of dry weight comparing the same treatment 5 days after biofilm formation and 24 h after the treatment (regrowth- 6 days) on Enamel. Data was analyzed by one-way ANOVA with Tukey post-hoc test (p≤0.05). Different letters represent statistical difference. *value equal to zero

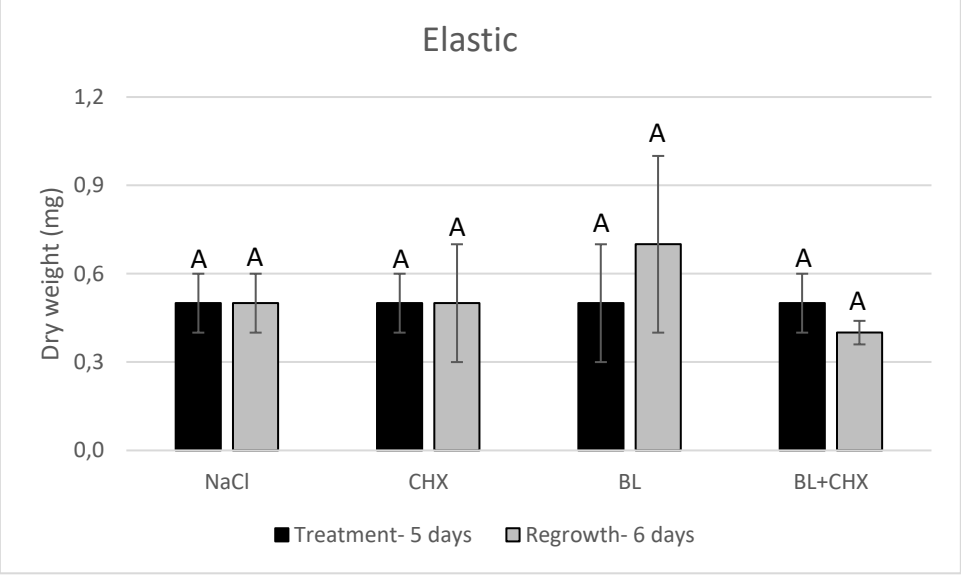

Figure 12. Mean ± standard deviation of dry weight comparing the same treatment 5 days after biofilm formation and 24 h after the treatment (regrowth- 6 days) on Elastic. Data was analyzed by one-way ANOVA with Tukey post-hoc test (p≤0.05). Different letters represent statistical difference.

