UNIVERSIDADE ESTADUAL DE CAMPINAS
FACULDADE DE ODONTOLOGIA DE PIRACICABA

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Avaliação das propriedades físicas e microbiológicas de materiais CAD-CAM: efeitos de desafios erosivos e abrasivos e incorporação de nanotubos de dióxido de titânio no glaze cerâmico.

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Tese apresentada à Faculdade de Odontologia de Piracicaba da Universidade Estadual de Campinas como parte dos requisitos exigidos para a obtenção do título de Doutora em Clínica Odontológica, na Área de Dentística.

Thesis presented to the Piracicaba Dental School of the University of Campinas in partial fulfillment of the requirements for the degree of Doctor in Restorative Dentistry

Orientadora: Profª Drª Vanessa Cavalli Gobbo

ESTE EXEMPLAR CORRESPONDE À VERSÃO FINAL
TESE DEFENDIDA PELA ALUNA MAYARA ZAGHI DAL PICOLO,
E ORIENTADA PELA PROFª. DRª. VANESSA CAVALLI GOBBO.

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Faculdade de Odontologia de Piracicaba

A Comissão Julgadora dos trabalhos de Defesa de Tese de Doutorado, em sessão pública realizada em 16 de fevereiro de 2023, considerou a candidata MAYARA ZAGHI DAL PICOLO aprovada.

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RESUMO

Os objetivos desta tese foram avaliar (1) o comportamento de diferentes materiais CAD-CAM frente a desafios erosivos e/ou abrasivos, e (2) desenvolver e analisar as propriedades de um glaze cerâmico incorporado com nanotubos de dióxido de titânio (n-TiO_2). No estudo 1, cerâmica de silicato de lítio reforçada por partículas de zircônia (***ZLS***), cerâmica infiltrada por polímero (***PICN***), cerâmica feldspática ***(FE)*** e duas resinas nanohíbridas ***(RG e RB)*** foram submetidos à erosão (E), abrasão (A), erosão associada à abrasão (E+A), ou mantidos sem tratamento (controle - C). os materiais foram testados quanto à resistência à flexão (Rf, n=10), microdureza (KHN) e rugosidade de superfície (Ra) (n=10), atividade antimicrobiana (*S. mutans*, *S. sanguinis* e *C. Albicans*) e a morfologia da superfície, em microscopia eletrônica de varredura (MEV). Os dados foram analisados por modelos lineares mistos pelo método REML. FE apresentou menor Rf entre os grupos, sendo que ZLS e RG apresentaram a maior Rf, e PICN e RK, valores intermediários ($p<0,05$). PICN, ZLS e FE apresentaram menor KHN após desafios E / E+A comparados aos materiais RG e RK. FE apresentou maior Ra após E / E+A, enquanto a resina RK após desafio A / E+A ($P<0,05$). ZLS apresentou maior formação de biofilme que FE, RK e RG, enquanto RK e RG apresentaram os menores valores de formação de biofilme. E+A promoveram alterações morfológicas, sendo as mais severas na superfície dos materiais cerâmicos ou híbridos. Concluiu-se que os desafios erosivos associado à abrasão causam alterações nas propriedades mecânicas e topografia dos materiais CAD/CAM, além de promover maior adesão de biofilme. No estudo 2, foi avaliada a alteração de cor, rugosidade de superfície, e efeito antibacteriano de um glaze cerâmico dopado com n-TiO_2. Ao pó do glaze foi incorporado 1%, 2,5% e 5 wt% de n-TiO_2 ou 0% (controle) e aplicado à superfície de corpos de prova da FE (n=10). A alteração de cor (ΔE_{00}, ΔE_{ab}, ΔW_{ID}) e rugosidade (Ra) da cerâmica, foram determinadas antes (T0) e após a aplicação do glaze (T1). O efeito antibacteriano contra *S. mutans* e *S. sanguinis* (UFC/mL) foi avaliado em T1. ΔE_{00}, ΔE_{ab}, Ra, e UFC/mL foram analisados por ANOVA dois fatores de medidas repetidas, e teste de Bonferroni ($\alpha= 0,05$). Não houve diferenças nos parâmetros de cor ΔE_{00}, ΔE_{ab} entre os grupos ($p>0,05$) e o W_{ID} apenas foi afetado com adição de 5% de n-TiO_2. Todos os grupos apresentaram valores médios superiores a 1,8 (ΔE_{00}) e 2,3 (ΔE_{ab}), ultrapassando os limites de percepção. Em T0, não foram detectadas diferenças de Ra entre os grupos, mas em T1, houve redução de Ra para os grupos testados, exceto para o glaze contendo 5% de n-TiO_2, no qual houve aumento de Ra em relação ao controle ($p<0,05$). A incorporação de 0%, 1% e 2,5% de n-TiO_2 no glaze não interferiu na formação de biofilme e não houve diferenças entre as concentrações ($p<0{,}05$). Concluiu-se que o glaze contendo 5% de n-TiO_2 promoveu mínima interferência na cor e rugosidade da cerâmica, porém as concentrações estudadas não impediram a formação de biofilme bacteriano.

Palavras-chave: Cerâmica. CAD-CAM. Biofilme.

ABSTRACT

The aim of these thesis was evaluated through two distinct studies (1) the behavior of different CAD-CAM materials against erosive and/or abrasive challenges, and (2) to develop and analyze the properties of a ceramic glaze incorporated with titanium dioxide nanotubes (n- TiO2). For study 1, lithium silicate reinforced with zirconia ceramic (ZLS), polymer-infiltrated ceramic network (PICN), feldspathic ceramic (FE) and two nanohybrid resins (RG and RB) were subjected to erosion (E), abrasion (A), erosion associated with abrasion (E+A), or left untreated (control - C). The materials were tested for flexural strength (Rf, n=10), microhardness (KHN) and surface roughness (Ra) (n=10). Antimicrobial activity (S. mutans, S. sanguinis and C. albicans) and surface morphology in scanning electron microscopy (SEM) were also performed. Data were analyzed by mixed linear models using the REML method. FE had the lowest Rf between the groups. ZLS and RG showed the highest Rf, and PICN and RK, intermediate values ($p<0.05$). PICN, ZLS and FE showed lower KHN after E / E+A challenges compared to RG and RK. FE presented higher Ra after E / E+A, while the RK resin after A / E+A challenge ($P<0.05$). ZLS showed highest biofilm formation than FE, RK and RG, while RK and RG showed the lowest values of CFU/mL. E+A promoted morphological changes, the most severe was observed on the surface of ceramic or hybrid materials. Could be concluded that the erosive challenges associated with abrasion could damage the mechanical properties and topography of CAD/CAM materials, in addition to promoting greater biofilm adhesion. In study 2, the color change, surface roughness, and antibacterial effect of a ceramic glaze doped with n-TiO_2 was evaluated. 1%, 2.5% and 5 wt% of n-TiO_2 or 0% (control) were added to the glaze powder and applied to the surface of FE specimens (n=10). The color change (ΔE_{00}, ΔE_{ab}, W_{ID}) and roughness (Ra) of the ceramic were determined before (T0) and after glaze application (T1). The antibacterial effect against *S. mutans* and *S. sanguinis* was evaluated by counting CFU/mL in T1. ΔE_{00}, ΔE_{ab}, Ra, and CFU/mL were analyzed by two-way repeated measures ANOVA and Bonferroni test ($\alpha= 0.05$). There were no differences in color parameters ΔE_{00}, ΔE_{ab} between groups ($p>0.05$) and W_{ID} was only affected with the addition of 5% n-TiO_2. All groups had mean values greater than 1.8 (ΔE_{00}) and 2.3 (ΔE_{ab}), exceeding the limits of perception. At T0, no differences in Ra were detected between the groups, but at T1, there was a reduction in Ra for the tested groups, except for the glaze containing 5% n-TiO_2, in which there was an increase in Ra compared to the control ($p <0.05$). The incorporation of 0%, 1% and 2.5% of n-TiO_2 in the glaze did not interfere with the biofilm formation and there were no differences between the concentrations ($p<0.05$). In conclusion, glaze containing 5% n-TiO_2

caused minimal interference in the color and roughness of the ceramic, but the tested concentrations did not inhibit biofilm formation.

