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ADESÃO MICROBIANA À SUPERFÍCIE RESTAURADORA: INFLUÊNCIA DAS CARACTERÍSTICAS DO MATERIAL NA FORMAÇÃO DE BIOFILME

Santa Maria, RS
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Tese apresentada ao Curso de Doutorado do Programa de Pós-Graduação em Ciências Odontológicas, Área de Concentração em Odontologia, ênfase em Periodontia, da Universidade Federal de Santa Maria (UFSM, RS), como requisito parcial para obtenção do grau de **Doutor em Ciências Odontológicas**.

Orientador: Prof. Dr. Fabrício Batistin Zanatta

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Santa Maria, RS
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RESUMO

ADESÃO MICROBIANA À SUPERFÍCIE RESTAURADORA: INFLUÊNCIA DAS CARACTERÍSTICAS DO MATERIAL NA FORMAÇÃO DE BIOFILME

AUTOR: Danilo Antonio Milbradt Dutra
ORIENTADOR: Fabrício Batistin Zanatta

A presente tese foi estruturada em dois estudos, apresentados em forma de artigo, que investigaram o efeito de características de superfície na adesão bacteriana e formação de biofilme sob materiais restauradores. O primeiro artigo avaliou o efeito do desgaste com pontas diamantadas e envelhecimento hidrotérmico sobre as características superficiais do material e adesão bacteriana sobre uma superfície de cerâmica Y-TZP. Para isso, espécimes Y-TZP foram divididos em 6 grupos de acordo com dois fatores: desgaste (3 níveis: apenas sinterizado [controle], desgaste ponta diamantada extra-fina [grão de 25 μm] e desgaste com ponta diamantada grossa [grão de 181 μm]) e envelhecimento hidrotérmico (presença/ausência). Foram analisadas a transformação de fase (difractômetro de raios X), rugosidade superficial, padrões micromorfológicos (microscópio de força atômica) e ângulo de contato (goniômetro). A adesão bacteriana (UFC/biofilme) foi quantificada utilizando um modelo de biofilme polimicrobiano *in vitro*. Tanto o tratamento superficial quanto o envelhecimento hidrotérmico promoveram um aumento no conteúdo de *m-fase*. Os valores de rugosidade aumentaram em função do aumento do tamanho do grão. A desgaste com ponta diamantada grossa resultou em valores significativamente menores de ângulo de contato ($P < 0,05$) quando comparados com os grupos de granulação fina e controle, enquanto não houve diferenças ($P < 0,05$) após a simulação de envelhecimento hidrotérmico. Os resultados de UFC/biofilme mostraram que nem o tratamento superficial nem a simulação de envelhecimento hidrotérmico afetaram significativamente a aderência bacteriana ($P > 0,05$). Assim, concluiu-se que o desgaste com pontas diamantadas e o envelhecimento hidrotérmico modificaram as propriedades de superfície da cerâmica Y-TZP; Contudo, estas propriedades não tiveram efeito significativo na adesão bacteriana na superfície do material. Posteriormente, o segundo artigo sumarizou os dados disponíveis a respeito de como os métodos de acabamento e polimento afetam as propriedades superficiais de diferentes materiais restauradores em relação à adesão bacteriana e à formação de biofilme através de uma revisão sistemática. As buscas foram realizadas nas bases de dados MEDLINE-PubMed, EMBASE, Cochrane-CENTRAL e LILACS. Dos 2.882 artigos potenciais encontrados nas buscas iniciais, apenas 18 preencheram os critérios de elegibilidade e foram incluídos na revisão (12 estudos *in vitro*, 4 estudos *in situ* e 2 ensaios clínicos). A análise do risco de viés apresentou apenas 2 estudos como baixo risco (enquanto 11 alto risco, 5 médio risco), bem como alta heterogeneidade entre os estudos. Assim, somente análises descritivas, considerando o desenho do estudo, materiais, intervenção (acabamento / polimento), rugosidade e protocolo de formação de biofilme (adesão bacteriana) puderam ser executadas. Algumas conclusões puderam ser extraídas: o impacto da rugosidade sobre a adesão bacteriana parece não estar totalmente relacionado com um limiar de rugosidade, principalmente em estudos laboratoriais; A variação de rugosidade da superfície entre diferentes métodos de polimento é ampla e material-dependente; Acabamento/desgaste invariavelmente cria uma superfície mais rugosa e deve ser sempre seguido por um método de polimento; O desenho de estudo *in vitro* parece não ser uma ferramenta efetiva para obter informação com relevância clínica sobre o tema de estudo, enquanto os desenho de estudo *in vivo e in situ* são mais recomendados.

Palavras-chave: Adesão de bactérias. Biofilme dental. Características da superfície. Degradação a baixa temperatura. Materiais restauradores. Microbiologia. Tratamentos de superfície.

ABSTRACT

MICRO-ORGANISM ADHESION ON RESTORATIVE SURFACE: INFLUENCE OF MATERIAL CHARACTERISTCS ON BIOFILM FORMATION

AUTHOR: Danilo Antonio Milbradt Dutra
ADVISOR: Fabrício Batistin Zanatta

The present thesis was divided into two studies, presented in form of articles, that evaluated the effect of materials characteristics on bacteria adhesion and biofilm formation. The first article aimed to evaluate the effect of grinding with diamond burs and low temperature aging on the material surface characteristics and bacteria adhesion on a Y-TZP surface. Y-TZP specimens were assigned into 6 groups according to two factors: grinding (3 levels: as-sintered; grinding with extra-fine diamond bur [25 µm grit] and grinding with coarse diamond bur [181 µm grit]), and hydrothermal aging to promote low temperature degradation (2 levels: presence/absence). Phase transformation (X-ray diffractometer), surface roughness, micromorphological patterns (atomic force microscope), and contact angle (goniometer) were analyzed. Bacterial adhesion (CFU/biofilm) was quantified using an *in vitro* polymicrobial biofilm model. Both the surface treatment and hydrothermal aging promoted an increase in *m*-phase content. Roughness values increased as a function of increasing bur grit sizes. Grinding with a coarse diamond bur resulted in significantly lower values of contact angle ($P<0.05$) when compared with the Xfine and control groups, while there were no differences ($P<0.05$) after hydrothermal aging simulation. The CFU/biofilm results showed that neither the surface treatment nor hydrothermal aging simulation significantly affected the bacteria adherence ($P>0.05$). Thus, based on the data of the first article we concluded that grinding with diamond burs and hydrothermal aging modified the Y-TZP surface properties; however, these properties had no effect on the amount of bacteria adhesion on the material surface. Later, the second article summarized the available data about how finishing and polishing methods affect the surface properties of different restorative materials with regard to bacterial adhesion and biofilm formation through a systematic review. Searches were carried out in MEDLINE-PubMed, EMBASE, Cochrane-CENTRAL and LILACS databases. From 2,882 potential articles found in the initial searches, only 18 met the eligible criteria and were included in this review (12 with *in vitro* design; 4 *in situ*; 2 clinical trials). Risk of bias analysis showed that only two studies presented low-risk (while 11 high, 5 medium). Thus, only descriptive analyses considering study design, materials, intervention (finishing/polishing), surface characteristics (roughness and SFE), and protocol for biofilm formation (bacterial adhesion) could be executed. Some conclusions could be drawn: the impact of roughness on bacterial adhesion seems not to be not fully related to a roughness threshold, especially in laboratory studies; the range of surface roughness among different polishing methods is wide and material dependent; finishing invariably creates a rougher surface and should be always followed by a polishing method; in *vitro* design seems not to be a powerful tool to draw clinical relevant evidences about the studies field, while *in vivo* and *in situ* designs are more recommended.

Keywords: Bacteria adhesion. Dental biofilm. Low temperature degradation. Microbiology. Restorative Materials. Surface treatments. Surface characteristics

SUMÁRIO

1 INTRODUÇÃO

Nas últimas décadas a preocupação com a aparência do sorriso fez aumentar o interesse por procedimentos restauradores estéticos (GUESS et al., 2011). Para satisfazer a crescente demanda dos pacientes e dentistas por restaurações altamente estéticas, biocompatíveis e duradouras, diversos tipos de sistemas cerâmicos têm sido desenvolvidos e aprimorados para uso na odontologia (DENRY; KELLY, 2008; GUESS et al., 2011), o que resultou em um grande número de sistemas cerâmicos disponíveis (LI; CHOW; MATINLINNA, 2014).

Além disto, com o advento dos procedimentos de usinagem CAD/CAM (*computer assisted design/computer assisted machining*), novas alternativas aos sistemas convencionais foram desenvolvidas, otimizando ainda mais o uso de materiais cerâmicos (KAMADA; YOSHIDA; ATSUTA, 1998; LI; CHOW; MATINLINNA, 2014). Dentre estes, a zircônia tem se destacado, sendo o material de escolha para os tratamentos protéticos reabilitadores quando se deseja associar estética e resistência mecânica (DENRY; KELLY, 2008).

O óxido de zircônio (ZrO_2), ou zircônia, é um material polimorfo que existe em três principais fases cristalinas: monoclínica (m), tetragonal (t) e cubica (c), as quais são estáveis em diferentes faixas de temperatura (GUAZZATO et al., 2005). A zircônia pura é monoclínica a temperatura ambiente até 1170°C. Acima desta temperatura, as partículas de zircônia sofrem uma transformação para a fase tetragonal (m→t), resultando numa redução volumétrica de aproximadamente 4% (como observado no processo de sinterização do material). Ainda, em temperaturas superiores a 2370ºC, as partículas de zircônia sofrem uma nova transformação, desta vez para a fase cúbica (t→c), a qual se mantem estável somente em altíssimas temperaturas.

Entretanto, uma transformação reversa (t→m) ocorre durante o resfriamento pós-sinterização da zircônia pura. Esta transformação reflete em uma expansão volumétrica (na ordem de 4%) suficiente para gerar microfraturas na estrutura da cerâmica, tornando-a imprópria para o uso (DENRY; KELLY, 2008; PICONI; MACCAURO, 1999). Por este motivo, óxidos estabilizadores (CaO, MgO, CeO_2, Y_2O_3) foram adicionados à zircônia pura, o que permitiu a manutenção da estrutura tetragonal à temperatura ambiente e, por conseguinte, o controle da transformação t→m, impedindo a propagação de fissuras de forma eficaz e conduzindo a uma elevada tenacidade (GARVIE; NICHOLSON, 1972).

Entre os diferentes tipos de zircônia presentes no mercado, as policristalinas de zircônia tetragonal parcialmente estabilizada com óxido de ítrio (Y-TZP) tem recebido destaque (DENRY; KELLY, 2008). A zircônia Y-TZP apresenta excelente biocompatibilidade, estabilidade química e propriedades mecânicas (resistência e tenacidade à fratura) superiores aos demais materiais cerâmicos, além de módulo de Young na mesma ordem de grandeza das ligas de aço inoxidável (PICONI; MACCAURO, 1999). Essas propriedades viabilizaram sua inserção na prática clínica, sendo utilizada na confecção de infraestrutura de próteses parciais fixas e de coroas unitárias (DENRY; KELLY, 2008), implantes dentários e pilares protéticos (WENZ et al., 2008) e próteses parciais fixas adesivas (KILIÇARSLAN et al., 2004).

Contemporaneamente, a zircônia Y-TZP vem sendo utilizada na confecção de coroas monolíticas (coroas totais) em dentes posteriores (SABRAH et al., 2013). Como vantagens tem-se o preparo coronário protético mais conservador, uma vez que este tipo de restauração não recebe porcelana de cobertura e, consequentemente, pode apresentar uma menor espessura. Além disso, essa abordagem restauradora parece solucionar um dos principais problemas das restaurações livres de metal em zircônia recobertas por porcelana: fratura e/ou delaminação (*chipping*) da cerâmica de cobertura, conforme tem sido relatado por estudos clínicos (CHRISTENSEN; PLOEGER, 2010; RAIGRODSKI et al., 2006).

Entretanto, a zircônia Y-TZP apresenta limitações quando exposta ao meio bucal, como na presença de água, onde fica sujeita ao envelhecimento. O fenômeno de envelhecimento, também conhecido como degradação a baixa temperatura (*low temperature degradation* - LTD), pode gerar consequências a estrutura do material, com a diminuição da resistência (LUGHI; SERGO, 2010).

A LTD foi primeiramente evidenciada por Kobayashi e colaboradores (1981), os quais demonstraram ocorrer espontaneamente uma degradação das partículas de zircônia quando exposta à umidade e a baixas temperaturas (150 – 400º C) por longo período de tempo (KOBAYASHI; KUWAJIMA; MASAKI, 1981). Em estudo recente, Lughi e Sergo (2010) demonstraram ocorrer inicialmente uma transformação de fase (t→m) na superfície, penetrando posteriormente para o corpo do material. O crescimento da área de transformação resulta em perda do material (*grain pull-out*), aumento da rugosidade superficial e presença de falhas, resultando em uma diminuição da resistência do material, o que é acelerada pelo vapor de água e umidade (LUGHI; SERGO, 2010). Entretanto, devido ao pouco tempo de acompanhamento clínico de

restaurações em zircônia, o entendimento sobre seu comportamento de degradação hidrotérmica ainda não está completamente elucidado na Odontologia.

Além disso, a aplicação de estímulos mecânicos tensionais tem sido outro fator associado a uma transformação de fase (t→m) da zircônia, como os decorrentes da realização de desgastes/ajustes clínicos da peça cerâmica (AMARAL et al., 2013; PEREIRA et al., 2016; PEREIRA et al., 2016). Apesar dos sistemas atuais de usinagem CAD/CAM propiciarem alta precisão, pequenos ajustes clínicos das peças de Y-TZP podem ser necessários para refinar a adaptação da peça e obter uma anatomia e adaptação desejáveis (ex., perfil de emergência, adaptação da margem restauradora).

Os ajustes podem incorporar diferentes tipos de defeitos na cerâmica (riscos e trincas), os quais apresentam variações de espessura e profundidade, podendo se estender por toda a peça (KIM et al., 2009; PAPANAGIOTOU et al., 2006; PEREIRA et al., 2014). Pereira e colaboradores (2014) observaram que desgastes realizados com pontas diamantadas promoveram um aumento de fase monoclínica na superfície da cerâmica Y-TZP, demonstrando que o desgaste com pontas diamantadas de maior granulação potencializaram os efeitos da LTD. Isso indica que tais ajustes podem atuar como fatores de concentração de tensão e levar a fratura catastrófica do material ao longo do tempo.

Ainda, os ajustes são geralmente realizados com instrumentos de corte - pontas diamantadas de granulação fina - que quebram a camada de polimento e modificam as características superficiais da restauração, tais como rugosidade, resultando em uma superfície mais rugosa e mais suscetível a formação de biofilme (AZEVEDO et al., 2012; BRENTEL et al., 2011; KANTORSKI et al., 2009; SCOTTI et al., 2007). A adesão microbiana pode ser crítica especialmente em regiões de maior exposição ao meio bucal, como na porção cervical de conectores e pônticos (área referente à “cinta cerâmica” de coroas protéticas), bem como em *abutments* para implantes.

O acúmulo de biofilme sobre as superfícies dentais e restauradoras pode favorecer a ocorrência de prevalentes doenças bucais, como cárie, doenças gengivais e periodontais (AXELSSON; LINDHE, 1978; GIBBONS, 1989; HOUTE, VAN, 1994; LOE; THEILADE; JENSEN, 1966), sendo este um aspecto importante relacionado à longevidade das restaurações. Além disso, uma maior adesão bacteriana em *abutments* para implantes poderia, em tese, favorecer ao desenvolvimento de doenças periimplantares, especialmente em sujeitos mais susceptíveis a

doença periodontal. Posto isso, materiais restauradores que apresentem baixa susceptibilidade a adesão bacteriana são desejáveis (ROSENTRITT et al., 2009).