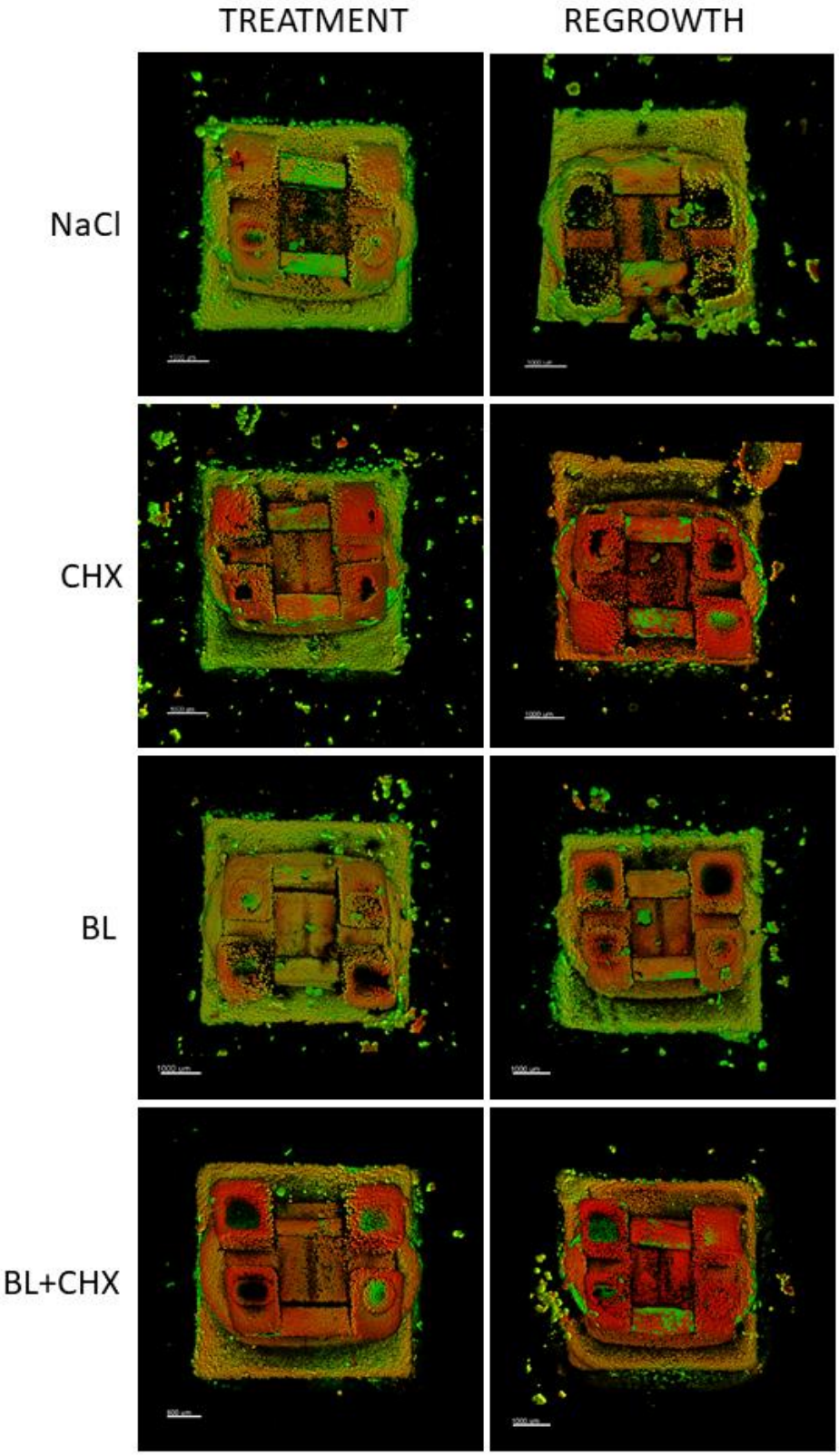

Figure 13. Confocal laser scanning microscopy images showing differences on the amount of live (green) and dead (red) cells on the samples immediately after treatments and 24-h after treatments (regrowth).

3.2 Publicação 2*

Influence of the association between blue light and chlorhexidine on the shear bond strength between bracket and enamel

Eder A. M. Cavichioli[1], Beatriz H. D. Panariello[2], Sabrina Feitosa Sochacki[3], Luiz Gonzaga Gandini Junior[1], Simone Duarte*[2]

[1] Department of Orthodontics and Pediatric Dentistry, School of Dentistry at Araraquara, UNESP São Paulo State University, Araraquara, São Paulo, Brazil

[2] Department of Cariology, Operative Dentistry and Dental Public Health, Indiana University School of Dentistry, Indianapolis, Indiana, USA

[3] Department of Biomedical Sciences and Comprehensive Care, Indiana University School of Dentistry, Indianapolis, Indiana, USA

*Corresponding Author
Simone Duarte
Department of Cariology, Operative Dentistry and Dental Public Health
Indiana University School of Dentistry
1121 W. Michigan Street, DS 406
Indianapolis, IN, 46202, USA
Tel: 317-274-5437
E-mail: siduarte@iu.edu

* O artigo segue as normas da revista *American Journal of Orthodontics and Dentofacial Orthopedics*, para o qual pretende-se submeter.

ABSTRACT

The frequent fall of orthodontic brackets interferes with the course of the orthodontic treatment. In addition, orthodontic brackets creates retention areas for biofilm, therefore, white spots or even carious cavitation lesions may occur, especially in patients at high risk for caries. Thus, alternative therapies are needed for those patients, and a previous study from our group showed that blue light phototherapy followed by 0.12% chlorhexidine is a promising one. The aim of the present study was to verify if the application of blue light prior to 0.12% chlorhexidine (CHX) would interfere with bracket adhesion to the enamel. The specimens were treated with 0.89% NaCl for 1 min (NaCl), blue light for 12 min (BL), 0.12% chlorhexidine for 1 min (CHX) and BL for 12 min + 0.12% CHX for 1 min (BL+CHX). Shear bond strength (SBS) between bracket and enamel was evaluated after specimens were thermocycled for 500 cycles (5° and 55 °C), treated and thermocycled again in the same conditions. Shear forces (N/mm^2) were applied to the specimens with a universal testing machine at a crosshead speed of 1 mm/min. There was no difference on the SBS between the treatments ($p \geq 0.932$). The association between BL and CHX did not influence on the bonding strength between bracket and enamel.

Keywords: Orthodontics*;* Enamel; Orthodontic Brackets; Shear Bond Strength.