Keywords: Ceramics. CAD-CAM. Biofilm.

SUMÁRIO

1 INTRODUÇÃO

A produção industrial de blocos CAD-CAM (computer-aided design/computer aided manufacturing) parcialmente ou totalmente cristalizados promoveu o desenvolvimento de materiais homogêneos e com alta resistência mecânica, constituídos dos mais variados tipos de cerâmicas odontológicas ou a base de matriz resinosa (Mainjot et al., 2016). A composição destes materiais possibilitou alcançar altos padrões estéticos sem impactar no desempenho mecânico, facilitando o emprego em diversos tipos de restaurações indiretas (Silva et al., 2017).

De acordo com Gracis et al 2015, os materiais cerâmicos podem ser classificados como materiais a base de matriz vítrea ou resinosa e policristalinos. Portanto, a seleção do sistema cerâmico utilizado clinicamente baseia-se em suas propriedades mecânicas e óticas, geralmente determinadas pela sua composição (Gracis et al., 2015). Cerâmicas vítreas como a silicato de lítio reforçada por zircônia combinam a fase cristalina de silicato de lítio com uma matriz vítrea reforçada com cristais de dióxido de zircônio (8 a 12%), característica que melhora as propriedades mecânicas da cerâmica. Ainda, a adição de zircônia é descrita como capaz de interferir no processo de cristalização reduzindo possíveis trincas, dessa forma após cristalização o material pode atingir altos padrões estéticos (Elsaka et al., 2016).

Os blocos CAD/CAM compostos a base de matrizes resinosas apresentam melhorias nas propriedades físicas e óticas em relação à resina composta direta, pois a fabricação industrial garante homogeneidade e diminui a presença de falhas intrínsecas (Mainjot et al., 2016). Alguns materiais apresentam maior teor de carga do que compósitos diretos, influenciando diretamente na resistência mecânica à flexão (Giordano et al., 2006). Além disso, estes materiais são facilmente fresados, polidas e reparados, sendo classificadas de acordo com sua composição microestrutural em resinas nanocerâmicas (ex: Katana Avencia e Grandio Block), compostas por nanopartículas silanizadas combinadas com silício e partículas de alumínio, ou cerâmicas híbridas (cerâmica infiltrada de polímero – PICN, Vita Enamic, Vita Zanfabrik), que apresenta uma mistura de monômeros infiltrados de UDMA e TEGDMA (14% em peso) em uma rede de cerâmica vítrea (86% em peso) (Mainjot et al., 2016).

Embora estudos relatem o excelente desempenho mecânico e óptico de materiais indiretos CAD/CAM avaliando sua estabilidade química e mecânica, desafios clínicos orais exógenos (bebidas ácidas e alimentos) e endógenos (biofilme cariogênico, enzimas salivares, ácido gástrico) podem promover a degradação do material e defeitos estruturais, devido ao declínio do pH e lixiviação seletiva dos íons alcalinos (Esquivel-Upshaw et al., 2018, Kukiattrakoon et al., 2010, Alnasser et al., 2019). Nesse contexto, os distúrbios gástricos representam uma condição sistêmica de alto risco que pode intensificar a degradação das restaurações indiretas do sistema CAD-CAM. A bulimia nervosa, que é uma condição grave e potencialmente fatal, consiste em um distúrbio alimentar seguido por comportamentos compensatórios, como vômito autoinduzido, causando a doença do refluxo gastresofágico (DRGE) (Castillo et al., 2017).

A DRGE provoca alterações na mucosa do esôfago resultando no refluxo do conteúdo estomacal (ácido clorídrico - HCl) para o esôfago e, finalmente, para a cavidade oral, onde altera abruptamente o pH oral (Kulkarni et al., 2020). Portanto, pacientes com bulimia ou DRGE podem apresentar erosão dentária causada pela exposição ao ácido clorídrico, especialmente nas superfícies palatinas maxilares dos dentes anteriores e/ou nas superfícies oclusais dos dentes posteriores (Harwood et al., 1995). Adicionalmente, os desafios ácidos decorrentes da bulimia ou DRGE são capazes de degradar os materiais restauradores diretos ou indiretos, promovendo diminuição da microdureza de superfície e aumento da rugosidade, favorecendo o acúmulo de biofilme. Estas alterações ocorrem em maior ou menor grau de severidade a depender das características e composição destes materiais (Backer et al., 2017).

Embora a maioria dos materiais CAD-CAM de vitrocerâmica, à base de polímeros e híbridos possuam alta resistência mecânica e propriedades ópticas comparados aos materiais restauradores diretos convencionais, a exposição repetida e contínua ao pH ácido combinada com a abrasão mecânica promovida pela escovação, pode comprometer a resistência à flexão, a microdureza, a rugosidade superficial e influenciar a formação de biofilme (da Cruz et al., 2022, Picolo et al., 2023). Portanto, determinar o comportamento de materiais CAD-CAM indiretos expostos a severos desafios orais é primordial para o planejamento reabilitador. Soma-se a esta necessidade, o fato de alguns materiais não requererem etapas adicionais de finalização após a fresagem, apenas polimento com o uso de discos e borrachas abrasivas, o que pode os tornar mais susceptíveis à degradação. Assim, o Artigo 1 desta Tese avaliou os efeitos da erosão promovida pelo ácido gástrico combinados com a escovação mecânica nas propriedades mecânicas, topografia de superfície e adesão do biofilme em diferentes materiais do sistema CAD/CAM.

Além das variações de acidez causadas fisiologicamente pela alimentação ou por condições extremas como os DRGEs, o ambiente clínico oral é desafiador para a manutenção de materiais restauradores. A constante mudança de coeficientes térmicos, desgastes oclusais fisiológicos ou patológicos e a presença de microorganismos, motiva a necessidade constante de evolução das características mecânicas, óticas e biológicas destes materiais (Kulkarni et al., 2020). A capacidade de inibição ou o controle da formação do biofilme, aliado à manutenção das características físico-mecânicas, é certamente um dos requisitos mais desejáveis para um material cuja longevidade é comprovadamente longa (Imazato et al., 2003). Neste sentido, estudos recentes têm proposto a incorporação de agentes com propriedades antimicrobianas em cerâmicas (Goldschmidt et al., 2021, Baptista et al, 2022). Em estudo *in vitro*, Oh et al., 2019 avaliou a incorporação de nanopartículas de prata e fluoreto de sódio para aplicação sobre o glaze e os resultados demonstraram diminuição da adesão e colônias de *Strepctococcus mutans* sobre estes materiais (Oh et al., 2019). Em outro relato, uma cobertura a base de carbeto de silício demonstrou redução na atividade microbiana contra *S. mutans* e *S. sanguinis* (Afonso Camargo et al., 2020), corroborando que a adição de partículas às cerâmicas pode favorecer o controle do biofilme, evento especialmente importante para restaurações indiretas com términos em áreas subgengivais.

Estudos indicam que nanoestruturas derivadas do dióxido de titânio (TiO_2) melhoram consideravelmente as propriedades físico-químicas e mecânicas de diversos materiais odontológicos, além de promover efeito antimicrobiano (Cibim et al., 2017, Kantovitz et al., 2020, Antunes et al 2022). A redução bacteriana proporcionada pelo TiO_2 ocorre devido às interações entre os íons metálicos (Ti) com a membrana celular bacteriana (Tavassoli Hojati et al., 2013). Ainda, observa-se que na fase anatase dos nanotubos de TiO_2 (n-TiO_2) com o meio aquoso, há formação de radical hidroxila por meio de uma reação de fotocatálise, capaz de danificar o DNA bacteriano (Liou et al., 2012). Estudos prévios demonstram que a incorporação de n-TiO_2 a cimentos de ionômero de vidro (Araujo et al., 2021) ou a polímeros a base de poli-metil-metacrilato (PMMA) (Abdulrazzaq Naji et al., 2018) resultou em atividade antimicrobiana nestes materiais, sugerindo que a incorporação de n-TiO_2 em materiais odontológicos pode resultar em inibição ou controle de formação de biofilme.