Estudos *in vivo* e *in vitro* avaliando a capacidade de adesão microbiana a superfície restauradora têm demonstrado diferenças na formação de biofilme entre diferentes materiais restauradores (AZEVEDO et al., 2012; BRENTEL et al., 2011; KANTORSKI et al., 2009; SCOTTI et al., 2007). Estas variações na adesão bacteriana parecem ser consequência das propriedades dos materiais restauradores, como rugosidade superficial (KANTORSKI et al., 2009; QUIRYNEN et al., 1990; RIMONDINI et al., 1997) e a energia livre de superfície (QUIRYNEN et al., 1990; QUIRYNEN, M; BOLLEN, 1995).

A energia livre de superfície parece influenciar nas características da película adquirida adsorvida sobre a superficie das restaurações, influenciando nas composições da película e do biofilme dentário, dependendo do tipo de material utilizado (QUIRYNEN; BOLLEN, 1995). Neste sentido, evidências prévias tem demonstrado que substratos com alta energia livre superficial (hidrofílicos) apresentam maior acúmulo de biofilme quando comparados substratos com baixa energia (hidrofóbicos) (QUIRYNEN et al., 1990; QUIRYNEN; BOLLEN, 1995).

Em relação a rugosidade superficial, estudos prévios sugerem que o biofilme forma-se mais rapidamente e em maior quantidade em superfícies rugosas quando comparado às superfícies lisas (QUIRYNEN; BOLLEN, 1995). Estudos *in situ* com microscopia eletrônica de varredura (MEV) revelaram que a adesão inicial de micro-organismos começa em irregularidades e, sequencialmente, expande-se para o resto da superfície (SCOTTI et al., 2007). Ainda, evidências prévias demostraram uma associação positiva entre a quantidade de biofilme e a rugosidade da superfície em diferentes materiais dentários. Isso pode ser explicado pelo fato de que uma superfície mais rugosa proporciona nichos onde as bactérias podem se aderir e se desenvolver protegidas da desestruturação mecânica, da ação muscular e do fluxo salivar (BRENTEL et al., 2011; QUIRYNEN; BOLLEN, 1995).

Com isso, uma restauração mal finalizada (mais rugosa) pode favorecer a aderência do biofilme à superfície restauradora e às áreas adjacentes. Objetivando minimizar este efeito, vários kits de polimento encontram-se disponíveis para eliminar as ranhuras e irregularidades para alcançar uma superfície mais lisa (procedimentos de polimento). Dentre os dispositivos utilizados, discos de lixa, discos de borracha e discos de borracha com pasta diamantada são comumente

disponíveis. O efeito de cada método na superfície restauradora é relatado como material-dependente e sua eficácia sistema-dependente (YADAV et al., 2016).

Algumas revisões da literatura têm sido realizadas para avaliar o impacto desses procedimentos sobre as características superficiais das restaurações e formação de biofilme. Bollen e colaboradores (1997) avaliaram a rugosidade superficial inicial de vários materiais restauradores, bem como as mudanças na rugosidade superficial como consequência de diferentes modalidades de tratamento (BOLLEN; LAMBRECHTS; QUIRYNEN, 1997). Após busca eletrônica no Medline, os autores verificaram que a faixa de rugosidade superficial de diferentes superfícies duras intra-orais é ampla e que o impacto dos tratamentos na rugosidade superficial é material-dependente. Esses achados indicaram que todo material odontológico precisa de sua própria modalidade de tratamento para obter e manter uma superfície tão lisa quanto possível. Entretanto, poucos estudos incluídos na revisão apresentaram o desfecho de formação de biofilme.

Teughels e colaboradores (2006) avaliaram o impacto das características de superfície (energia livre, rugosidade e composição química) na formação de biofilme (TEUGHELS et al., 2006). Uma busca eletrônica por estudos clínicos foi realizada no Medline (de 1966 até julho de 2005). Os autores concluíram que um aumento da rugosidade superficial acima de 0,2 µm e/ou da energia livre superficial facilitam a formação de biofilme em materiais restauradores. Entretanto, quando ambas as características de superfície interagem entre si, a rugosidade superficial parece produzir um efeito preponderante. Ainda, pôde ser observado que a formação de biofilme também foi influenciada pelo tipo (composição química) do biomaterial ou pelo tipo de revestimento. Com isso, estudos avaliando o efeito de diferentes tratamentos sobre o mesmo substrato são desejáveis para se obter evidências da influência dos tratamentos de superfície na adesão bacteriana, considerando cada material avaliado.

Assim, diante dos pressupostos teóricos apresentados acima, a presente Tese teve como justificativa e objetivos:

- Estudo 1: Apesar de haver evidências sobre a influência das características da superfície cerâmica na capacidade de adesão bacteriana sobre estes materiais, bem como do importante papel destas variáveis para a longevidade dos procedimentos restauradores, de acordo com nosso conhecimento, não há evidências que tenham investigado o efeito do desgaste/ajuste com pontas diamantadas e da LTD na capacidade de adesão bacteriana (formação de biofilme) sobre uma superfície cerâmica Y-TZP. Assim, o primeiro estudo desta tese teve como objetivo a avaliar o

efeito do desgaste com pontas diamantadas e da LTD na caracterização de superfície e adesão bacteriana *in vitro* sobre a superfície cerâmica Y-TZP.

- Estudo 2: Os procedimentos de acabamento e de polimento podem modificar as características de rugosidade das restaurações, e assim influenciar na capacidade de adesão bacteriana sobre as restaurações. Com isso, o segundo estudo desta tese se propôs a avaliar, por meio de uma revisão sistemática, o efeito dos métodos de polimento e acabamento nas propriedades superficiais de diferentes materiais restauradores e sua influência em relação à adesão bacteriana e formação de biofilme.

2 ARTIGO 1 – GRINDING WITH DIAMOND BURS AND HYDROTHERMAL AGING OF A Y-TZP CERAMIC: EFFECT ON THE MATERIAL SURFACE CHARACTERISTICS AND BACTERIAL ADHESION ON Y-TZP SURFACE.

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Grinding with diamond burs and hydrothermal aging of a Y-TZP ceramic: effect on the material surface characteristics and bacterial adhesion on Y-TZP surface.

Effect of Y-TZP Surface Treatment on Bacterial Adhesion

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Grinding with diamond burs and hydrothermal aging of a Y-TZP material: effect on the material surface characteristics and bacterial adhesion on a Y-TZP.

Effect of surface treatment on bacterial adhesion on a Y-TZP

Clinical Relevance

Finishing of Y-TZP restorations with diamond burs altered the material surface characteristics, but neither the grinding nor an aging condition affected biofilm formation.

SUMMARY

The aim of this study was to evaluate the effect of grinding with diamond burs and low temperature aging on the material surface characteristics and bacteria adhesion on a yttrium-stabilized tetragonal zirconia polycrystalline (Y-TZP) surface. Y-TZP specimens were made from presintered blocks, sintered as recommended by the manufacturer and assigned into 6 groups according to two factors: grinding (3 levels: as-sintered; grinding with extra-fine diamond bur [25 µm grit] and grinding with coarse diamond bur [181 µm grit]), and hydrothermal aging to promote low temperature degradation (LTD - 2 levels: presence/absence). Phase transformation (X-ray diffractometer), surface roughness, micromorphological patterns (atomic force microscopy), and contact angle (goniometer) were analyzed. Bacterial adhesion (CFU/biofilm) was quantified using an *in vitro* polimicrobial biofilm model. Both the surface treatment and hydrothermal aging promoted an increase in *m*-phase content. Roughness values increased as a function of increasing bur grit sizes. Grinding with a coarse diamond bur resulted in significantly lower values of contact angle ($P<0.05$) when compared with the Xfine and control groups, while there were no differences ($P<0.05$) after hydrothermal aging simulation. The CFU/biofilm results showed that neither the surface treatment nor hydrothermal aging simulation significantly affected the bacteria adherence ($P>0.05$). Grinding with diamond burs and hydrothermal aging modified the Y-TZP surface properties; however, these properties had no effect on the amount of bacteria adhesion on the material surface.

Keywords: Ceramics, Polycrystalline zirconia, Biofilm, Surface alterations, Low temperature degradation, Surface properties.

INTRODUCTION

Zirconia-based ceramics are a contemporary option for fixed dental prosthesis, dental implants and abutments[1] because of their aesthetic and superior mechanical strength.[2, 3] Among the different types of zirconia-based ceramics, the yttrium-stabilized tetragonal zirconia polycrystalline (Y-TZP) has been highlighted. [4, 5] Y-TZP ceramic shows high biocompatibility, chemical stability and a fracture strength/toughness higher than other ceramic systems.[6] More recently, it has been used to produce monolithic zirconia crowns in posterior teeth.[7]

Zirconia is a polymorphic material that has three crystalline forms that are stable at different temperatures: monoclinic (*m* - up to 1170 °C), tetragonal *(t* - above 1170 °C and up to 2370 °C) and cubic (*c* - above 2370 °C).[8] Phase transformation from monoclinic to tetragonal zirconia (*m* → *t*) occurs during the sintering process and is associated with a volume decrease of approximately 4%. After sintering, stabilizing oxides (i.e. Y_2O_3) are added to pure zirconia, keeping the tetragonal form stable at room temperature and avoiding the deleterious effects of volume expansion during the cooling process due to *t* → *m* transformation.[6]

Some factors associated with the clinical use of Y-TZP may also induce a " *t* → *m*" transformation of this material, such as intermittent mechanical loading (stress) and corrosion in the presence of humidity (low temperature degradation - LTD).[9,10] Additionally, stress concentration with subsequent phase transformation will occur to Y-TZP after adjustment of the Y-TZP surface (outer or intaglio) by grinding and/or polishing.[11] These procedures introduce different types of damage to the Y-TZP surface, such as scratches and cracks of various depths, which penetrate toward the bulk of the material.[12,13]

These damages to the Y-TZP surface (scratches and cracks) may be limited by a mechanical property of Y-TZP ceramic known as transformation toughening. The "t → m" transformation associated with localized volumetric expansion results in compressive stresses at an existing crack, which counteracts tensile stresses in this region and limits crack propagation. However, the increase in the transformation area may also result in material loss (grain pull-out), a rougher surface, and a higher incidence of cracks, all of which decrease the material strength.[14]

Moreover, grinding with diamond burs produces a modification of the surface characteristics of the Y-TZP material, which might increase bacterial adhesion[15-19] and favor the incidence of secondary caries and periodontal inflammation,[20] relevant aspects to the longevity of restorations. The restoration surface properties, such as roughness and the surface free energy,

seem to play a key role in this process.[21] The surface free energy influences the acquired film formed over the restorative surface.[22,23] The increase in free energy of the substratum surface can result in a higher plaque growth rate and plaque retention capacity of the surface and the selection of specific organisms.[21] Regarding the surface roughness, previous studies suggest that the biofilm is formed in larger amounts and more rapidly on rough surfaces when compared to smooth surfaces.[23] *In situ* studies using scanning electron microscopy (SEM) revealed that the initial adhesion of microorganisms starts on irregularities and sequentially expands to the rest of the surface.[19] Additionally, previous studies have demonstrated a positive association between the amount of biofilm and the surface roughness in different dental materials such as ceramics, composite resin, acrylic resin and titanium.[18,24 25]

Although there is evidence regarding the influence of surface characteristics on bacteria adhesion to restorative materials and the importance of these factors on the longevity of prosthetic restorations, there are no studies that have investigated the effects of grinding with diamond burs and hydrothermal aging (Y-TZP under LTD) on bacterial adhesion (biofilm formation) on a Y-TZP surface. These conditions can be clinically relevant when utilizing a Y-TZP ceramic for implant abutments, which are kept subgingival, and at th areas close to gingival tissues (marginal and connector zones). Thus, the present study aimed to evaluate the effect of grinding with diamond burs and hydrothermal aging on the material surface characteristics (m-phase transformation, surface roughness, superficial topography and surface free energy), and bacteria adhesion on a Y-TZP ceramic surface. The null hypothesis (H_0) was that grinding with diamond burs of different grit sizes and hydrothermal aging conditions would yield equivalent bacteria adhesion on the Y-TZP surface.

MATERIALS AND METHODS

Specimen preparation

Y-TZP specimens (In-Ceram YZ, Vita Zahnfabrik, Bad Sackingen, Germany) were prepared from prefabricated blocks. For the complementary analysis of surface characterization, specimens were manufactured with a final size of 14x14x2 mm, while for the microbiological evaluation with an *in vitro* biofilm formation model, specimens were used with a final size of 7x6x2 mm.

To remove the cutting irregularities, the pre-sintered specimens were polished with 1200 grit SiC paper and cleaned in an ultrasonic bath (1440 D – Odontrobras, Ind. E Com. Equip. Méd.

Odonto. LTDA, Ribeirão Preto, Brazil) using 78% isopropyl alcohol for 10 minutes. Then, the specimens were sintered as recommended by the manufacturer (Zyrcomat T, Vita Zahnfabrik).

Experimental groups

After sintering, the Y-TZP specimens were allocated into 6 groups according to 2 factors: grinding with diamond burs and low temperature aging to simulate LTD, as shown in Table 1.

Surface Treatment

Specimens from the control groups (Control and Control Aging) remained untreated after the sintering process. For the other groups, a single trained operator performed the grinding procedures using diamond burs (Xfine #3101FF, 25 µm grit size; and Coarse #3101G; 181 µm grit size; KG Sorensen, Cotia, SP, Brazil) coupled to a low-speed motor (Kavo Dental, Biberach, Germany) associated with a contra-angle handpiece (T2 REVO R 170 contra-angle handpiece up to 170,000 rpm, Sirona, Bensheim, Germany) under constant water-cooling (≅ 30ml/min). The diamond bur was replaced after each specimen.

A marking with permanent marking pen (Pilot, Sao Paulo, SP, Brazil) was made over the entire surface of each specimen prior to the grinding procedures. Afterward, the specimens were fixed to a device which ensured parallelism between the specimen and diamond bur. Grinding was carried out by similar horizontal movements until the pen mark was eliminated. This protocol standardized the grinding thickness while ensuring that the entire specimen surface was subjected to bur-grinding.[26]

Low temperature aging

The hydrothermal aging was simulated in an autoclave (Sercon HS1-0300 nº1560389/1) at 134ºC, under 2 bar, for 20 hours.[27]

Phase analysis by X-ray diffraction

Quantitative analysis of phase transformation was conducted (1 specimen/group) to determine the relative amount of m-phase and depth of the transformed layer under each condition. This analysis was performed using an X-ray diffractometer (Bruker AXS, D8 Advance, Karlsruhe, Germany). Spectra were collected into the 2θ, with a range of 25–35º, at a step interval of 1 s and step size of

0.03º. The amount of m-phase was calculated using the method introduced by Garvie & Nicholson[28]:

$$X_M = \frac{(\text{-}111)_M + (111)_M}{(\text{-}111)_M + (111)_M + (111)_T} \quad (1)$$

Where: $(\text{-}111)_M$ and $(111)_M$ represent the intensity of the monoclinic peaks (2θ = 28° and 2θ = 31.2°, respectively) and $(101)_T$ indicates the intensity of the respective tetragonal peak (2θ = 30°). The volumetric fraction of the m-phase was calculated according to Toraya et al.[29]:

$$F_M = \frac{1.311 \cdot X_M}{1 + 0.311 \cdot X_M} \quad (2)$$

The depth of the transformed layer was calculated based on the amount of the m-phase, considering that a constant fraction of grains had symmetrically transformed to the m-phase along the surface, as described by Kosmac et al.[30]:

$$PZT = \left(\frac{sen\theta}{2\mu}\right)\left[ln\left(\frac{1}{1-FM}\right)\right] \quad (3)$$

Where θ=15° (the angle of reflection), μ=0.0642 is the absorption coefficient, and F_M is the amount of m-phase obtained using Eqs. (1) and (2).