1. INTRODUCTION

One of the most common unwanted effects associated with orthodontic treatments is enamel desmineralization around fixed appliances[1]. A study showed that 72.9% of the patients who used orthodontic braces had white spot lesions and 2.3% had cavitated lesions[2]. A significant association was evidenced with increased treatment duration[2]. Instructions on oral hygiene and eating habits, as well as the prescription of fluoride dentifrices (≥ 1000 ppm) and mouthwashes are preventive methods that can be used to avoid developing caries[3]. Patients and professionals need to emphasize caries prevention methods since the development of white spot lesions during the orthodontic phase is an alarming problem[2]. The effective control of dental biofilm by mechanical methods suffers some limitations in patients with fixed orthodontic appliances[4,5], so it is necessary to implement a preventive oral hygiene program for orthodontic patients, since oral hygiene is more difficult to maintain when brackets, wires and other accessories are present[6,7]. Thus, we should considered the important role of chemical agents used to improve the oral health of patients undergoing orthodontic treatment[8].

Photodynamic antimicrobial therapy has been indicated as an alternative to conventional therapies for treating oral diseases of microbial origin. This therapy is based on the use of photosensitizers that initiate a photochemical response when exposed to light of a specific wavelength. In a study investigating the antimicrobial effects of blue (400-440 nm) and red (570-690 nm) lights in combination with the curcumin and blue toluidine photosensitizers on *S. mutans*, it was observed that lights in combination with photosensitizers promoted the inactivation of planktonic cells of *S. mutans* in very short times of illumination[9]. However, the major limitation of antimicrobial photodynamic therapy is the challenge for the photosensitizer to penetrate the depth of the biofilm[9]. Studies that evaluate the effectiveness of antimicrobial photodynamic therapy in *S. mutans* suggest that the therapy is effective and promotes eradication of the microorganisms in their planktonic form[10]; however, the eradication of microorganisms organized in biofilms has not yet been observed. This is probably due to the protective effect of the biofilm matrix, which hinders the action of the photosensitizing agent. Thus, blue-light phototherapy without the presence of a photosensitizer appears to be a promising alternative to antimicrobial photodynamic therapy to overcome this problem[11]. Phototherapy without exogenous

photosensitizers demonstrated that *S. mutans* lost the ability to reorganize into biofilm after exposure to blue light (68-680 J/cm^2; 400 to 500 nm)[12]. In addition, it was demonstrated that the application of blue light (72 J/cm^2; 400-440 nm) twice daily prevented the development of the EPS matrix in *S. mutans* biofilms[11]. Thus, it can be hypothesized that the association of blue light, which significantly reduces EPS from the *S. mutans* biofilm matrix, may facilitate the penetration of CHX by biofilm, since CHX, that is cationic, interacts with the negative charge of the matrix of EPS and diffusion is difficult through biofilm[13].

A reliable bonding force between bracket and tooth is essential for successful orthodontic treatment[14]. Reynolds[15] found that a shear bond force between 5.9 and 7.8 MPa is adequate for maintaining bracket adhesion to the tooth. Falling orthodontic brackets can frustrate the clinician, significantly affect treatment efficiency and have a financial impact on treatment[16], and therefore we should do our best to avoid it. The effects of CHX applications on the SBS of orthodontic brackets was assessed in vitro previously[17,18], showing that CHX do not interfere with bond strength between brackets. On the other hand, it was demonstrated that the application of 445-nm Diode Laser for 15 seconds on the bracket base significantly reduced the SBS values[19]. However, to our knowledge, no studies evaluated the effects blue light combined with chlorhexidine on the SBS of orthodontic brackets.

2. MATERIALS AND METHODS

2.1 Specimen preparation for Shear Bond Strength (SBS) test

The support used in this study to hold the bovine enamel chip was made using a polyvinyl chloride (PVC) tube, a double-sided tape (Permanent Double Sided Tape, Scotch, 3M) and an acrylic resin (Bosworth Fastray, The Harry J. Bosworth Company, a division of Keystone Industries, Myerstown, PA, USA, lot DEN-001782). The double-sided tape was glued to the base of the PVC tube and the bovine enamel chip was glued to the center of the double-sided tape. The inner side of the PVC tube was insulated with Vaseline and the acrylic resin was poured inside it. Later, the specimen was polished with a polishing paper (Leco SS1000 Grinder/Polisher, Leco Corporation, St. Joseph, MI, USA) and silicon carbide paper (1200 grit) (Leco 8`` DIA (203 mm) 1200 psa silicon carbide wet or dry, Leco Corporation, St. Joseph, MI, USA, lot

112370562), under water irrigation. Once the aforementioned steps were finished, specimens were washed in ultrasonic bath for 5 min.