O glazeamento consiste em um método de acabamento e polimento das restaurações cerâmicas, e é composto de cerâmica de baixa fusão indicada para aplicação sobre a superfície de cerâmica vítrea ou vítrea reforçada, para minimizar defeitos provenientes do processo de sinterização ou fresagem do material, o que o torna responsável por diminuir a rugosidade de superfície, minimizar a propagação de trincas e aumentar a resistência intrínseca da cerâmica (Sagsoz et al., 2016, Willers et al., 2020). Entretanto, o desgaste clínico decorrente da condição oral, promove degradação do glaze e aumento de rugosidade nestas regiões (Sarikaya et al., 2010). Por sua vez, o aumento de rugosidade favorece o acúmulo de biofilme, desencadeando respostas biológicas nos tecidos periodontais, além de propiciar maior risco de lesões de cárie recorrente na interface dente-restauração

(Mohammadibassir et al., 2019). Sugere-se, portanto, que a incorporação de nt-TiO_2 a um glaze cerâmico possa inibir ou controlar a formação de biofilme, fornecendo propriedades antimicrobianas ao glaze cerâmico, impactando positivamente no desempenho clínico deste material em longo prazo. Desta forma, o Artigo 2 propõe a modificação de um glaze cerâmico comercial a partir da incorporação de diferentes concentrações de nanotubos de dióxido de titânio (nt-TiO_2), e a avaliação de seu impacto nas propriedades óticas e antimicrobianas do glaze cerâmico.

Os dois estudos apresentados nesta tese são independentes, porém apresentam como temática principal o estudo da estabilidade de materiais do sistema CAD-CAM. O objetivo primário do Artigo 1 foi a avaliação da estabilidade físico-mecânica de materiais do sistema CAD-CAM de diferentes composições submetidos a desafios erosivos e/ou abrasivos severos, enquanto do Artigo 2, o impacto da incorporação de nanotubos de dióxido de titânio (n-TiO_2) nas propriedades físicas e antibacterianas de um glaze cerâmico aplicado sobre cerâmica vítrea.

2 ARTIGOS

2.1 Artigo 1

Effects of gastric acid and mechanical toothbrushing in CAD-CAM restorative materials: mechanical properties, surface topography and biofilm adhesion

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Abstract

Objective: To evaluate the effects of simulated gastric acid erosion combined with mechanical toothbrushing abrasion on the mechanical properties, surface topography, and biofilm adhesion of different CAD/CAM systems.

Material and methods: Specimens of zirconia reinforced lithium silicate glass-ceramic (ZLS), polymer-infiltrated ceramic network (PICN), feldspathic glass-ceramic (FE), and two nanoceramic resins (RK, RG), were submitted to the following challenges: erosion (E), abrasion (A), erosion combined with abrasion (EA), or remained untreated (control- C). After challenges, flexural strength (0.5 mm/min) was evaluated, while microhardness (KHN) and surface roughness (Ra) were tested before and after treatments. The biofilm adhesion (*Streptococcus mutans*, *Streptococcus sanguinis* e *Candida albicans*) was determined by the counting of colonies forming units per milliliters (UFC/mL) after erosive and abrasive challenges.

Results: FE showed the lowest flexural strengths, while ZLS and RG exhibited the highest and PICN and RK, intermediate values. PICN, ZLS, and FE showed lower microhardness after E and E+A challenges than the polymer-based materials (RG and RK). FE surface roughness increased after E / E+A challenges and after A / E+A challenges for RK. Biofilm formation after erosive/abrasive challenges was higher on ZLS than FE, RK, and RG, but no different than PICN. RK and RG exhibited the lowest biofilm formation among the groups. Furthermore, E + A challenges held significant changes in the surface of the materials, which were more severe on the surface of glass-ceramics or hybrid materials.

Conclusion: Erosive challenges combined with abrasion negatively influenced the mechanical properties and surface topography of most CAD/CAM materials and increased the biofilm adhesion on ZLS. Besides, the severity of the damage is related to the type and composition of each material.

Keywords: erosion, abrasion, CAD/CAM, ceramic, biofilm

ACKNOWLEDGEMENTS

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1. Introduction

The high homogeneity, mechanical strength, and reliability of industrially fabricated milled CAD/CAM blocks resulted in crucial advantages for processing glass-matrix ceramics, resin-matrix, hybrid, and polycrystalline indirect materials.[1] For achieving high aesthetic requirements without decreasing the mechanical behavior, fully crystallized ceramic blocks have been introduced into the market.[2] The monolithic feldspathic ceramic Vita Mark II is an example of a fully sintered glass-ceramic in which the extra steps of the stratification process favor material manipulation.[3] Nevertheless, because the use of glass-ceramics in posterior restorations presents strength limitations, various filler types have been added to these materials aiming to improve the mechanical behavior without downgrading the esthetic features.[4]

Zirconia reinforced lithium silicate glass-ceramic (Celtra® Duo, Degudent, Hanau-Wolfgang, Germany) is a material that combines lithium silicate crystalline phase with a glassy matrix reinforced with zirconium dioxide crystals (8 to 12%).[5] The presence of the zirconium compound improves the mechanical properties of the material and interferes in the crystallization process by preventing crystal growth.[6] The reduced crystal size allegedly enhances polishing compared to the lithium disilicate glass-ceramics.[7]

CAD/CAM resin-matrix ceramics exhibit improvements compared to the direct resin composite because the industrial fabrication ensures homogeneity and decreases the presence of flaws and pores.[8] Besides, resin-matrix ceramics blocks exhibit superior polymerization under high temperatures (180º C), and some materials exhibit higher filler content than direct composites, directly influencing the mechanical flexural strength. In addition, these materials are easily milled, polished, and repaired, being classified according to their microstructure composition as nanoceramic resins (e.g. Katana Avencia and Grandio Block), composed of silanized nanoparticles combined with silicon and aluminum particles, or hybrid ceramics (e.g. *polymer-infiltrated ceramic-network* – PICN, Vita Enamic, Vita Zanfabrik), which presents a blend of UDMA and TEGDMA (14% weight) infiltrated monomers in a vitreous ceramic network (86% weight).[9,10,11]

Although studies report the excellent mechanical and optical performance of CAD/CAM indirect materials appraising its chemical and mechanical stability, the occurrence of exogenous (acidic beverages and food) and endogenous (cariogenic biofilm, salivary enzymes, gastric acid) clinical oral challenges might lead to material degradation and structural restorative defects due to pH decline and selective lixiviation of the alkaline ions.[12,13] In this context, gastric disorders stand as a high-risk systemic condition that could intensify the degradation of the indirect CAD-CAM restorations.[14] Bulimia nervosa, which is a serious, potentially life-threatening condition, consists of a diet disorder followed by compensatory behaviors such as self-induced vomit, leading to gastroesophageal reflux disease (GERD).[15]

The GERD provokes esophagus mucosa alterations resulting in the reflux of the stomach content (hydrochloric acid - HCl) to the esophagus and finally, to the oral cavity, where it abruptly changes the oral pH.[16] Therefore, patients with bulimia or GERD might exhibit severe dental erosion caused by hydrochloric acid exposure, especially on maxillary palatal surfaces of anterior teeth and/or on the occlusal surfaces of posterior teeth.[17] These patients frequently require indirect rehabilitations to reestablish dental morphology, occlusal function, and esthetics, which could be accomplished with CAD-CAM indirect materials.[18] However, acid challenges are likely to degrade the indirect material by decreasing the surface microhardness, increasing the surface roughness, and favoring the biofilm accumulation.[14]

Indeed, previous studies reported the effects of GERD on CAD-CAM blocks, observing that 5% HCl immersion challenges (45 or 91 hours), without toothbrushing simulation, did not significantly affect lithium disilicate glass-ceramics (LDS) and polycrystalline zirconium (Zr) materials, but increased the surface roughness of feldspathic, leucite and polymer-based materials (Alnasser et a., 2019).[14] Others observed that even though the gastric acid simulation (pH 1.2) for 6 and 24 h did not change the surface roughness, it decreased the microhardness of two polymeric blocks.[18] Although toothbrushing is an indispensable habit to maintain oral health, its combination with severe episodes of extremely low pH, like those caused by GERD, could potentialize the aging and degradation of the CAD-CAM materials, thereby compromising the flexural strength, microhardness, surface roughness, and biofilm formation.[19,20]

Although most glass-ceramics, polymer-based, and hybrid indirect CAD-CAM materials are reported as having high mechanical strength and appropriate optical properties over the conventionally fabricated restorations, repeated and continuous exposure to low pH combined with the mechanical abrasion promoted by toothbrushing might compromise the flexural strength, microhardness, surface roughness, and biofilm formation.