Surface roughness and micromorphological analysis

Y-TZP specimens were evaluated for quantitative (10 specimens/group) and qualitative (2 specimens/group) analysis of the micromorphological pattern generated by the grinding procedure. Specimens were analyzed using a surface roughness tester (Mitutoyo SJ-410, Tokyo, Japan) and atomic force microscopy (AFM; Agilent Technologies 5500 equipment, Chandler, Arizona, USA), respectively.

For the roughness analysis, four measurements were made for each specimen (2 following the grinding direction, two in the opposite direction) according to the ISO 1997 parameters (Ra – arithmetical mean of the absolute values of peaks and valleys measured from a medium plane (μm)

and Rz – average distance between the five highest peaks and five major valleys (μm))[31] with a cut-off (n=5), λC 0.8mm and λS 2.5μm. After that, the arithmetic mean of all measurements from each specimen were obtained.

Afterwards, two specimens of each group were randomly selected for qualitative analysis of superficial topography using AFM. First, all selected specimens were submitted to the cleaning protocol in an ultrasonic bath as previously described. The AFM images were obtained by non-contact methodology and specific probes from an area of 20x20 μm (PPP-NCL probes, Nanosensors, Force constant = 48 N/m) and evaluation using specific computer software (Gwyddion™ version 2.33, GNU, Free Software Foundation, Boston, MA, USA).

Contact angle

The contact angle was measured (10 specimens/group) using the sessile drop technique and a goniometer (DSA30S – Drop Shape Analyzer, KRÜSS, Hamburg, Germany) associated with a computer device using a specific software (Advanced Drop Shape Analysis, KRÜSS, Hamburg, Germany). For the contact angle measurement, a syringe was used to place a drop (10 μl) of pre-selected liquid (deionized water) on the treated surface of the specimen and the contact angle (angle between the drop and the surface plane) was measured after 5 seconds.[32] The software carried out 5 measurements and the average value from each specimen was calculated.

The biofilm model

In vitro biofilms were grown using the Amsterdam Active Attachment model (AAA-model[33]). This model consisted of a custom-made stainless steel lid with 24 clamps in which the substratum was fixed.

Saliva Collection

Stimulated saliva was previously collected from a single donor (DAMD) who refrained from dental hygiene for 24 hours before the collection procedure. The saliva was diluted 2-fold with 60% sterile glycerol to protect the bacterial cells from cryodamage and stored at –80° C.

Initial bacterial attachment

The inoculation medium for the polymicrobial biofilms was 50-fold diluted saliva in a semidefined medium,[34] with 0.2% sucrose and 50 mmol/l PIPES at pH 7.0.

Y-TZP specimens (6 specimens/group) were fixed in the lid clamps and placed onto standard polystyrene 24-well plates (multiwell plates; Greiner Bio One, Alphen aan den Rijn, The Netherlands). Biofilms were produced by adding 1.7 ml of the inoculation medium to each well and the model was subsequently incubated anaerobically (10% CO_2, 10% H_2, e 80% N_2) at 37 °C for 6 hours.

Determination of CFU

After allowing for biofilm growth, the specimens with the biofilms were removed from the lid and transferred into 2 ml cysteine peptone water (CPW). The biofilms were dispersed by sonication for 2 minutes, 1 second pulsations at an amplitude of 40 W (Vibra Cell; Sonics & Materials Inc, Newtown, CT), vortex-mixing for 30 seconds and then a series of dilutions were made.

The polymicrobial biofilm suspensions were plated on tryptic soy agar blood plates for total counts. Plates were incubated for 96 hours at 37°C under anaerobic conditions (10% CO_2, 10% H_2, 80% N_2).

Data Analysis

Statistical analysis was executed using SPSS 18. Roughness (Ra and Rz) and contact angle data were analyzed using two-way ANOVA considering two factors (grinding and aging) and the interaction of both factors. CFU/biofilm counts were compared using one-way ANOVA and Tukey's test. All statistical tests were performed considering a 5% significance level.

RESULTS

Phase Analysis

Surface treatment alone promoted an increase in the *m*-phase content and transformation depth, showing higher values for both the bur grit sizes (Xfine and Coarse) when compared to the control (Table 2). Furthermore, all groups showed a higher amount of *m*-phase content and transformation depth after hydrothermal aging, and these differences were more pronounced for the as-sintered control group (0 to 54% and 0 to 3.97 μm, respectively).

Surface roughness and micromorphological analysis

The bur grit size directly affected the Ra and Rz parameters on the material surface (Table 2). These results showed an increase ($P<0.05$) in the roughness parameters as a function of increasing bur grit size. Additionally, there was an effect of hydrothermal aging on the roughness parameters for the treated groups (Xfine and Coarse groups), with a decrease ($P<0.05$) of the Ra parameter after aging for the Xfine (0.70 to 0.53 μm) and Coarse (1.16 to 0.99 μm) groups. There was no difference between Control groups, either with or without aging ($P>0.05$).

Micromorphological analysis showed that grinding with a diamond bur (Xfine and Coarse) resulted in similar surface patterns, with scratches parallel to the direction of the grinding tool motion and a depth proportional to the grit size of the diamond bur used. The untreated surface showed a distinct micromorphological pattern, with a smoother surface where superficial Y-TZP grains can be seen.

Contact angle measurements

The data from contact angle measurements indicated that the surface treatment alone also modified the surface free energy (Table 2). This result indicates that the specimens ground with the coarse diamond bur had significantly lower values of contact angle measurement ($P<0.05$) when compared with the xfine and control groups. Moreover, hydrothermal aging significantly affected ($P<0.05$) the contact angles values between the Control groups (81 to 59°), but no difference ($P>0.05$) was observed between the Xfine and Coarse groups. When only the aged groups were compared, the contact angle values showed no significant differences ($P>0.05$) between the groups.

Bacteria Adherence

The bacteria adherence was evaluated using an *in vitro* model of biofilm formation. The CFU/biofilm results showed that neither the surface treatment nor hydrothermal aging simulation significantly affected ($P>0.05$) bacteria adherence on the material surface (Figure 2).

DISCUSSION

In the present study, grinding with diamond burs (Xfine and coarse) promoted higher *m*-phase content when compared to the as-sintered condition (control). Additionally, the grinding procedures altered the superficial topography, roughness, and surface free energy of the Y-TZP ceramic. Regarding aging, distinct effects were observed depending on the presence/absence of grinding. However, despite these differences observed regarding the surface treatment, no significant effect was observed on the bacterial adhesion to Y-TZP surface using an *in vitro* model of biofilm formation.

As indicated previously, some conditions associated with the clinical use of Y-TZP may induce phase transformation ($t \rightarrow m$), such as intermittent loading, humidity, and adjustment by grinding of the Y-TZP surface.[11] In this study, the clinical adjustment was simulated by grinding using diamond burs with different grit sizes (Xfine and coarse), and LTD was artificially induced by hydrothermal aging. In agreement with the literature,[26,35-36] the current data indicate that grinding increased the *m*-phase content (control: 0%; Xfine: 8.9%; coarse: 10.6%), and it decreased the susceptibility of Y-TZP to phase transformation during aging (control aging: 54.3%; Xfine aging: 12.7%; coarse aging: 19.9%). Muñoz-Tabares and Anglada[37] stated that grinding induces a recrystallization of a very thin surface layer of tetragonal nanograins from the highly deformed surface, whose size is smaller than the critical size for phase transformation in a humid environment, such that this process may decrease Y-TZP susceptibility to $t \rightarrow m$ transformation.

Surface topography (AFM images) and roughness analysis (Ra and Rz parameters) were conducted to evaluate the direct effect of grinding on the Y-TZP surface. Roughness results from nonaged groups showed that Ra and Rz values increased with increasing bur grit sizes, and these differences among groups can also be observed in the surface topography images obtained using AFM (Figure 1). The as-sintered condition (control) presented a smoother topographical pattern (superficial Y-TZP grains can be seen), and that grinding, regardless of grit size, changed this pattern by introducing scratches and promoting deformations in the direction of the bur movement.

Previous studies have suggested that the increase in the transformation area ($t \rightarrow m$) would result in material loss (by grain pullout) and increasing surface roughness.[10,14,37,38] However, even with the higher *m*-phase content presented after aging for all groups of this study, higher roughness values did not present as a result. Both the Xfine and the coarse groups had lower Ra (Xfine: 70 to 53 μm; coarse: 1.16 to 0.99 μm) and Rz (Xfine 4.56 to 3.47 μm; coarse: 6.87 to 6.11 μm) values

after hydrothermal aging, and the difference between the control groups was not significant (p>0.05), even with an extensive increase in *m*-phase (0% to 54%). On the other hand, Deville and others[39] stated that $t \rightarrow m$ transformation is triggered preferentially on surrounding areas of superficial defects and residual stress concentration. Thus, it is possible to hypothesize that effects of aging (ie, grain pullout) on ground surfaces occur initially around the highest topographical grains (superficial layer), which are also more susceptible to water contact, resulting in a less rough surface when compared to nonaged ground surfaces. This fact could also be indicative that aging by autoclave for 20 hours at 134°C with 2 bars of pressure was not significant enough to promote the deleterious effects described by Lughi and Sergo[14] on the Y-TZP ceramic used here.

Moreover, the effect of surface roughening on the material surface wettability has been previously reported.[40] In this study, the grinding effect on the contact angle analysis of the Y-TZP surface was observed only for the coarse group, which presented higher surface free energy than the Xfine and control groups. Additionally, it is important to notice that, after aging, there was no difference between the groups regardless of the presence or absence of surface treatment.

The relationship between material surface characteristics and bacteria adhesion has been studied extensively;[40,41] however, few studies have been performed on ground Y-TZP. The understanding of bacteria–surface interactions and how grinding using diamond burs and aging affect biofilm accumulation becomes an important tool for biofilm control and a relevant aspect to preview the longevity of Y-TZP restorations and implant abutments. Regarding the surface characteristics, previous studies have reported that roughness and surface free energy seem to play an important role in the process of bacteria adhesion on restorative surfaces.[40-42] Quirynen and Bollen[23] found that increased surface free energy attracts more bacteria when compared to more hydrophobic surfaces. Likewise, Al-Radha and others[43] concluded that the influence of surface free energy on initial bacterial adhesion to smooth implant materials *in vitro* appears to be the most important factor, in addition to the material type. However, these studies have compared materials with similar patterns of surface roughness. When both the roughness and the surface free energy were evaluated together, the influence of surface roughness on the accumulation and composition of biofilm is more important than the influence of surface free energy.[44]

In general, an increase in surface roughness promotes an increase in bacterial attachment due to the initial adhesion of bacteria at locations where they are sheltered against shear forces[40] and also because roughening of the surface increases the contact area between the material surface

and bacterial cells available for adhesion.[45] It is accepted that an increase in surface roughness above a threshold of 0.2 μm facilitates biofilm formation on restorative materials, while bacterial adhesion to surfaces below the threshold of 0.2 μm cannot be reduced.[46] On the other hand, while both the Xfine and the coarse groups presented Ra values higher than the threshold of 0.2 μm (0.70 and 1.16 μm, respectively), they did not present an increase in bacterial adhesion when compared with the control group (0.13 μm). Hence, it is possible to hypothesize that the range of surface roughness observed in our results is not the main factor for promoting bacterial adhesion on the Y-TZP ceramic *in vitro* and that this low susceptibility to bacterial adhesion can be considered an advantage of this material. This result is in agreement with other studies that indicated that bacteria adhesion cannot be fully explained by small differences in the surface roughness and surface free energy.[47,48]

This inconsistency regarding the effect of surface characteristics on bacteria adhesion on material surface may be explained mainly by 1) characteristics derived from the distinctive materials, such as material chemical composition; 2) the range of roughness promoted on the material surface; and 3) culture conditions used in the tests. In relation to culture conditions, this study evaluated a complex *in vitro* polymicrobial biofilm consisting of diluted-saliva inoculation medium, which differs from other studies with similar purposes that used a single-specimen biofilm, with less varied modes of attachment and without a significant degree of interspecies interactions.[49] The protocol of 6 hours of biofilm growth was chosen in order to evaluate early bacteria adhesion. Additionally, the current study evaluated bacteria adhesion on a Y-TZP surface using the AAA model,[33] a validated and extensively studied polymicrobial model of biofilm formation *in vitro*. However, the use of the AAA model can be considered a limitation of this study, as this *in vitro* model does not simulate some factors from a typical oral environment, such as low shear forces, which can limit the roughness effect on bacteria adherence capacity.

The findings of the current study indicate that grinding with diamond burs and hydrothermal aging modify the surface properties (ie, *m*-phase content, surface roughness, and surface free energy) of the assessed Y-TZP material; however, those properties/characteristics did not significantly affect bacterial adhesion when using the AAA model of *in vitro* biofilm formation. These results suggest that the Y-TZP ceramic may have low susceptibility to bacterial adhesion regardless of the surface condition. However, even if our results have shown no differences between the control and other groups with regard to bacterial adhesion, the surface

roughness may affect other properties of the material, such as its mechanical behavior and wear of antagonist teeth, so a smoother surface is clinically preferable. Thus, when clinical grinding is necessary, it should be made using extra-fine diamond burs followed by polishing.[50] Further studies should be performed to provide additional information regarding the behavior of this material using biofilm models that simulate clinical conditions and/or clinical studies to better understand the influence of these factors on the longevity of the prosthetic restorations.

CONCLUSION

- Grinding with diamond burs and hydrothermal aging promoted *m*-phase content, surface roughness, and surface free energy alterations of the assessed Y-TZP material.
- Bacterial adhesion was not affected by grinding with different diamond burs.

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Regulatory Statement

This study was conducted in accordance with all the provisions of the local human subjects oversight committee guidelines and policies of the Federal University of Santa Maria, Brazil.

Conflict of Interest

The authors of this article certify that they have no proprietary, financial, or other personal interest of any nature or kind in any product, service, and/or company that is presented in this article.