2.2 Bracket-Enamel Adhesion Protocol for Shear Bond Strength Test

Specimens were cleaned with a pumice paste (Nada Pumice Paste, Preventech, Matthews-Indian Trail Road, Indian Trail Road, North Carolina, lot 050817AP) for 5 sec, rinsed with Milli-Q water for 5 sec and dried with an oil-free and moisture-free jet of air for 5 sec. Then, the enamel was treated with 35% phosphoric acid conditioner gel for 30 sec, rinsed with water for 30 sec and dried for 5 sec. After, a small layer of primer was applied for 20 sec using a micro brush, dried for 5 sec and photo polymerized with a 1600 LED mW/cm² polymerization lamp for 10 sec[20]. The intensity of the light was checked (~1750) with a radiometer as previously described. The bracket base area was measured with a digital caliper. A small amount of the orthodontic adhesive was applied to the base of the bracket (Roth Bracket 0.022”, Henry Schein Inc., Melville, New York, USA, lot #4370086). The bracket was seated on the previously prepared enamel. The excess of resin was removed with a dental explorer. The 1600 LED mW/cm² polymerization lamp was placed at a distance of 2-3 mm above the bracket by mesial and this region was polymerized for 5 sec. This step was repeated for the distal. Specimens were stored in ultrapure water in an incubator at 37°C for 24 h.

2.3 Aging (thermocycling) and treatments

The specimens were thermocycled (500 cycles, 5–55 °C, dwell time of 30 s, transfer time of 15 s) and then treated with 0.89% NaCl for 1 min (NaCl), 0.12 % Chlorhexidine for 1 min (CHX), Blue Light for 12 min (BL) and the combination of Blue Light for 12 min and Chlorhexidine for 1 min (BL+CHX). A commercially available, non-coherent blue light (LumaCare Model L-122, Medical Group, Newport Beach,CA) was used in this study with a wavelength of 420 nm, spot size of 113.1 mm^2, and fixed output of 95.5 mW/cm^2. After the treatments, specimens were thermocycled again (500 cycles, 5–55 °C, dwell time of 30 s, transfer time of 15 s).

2.4 Shear Bond Strength (SBS)

Shear bond strength test was performed using a Universal Testing Machine (MTS). The load was applied to the adhesive interface until failure (1 mm/min). The maximum stress to produce fracture was recorded (N/mm^2= MPa).

2.5 Type of failure analysis

After the SBS test, the bonding interface between the enamel and the bracket was examined under optical magnification (×10) and the type of failure was characterized according to the Adhesive Remnant Index (ARI)[21] into: Enamel adhesion failure (i.e. absence of resin in the enamel); bracket base adhesion failure (i.e. absence of resin at the bracket base); cohesive failure (i.e. when there is a portion of resin at the base of the bracket and another portion of resin in the teeth); and enamel fracture (i.e. when the enamel part of the teeth is broken)[22]. The criteria for ARI scoring were as follows: 0, no adhesive on the tooth; 1, less than 50% adhesive on the tooth; 2, more than 50% adhesive on the tooth; and 3, all adhesive remained on the tooth.

2.6 Statistical Analysis

Shear bond strength data were analyzed using one-way ANOVA and Tukey's test ($\alpha = 0.05$) to examine the effects of group and condition on shear bond strength.

3. RESULTS

3.1 Shear bond strength (SBS)

Figure 1 show the SBS results after the specimens being thermocycled for 500 cycles (5–55 °C, dwell time of 30 s, transfer time of 15 s), treated with NaCl, BL, CHX, and BL+CHX and thermocycled again for 500 cycles (5–55 °C, dwell time of 30 s, transfer time of 15 s). Statistics analysis showed that there was no difference on the SBS between the treatments ($p \geq 0.932$).

3.2 Type of failure

The type of failure observed for all of the specimens (n=12) tested after treatment with NaCl, BL and BL+CHX presented cohesive failure with prevalence of resin in bracket (ARI score 1). For the treatment with CHX, eleven (11) specimens presented cohesive

failure with prevalence of resin in bracket (ARI score 1) while only one presented cohesive failure with prevalence of resin in enamel (ARI score 2).