In the United States alone, 1 to 3% and 18 to 25% of the population are diagnosed with bulimia and GERD, respectively.[21] Therefore, it is crucial to determine the behavior of indirect CAD-CAM materials exposed to these severe oral challenges. Given these facts, this study investigated the effects of gastric acid combined with mechanical toothbrushing abrasion challenge on the flexural strength, microhardness, surface roughness, and biofilm accumulation on different types of CAD-CAM materials. The null hypotheses tested were that the erosive/abrasive challenges would not change (1) the flexural strength, (2) the surface microhardness and surface roughness, and (3) the biofilm formation of CAD-CAM materials tested.

2. Materials and Methods

2.1. Experimental Design

This randomized *in vitro* study used CAD-CAM blocks (ZLS- zirconia-reinforced lithium silicate glass-ceramic, PICN- polymer-infiltrated ceramic network, FE- feldspathic glass-ceramic, and two polymer-based materials - RK and RG) as experimental units, submitted to following acid-abrasion challenges (n=10): erosion (E), abrasion (a), erosion with abrasion (EA), or kept without treatment (control- C). Table 1 displays the composition of the materials.

The flexural strength (MPa) was tested after erosion and/or abrasion, while microhardness (KNH) and surface roughness were determined at baseline (T_0) and after erosion and/or abrasion (T_1). The biofilm adhesion through viable colony counting (UFC/mL) was performed after challenges.

Table 1: Material, Material type, composition, and manufacturer of all tested materials

Material	Material type	Composition	Manufacturer
Celtra Duo- ***ZLS***	Zirconia reinforced lithium silicate glass-ceramic	SiO_2, P_2O_5, Al_2O_3, Li_2 O, K_2O, ZrO_2, CeO_2, Na_2O, Tb_4O_7, V_2O_5, Pr_6O_{11}, Cr, Cu, Fe, Mg, Mn, Si, Zn, Ti, Zr, Al.	Dentsply Sirona-Degudent GmbH, Hanau-Wolfgang, Germany
Vita Blocks Mark II- ***FE***	Glass Ceramic	Fine particle feldspar ceramic material (56-64% SiO_2, 20-23% Al_2O_3, 6-9% Na_2O, 6-8%K_2O, and other oxides)	Vita Zahnfabrik, Bad Säckingen, Germany
Vita Enamic- ***PICN***	Hybrid Ceramic	86 wt% of feldspar ceramic, 14 wt% polymer $Si0_2$, Al_2O_3, Na_2O, K_2O, B_2O_3, CaO, TiO_2, TEGDMA, UDMA	Vita Zahnfabrik, Bad Säckingen, Germany
Katana Avencia Block -***RK***	Resin Nanoceramic	Mixed filler with colloidal silica (40 nm) and aluminum oxide (20 nm), cured resins consisting of methacrylate monomer (Copolymer of Urethane dimethacrylate and other methacrylate monomers), pigments, filler content 62 (wt%)	Kuraray Noritake Dental, Tokyo, Japan.
Grandio Blocs- ***RG***	Resin Nanoceramic	86 wt% nanohybrid fillers, 14% UDMA + DMA.	VOCO, Cuxhaven Germany

2.2.Specimen Fabrication

Forty slices (14 mm × 14 mm × 1 mm) of each material listed in Table 1 were obtained in a speed saw machine (Isomet 1000, Buehler), followed by polishing (Arotec Ind. Com., Sao Paulo, Brazil) with silicon carbide sandpapers (Carbimet Paper Discs; Buehler, IL, USA) (#600, #800 and #1200) under copious water, and sonicated in an ultrasonic bath for 10 min. This polishing procedure was adopted to standardize the surface since the CAD/CAM blocks used did not required glaze coating, the manufacturers only recommend finishing and polishing with rubbers and discs. For the flexural strength test (n=40), the slices were sectioned in 12 mm × 2 mm × 1 mm bars.[22] For the microhardness, roughness, and biofilm adhesion analyses, square-shaped 4 mm × 5mm × 1.5mm were obtained.

2.3.Experimental groups and erosive/abrasive challenges

Specimens of each material were allocated according to the following erosive and/or abrasive challenges (n=10):

Erosion (E): Specimens were immersed in a simulated acid gastric solution composed of 0.2% (w/v) of sodium chloride in a 0.7% (v/v) hydrochloric acid (pH= 1,2 ±0,2 em 25^{O} C) in a pepsin-free solution (Backer et al., 2015), for 91 h as previously proposed (Alnasser et al, 2019),[14] to simulate one-year of clinical exposure. This procedure was repeated three times, totalizing 273 h and 3 y of clinical exposure.

Abrasion (A): The abrasive challenge was performed by a mechanical toothbrushing simulator (MEV4T 10x- Odeme Dental Research), using soft dental brushes (Oral B classic 40) under 200 g load, and a slurry with 45g of dentifrice (Colgate Triple Action®, Colgate-Palmolive. Abrasive agent: calcium carbonate; sodium bicarbonate and sodium silicate) diluted in 90 mL of distilled water (1:2). To simulate 3 years of toothbrushing, 30.000 cycles were performed.[23] Specimens were cleaned with distilled water and air-dried at the end of cycles.

Erosion/Abrasion (E+A): The specimens were submitted to erosion followed by abrasion procedure.

Control (C): Specimens were kept under humidity in a bacteriological oven at 37^{o}C, while the erosion/abrasive challenges were performed in the other groups.

2.4.Flexural Strenght Test

After challenges (T_1), specimens were subject to a three-point flexural strength assay in a universal testing machine (Instron 4411, Buheler) with 12 mm of distance between the supports and a crosshead speed of 0.5 mm/min. The flexural strength was calculated according to the formula: L × D3 −3 Mf = 4 × l × h3 × d × 10, where *Mf* is the flexural module (MPa), *L* is the load registered (N), *D* is the interval between the supports, *w* specimen length, *h* the high and *d* represents the *L* deflection.

2.5.Microhardness

To access the material's deformation after the erosive/abrasive challenges, Knoop microhardness (Future Tech-FM-1e, Tokyo, Japan) was performed before (T_0) and after (T_1) challenges. Three measurements under 200 g load for 15 s, and a distance of 50 μm between them, were used as test parameters.

2.6.Surface Roughness

Surface roughness (Ra) was determined by a surface tester (Mitutoyo Surftest SJ-410, São Paulo, SP, Brazil) at T_0 and T_1. Three measurements were performed on each specimen by rotating the specimen 45° with a cut-off set at 0.8 mm and speed of 0.2 mm/s.

2.7.Biofilm Adhesion

The biofilm adhesion was performed with a multispecies biofilm composed of standard strains of *Streptococcus mutans* (ATCC 700610), *Streptococcus Sanguinis* (ATCC 10556) and *Candida Albicans* (MYA 2876) incubated in an infusion broth of BHI (Difico) for 24h in CO_2 chamber. The inoculum was standardized by spectrophotometry at a concentration of 1x108 cells using BHI broth supplemented with 1% of sucrose. Aliquots of 500 μL of multispecies biofilm inoculum were added to a sterile 24-well plate with the specimens previously sterilized by UV light. The biofilm was grown for 24 h in a CO_2 chamber and each specimen was transferred to an Eppendorf with 1mL of a NaCl (0.9%) sterile solution and sonicated for 5 min. The aliquots were plated in the 10^{-1} until 10^{-4} dilutions after 24 h of incubation and the viable colony counting (UFC/mL) was performed and transformed in log10.