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Figure 1. *Atomic force micrographs from zirconia samples of different groups considering the two factors (grinding and aging). It can be noticed that the grinding procedures promoted surface alterations compared to the as-sintered group and that the low-temperature aging did not change the micromorphological pattern.*

Figure 2. *CFU/biofilm counts of bacteria grown* in vitro *on zirconia surfaces. Two-way ANOVA was performed considering the two factors (grinding and aging) and showed no significant differences between the experimental groups (*$p>0.05$*). Error bars show the standard deviation from the average value.*

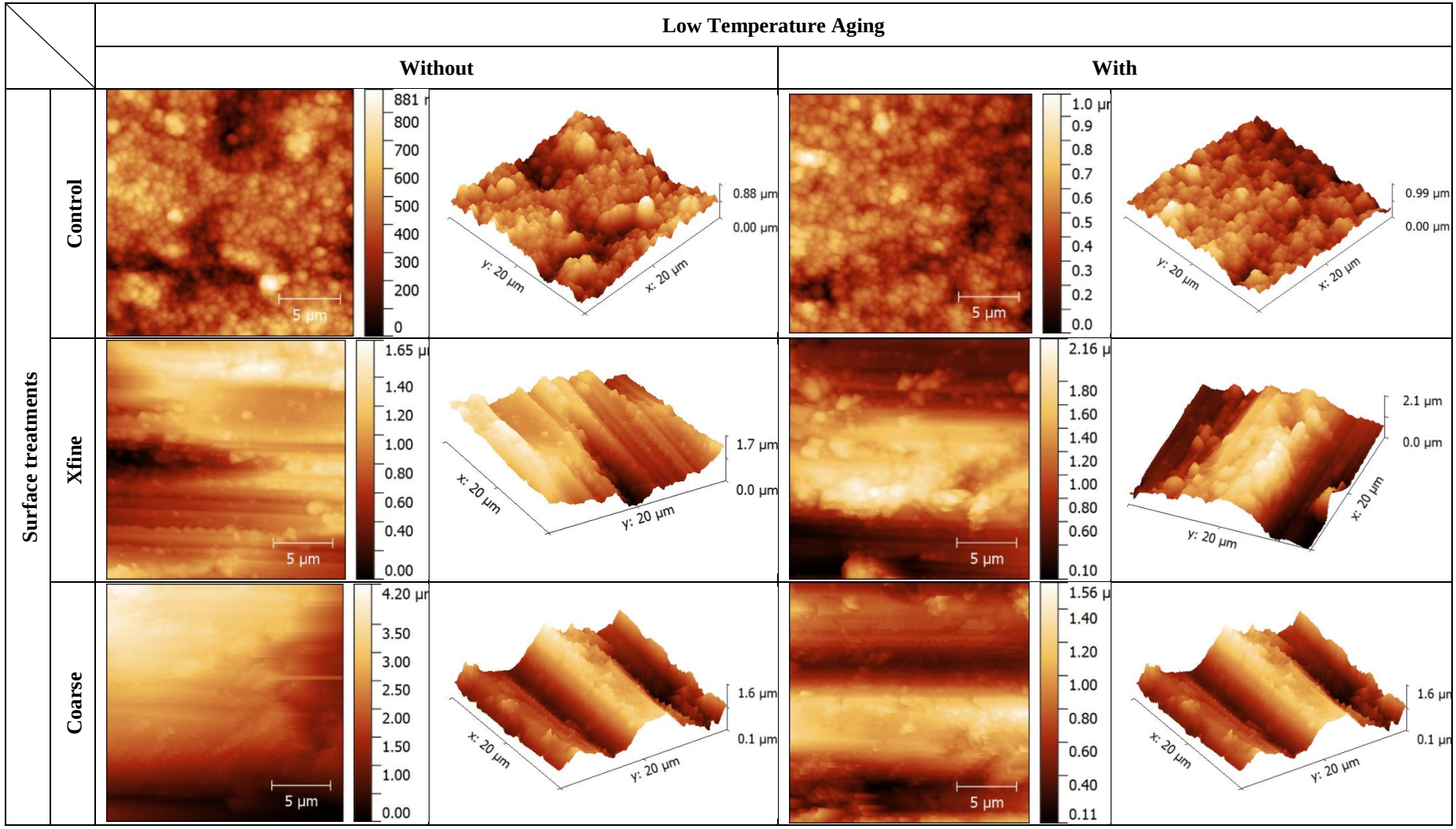

Figure 1. Atomic force micrographs from zirconia samples of different groups considering the 2 factors (grinding and aging). It can be notice that the grinding procedures promoted surface alterations compared to the as-sintered group, and the low-temperature aging did not change the micromorphological pattern.

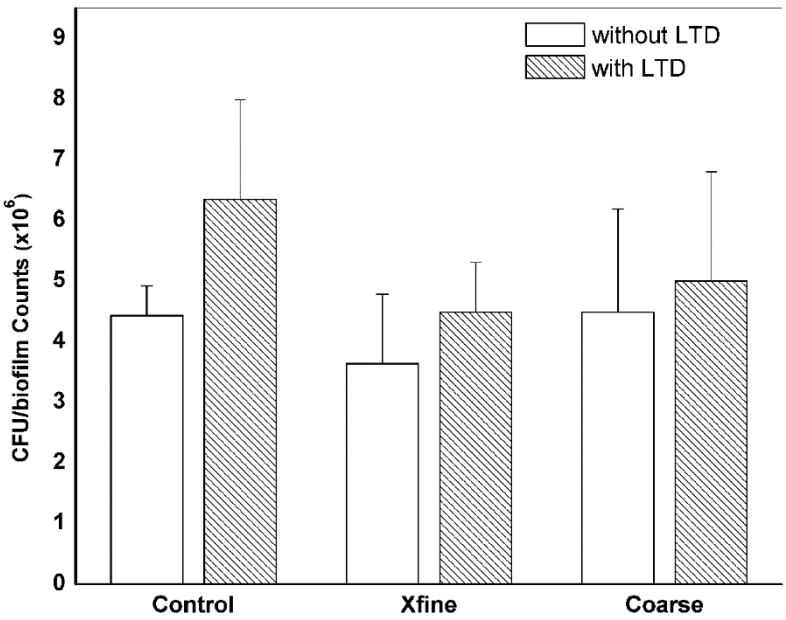

Figure 2. CFU/biofilm counts of bacteria grown *in vitro* on zirconia surfaces. Two-way Anova was performed considering the two factors (grinding and aging) and showed no significant differences between the experimental groups (P>0.05). Error bars show the standard deviation from average value.

Table 1. Experimental Groups.

Groups	Surface treatment	Low temperature aging
Control	As-sintered (untreated)	Without
Control Aging		With
Coarse	Coarse Diamond Bur #3101G (average grit size 181 µm)	Without
Coarse Aging		With
Xfine	Extra-fine Diamond Bur #3101FF (average grit size 25 µm)	Without
Xfine Aging		With

Table 2. X-ray Difractometry analysis (F_m: % of monoclinic phase; PTZ: depth of transformed layer), Roughness (Ra and Rz) and Contact angle results for grinding and aging factors.

	Difractometry analysis*		**Ra (µm)****	**Rz (µm)****	**Contact Angle****
Groups	**F_m (%)**	**PTZ (µm)**	**Mean (SD)**		
Control	0.00	0.00	0.13 (0.02)^{A}	1.17 (0.19)^{A}	81.02 (9.83)^{A}
Control Aging	54.38	3.97	0.14 (0.02)^{A}	1.32 (0.27)^{A}	59.55 (8.30)$^{C.D}$
Xfine	8.93	0.47	0.70 (0.21)^{B}	4.56 (0.94)^{B}	75.88 (11.90)$^{A.B}$
Xfine Aging	12.72	0.68	0.53 (0.11)^{C}	3.47 (0.65)^{C}	67.71 (9.01)$^{B.C}$
Coarse	10.66	0.57	1.16 (0.14)^{D}	6.87 (0.71)^{D}	53.75 (7.27)^{D}
Coarse Aging	19.95	1.12	0.99 (0.08)^{E}	6.11 (0.54)^{D}	60.01 (14.12)$^{C.D}$

*Difractometry analysis: F_m: % of monoclinic phase; PTZ: depth of transformed layer;
**Two-way Anova and Tukey's test: same letters show no statistical difference between the groups ($p>0.05$). Different letters represent differences between groups ($p<0.05$).

3 ARTIGO 2 – DOES FINISHING AND POLISHING OF RESTORATIVE MATERIALS AFFECT BACTERIAL ADHESION AND BIOFILM FORMATION? A SYSTEMATIC REVIEW

Este artigo foi submetido para publicação no periódico Operative Dentistry (Print ISSN: 0361-7734, Fator de impacto = 2.819; Qualis A1), e aceito no dia 10 de maio de 2017. As normas para publicação estão descritas no Anexo A.

Does finishing and polishing of restorative materials affect bacterial adhesion and biofilm formation? A systematic review

Influence of surface properties on bacterial adhesion.

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Does finishing and polishing of restorative materials affect bacterial adhesion and biofilm formation? A systematic review

Influence of surface properties on bacterial adhesion.

Clinical Relevance

Polished/smooth surface is mandatory for maintaining clinical health status on restored teeth. However, this review depicts the absence of reliable data on literature that characterize and elucidate the mechanism related to the effect of surface properties on bacterial adhesion / biofilm formation.

SUMMARY

Biofilm (bacterial plaque) accumulation on the surface of restorative materials favors the occurrence of secondary caries and periodontal inflammation. Surface characteristics of restorations can be modified by finishing and/or polishing procedures and may affect the bacteria adhesion. The aim of this systematic review was to characterize how finishing and polishing methods affect the surface properties of different restorative materials with regard to bacterial adhesion and biofilm formation. Searches were carried out in MEDLINE-PubMed, EMBASE, Cochrane-CENTRAL and LILACS databases. From 2882 potential articles found in the initial searches, only 18 met the eligible criteria and were included in this review (12 with *in vitro* design; 4 *in situ*; 2 clinical trials). However, they present high heterogeneity regarding materials considered and methodology for evaluate the desired outcome. Besides, risk bias analysis shows that only 2 studies presented low-risk (while 11 high, 5 medium). Thus, only descriptive analyses considering study design, materials, intervention (finishing/polishing), surface characteristics (roughness and SFE), and protocol for biofilm formation (bacterial adhesion) could be executed. Some conclusions could be drawn: the impact of roughness on bacterial adhesion seems not to be related to a roughness threshold (as previously believed), but rather to the range; the range of surface roughness among different polishing methods is wide and material dependent; finishing invariably creates a rougher surface and should be always followed by a polishing method; each dental material requires its own treatment modality to obtain and maintain the surface as smooth as possible; in vitro design seems not to be a powerful tool to draw relevant conclusions, thus in vivo and in situ designs become strongly recommended.

Key words: Surface treatments. Surface characteristics. Microbiology. Bacterial adhesion. Restorative Materials. Dental biofilm.

INTRODUCTION

Biofilm (bacterial plaque) accumulation on the surface of restorative materials favors the occurrence of secondary caries and periodontal inflammation,[1] which is an important aspect related to the longevity of restorations. Greater bacterial adhesion to dental abutments also favors the development of peri-implant diseases,[2] especially in individuals who are susceptible to periodontal disease. Therefore, restorative materials with low susceptibility to bacterial adhesion are desirable.

In vivo and *in vitro* studies evaluating microbial adhesion to restorative materials have shown differences in biofilm formation.[3–5] The variation in microbial adherence among different materials is related to the properties of the material, such as chemical composition and its surface characteristics.[6–8] Substrates with high surface free energy [SFE], i.e. hydrophilic surface, exhibit more biofilm than substrates with low SFE (hydrophobic). Moreover, rough surfaces provide niches in which microorganisms are protected from brushing, muscle action and salivary flow. While both SFE and roughness influence in microbial adherence and the formation of biofilm, roughness seems to be more important to the accumulation and composition of biofilm, whereas the impact of SFE is greater when comparing surfaces with similar pattern of roughness.[9]

From a clinical standpoint, dentists sometimes need to carry out clinical adjustments of the restoration (e.g., occlusal adjustments, contouring of the restoration or cementation areas) with the use of finishing procedures. The aim of finishing is to obtain the desired anatomic shape and adaptation by contouring the restoration (e.g., emergence profile, restoration marginal fit). Such adjustments are usually performed with fine-grained diamond rotary cutting instruments that break the polished layer and modify the surface characteristics of the restoration, changing the surface topography and causing an increase in surface roughness.[6]

A poorly finished restoration can therefore favor the adherence of biofilm to the surface and adjoining areas in the oral cavity. To minimize this effect, several polishing kits are available to eliminate the grooves and achieve a smoother surface (polishing procedures). Sandpaper discs, rubber wheels and wheels with diamond paste are commonly used. Some literature reviews have been conducted to evaluate the impact of these procedures on the surface characteristics of restorations, as well as biofilm formation as the outcome.[7,8]

Therefore, finishing and polishing procedures can modify roughness characteristics of restorations, thereby either promoting or inhibiting/decreasing the formation of biofilm. The aim of the present systematic review was to characterize how these methods affect the surface

properties of different restorative materials with regard to bacterial adhesion and biofilm formation.

METHODS

Focused question

This systematic review was conducted to answer the following question: based on clinical, *in vitro* or *in situ* studies, do restorative finishing and/or polishing procedures decrease bacterial adherence to the surface of dental materials?

This study was conducted in accordance with the Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA).[10] The protocol is registered with the Prospective Register of Systematic Reviews (PROSPERO: CRD42016036234).

Search strategy

Four internet sources were searched for eligible papers published by November 4, 2016: the MEDLINE-PubMed, EMBASE, Cochrane-CENTRAL and LILACS databases. The structured search was performed using a combination of controlled vocabulary and key words (Table 1) and a similar search strategy was adapted for the other databases. Searches for relevant ongoing trials from the US clinical trials register (http://www.clinicaltrials.gov) and grey literature (OpenGrey repository) were also performed. Manual searches of all references of the selected studies were performed in an attempt to find further relevant reports.

Screening and study selection

Two reviewers (DAMD and GKRP) independently screened the articles. Study selection was performed in two steps: 1) evaluation of title and abstract; and 2) full-text analysis. Titles and abstracts were evaluated for the pre-selection of i*n vitro, in situ* or clinical studies published in the English language that evaluated bacterial adhesion to the surface of dental restorative materials. The reviewers then performed the full-text analysis of the selected studies using the following inclusion criteria:

- Intervention: Finishing/polishing procedures on the surface of dental restorative materials;
- Comparison: Unmodified or treated surfaces with the same material as the intervention group. Thus, studies that only evaluated finishing/polishing procedures among different restorative materials were not considered eligible;
- Quantitative assessment of bacterial adhesion to the surface of restorations;

- Surface characteristics (e.g., roughness, free energy) determined using profilometry, scanning electron microscopy or atomic force microscopy.

Papers that fulfilled all selection criteria were considered eligible for this investigation and submitted to the data extraction process. The concordance between the reviewers for full-texts analysis was statistically assessed showing a 0.9 kappa score. Divergences between the reviewers were discussed and resolved by consensus. If a disagreement persisted, the judgment of a third reviewer (FBZ) was decisive.

Data collection

Both reviewers independently collected the following data from eligible studies: Study identification (authors, year of publication, country in which study was conducted), study design, description of methods (restorative material evaluated and description of finishing/polishing procedures) and type of biofilm formation (e.g., type of microorganism). The main results and conclusions of the studies were recorded. For cases in which the paper that did not provide enough data for inclusion in the analysis, the first or corresponding authors were contacted to determine whether additional data could be provided. If contact with the authors was not achieved after three attempts, the paper was excluded.

Risk of bias (quality assessment)

The quality assessment of the selected studies was adapted from previous investigations.[11,12] The evaluation of the risk of bias involved the use of a chart considering the following aspects for each study design: description of sample-size calculation; randomization of the sample; untreated control group; materials used according to manufacturer's instructions; description of finishing/polishing standardization; blinding of the examiner of the outcome and repetition of biofilm experiment (*in vitro*). If the authors reported the parameter, the article had a Y (yes) on that specific parameter; if it was not possible to find the information, the article received an N (no). Articles that reported 0 to 2 items were classified as high risk of bias, 3 or 4 as medium risk, and 5 to 7 as low risk.

Data analysis

Due to the considerable variability in the methodologies used to evaluate the effect of the finishing/polishing of different restorative materials on biofilm formation and the consequent heterogeneity of the results, meta-analysis was not possible. Thus, descriptive analysis was

performed considering study design, materials, intervention on the surface of the restoration, surface characteristics (roughness and SFE) and the evaluation protocol for biofilm formation and bacterial adhesion.

RESULTS

Figure 1 shows the flowchart of the systematic review as well as the reasons for the exclusion of studies. A total of 2882 articles were found in the initial searches of the electronic databases. After reading the titles and abstracts, 2840 were excluded and 42 articles were submitted to full-text analysis. The manual search yielded no additional studies and one study was selected by direct contact with the authors. Among the 43 articles, 18 met the eligible criteria and were included in the review. Table 2 displays the characteristics of the studies selected.

All studies evaluated the effect of finishing/polishing methods on the surface properties of restorative materials as well as the impact on bacterial adhesion and biofilm formation. Most studies[2,13–23] were developed using an *in vitro design* (n = 12), four studies[5,24–26] used an *in situ* model and two studies[27,28] were clinical trials.

Different materials were evaluated in the studies analyzed. Eighteen experimental groups[14,16–20,22,26] were used to test direct and indirect resin composites. Four experimental groups[14,22,26] were used to evaluate glass ionomer cements. Five experimental groups were used to evaluate dental ceramics (two porcelain[13,25], two feldspar ceramic[5,19] and one Y-TZP ceramic[23]) and six experimental groups[2,15,21,24,27,28] were used to evaluate titanium samples.