4. DISCUSSION

Since fixed orthodontic appliances create areas of stagnation for the dental biofilm and make it difficult to clean the teeth, our group studied the association between blue light and chlorhexidine to reduced *Streptococcus mutans* biofilm and its regrowth on an *in vitro* orthodontic model (Publicação 1). It was observed that this association reduced *S. mutans* biofilm and its regrowth on an in vitro orthodontic model. However, it is important that the therapy does not interfere with the adhesion between teeth and bracket. Therefore, the shear bond strength (SBS) between bracket and enamel was evaluated after treatments with blue light and chlorhexidine, alone and associated.

Bracket material and base structure, type of bonding agent used and enamel quality interfere with the SBS between bracket and enamel[23]. There are other factors that influence the adhesion of brackets to the enamel, such as 445 nm Diode Laser application for 15 seconds, that was previously observed toreduce SBS values[19].

In the present study, it was demonstrated that the applied treatments did not interfere with the bracket adhesion to the enamel. This is a positive result, since unattached brackets can make the orthodontic treatment longer and financially more costly, and might frustrate the clinician because of the necessity of reattaching the brackets[16].

Most specimens presented cohesive fracture with most of the resin remaining in the bracket (ARI score 1), which is in agreement with other reports in the literature[25,26]. This type of failure provides the clinician with additional time at the end of treatment to remove adhesive remnants from the patient's tooth, which need to be carefully executed, as it may damage the dental enamel[22]. These low ARI score have been considered favorable for some authors since there is less adhesive to remove from the enamel compared to ARI 2 and 3 and, thus, less risk of damage the tooth during polishing[27,28,29]. Therefore, the association between blue and chlorehexidine in vitro showed to BE effective.

5. CONCLUSION

In conclusion, the association between blue light and chlorhexidine did not influence on the bonding between the bracket and the enamel.

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FIGURE

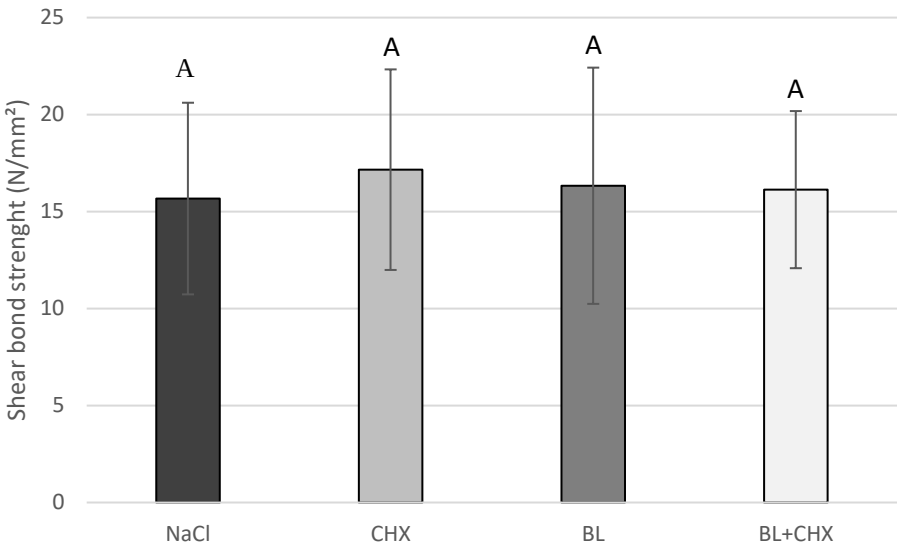

Figure 1: Mean ± standard deviation of shear bond strength between bracket and enamel after the treatments with NaCl, CHX, BL and BL+CHX. Data was analyzed by one-way ANOVA with Tukey post-hoc test (p≤0.05). Different letters represent statistical difference.

4 CONCLUSÃO

Como o tratamento ortodôntico é uma fase crítica para alguns pacientes com dificuldade no controle da higiene bucal e considerando os bons resultados imediatos e prolongados obtidos neste estudo, o tratamento com BL+CHX pode ser considerado uma alternativa aos métodos tradicionais, a fim de reduzir o risco de manchas brancas devido a desmineralização do esmalte. No entanto, evidências derivadas de observações clínicas são necessárias para confirmar nossos achados. Em conclusão, a associação entre luz azul e clorexidina reduziu o biofilme de *Streptococcus mutans* e seu recrescimento em um modelo ortodôntico in vitro e não influenciou na força de adesão entre bráquete e esmalte.

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Não autorizo a publicação deste trabalho até 03 de abril de 2022.

Araraquara, 03 de abril de 2020.

Eder Augusto Mastropietro Cavichioli