2.8.Scanning Electron Microscope analysis

Additionally, two specimens of each group were sputtered with gold coating (Blazers-SCD 050 Sputter Coater, Liechtenstein) and scanning electron microscopy (SEM, JSM 5600V, JEOL, Tokyo, Japan) images at 3000x magnification were obtained.

2.9.Statistical Analyses

For the response variables *microhardness* and *roughness*, statistical analyses were performed using mixed linear models, under the REML method, with an analysis of the model's adjustment using the AIC. For the *flexural strength* and *biofilm adhesion* variables, generalized linear models (GLM) were used, adjusting the distribution through the AIC analysis. Tables 2 to 4 and Figure 1 display the original mean and standard deviation. Multiple comparisons of the results were performed using HSD Tukey test. For all comparisons, a significance level of 5% was adopted.

3. Results

3.1.Flexural Strength

The results presented in Table 2 describe the flexural strength. A significant difference was found for the factor "material" ($p<0.001$) and "challenge" ($p<0.001$), but with no interactions between the factors (Wald= 17.82 and $p=0.121$). Regardless of the erosive-abrasive challenge, ZLS and RG presented the highest flexural strength values, with no differences from each other, while FE exhibited the lowest flexural strength.

Irrespective of the material, the flexural strength of the materials tested was higher in the control group, with no challenges (C). The indirect materials submitted to E or E+A displayed the lowest flexural strengths ($p<0.05$), with no differences from each other ($p>0.05$). The abrasion (A) promoted an intermediate mean value with no differences from the other treatments (E, E+A, and C, $p>0.05$).

Table 2. Mean values and standard deviation of tested material flexural strength after challenges.

Material	E	A	E+A	C - Without treatment	Materials Marginal means (SE)
ZLS	189.8 (56.75)	197.4 (66.67)	211.2 (30.81)	203.8 (48.32)	204.6 (6.05) **A**
FE	100.2 (12.39)	100.2 (18.51)	100.3 (10.72)	107.3 (13.04)	102.1 (3.02) **C**
PICN	105.8 (15.81)	124 (12.14)	113.7 (15.82)	135.8 (15.76)	119.3 (3.53) **B**
RK	103.5 (14.95)	127.8 (13.92)	95.5 (15.02)	127. (15.79)	113.6 (3.36) **B**
RG	195.9 (22.41)	198.2 (32.78)	175.8 (53.70)	222 (36.56)	198.3 (5.86) **A**
Marginal Means (SE)	133.6 (3.53) **b**	144.8 (3.83) **ab**	134.1 (3.55) **b**	153.2 (4.05) **a**	

Upper case letters compare the materials regardless of the treatment. Lowercase letters compare the treatments regardless of the material. Marginal means are followed by standard error (SE) values

3.2.Microhardness

Table 3 displays the results of the microhardness test. A significant difference for the factor “material” ($p<0.001$), “challenge” ($p<0.001$), “time” ($p<0.001$), and a triple interaction material*challenge*time ($p<0.001$) were found. Erosion (E) and Erosion and abrasion (E+A) decreased ZLS, FE, and PICN's microhardness. Abrasion (A) did not change the microhardness values of ZLS, FE, and PICN. Nevertheless, abrasion led to an intermediate value for PICN with no differences compared to the other challenges ($p>0.05$). RK and RG displayed no microhardness differences, irrespective of the challenge performed.

The materials tested exhibited different KHN values, regardless of the challenge or evaluation time ($p<0.05$). ZLS glass-ceramic showed the highest KHN, followed by FE, PICN, and RG, while RK displayed the lowest microhardness. ZLS, FE, and PICN exhibited a significant KHN decrease after E and E+A challenges ($p<0.05$), while RK and RG showed no differences in T_1, irrespective of the challenges ($p>0.05$).

Table 3. Mean values and standard deviation of Knoop microhardness test for CAD/CAM blocks before and after challenges.

Material	**Time**	**E**	**A**	**E+A**	**C - Without treatment**
ZLS	T_0	642.0 (18.00) **Aa***	640.0 (18.76) **Aa***	643.4 (20.42) **Aa***	642.0 (26.27) **Aa***
	T_1	475.3 (39.24) **Bb#**	637.7 (26.93) **Aa#**	490.4 (26.41) **Bb#**	644.0 (14.11) **Aa#**
FE	T_0	493.6 (20.14) **Aa***	491.0 (18.78) **Aa***	493.7 (26.40) **Aa***	491.1 (32.92) **Aa***
	T_1	187.6 (41.18) **Bb#**	480.5 (20.43) **Aa#**	189.0 (44.19) **Bb#**	482.5 (25.37) **Aa#**
PICN	T_0	172.7 (15.541) **Aa***	171.9 (13.48) **Aa***	173.1 (6.99) **Aa***	171.5 (10.63) **Aa***
	T_1	123.3 (21.38) **Bb#**	162.8 (19.86) **Aab#**	136 (18.33) **Bb#**	174.7 (6.78) **Aa#**
RK	T_0	56.5 (2.06) **Aa***	57.8 (3.00) **Aa***	56.6 (7.48) **Aa***	57.2 (3.60) **Aa***
	T_1	46.4 (4.12) **Aa#**	58.0 (3.74) **Aa#**	50.4 (7.50) **Aa#**	58.5 (2.67) **Aa#**
RG	T_0	96.0 (7.95) **Aa***	96.4 (10.66) **Aa***	95.7 (8.97) **Aa***	95.8 (9.98) **Aa***
	T_1	87.3 (10.17) **Aa#**	98.5 (6.61) **Aa#**	93.2 (8.08) **Aa#**	94.8 (8.63) **Aa#**

Different letters mean significant statistical differences. Upper case letters compare T_0 and T_1 within the same material. Lower case letters compare different treatments within the same material and the same time. Asterisk symbols indicate statistical differences between the materials in T_0 for each treatment. Hashtag symbols indicate statistical differences between the materials in T_1.

3.3.Surface Roughness

Table 4 exhibits the Ra values of the materials tested. Statistical analysis showed a statistically significant difference for the material factors ($F = 67.19$ and $p<0.001$); Treatment ($F=31.55$ and $p<0.001$), Time ($F=124.60$ and $p<0.001$) and a triple interaction among ceramic*treatment*time ($F=21.21$ and $p=0.000$).Before treatments, ZLS and FE exhibited the lowest RA mean values, but no differences were observed between FE and RK ($p>0.05$), while ZLS showed lower Ra than PICN, RK, and RG ($p<0.05$). No differences in Ra were noted between PICN and RG and these materials exhibited higher mean values at T0.

The surface roughness of RK and RG increased after A and E+A ($p<0.05$), while E and E+A treatments caused a significant increase in Ra for the FE glass-ceramics. Indeed, the highest Ra mean values among groups were found for FE after E+A (0.249 μm) followed by E (0.142 μm) ($p<0.05$)

Table 4. Mean values and standard deviation of CAD/CAM materials surface roughness (Ra, in µm) obtained before and after treatments