The studies demonstrated considerable variability regarding the biofilm formation model. Two clinical studies[27,28] evaluated supragingival and subgingival biofilm formation. Four studies[5,24,25] used natural human biofilm formed on dental appliances *in situ*. Synthesized biofilm was used in 11 studies[2,13–22] and biofilm was cultivated from human saliva in one study[23]. Moreover, different methods were used to quantify biofilm formation, such as the percentage of area covered [2,15,22,25,26,28], total counts of colony-forming units [13,18,19,23,27], counts per minute[14], hemocytometer[29], optical density[16,17] and the quantification of viable biomass and biovolume[5,20,24].

Table 3 displays the descriptive analyses of the effect of finishing/polishing methods on the surface roughness of different materials and the impact on bacterial adhesion. A wide variety was found regarding the finishing or polishing method for each material evaluated, with varied results. In all 13 experimental groups[5,17,23,26,28,30] evaluating a finishing method, an increase in surface roughness was found in comparison to polished and control groups. Fifty

experimental polishing groups[2,6,13–18,20,22–26,29,30] were evaluated and exhibited a tendency toward a smother surface compared to the control. However, some studies found no difference between polishing and control groups.[2,13,14,18,20,26,27,30] The impact of roughness on bacterial adhesion seems not to be related to a roughness threshold, but rather to the range.

In the analysis of the risk of bias (Table 4), 11 studies presented high risk[13–18,20,22,28,30], five studies presented medium risk[2,5,24,25,29] and two studies presented low risk[23,27]. The main aspects related to a higher risk of bias were the description of sample size calculation, randomization of the samples and the blinding of the operator.

DISCUSSION

The present systematic review offers a summary of data regarding the effect of finishing and polishing methods on different materials as well as the impact on bacterial adhesion and biofilm formation. Several restorative materials and finishing/polishing methods have been evaluated and exhibit different degrees of surface roughness. There was a tendency for polishing protocols to produce similar pattern of surface roughness in comparison to untreated or glazed (control) surfaces, whereas finishing methods seem to increase the surface roughness significantly. The impact of surface roughness on bacterial adhesion differs depending on the type of material, study design and range of surface roughness, but does not seem to be strongly related to a pre-established roughness threshold.

Effect of finishing and polishing on surface roughness

The studies included in the present systematic review tested a large variety of finishing and polishing methods, including diamond finishing burs, abrasive paper discs, silicon carbide (SiC) and silicone points, abrasive impregnated rubber, felt wheels and polishing pastes. The effect of each method on the restoration surface is reported to be material dependent and its effectiveness is mainly system dependent.[31] Thus, the effect of finishing and polishing systems in each material was explored individually, as follows:

Resin composite: Eight studies[14,16–18,20,22,26,30] evaluated the surface of resin composites. Only one study[14] tested an untreated surface (Ra = 0.15 µm) as the control group. In two other studies[20,26], the control group was a resin composite compressed against a Mylar matrix to create a smooth surface (Ra values up to 0.2 µm). When finishing and polishing groups were considered,[17,26] finishing by grinding with diamond burs was found to promote a drastic

increase in surface roughness, with Ra values ranging from 2.0 µm (Grandio, Voco)[17] to 4.5 µm (Grandio, Voco) [26]. In the study conducted by Ono et al. (2007)[17], polishing was performed using a diamond paste that reduced surface irregularities, with Ra values of approximately 0.2 µm. In the study by Perez (2008)[26], polishing was performed with the use of a BisCover™ resin polisher (Bisco), leading to a decrease in surface roughness, with Ra values up to 0.43 µm.

Most studies compared two different polishing protocols. When polishing was performed with SiC sandpaper, the surface roughness pattern was directly related to grit size and the range of Ra values was varied among studies. Dezelic et al. (2007)[18] found the smoothest surface using a sequence of 1200-grit, 2400-grit and 4000-grit SiC sandpaper (Ra values of 0.04 µm) compared to 320-grit SiC sandpaper (Ra value of 0.5 – Tetric, Ivoclar; and Ra value of 0.6 – Tetric flow, Ivoclar). Cárlen et al. (2001)[14] compared a 1000-grit SiC sandpaper to an untreated surface and found a rougher surface in the test group (Ra = 0.5 µm vs. 0.15 µm). Yuan et al. (2016)[22] report similar results using a 1200-grit wet abrasive sandpaper (Z250 and Z350, 3M/ESPE – Ra = 0.4 µm; Filtek P90, 3M/ESPE – Ra = 0.5 µm); the authors also tested a polishing method using a nano-silicon dioxide fabric (polishing pad) that achieved a smoother surface (Ra = 0.02 µm) in comparison to the sandpaper group.

Glass ionomer cement: Three studies[14,22,26] assessed a glass ionomer experimental group. Perez (2008)[26] compared three surface treatment methods: 1) compression against a Mylar matrix (control), 2) finishing with fine grain diamond points and 3) polishing with the application of the BisCover™ resin polisher (Bisco) after finishing. The results showed that finishing led to a significant increase in surface roughness (up to Ra = 4.39 µm), whereas polishing reestablished a degree of roughness similar to that in the control group (Ra values = 0.2 to 0.8 µm). Cárlen et al. (2001)[14] found that polishing with 1000-grit Sic sandpaper created a rougher surface (Ra = 1.05 µm) in comparison to an untreated group (Ra = 0.86 µm). Recently, Yuan et al. (2016)[22] achieved a very smooth surface (Ra = 0.03 µm) polishing with a nano-silicon dioxide fabric (polishing pad).

Ceramics: Different dental ceramics were evaluated in four studies.[5,13,25] Dutra et al. (2017)[25] evaluated the effect of finishing by grinding with diamond burs on a Y-TZP surface and found an increase in surface roughness with the increase in bur grit size (up to Ra = 1.16 µm) compared to an untreated control group (Ra = 0.13 µm). Two studies[5,13] that used a glazed group (Ra = 0.5 µm) as control found that no polishing method tested was fully effective at

reestablishing the surface roughness pattern of the control group after finishing. Likewise, Aykent et al. (2010)[19] tested three different polishing methods on feldspar ceramic after finishing (Ra = 1.1 µm) and found a surface roughness pattern with the Ra value ranging from 0.6 to 0.9 µm.

Titanium: Six studies[2,15,21,24,27,28] evaluated titanium samples. In a clinical trial, Quirynen et al. (1997)[27] tested machined and manual polishing methods on titanium abutments and found that both methods created a smoother surface (Ra = 0.11 and 0.06 µm, respectively) in comparison to the control group (Ra = 0.12 µm). In another clinical trial, Elter et al. (2008)[28] evaluated the effect of a finishing method on implant abutments and found a slightly rougher surface (Ra = 0.4 µm) in comparison to the control (Ra = 0.2 µm). In an *in situ* study, Rimondini et al. (1997)[24] evaluated the effect of polishing with grinding paper and diamond paste with and without SiO_2 suspension and found very smooth surface patterns (Ra = 0.09 and 0.2 µm, respectively). In an *in vitro* study, Li et al. (2013)[21] evaluated three different polishing protocols (manual, electrolytic and centrifugal) and found similar surface roughness patterns (Ra values of 0.35, 0.19 and 0.18 µm, respectively). Likewise, Pier-Francisco et al. (2006)[15] compared manual and machined polishing methods and found Ra values of 0.03 µm and 0.16 µm, respectively.

Impact of surface roughness on bacterial adhesion

The data collected in this systematic review showed that finishing and polishing affect the surface roughness and it promotes a heterogeneous impact to bacterial adhesion considering each material evaluated and the method of evaluation of bacterial adhesion outcome (thickness, covered area, biomass, colony-forming units).

In general, smoother surfaces are less likely to lead to the formation of biofilm regardless of restorative material and are therefore desirable. Based on the present findings it may be concluded that: (1) finishing procedures when do not followed for a polishing system provides greater adhesion and retention of bacteria; (2) Some studies showed that polishing successfully reestablished the level of biofilm formation observed on untreated or glazed control groups, regardless of not achieve the same pattern of surface roughness (3) and other studies showed significant differences of biofilm formation among polishing groups even when similar patterns of surface roughness were compared.

The impact of finishing and polishing methods to titanium abutments was evaluated in two clinical studies[27,28] included in this review. Quirynen et al., (1997)[27] evaluated the influence

of the surface smoothing on supra and sub-gingival biofilm formation comparing titanium abutments with different surface roughness (untreated, machined and manually polishing protocols) in six partially edentulous patients. The data showed no significant differences on colony-forming units counts between the control (Ra = 0.2 μm) and polished groups (manual, Ra = 0.06 μm; machined, Ra = 0.11 μm). These results indicated that a reduction in surface roughness (less than a roughness of 0.2 μm) had no major effect on the microbiologic composition, supra-gingivally or sub-gingivally. Based on these observations, the authors suggested an existence of a threshold roughness (Ra = 0.2 μm) below of which no further impact on the bacterial adhesion and/or colonization should be expected. This threshold roughness has been extensively used in the literature. Later, Elter et al. (2008)[28] evaluated supra- and sub-gingival natural human biofilm formation to finishing and untreated titanium abutment surface. Their results showed that finishing the surfaces (Ra = 0.4 μm) retained more supra-gingival biofilm compared to the control (Ra = 0.2 μm) analyzed using scanning electron microscopy, while no differences were observed to the sub-gingival biofilm. These results corroborated the threshold roughness, especially when supra-gingival biofilm was considered. The greater impact of roughness on supra- than sub-gingival biofilm may be explained because the clinical impact of surface roughness becomes especially important when larger shear forces are active.[32]

In agreement with the previous studies, Rimondini et al. (1997)[24] evaluated the surface roughness necessary to reduce early (24h) *in vivo* biofilm colonization on titanium disks assigned to different polishing groups. The results showed no significant differences in bacteria biomass among the polishing groups below the threshold roughness. All others *in situ* studies included in this systematic review[5,25,26] compared finished and polished surfaces with roughness above the threshold roughness. Brentel et al. (2010)[5] assessed the in situ biofilm formation on feldspar ceramic (VM7, Vita). The biomass assessment showed greater bacteria adhesion when the ceramic surface was only ground (finished) by diamond burs (Ra= 2.0 μm) compared to glazed group (Ra= 0.5 μm). In the other hand, when the feldspar ceramic was polished after ground [F&P(2), Ra= 0.8 μm] it successfully reestablish the bacteria adhesion level to the control groups even with a light rougher surface. Controversially results was related by Haralur et al., (2012)[25] evaluating the percentage of covered area by natural biofilm to porcelain (Vita VMK) ceramics. Their results showed that polished groups (Ra= 0.6 and 0.9 μm) failed to achieve similar percentage of bacteria accumulation compared to the smoother groups (auto-glazed: Ra= 0.4; and over-glazed: 0.3 μm).

Perez (2008)[26] evaluated *in situ* bacteria adhesion to glass ionomer and resin composite specimens submitted to finishing and polishing protocols. The author found that the ground surfaces (finishing group) always showed drastically rougher surfaces and presented higher biofilm formation compared to the control group, while polished surfaces presented no differences to the control regarding the biofilm accumulation. Based on the data from these *in situ* studies, it may be stated that mild differences of roughness are not enough to affect the amount of biofilm accumulation even when comparing surfaces above of threshold roughness, once polishing surfaces achieved similar results of biofilm accumulation compared to the pre-treatment surfaces without presenting the same level of surface roughness.

Additionally to the data from clinical and *in situ* studies, most of the articles included in this systematic review used *in vitro* experiments. In brief, it was observed that several articles found no differences in biofilm formation when surfaces with Ra values above the threshold of 0.2 µm were compared,[2,13,14,18,23,30] whereas significant differences in biofilm formation were found in other studies in which only smooth surfaces (Ra values up to 0.2 µm) were evaluated.[15,20] Thus, based on data from these laboratory studies, the threshold roughness of 0.2 µm was not fully corroborated and it should be used cautiously among the different materials evaluated. This divergence may be explained for the intrinsic limitations of laboratory studies, which does not offer the strongest evidence.

Only one *in vitro* study[23] used a polymicrobial biofilm model formed from human saliva, while all other studies used synthesized biofilm. Despite of mono-specimens studies have enhanced the knowledge about the mechanisms of bacteria adhesion to surfaces and differentiate into multicellular biofilms, the use of polymicrobial biofilms models should be incentivized once the majority of chronic infections harboring polymicrobial communities. Although *in vitro* models have been extensively used to study dental biofilm, there are limitations when trying to simulate the oral environment and *in vivo* conditions. It has to be highlighted that during *in vivo* chronic infection, there is a complex interplay between host and pathogen, with species not directly mixing, but residing within their own ecological space, which is not easily replicated *in vitro*, and leads to observable differences between *in vitro* and *in vivo* "chronic infections".[33]

It is well accepted that hard tissues with rougher surfaces in the oral cavity contribute to microorganism retention, since rougher surfaces have a greater area for the development of biofilm as well as topographical irregularities that produce niches in which microorganisms are protected from shear forces and salivary flow. Such factors affect microorganism retention only

in clinical and *vivo* studies, once these factors are rarely simulated in laboratory studies. Therefore, the impact of topographical irregularities on bacterial retention in *in vitro* studies appears to be limited and the amount of biofilm in such studies may be strongly related to other factors linked to the biofilm protocol, such as the type of inoculum (bacterial strain and human saliva) and culture conditions (temperature, pH, nutritional status and nutrient flow, presence of salivary pellicle and incubation time).

Limitations of the study

The results of the present review should be interpreted cautiously, once most of the included studies were carried out using laboratory studies that do not represent the same evidence from clinical studies. Roberts et al (2015)[33] stated that "whilst there is no "gold-standard" for the study of *in vivo* and *in vitro* biofilm formation, it is crucial to know the limiting factors of selected models so as to not over-extrapolate data, and generate assumptions beyond the capabilities of the model". For this reason we discussed the results from each study design individually.

Moreover, it must be mentioned that the assessment of the risk of bias showed the most of included studies had high risk (61%). It was specially critic for *in vitro* studies, once nine from the 12 articles had high risk, while only one had low risk. This result highlight that *in vitro* studies had poor control regarding the methodological variables that could influence the results, Which directly affects the validity of the studies and explains in part the results heterogeneity.

CONCLUSIONS

Based on the findings of this systematic review, it may be concluded that:

- Finishing invariably creates a rougher surface and should be always followed by a polishing method.
- The range of surface roughness among different polishing methods is wide and material dependent.
- Each dental material requires its own treatment modality to obtain and maintain as smooth a surface as possible.
- Topographical irregularities of restorative surface played a limited effect on the *in vitro* bacterial retention, while higher impact was observed on *in vivo* studies.

Additionally, it became evident a wide methodological heterogeneity and poor bias control in the major of studies included in this review. These study limitations difficulty the inter-study comparison and the summarization of the related-evidence. Future investigations targeting to characterize the bacterial adhesion capacity on restorative materials, as well as to evaluate the effect of surface treatments and topographical irregularities on bacteria adhesion and biofilm formation, must be planned considering each study design restriction and predicting the validity and relevance of the evidence to be generated. Thus, in order to better standardization of the studies in this area and to produce evidence of greater clinical relevance, well-designed *in vivo* studies are strongly recommended.

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Figure 1. Selection of studies for systematic review.