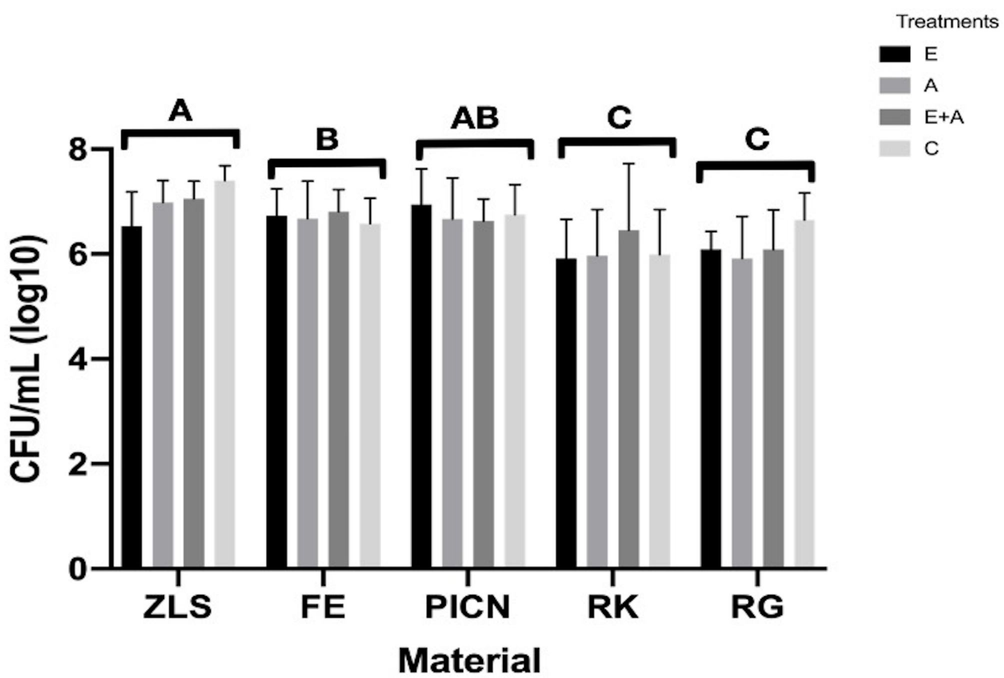
Material	Time	E	A	E+A	C - Without treatment
ZLS	T_0	0.019 (0.004) **Aa**$^{\&}$	0.018 (0.004) **Aa**$^{\&}$	0.019 (0.005) **Aa**$^{\&}$	0.019 (0.006) **Aa**$^{\&}$
	T_1	0.021 (0.006) **Aa**$^{\spadesuit}$	0.017 (0.003) **Aa**$^{\spadesuit}$	0.017 (0.004) **Aa**$^{\spadesuit}$	0.020 (0.005) **Aa**$^{\clubsuit}$
FE	T_0	0.028 (0.007) **Ba**$^{*\&}$	0.028(0.008) **Aa**$^{*\&}$	0.028 (0.006) **Ba**$^{*\&}$	0.028 (0.008) **Aa**$^{*\&}$
	T_1	0.142 (0.016) **Ab**$^{\Phi}$	0.031 (0.005) **Ac**$^{\Phi}$	0.249 (0.078) **Aa**$^{\Phi}$	0.025 (0.006) **Ac**$^{\clubsuit}$
PICN	T_0	0.065 (0.012) **Aa**$^{\#}$	0.066 (0.007) **Aa**$^{\#}$	0.067 (0.007) **Aa**$^{\#}$	0.066 (0.009) **Aa**$^{\#}$
	T_1	0.069 (0.012) **Aa**$^{\heartsuit}$	0.075 (0.008) **Aa**$^{\clubsuit}$	0.076 (0.005) **Aa**$^{\clubsuit}$	0.063 (0.007) **Aa**$^{\heartsuit}$
RK	T_0	0.036 (0.006) **Aa***	0.035 (0.009) **Ba***	0.037(0.007) **Ba***	0.036 (0.008) **Aa***
	T_1	0.041 (0.010) **Ab**$^{\clubsuit}$	0.096 (0.044) **Aa**$^{\clubsuit}$	0.069 (0.013) **Aa**$^{\clubsuit}$	0.031 (0.005) **Ab**$^{\clubsuit}$
RG	T_0	0.053 (0.011) **Aa**$^{\#}$	0.052 (0.010) **Ba**$^{\#}$	0.052 (0.008) **Ba**$^{\#}$	0.054 (0.011) **Aa**$^{\#}$
	T_1	0.054 (0.014) **Ab**$^{\diamondsuit}$	0.081 (0.014) **Aa**$^{\clubsuit}$	0.097 (0.021) **Aa**$^{\diamondsuit}$	0.055 (0.009) **Ab**$^{\heartsuit}$

Different letters mean significant statistical differences. Uppercase letters compare T_0 with T_1 within the same material and the same treatment (vertical). Lowercase letters compare different treatments within the same material and at the same time (horizontal). Different superscript symbols indicate statistically significant differences. Superscript symbols compare materials within the same time in the same treatment

3.4.Biofilm adhesion

A significant difference in biofilm adhesion was found among the materials tested ($p<0.001$), but no differences were observed among challenges ($p=0.306$) or in the interaction between the factors ($p=0.356$) (Figure 1). ZLS glass-ceramic displayed higher biofilm formation than FE, RK, and RG. PICN exhibited intermediate values with no differences from ZLS and FE, while RK and RG exhibited the lowest biofilm formation among the materials.

Figure 1. Means and standard deviation of CFU/mL for each material and treatment. Upper case letters represent the statistical differences of marginal means for each material**.**



Letters represent differences in the marginal means of all treatments for each material

3.5.Surface morphology

Representative SEM images of the materials tested after challenges and the comparison with the control group are shown in Figures 1 to 5. Erosion and abrasion promoted surface alterations in all materials, but the contact with the acid solution significantly increased the damage in the ceramic-based materials. For ZLS (Fig. 1A and C) and FE (Fig. 2C) deterioration areas are present on the surface especially when both challenges were associated. PICN showed a massive loss of ceramic and polymeric phase, increase in surface irregularities and in materials' porosity, and particles exposure (Fig. 3C). In the nanoceramic resins RK (Fig. 4) and RG (Fig. 5), the long contact with acid medium promotes an increase on the surface texture of those materials, but still less damage than ceramic based blocks.

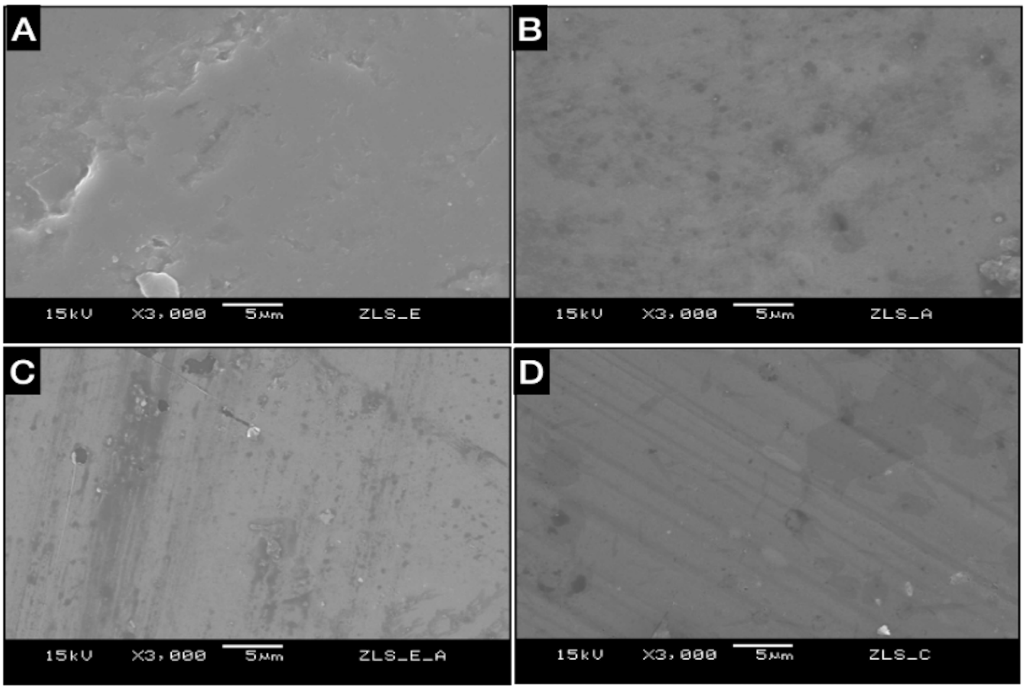


Figure 2- SEM representative images for Celtra Duo (ZLS) after erosive and/or abrasive challenges. **A:** ZLS+ erosion; **B:** ZLS+ abrasion; **C:** ZLS+ erosion/abrasion and **D:** control (without treatment).

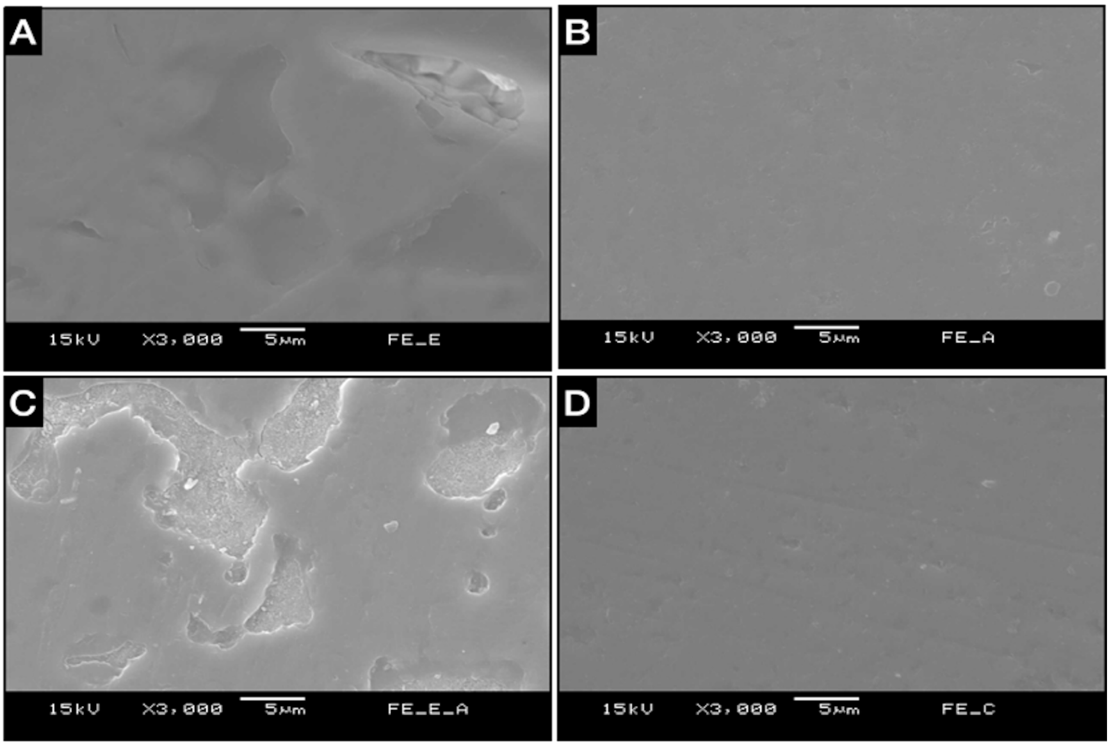


Figure 3- SEM representative images for Vita Mark II (FE) after erosive and/or abrasive challenges. **A:** FE+ erosion; **B:** FE+ abrasion; **C:** FE+ erosion/abrasion and **D:** control (without treatment).

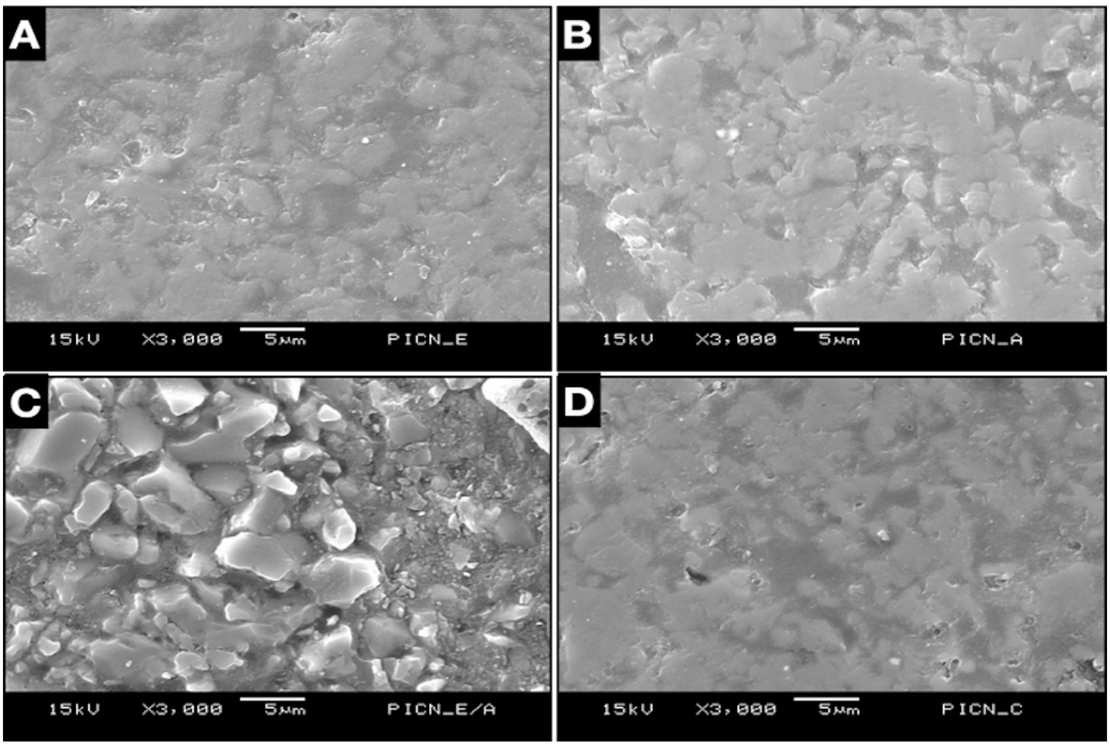


Figure 4- SEM representative images for Vita Enamic (PICN) after erosive and/or abrasive challenges. **A:** PICN+erosion; **B:** PICN+ abrasion; **C:** PICN+ erosion/abrasion and **D:** baseline (without treatment).

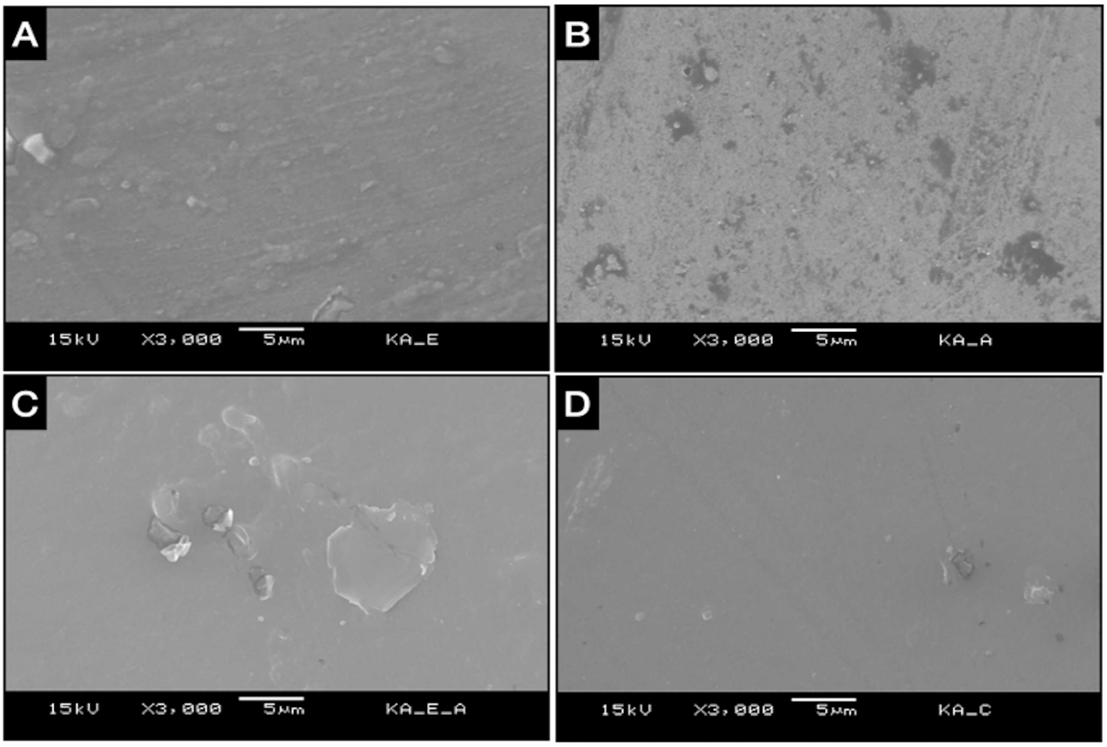


Figure 5- SEM representative images for Katana Avencia (RK) after erosive and/or abrasive challenges. **A:** RK +erosion; **B:** RK+ abrasion; **C:** RK+ erosion/abrasion and **D:** baseline (without treatment).