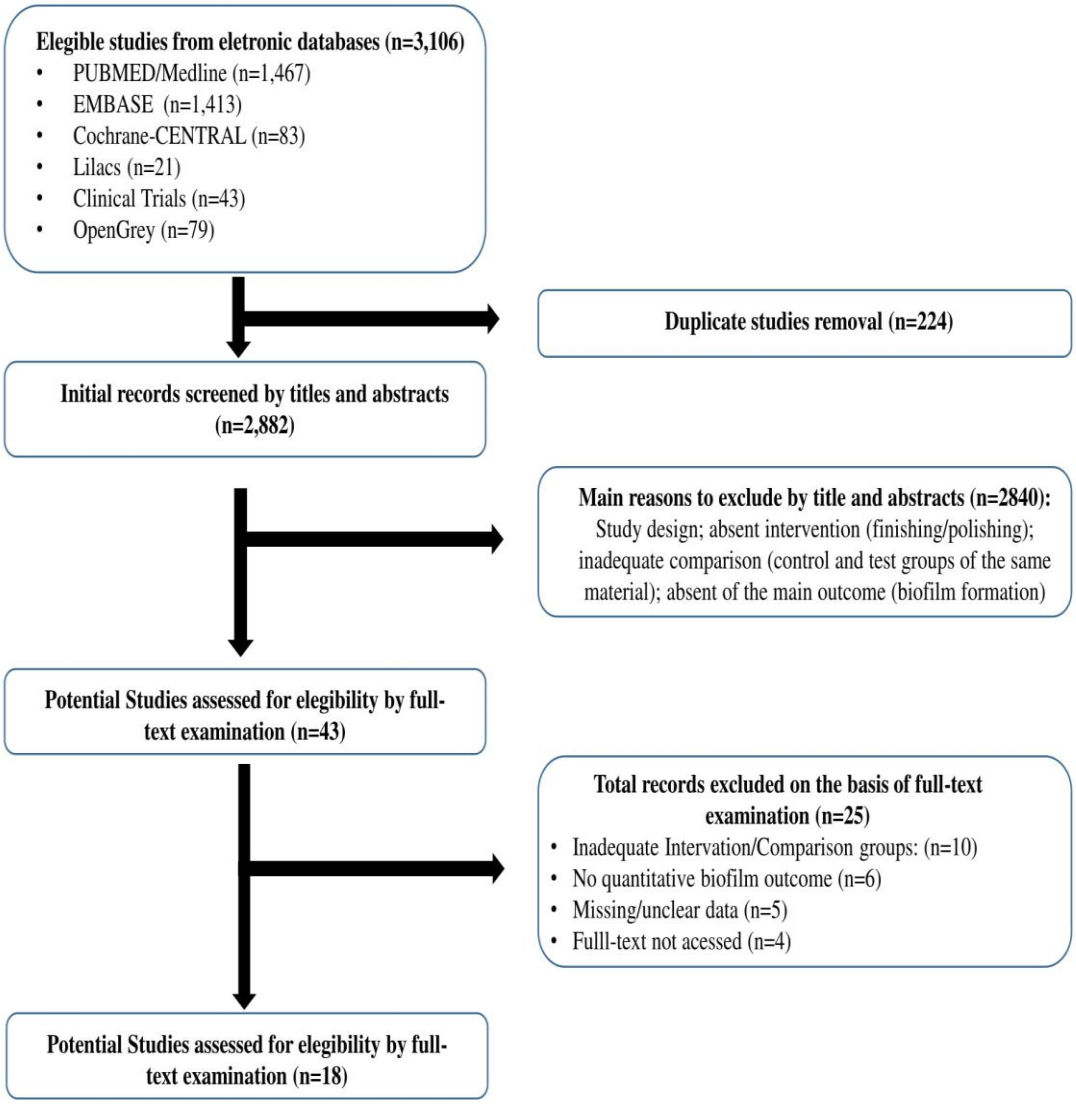

Figure 1. Selection of studies for systematic review.

Table 1. Search Keyword: <Intervention AND Control AND Outcome>
Intervention: Surface treatment (472.425 titles – 07/03)
#1 Population: dental restorations
[MeSH Terms]: "Dental Prosthesis" OR "Dental Restoration, Permanent" OR "Crowns" OR "Dental Abutments" OR "Ceramics" OR "Metal Ceramic Alloys" OR "Dental Porcelain" OR "Dental Materials" OR "Composite Resins" OR "Compomers" OR "Glass Ionomer Cements" OR "Dental Amalgam" [text/words]: dental crown OR dental crowns OR dental restoration OR dental restorations OR dental filling OR dental ceramic OR metal ceramic alloys OR porcelain-metal alloys OR metallo ceramic alloys OR metalloceramic OR metal ceramic restorations OR dental porcelain OR dental material OR composite OR resin OR resin composite OR compomers OR glass-ionomer OR dental amalgam OR abutments OR lithium disilicate OR lithium dislocate glass-ceramic OR feldspathic ceramic OR feldspathic porcelain OR feldspathic veneers OR glass ceramic OR porcelain OR alumina OR alumina ceramic OR alumina zirconia OR zirconia OR Y-TZP or zirconium
#2 Intervantion: Finishing/Polishing (299.283 titles)
[MeSH Terms]: "dental polishing" OR "Prosthesis Fitting" OR "Restore polishing paste" [Supplementary Concept] [text/words]: prosthesis fitting OR prosthesis adjustment OR grinding OR gross OR glaze OR texture OR abrasive OR abrasives OR polish* OR finish* OR burnish*
#3 Outcome: Dental Plaque OR Gingival parameters
[MeSH Terms]: "biofilms" OR "dental plaque" OR "dental plaque index" OR "dental plaque indexes" OR "dental plaque indices" OR "'bacterial adhesion"

Table 2. Summary of the description of the included studies

Study Design	Author/year	Material	Intervention	Sample/ Biofilm	Bacteria Adhesion Outcome
Clinical					
	Elter et al, 2008	▪ Titanium abutments (Nobel Biocare)	▪ Control: Untreated surface ▪ Finishing: grinding with a smooth diamond bur	Supra- and Subgingival natural human biofilm adhered to the abutments surface (n=15)	Percentage of biofilm covering the abutment surface analyzed by SEM
	Quirynen et al, 1997	▪ Titanium (Nobelfarma)	▪ Control: Untreated ▪ Polishing (machined): machine-polished using diamond material ▪ Polishing (manual): manually polished using diamond material	Supra- and Subgingival natural human biofilm adhered to the abutments surfaces (6 volunteers)	Mean CFUs of Supra- and subgingival biofilm formation around the abutment surface
In situ					
	Haralur et al, 2012	▪ Porcelain (VitaVMK, Vident)	▪ Control: Autoglazed & Overglazed ▪ Polishing (Shofu): Shofu kit ▪ Polishing (DFS): DFS kit Polishing (Eve): Eve kit	Natural human biofilm adhered to the samples using oral appliances *in situ* (12 volunteers)	Percentage of biofilm covering the surface area calculated by placing the OHP graph sheet on the test specimen.
	Brentel et al, 2010	▪ Feldspar Ceramic (VM7, Vita Zahnfabrik)	▪ Control: Glazed Surface ▪ Finishing (grind): coarse diamond bur; ▪ F&P (1): coarse diamond bur + silicon rubber tips; ▪ F&P (2): coarse diamond bur + silicon rubber tips + felt disk impregnated with	Natural human biofilm adhered to the samples using oral appliances *in situ* (10 volunteers)	Mean biovolume analyzed by CLSM and CONSTAT software.

			a fine-diamond particle based paste		
	Perez, 2008	▪ Glass ionomer (Ionofil plus, Voco) ▪ Glass ionomer (Vitremer, 3M ESPE) ▪ Resin composite (Filtek Supreme, 3M ESPE) ▪ Resin composite (Grandio, Voco)	▪ Control: Mylar Stips ▪ Finishing (grind): extra-fine diamond burs ▪ Polishing: BisCover™ resin polisher (Bisco)	*S. mutans* Natural human biofilm adhered to the samples using oral appliances *in situ* (1 volunteer)	Percentage area coverage by adherent bacteria.
	Rimondini et al, 1997	▪ Titanium discs	▪ Polishing (1): Grinding paper + diamond paste (3 um) + suspension of SiO_2 (0.04 um) ▪ Polishing (2): Grinding paper + diamond paste (6 um)	Natural human biofilm adhered to the samples using oral appliances *in situ* (8 volunteers)	Total bacteria amount (Biomass – optical density) by spectroscopy
In vitro					
	Dutra et al, 2017	▪ Y-TZP (InCeram, VITA)	▪ Control: Untreated ▪ Finishing: grinding with a fine diamond bur ▪ Finishing: grinding with a coarse diamond bur	Polymicrobial biofilm formed from human saliva (single donor)	Mean CFU of biofilm formation
	Yuan et al, 2016	▪ Nanoparticle Resin Composite (FiltekTM Z350, 3M ESPE, USA) ▪ Nano-hybrid Resin Composite	▪ Polished: polished with 11-um grit (grain 1200 wet abrasive paper disc) ▪ Polished: polished with nano-silicon dioxide fabric	*S. mutans* Single specie synthesized biofilm	Area of bacteria adhesion (A%) by CLSM images.

	(FiltekTM Z250 XT, 3M ESPE, USA) ▪ Low-shrink Resin Composite (FiltekTM P90, 3M ESPE, USA) ▪ Polymer-based pre-reacted glass ionomer (Beautifil II, Shofu, Japan).			
Li et al, 2013	▪ Titanium (Chinese national (GB/T 3623-1998))	▪ Control: Untreated surface ▪ Polishing (manual): manual polishing (carborundum point + silicone points + hard rubber wheel) ▪ Polishing (Electrolytic): electrolytic polishing ▪ Polishing (Centrifugal): centrifugal mill polishing	*Candida Albicans* Single specie synthesized biofilm	Total counts by a hemocytometer under the objective (40x) of an optical microscope
Ionescu et al, 2012	▪ Resin composite (Filtek Supreme XT, 3M ESPE) ▪ Resin composite (Filtek Silorano, 3M ESPE)	▪ Control: Mylar Stips ▪ Polishing (4000 Sic): 1,000 + 4,000-grit SiC paper	*S, mutans* Single specie synthesized biofilm	Viable biomass assessment by MTT assay
Aykent et al, 2010	▪ Direct Resin Composite (Tetric Evo-Ceram, Ivoclar, Vivadent) ▪ Indirect Resin Composite Estenia	▪ Finishing (grind): Fine (46 μm) and extra-fine (25 μm) diamond rotatory cutting instruments. ▪ Polishing (sof-lex): Sequence of 3 sandpaper	*S. mutans* Single specie synthesized biofilm	Total counts of vital adhered bacteria analyzed by CLSM.

	(KL 100; Kuraray Co Ltd) ▪ Indirect Resom Composite (SR Adoro) ▪ Feldspar Ceramic (VITABLOCS Mark II)	discs (Sof-Lex coarse: 100 μm, medium: 29 μ, and fine: 14 μm). ▪ Polishing (diamond paste): Felt wheel with diamond paste. ▪ F&P: Finished with a white stone + polished with a sequence of 3 SiC rubber points (Ceramiste Standard: 48 μm, Ultra: 28 μm, and Ultra II: 6.3 μm).		
Barbour et al, 2007	▪ Standard Titanium abutments (NobelBiocare)	▪ Control: Untreated surface ▪ Polishing: Fine diamond grit rotary bur + green carborundum stone rotary point + brown impregnated silicon rubber point + green impregnated silicon rubber point + cloth mop and polishing compound containing amorphous silica/silicon carbide ▪ Polishing: Brown impregnated silicon rubber point + green impregnated silicon rubber point + cloth mop and polishing compound containing amorphous silica/silicon carbide.	*S. mutans* Single species synthesized biofilm	Median values of percentage coverage area analyzed by AFM

Dezelic et al, 2007	▪ Resin composite (Tetric, Ivoclar Vivadent) ▪ Flowable Resin composite (Tetric Flow, Ivoclar Vivadent) ▪ Unfilled resin composite (Heliobond, Ivoclar Vivadent)	▪ Control (320-Sic): 320-grit SiC sandpaper. ▪ Polishing (4000-Sic): Sequence of 1200-grit, 2400-grit and 4000- grit SiC sandpaper.	*A. naeslundii, V. dispar, F. nucleatum, S. sobrinus, S. oralis* and *Candida albicans* Single species synthesized biofilm	Mean CFUs (log10) of biofilm formation
Ono et al, 2007	▪ Resin composite (Clearfil AP-X, Kuraray Medical) ▪ Resin composite (Grandio, Voco)	▪ Polishing (800-SiC): polished with 800-grit SiC sandpaper ▪ Polishing (diamond paste): diamond paste of up to 1 μm particle size	*S. mutans* Single species synthesized biofilm	Total count of bacteria/mm^3 quantified by turbidimetric analisys (OD_{550nm})
Ikeda et al, 2007	▪ Resin Composite (Estenia C&B, Kuraray) ▪ Resin Composite (Gradia, GC)	▪ Polishing: Ground with 800-grit SiC paper ▪ Polishing: Diamond Pastes up to 1 um	*S. mutans* Single species synthesized biofilm	Amount of bacteria (optical density) by infrared spectroscopy
Pier-Francesco et al, 2006	▪ Titanium (Goodfellow Cambridge Limited)	▪ Polishing (brushes): A hand polished with rotary brushes ▪ Polishing (machine): Eco mini dry machine	*P. gingivalis* Single species synthesized biofilm	Median values of percentage coverage area analyzed by fluorescent microscopy
Cárlen et al, 2001	▪ Glass ionomer (KetacFilt Aplicapt; ESPE)	▪ Control: Untreated surface ▪ Polishing (1000-SiC): 1000 grit SiC sandpaper	*S. mutans S. sanguis* and *A.naeslundii* Single species synthesized biofilm	Total number of cells were calculated from the CPM values.

	▪ Resin Composite (TPH SpectrumTM; Dentsply DeTrey)			
Kawai et al, 2000	▪ Porcelain (Vita Celay blanks, A3M-9, Vita Zahnfabrik).	▪ Control: Glazed ▪ Polishing (120 EP): emery paper of 120-grit ▪ Polishing (600-EP): emery paper of 600-grit ▪ Polishing (Diamond paste): felt wheel with diamond paste	*S. sobrinus* Single species synthesized biofilm	Total counts of adhered cells were measured by using a liquid scintillation method

CLSM: Confocal laser scanning microcopy; AF: Atomic force microcopy; SEM: Scanning electric microscopy; CPM: Count per minute; CFU: Colony-forming units; OHP: Overhead projection

Table 3. Summary of the results of roughness and bacteria adhesion

Author (year)	Material	Intervention	Surface Roughness(µm)	Bacteria adhesion Outcome	Statement
Clinical					
Elter et al, 2008	Titanium (Nobel Biocare)	▪ Control ▪ Finishing (grind)	0.2 0.4	▪ Supragingival biofilm: Finishing > Control ▪ Subgingival biofilm: No significant difference	▪ Finished surfaces (rougher surface) retained more supragingival biofilm compared to the control ▪ Regardless of the roughness differences, finishing surfaces did not retained more subgingival biofilm compared to the control
Quirynen et al, 1997	Titanium (Nobelfarma)	▪ Control ▪ Polishing (machined) ▪ Polishing (manual)	0.21 0.11 0.06	▪ No significant differences were observed between Control and Polishing groups	▪ Roughness values below the threshold of 0.2 µm did not present a significant amount of bacteria adhesion ▪ Both the polishing protocols were effective to establish the level of bacteria adhesion observed on control.
In situ					
Haralur et al, 2012	Porcelain (VitaVMK, Vident)	▪ Control (Autoglazed) ▪ Control (Overglazed) ▪ Polishing (Shofu) ▪ Polishing (DFS)	0.42 (0.06) 0.34 (0.07) 0.62 (0.01) 0.91 (0.02)	▪ Significant less percentage of plaque accumulation was found on surfaces of the control Groups (auto- and overglazed) than polished surfaces	▪ All Polishing protocols failure on prevent the bacteria adhesion when compared to the control groups
Brentel et al, 2010	Feldspar ceramic (VM7, Vita Zahnfabrik)	▪ Control (glazed) ▪ Finishing (grind) ▪ F&P (1) ▪ F&P (2)	0.53 (0.11) 2.02 (0.12) 1.27 (0.14) 0.88 (0.11)	▪ Finishing and F&P (1) > Control ▪ No significant differences were observed between F&P (2) and Control	▪ Differences between 0.53 and 0.88 µm did not result in an increased bacteria adhesion ▪ F&P (2) protocol successfully reestablished the level observed on control.
Perez, 2008	Glass ionomer	▪ Control	0.78	▪ Finishing > Polishing > Control	

	(Ionofil plus, Voco)	▪ Polishing ▪ Finishing (grind)	0.88 4.39		▪ The increase on biofilm formation was related to the increase on surface roughness. ▪ None finishing/ polishing protocol successfully reestablished the level of bacteria adhesion observed on control.
	Glass ionomer (Vitremer, 3M ESPE)	▪ Control ▪ Polishing ▪ Finishing (grind)	0.23 0.79 1.67	▪ Finishing > Control ▪ Polishing presented no statistical differences to the Control.	▪ Differences between 0.2 and 0.8 μm did not result in an increased bacteria adhesion ▪ Polishing protocol successfully reestablished the level observed on control.
	Resin composite (Filtek Supreme, 3M ESPE)	▪ Control ▪ Polishing ▪ Finishing (grind)	0.19 0.61 3.20	▪ Finishing > Control ▪ Polishing presented no statistical differences to the Control.	▪ Differences between 0.2 and 0.6 μm did not result in an increased bacteria adhesion ▪ Polishing protocol successfully reestablished the level observed on control
	Resin composite (Grandio, Voco)	▪ Control ▪ Polishing ▪ Finishing (grind)	0.04 0.43 (0.07) 4.54 (0.23)	▪ Finishing > Control ▪ Polishing presented no statistical differences to the Control.	▪ Differences between 0.04 and 0.4 μm did not result in an increased bacteria adhesion ▪ Polishing protocol successfully reestablished the level observed on control
Rimondini et al, 1997	Titanium discs	▪ Polishing (1) ▪ Polishing (2)	0.09 (0.01) 0.2 (0.06)	▪ No significant differences were observed between Polishing (1) and Polishing (2) groups	▪ Roughness values below the threshold of 0.2 μm did not present a significant amount of bacteria adhesion
In vitro					
Dutra et al, 2017	Y-TZP (InCeram, VITA)	▪ Control ▪ Finishing (fine-bur) ▪ Finishing (coarse-bur)	0.13 (0.02) 0.70 (0.21) 1.16 (0.14)	▪ No significant differences were observed between control and finishing groups	▪ Differences between 0.13 and 1.16 μm did not result in an increased bacteria adhesion

Yuan et al, 2016	Nanoparticle restorative (FiltekTM Z350, 3M ESPE, USA)	▪ Polished (1200 grit) ▪ Polished (nano-silicon)	0.44 0.02	▪ Polished (1200 grit) > Polished (nano-silicon)	▪ Results showed a significant increase on bacterial adhesion as an increase on surface roughness ▪ Polishing (nano-silicon) protocol was more effective to prevent bacteria adhesion in comparison to Polishing (1200 grit) protocol
	Nano-hybrid universal restorative (FiltekTM Z250 XT, 3M ESPE, USA)	▪ Polished (1200 grit) ▪ Polished (nano-silicon)	0.43 0.02	▪ Polished (1200 grit) > Polished (nano-silicon)	▪ Results showed a significant increase on bacterial adhesion as an increase on surface roughness ▪ Polishing (nano-silicon) protocol was more effective to prevent bacteria adhesion in comparison to Polishing (1200 grit) protocol
	Low-shrink posterior restorative based on siloxane and oxirane (FiltekTM P90, 3M ESPE, USA)	▪ Polished (1200 grit) ▪ Polished (nano-silicon)	0.53 0.02	▪ Polished (1200 grit) > Polished (nano-silicon)	▪ Results showed a significant increase on bacterial adhesion as an increase on surface roughness ▪ Polishing (nano-silicon) protocol was more effective to prevent bacteria adhesion in comparison to Polishing (1200 grit) protocol
	Polymer-based pre-reacted glass ionomer (Beautifil II, Shofu, Japan).	▪ Polished (1200 grit) ▪ Polished (nano-silicon)	0.67 0.03	▪ Polished (1200 grit) > Polished (nano-silicon)	▪ Results showed a significant increase on bacterial adhesion as an increase on surface roughness ▪ Polishing (nano-silicon) protocol was more effective to prevent bacteria adhesion in comparison to Polishing (1200 grit) protocol
Li et al, 2013	Titanium (Chinese national (GB/T 3623-1998))	▪ Control ▪ Polishing (manual) ▪ Polishing (eletrolytic) ▪ Polishing (centrifugal)	2.04 (0.42) 0.35 (0.12) 0.19 (0.09) 0.18 (0.08)	▪ Control > Polishing (manual and eletrolytic) > Polishing (centrifugal)	▪ Roughness values below the threshold of 0.2 μm presented significant amount of bacteria adhesion

					▪ Polishing using centrifugal mill protocol was effective to prevent *C. albicans* adhesion
Ionescu et al, 2012	Resin composite (Filtek Supreme XT, 3M ESPE)	▪ Control (Mylar Stips) ▪ Polishing (4000-Sic)	0.05 (0.02) 0.06 (0.02)	▪ 48h incubation: No significant difference ▪ 96h incubation: Polishing > Control	▪ Polishing protocol was not successfully to establish the level observed on control considering 96h biofilm incubation.
	Resin composite (Filtek Silorano, 3M ESPE)	▪ Control (Mylar Stips) ▪ Polishing (4000-Sic)	0.06 (0.03) 0.07 (0.03)	▪ 48h incubation: No significant difference ▪ 96h incubation: No significant difference	▪ Similar roughness values resulted in no significant difference on bacteria adhesion regardless the incubation time
Aykent et al, 2010	Direct composite (Tetric Evo-Ceram, Ivoclar, Vivadent)	▪ Finishing (grind) ▪ Polishing (sof-lex) ▪ Polishing (diamond paste) ▪ F&P	1 (0.4) 0.58 (0.4) 0.9 (0.2) 0.7 (0.1)	▪ No significant differences among surface treatments and no significant interactions between restorative materials and surface treatments	▪ Differences between 0.58 and 1.0 μm did not result in an increased bacteria adhesion
	Indirect composite Estenia (KL 100; Kuraray Co Ltd)	▪ Finishing (grind) ▪ Polishing (sof-lex) ▪ Polishing (diamond paste) ▪ F&P	1.2 (0.4) 0.6 (0.4) 1 (0.4) 0.58 (0.4)	▪ No significant differences among surface treatments and no significant interactions between restorative materials and surface treatments	Differences between 0.58 and 1.2 μm did not result in an increased bacteria adhesion
	Indirect composite (SR Adoro)	▪ Finishing (grind) ▪ Polishing (sof-lex) ▪ Polishing (diamond paste) ▪ F&P	1.5 (0.2) 0.7 (0.4) 1.1 (0.4) 0.9 (0.4)	▪ No significant differences among surface treatments and no significant interactions between restorative materials and surface treatments	▪ Differences between 0.7 and 1.5 μm did not result in an increased bacteria adhesion
	Ceramic material (VITABLOCS Mark II)	▪ Finishing (grind) ▪ Polishing (sof-lex)	1.1 (0.8) 0.6 (0.2)	▪ No significant differences among surface treatments and no	

		▪ Polishing (diamond paste) ▪ F&P	0.9 (0.6) 0.7 (0.4)	significant interactions between restorative materials and surface treatments	▪ Differences between 0.6 and 1.1 μm did not result in an increased bacteria adhesion ▪
Barbour et al, 2007	Standard Titanium abutments (NobelBiocare)	▪ Control ▪ Polishing (1) ▪ Polishing (2)	0.25 (0.42) 0.27 (0.04) 0.25 (0.04)	▪ *S. mutans:* Polishing (2) < Control No significant differences were observed between Polishing (1) and Control ▪ *A. naeslundii: P*olishing (1) and (2) > Control	▪ Similar roughness values presented significant differences on bacteria adhesion ▪ Polishing (2) was effective to prevent bacteria adhesion for both the *S. mutans* and A. *naeslundii* strains.
Dezelic et al, 2007	Resin composite (Tetric, Ivoclar Vivadent)	▪ Control (320-Sic) ▪ Polishing (4000-Sic)	0.49 0.04	▪ 15 minutes' incubation: Polishing < Control ▪ 15h incubation: No significant difference	▪ Polishing protocol successfully reestablished the level observed on control using an very early biofilm formation (15 minutes). ▪ After 15h biofilm formation, polishing procedure was not effective to prevent bacteria adhesion
	Flowable composite (Tetric Flow, Ivoclar Vivadent)	▪ Control (320-Sic) ▪ Polishing (4000-Sic)	0.61 0.04	▪ 15 minutes' incubation: No significant differences ▪ 15h incubation: No significant difference	▪ Regardless of the roughness values and incubation time, the polishing procedure did not differ from the control condition
	Unfilled resin (Heliobond, Ivoclar Vivadent)	▪ Control (320-Sic) ▪ Polishing (4000-Sic)	0.82 0.07	▪ 15 minutes' incubation: No significant difference ▪ 15h incubation: No significant difference	▪ Regardless of the roughness values and incubation time, the polishing procedure did not differ from the control condition
		Finishing (grind)	2.22 (0.13)	▪ Finishing > Polishing	

Ono et al, 2007	Resin composite (Clearfil AP-X, Kuraray Medical)	Polishing (diamond paste)	0.25 (0.66)		▪ Results showed a significant increase on bacterial adhesion as an increase on surface roughness ▪ Polishing protocol was more effective to prevent bacteria adhesion in comparison to finishing procedures.
	Resin composite (Grandio, Voco)	Finishing (grind) Polishing (diamond paste)	2.01 (1.12) 0.22 (0.01)	▪ Finishing > Polishing	▪ Results showed a significant increase on bacterial adhesion as an increase on surface roughness ▪ Polishing protocol was more effective to prevent bacteria adhesion in comparison to finishing procedures.
Ikeda et al, 2007	Resin Composite (Estenia C&B)	▪ Polishing (600SiC) ▪ Polishing (Diamond paste)	11.7 (0.3) 6.4 (0.2)	▪ Polishing (Sic) > Polishing (Diamond Paste)	▪ Results showed a significant increase on bacterial adhesion as an increase on surface roughness ▪
	(Kuraray) Gradia (GC)	▪ Polishing (600SiC) ▪ Polishing (Diamond paste)	11.2 (0.4) 7.3 (0.5)	▪ Polishing (Sic) > Polishing (Diamond Paste)	▪ Results showed a significant increase on bacterial adhesion as an increase on surface roughness
Pier-Francesco et al, 2006	Titanium (Goodfellow Cambridge Limited)	▪ Polishing (brushes) ▪ Polishing (machined)	0.03 0.16	▪ Polishing (machined) > Polishing (brushes)	▪ Results showed a significant increase on bacterial adhesion as an increase on surface roughness ▪ Roughness values below the threshold of 0.2 μm presented significant amount of bacteria adhesion
Cárlen et al, 2000	Glass ionomer (KetacFilt Aplicapt; ESPE)	▪ Control ▪ Polishing (1000-Sic)	0.86 (0.06) 1.05 (0.12)	▪ No significant differences were observed between Control and Polishing groups	▪ Differences between 0.86 and 1.05 μm did not result in an increased bacteria adhesion

					▪ Polishing protocol successfully reestablished the level observed on control.
	Composite resin (TPH SpectrumTM; Dentsply DeTrey)	▪ Control ▪ Polishing (1000-Sic)	0.15 (0.05) 0.56 (0.06)	▪ Polishing > Control	▪ Polishing protocol was not successfully to reestablish the level observed on control.
Kawai et al, 2000	Porcelain (Vita Celay blanks, A3M-9, Vita Zahnfabrik).	▪ Control (glazed) ▪ Polishing (120-EP) ▪ Polishing (600-EP) ▪ Polishing (Diamond paste)	0.15 (0.04) 0.53 (0.09) 0.25 (0.07) 0.12 (0.02)	▪ 3h incubation: No significant difference ▪ 8h incubation: No significant difference ▪ 12h incubation: No significant difference ▪ 24h incubation: Polishing (Diamond paste) < Control (glazed)	▪ Considering short periods of incubation (3, 8 and 12h), roughness values ranging from 0.12 to 0.53 μm did not result in significant increase of bacteria adhesion ▪ Polishing with Diamond paste was successfully to prevent bacteria adhesion compared to the control.

Table 4. Risk of Bias of the studies included on systematic review considering the aspects reported in the Materials & Methods section.

Author/year	Sample	Ramdon	Control	Materials	Treatment	Blinding	Repetition	Risk of bias
Clinical studies								
Elter et al, 2008	N	N	Y	Y	N	N	NA	High
Quirynen et al, 1997	N	Y	Y	Y	Y	Y	NA	Low
In situ studies								
Haralur et al, 2012	N	N	Y	Y	Y	N	NA	Medium
Brentel as, 2010	N	Y	Y	Y	Y	N	NA	Medium
Perez, 2008	N	N	Y	N	Y	N	NA	High
Rimondini l, 1997	N	Y	N	Y	Y	N	NA	Medium
In vitro studies								
Dutra et al, 2017	N	Y	Y	Y	Y	N	Y	Low
Yuan et al, 2016	N	N	N	N	Y	N	N	High
Li et al, 2013	N	Y	Y	N	Y	N	N	Medium
Ionescu et al, 2012	N	Y	Y	N	N	N	N	High
Aykent et al, 2010	N	N	N	N	N	N	N	High
Barbour et al, 2007	N	N	Y	Y	N	N	Y	Medium
Dezelic et al, 2007	N	N	Y	N	Y	N	N	High
Ono et al, 2007	N	N	N	N	N	N	Y	High
Ikeda et al, 2007	N	N	N	Y	N	N	Y	High
Pier-francesco et al, 2006	N	N	N	Y	N	N	N	High
Cárlen et al, 2000	N	N	Y	Y	N	N	N	High
Kawai et al, 2000	N	N	Y	N	N	N	N	High

Sample-size calculation; randomization of the sample; untreated control group; materials used according to manufacturer's instructions; description of finishing/polishing standardization; blinding of the examiner of the outcome and repetition of biofilm experiment (in vitro); Y, yes; N, no; NA. not applicable.

4 DISCUSSÃO

Os estudos desta tese focaram na temática do efeito das características superficiais do material restaurador na formação do biofilme, tendo em vista o importante papel que este exerce na etiologia de prevalentes doenças bucais, como doença cárie, doença periodontal e periimplantar.

A partir dos dados do primeiro estudo pode-se inferir que os tratamentos de superfície empregados modificam a superfície do material avaliado (cerâmica Y-TZP). Onde o desgaste com as pontas diamantadas (*Xfine* e *Coarse*) promovem maior conteúdo superficial de fase monoclínica quando comparado com a condição apenas sinterizada (Controle – sem tratamento), além de, alterar a topografia superficial, a rugosidade e a energia livre de superfície da cerâmica Y-TZP. Em relação ao envelhecimento hidrotérmico, observa-se efeitos distintos dependendo da presença / ausência de desgaste. Entretanto, as alterações induzidas por ambos fatores não foram suficientes para resultar em diferenças na formação de biofilme utilizando um modelo *in vitro* (AAA *model*) (EXTERKATE; CRIELAARD; TEN CATE, 2010).

A relação entre as características de superfície do material e a adesão bacteriana tem sido estudada extensivamente (SONG; KOO; REN, 2015; TEUGHELS et al., 2006). Teughels e colaboradores (2006) avaliaram o impacto das características de superfície (energia livre, rugosidade, composição química) na formação de biofilme em diferentes materiais restauradores, entretanto estudos com cerâmica Y-TZP não foram abordados (TEUGHELS et al., 2006). Os autores concluíram que um aumento da rugosidade superficial acima do limiar de 0,2 µm e/ou da energia livre superficial facilitam a formação de biofilme em materiais restauradores. Quando ambas as características de superfície interagem entre si, a rugosidade superficial foi considerada predominante.

A utilização de um limiar de rugosidade (0,2 µm) foi proposta inicialmente por Quirynen e colaboradores (1997) que avaliaram a influência do alisamento superficial na formação de biofilmes supra e sub-gengival, comparando os pilares de titânio com diferentes rugosidades superficiais em seis pacientes parcialmente desdentados (QUIRYNEN et al., 1997). Os resultados não mostraram diferenças significativas nas contagens das unidades formadoras de colônias entre os grupos controle (Ra = 0,2 µm) e polidos (manual, Ra = 0,06 µm, usinado, Ra = 0,11 µm). Estes resultados indicaram que uma redução da rugosidade superficial (inferior a uma rugosidade de 0,2 µm) não teve um efeito importante na composição microbiológica, supra-gengival ou sub-gengival e, com base nessas observações, os autores sugeriram a

existência de uma rugosidade limiar (Ra = 0,2 μm) abaixo da qual não se deve esperar mais impacto na adesão bacteriana e/ou colonização. Este limiar de rugosidade tem sido amplamente utilizado na literatura.

De forma divergente, os resultados do artigo 1 desta Tese mostraram que mesmo com os grupos *Xfine* e *Coarse* apresentando valores de Ra superiores ao limiar de 0,2 μm (0,70 e 1,16 μm respectivamente), não foi observado um aumento na adesão bacteriana quando comparados com o grupo Controle (0,13 μm). Assim, foi possível supor que o intervalo de rugosidade superficial promovido pelo desgaste com pontas diamantadas não foi o principal fator para promover a adesão bacteriana na cerâmica Y-TZP. Logo, essa baixa susceptibilidade à adesão bacteriana pode ser considerada uma vantagem deste material. Este resultado está de acordo com outros estudos na literatura que indicaram que a adesão bacteriana não pode ser totalmente explicada por pequenas diferenças das características da superfície do material (HAHNEL et al., 2009; RIMONDINI et al., 2002).

Considerando a inconsistência na temática da caracterização superficial com a formação de biofilme, bem como a importância de procedimentos de acabamento e polimento para a prática clínica do cirurgião dentista, o artigo 2 sumarizou por meio de uma revisão sistemática os dados disponíveis sobre o efeito de métodos de acabamento e polimento nas características superfícies de diferentes materiais restauradores, bem como o impacto sobre a adesão bacteriana e a formação de biofilme. Visando diminuir fatores de confusão em relação ao desfecho principal, formação de biofilme, como a influência das propriedades químicas de diferentes materiais, apenas estudos que tenham avaliado diferentes tratamentos sobre o mesmo substrato foram incluídos.

Vários materiais restauradores e métodos de acabamento/polimento foram avaliados, apresentando diferentes níveis de rugosidade superficial. Houve uma tendência de que protocolos de polimento produzissem um padrão de rugosidade superficial semelhante as superfícies não tratadas ou com *glaze* (controle), enquanto que os métodos de acabamento aumentaram a rugosidade superficial significativamente. O impacto da rugosidade superficial na adesão bacteriana difere dependendo do tipo de material, do desenho do estudo e da faixa de rugosidade da superfície, mas não parece estar fortemente relacionado com um limiar de rugosidade pré-estabelecido. Ainda, os dados coletados na revisão sistemática mostraram que o acabamento e o polimento afetam a rugosidade superficial e promovem um impacto heterogêneo na adesão bacteriana considerando cada material avaliado e o método de avaliação

do resultado da adesão bacteriana (espessura, área coberta, biomassa, unidades formadoras de colônia).

Em geral, superfícies mais lisas são menos susceptíveis à formação de biofilme independentemente do material restaurador e são, por conseguinte, desejáveis. Com base nos dados da revisão sistemática observou-se que (1) procedimentos de acabamento quando não seguidos por um sistema de polimento proporciona maior aderência e retenção de bactérias (2) alguns estudos mostraram que o polimento restabeleceu com sucesso o nível de formação de biofilme observado em grupos de controle não tratado ou *com glaze*, independentemente de não atingir o mesmo padrão de rugosidade superficial (3) e outros estudos mostraram diferenças significativas na formação de biofilmes entre grupos de polimento mesmo quando padrões semelhantes de rugosidade da superfície foram comparados.

Outro fator importante de discussão referiu-se a influência do delineamento dos estudos incluídos na revisão sistemática em relação ao desfecho de adesão bacteriana e formação de biofilme. É bem aceito na literatura que tecidos duros com superfícies mais rugosas na cavidade bucal contribuem para a retenção de microrganismos (SONG; KOO; REN, 2015; TEUGHELS et al., 2006). Isto justifica-se pelo fato de que superfícies mais rugosas proporcionarem uma maior área para o desenvolvimento de biofilme, bem como irregularidades topográficas promoverem nichos em que os microrganismos ficam protegidos dos mecanismos de controle e regulação da microbiota bucal, como fluxo salivar, mastigação, deglutição (NEWMAN, 1974) e procedimentos de higiene bucal (QUIRYNEN et al., 1990). Tais fatores afetam a retenção de microrganismos apenas em modelos *in vivo*, uma vez que esses fatores raramente são simulados em estudos laboratoriais. Portanto, o impacto das irregularidades topográficas na retenção bacteriana em estudos *in vitro* parece ser limitado e a quantidade de biofilme em tais estudos pode estar fortemente relacionada a outros fatores ligados ao protocolo de biofilme, como o tipo de inóculo (estirpe bacteriana e saliva) e condições de cultura (temperatura, pH, estado nutricional e fluxo de nutrientes, presença de película salivar e tempo de incubação). Com isso, a observação geral dos resultados da revisão deve ser interpretada com cautela, considerando o delineamento de cada estudo.

Assim, apesar de os estudos *in vitro* terem colaborado fortemente para o atual entendimento sobre o processo de adesão bacteriana as superfícies dentárias e restauradoras, bem como a diferenciação em biofilmes multicelulares, esses apresentam limitações metodológicas que não permitem uma adequada simulação das condições *in vivo*, resultando em uma evidência sem forte relevância clínica (ROBERTS et al., 2015). Com isso, novas

investigações que visem caracterizar a capacidade de adesão bacteriana sobre a superfície de diferentes materiais restauradores, bem como avaliar a adesão bacteriana a superfície restauradora submetida a diferentes tratamentos superficiais, devem dar preferência para estudos com modelos de formação de biofilme *in vivo.*

5 CONCLUSÃO

Com base nos dados obtidos nos estudos da presente tese pode-se observar o efeito do tratamento de superfície (desgaste com pontas diamantadas) e do envelhecimento hidrotérmico (LTD) na caracterização de superfície e adesão bacteriana sobre uma superfície cerâmica Y-TZP, bem como, em uma abordagem mais abrangente, sumarizar o efeito de métodos de polimento e acabamento na adesão microbiana e formação de biofilme em diferentes materiais restauradores.

O desgaste com pontas diamantadas e o envelhecimento hidrotérmico promoveram maior teor de fase monoclínca, rugosidade superficial e alterações de energia livre superficiais do material Y-TZP avaliado. Entretanto, a adesão bacteriana e formação de biofilme pareceu não ser afetada pela diferença de rugosidade obtida com as pontas diamantadas de diferentes granulações.

Quando o efeito do tratamento de superfície (acabamento e polimento) na adesão bacteriana foi avaliado de forma extensiva, considerando diferentes materiais, pode ser observado que (1) O acabamento invariavelmente cria uma superfície mais áspera e deve ser sempre seguido por um método de polimento; (2) A variação de rugosidade da superfície entre diferentes métodos de polimento é ampla e material-dependente; (3) Cada material necessita de sua própria modalidade de tratamento para obter e manter uma superfície tão lisa quanto possível; (4) Um limiar de rugosidade superficial de Ra = 0,2 μm não foi efetivo para predizer a formação de biofilme em estudos não-clínicos. (5) Irregularidades topográ ficas da superfície restauradora apresentaram um efeito limitado na retenção bacteria *in vitro,* enquanto um impacto mais significativo foi observado em estudos com modelo *in vivo.*

Ainda, apesar dos estudos da presente tese terem focado na temática do efeito das características superficiais do material restaurador na formação do biofilme, cabe ressaltar que essas não afetam exclusivamente no desfecho de adesão bacteriana, podendo também influenciar em outras propriedades do material como comportamento mecânico e desgaste do antagonista (*wear*). Então, quando o acabamento das restaurações for necessário, este deve ser realizado com pontas diamantadas finas e seguidas pelo sistema de polimento adequado para o material (recomendado pelo fabricante).

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ANEXO A – NORMAS PARA PUBLICAÇÃO NO PERIÓDICO *OPERATIVE DENTISTRY*

GUIDE FOR AUTHORS
Manuscript submission

General Requirements

Operative Dentistry requires electronic submission of all manuscripts. All submissions must be sent to Operative Dentistry using the Allen Track upload site. A mandatory and nonrefundable $25.00 fee is required at submission. Your manuscript will only be considered officially submitted after it has been approved through our initial quality control check, and any quality problems have been resolved. You will have 6 days from when you start the process to submit and approve the manuscript. After the 6 day limit, if you have not finished the submission, your submission may be removed from the server. You are still able to submit the manuscript, but you must start from the beginning. Be prepared to submit the following manuscript files in your upload:

• A Laboratory or Clinical Research Manuscript file must include:

- a title
- a running (short) title
- a clinical relevance statement o a concise summary (abstract) 14 Current as of: 3-Sep-14
- introduction, methods & materials, results, discussion and conclusion
- references (see Below)

• The manuscript body MUST NOT include any:

- Author identifying information such as:
  - Authors names or titles
  - Acknowledgements
  - Correspondence information
  - Response to reviewer files should also NOT include any author identifying information, such as a signature at the end, etc.
- Figures
- Graphs
- Tables

• An acknowledgement, disclaimer and/or recognition of support (if applicable) must in a separate file and uploaded as supplemental material.

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Important Information

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• The author(s) retain(s) the right to formally withdraw the paper from consideration and/or publication if they disagree with editorial decisions.

• International authors whose native language is not English must have their work reviewed by a native English speaker prior to submission.

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- Manuscripts that have major revisions requested (i.e. For English correction) are entered as a resubmission of the original article.

• Spelling must conform to the American Heritage Dictionary of the English Language, and SI units for scientific measurement are preferred.

• While we do not currently have limitations on the length of manuscripts, we expect papers to be concise; authors are also encouraged to be selective in their use of figures and tables, using only those that contribute significantly to the understanding of the research.

• Acknowledgement of receipt is sent automatically upon acceptance through quality control. This may take up to 7 days. If you do not receive such an acknowledgement, please check your author homepage at http://jopdent.allentrack.net if the paper does not appear there please resend your paper.

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All Manuscripts

CORRESPONDING AUTHOR must provide a WORKING / VALID e-mail address which will be used for all communication with the journal. NOTE: Corresponding authors MUST update their profile if their e-mail or postal address changes. If we cannot contact authors within seven days, their manuscript will be removed from our publication queue.

AUTHOR INFORMATION must include:

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• complete mailing address for each author

• valid email address for each author • degrees (e.g. DDS, DMD, PhD)

• affiliation (e.g. Department of Dental Materials, School of Dentistry, University of Michigan)

MENTION OF COMMERCIAL PRODUCTS/EQUIPMENT must include:

• full name of product • full name of manufacturer

• city, state and country of manufacturer

MANUSCRIPTS must be provided as Word for Windows files. Files with the .doc and .docx extensions are accepted.

TABLES may be submitted as either Word (.doc and .docx) or Excel (.xls and .xlsx) files. All tables must be legible, with fonts being no smaller than 7 points. Tables have the following size limitations: In profile view a table must be no larger than 7 x 9 inches; landscape tables should be no wider than 7 inches. It is the Editor’s preference that tables not need to be rotated in order to be printed, as it interrupts the reader’s flow.

ILLUSTRATIONS, GRAPHS AND FIGURES must be provided as TIFF or high resolution JPEG files with the following parameters:

• line art (and tables that are submitted as a graphic) must be sized with the short edge being no shorter than 5 inches. It should have a minimum resolution of 600 dpi and a maximum resolution of 17 Current as of: 3-Sep-14 1200 dpi. This means the shortest side should be no smaller than 3000 pixels.

• gray scale/black & white figures must be sized with the short edge being no shorter than 5 inches. It should have a minimum resolution of 300 dpi and a maximum of 400 dpi. This means the shortest side should be no smaller than 1500 pixels.

• color figures and photographs must be sized with the short edge being no shorter than 3.5 inches. It should have a minimum resolution of 300 dpi and a maximum of 400 dpi. This means that the shortest side should be no smaller than 1050 pixels.

Other Manuscript Type – Additional Requirements

CLINICAL TECHNIQUE/CASE STUDY MANUSCRIPTS must include as part of the narrative:

• a running (short) title

• purpose

• description of technique

• list of materials used

• potential problems

• summary of advantages and disadvantages

• references (see below)

LITERATURE AND BOOK REVIEW MANUSCRIPTS must include as part of the narrative:

• a running (short) title

• a clinical relevance statement based on the conclusions of the review

• conclusions based on the literature review…without this, the review is just an exercise and will not be published

• references (see below)

References

REFERENCES must be numbered (superscripted numbers) consecutively as they appear in the text and, where applicable, they should appear after punctuation. The reference list should be arranged in numeric sequence at the end of the manuscript and should include:

1. Author(s) last name(s) and initial (ALL AUTHORS must be listed) followed by the date of publication in parentheses.

2. Full article title.

3. Full journal name in italics (no abbreviations), volume and issue numbers and first and last page numbers complete (i.e. 163-168 NOT attenuated 163-68).

4. Abstracts should be avoided when possible but, if used, must include the above plus the abstract number and page number.

5. Book chapters must include chapter title, book title in italics, editors' names (if appropriate), name of publisher and publishing address.

6. Websites may be used as references, but must include the date (day, month and year) accessed for the information.

7. Papers in the course of publication should only be entered in the references if they have been accepted for publication by a journal and then given in the standard manner with "In press" following the journal name.

8. DO NOT include unpublished data or personal communications in the reference list. Cite such references parenthetically in the text and include a date.

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Reference Style Guide

• Journal article-two authors: Evans DB & Neme AM (1999) Shear bond strength of composite resin and amalgam adhesive systems to dentin American Journal of Dentistry 12(1) 19-25.

• Journal article-multiple authors: Eick JD, Gwinnett AJ, Pashley DH & 19 Current as of: 3-Sep-14 Robinson SJ (1997) Current concepts on adhesion to dentin Critical Review of Oral and Biological Medicine 8(3) 306-335.

• Journal article: special issue/supplement: Van Meerbeek B, Vargas M, Inoue S, Yoshida Y, Peumans M, Lambrechts P & Vanherle G (2001) Adhesives and cements to promote preservation dentistry Operative Dentistry

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• Corporate publication: ISO-Standards (1997) ISO 4287 Geometrical Product Specifications Surface texture: Profile method – Terms, definitions and surface texture parameters Geneve: International Organization for Standardization 1st edition 1-25.

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OpDent believes in the free market and that it is in the best interest of the profession for the market to give back generously to those groups who promote continuing education of those professionals. There must be clear guidelines and expectations however, so that the goodwill and generosity of the Market do not taint the educational activities with bias, real or imagined. To this end we have adopted the following policies and guidelines.

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Speakers/presenters at any CE activity will be required to disclose any potential bias towards commercial supporters, or any other commercial entity that will be mentioned in their presentation.

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Authors of every accepted manuscript will be required to disclose any potential bias towards commercial supporters, or any other commercial entity that will be mentioned in their manuscript.

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