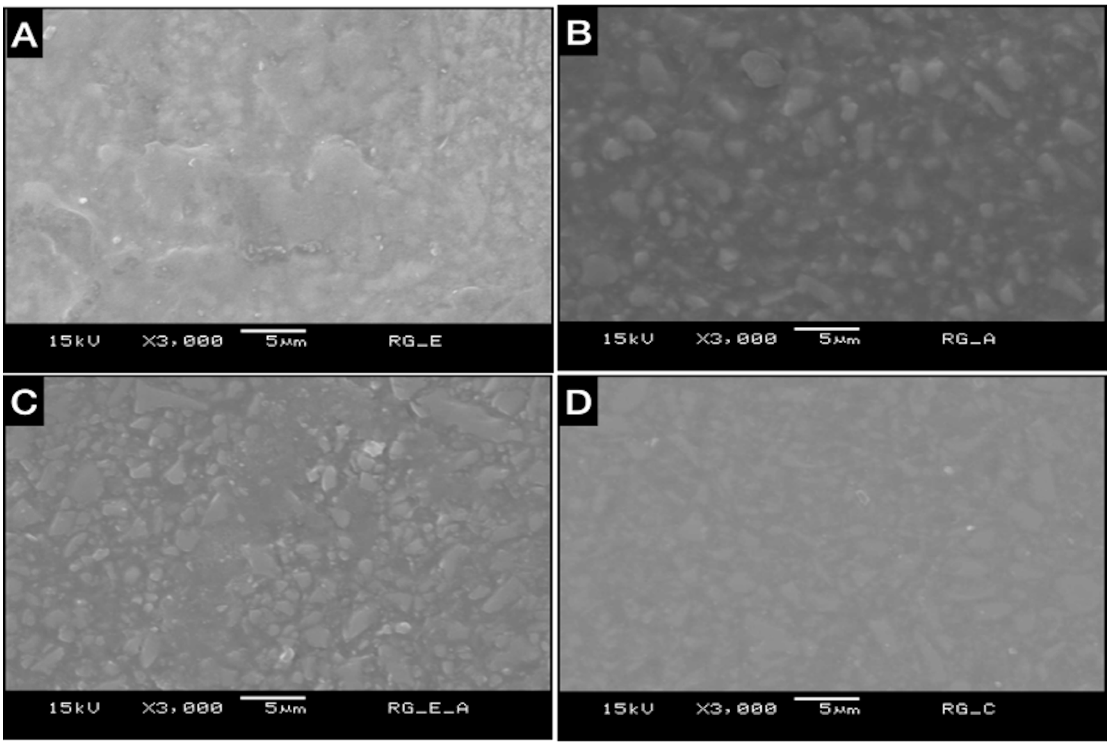


Figure 6- SEM representative images for Grandio Blocks (RG) after erosive and/or abrasive challenges. **A:** RG+erosion; **B:** RG+ abrasion; **C:** RG+ erosion/abrasion and **D:** control (without treatment).

4. **Discussion**

Indirect restorative materials are usually the first choice for rehabilitation of severe tooth destruction.[24] Therefore, erosion (caused by GERD condition) and/or abrasion (caused by toothbrushing) are existing clinical conditions that could accelerate the degradation of the glass-ceramics, hybrid materials, or composite restorations.[16] The flexural strength results confirm that these challenges damaged the tested materials. Regardless of the material type, the control groups showed higher flexural strength, while E and E+A challenges promoted the lowest mean values for all materials. Thus, the first null hypothesis was rejected.

The feldspathic ceramic (FE, Vita Mark II) showed the lowest flexural strength mean values among materials. This behavior is possibly caused by the extended immersion time in the HCl solution, with a very low pH (±1.2). The acid solution reacts with the silica of the vitreous matrix, and the aggressive removal of the vitreous matrix interferes with the glass strength performance.[25] The extended contact of the material with an aqueous solution promotes an ion exchange among the hydrogen ions from the solution and alkaline ions from the glass matrix, which could induce pore formation in the ceramic glassy matrix and also result in areas with a localized interruption in the Si–O–Si bond. Therefore, the silicon released from the glassy matrix would damage the entire ceramic structure[26]

ZLS and RG showed the highest flexural strength mean values, regardless of the challenge. The presence of 10 wt% of zirconium dioxide in the ZLS composition probably plays a role in the lithium silicate glass-ceramics by limiting the crack propagation. According to reports, zirconium dioxide can limit the growth of lithium silicate crystals under physical and chemical challenges. The ZrO2 granules from the tetragonal phase (metastable) change to the monoclinic phase (stable phase), and the larger volume of the monoclinic phase granules prevents crack propagation, thus increasing the resistance of this glass-ceramic under physical-chemical challenges.[27] The nanohybrid resin (RG) exhibited similar flexural strength to ZLS. RG displays a high inorganic concentration (86 wt%) and an organic phase formed by 14% of UDMA+DMA. According to Barutcigil et al,[28] the high temperature and pressure for the polymerization process of UDMA results in fewer amounts of monomer release because of its higher degree of conversion. Therefore, the controlled fabrication process of the nanohybrid CAD/CAM results in a material with reduced defects due to a more uniform polymer network[29], directly influencing its mechanical performance.

The erosive and/or erosive + abrasive challenges decreased the microhardness of CAD/CAM ceramic materials, particularly ZLS, FE, and PICN. The acidic solutions clearly changed the surface by reacting with the silica of the glass phase, as observed in the SEM images (Fig.2A and C; Fig. 3C and Fig. 4C). It is well established that HF etching (5 - 10%) reacts with the glass phase of the ceramic matrix, but no microhardness reduction occurs, as the contact time with the HF is controlled, and well-defined based on each material's protocol.[30] However, the prolonged gastroesophageal reflux simulation tested herein (3 cycles of 91 hours) has contributed to the decreased capacity of the material to resist deformation. Contrarily, the erosive challenges did not influence the nanohybrid resins' microhardness. These results are in accordance with those of Barker et al., 2015, who observed the maintenance of microhardness after challenges. In that report, the best results were found in a material composed of Bis-GMA and TEGDMA, with a strong organic phase, allowing a crosslinked structure formation. Even though the resin blocks tested in this study are composed of UDMA which are more flexible than Bis-GMA, this fact did not impact their behavior, probably due to the high inorganic content and manufacturing standardization of those materials.[18]

The surface roughness of the resin-based CAD/CAM materials (RK and RG) increased following abrasion (A) and erosion combined with abrasion (E+A). These materials exhibit lower hardness than the ceramic-based restoratives and, therefore, are more susceptible to abrasive wear promoted by simulated toothbrushing. Furthermore, immersion in an acidic solution with a considerably low pH, degrades the polymer, although it is suggested that the polymer degradation process is deeply influenced by the hydrophilicity of the polymer matrix and the presence and location of the hydrolysable groups in the matrix network.[31] The acidic solution can increase the rate of the material's hydrolysis, accelerating the degradation process and reducing the longevity of composites. The hydrolysis increases the matrix volume due to sorption, resulting in pores and intermolecular spaces within the matrix. In this process, the filler particles can be released, resulting in loss of mass, surface roughness, and biofilm accumulation.[32]

The feldspathic (FE) ceramic exhibited a significant roughness increase after erosion (E) and erosion + abrasion (E+A). As previously reported, immersion in an aggressive acidic solution for an extended time changed the material's topography because of the acid interaction with the vitreous phase, considerably reducing microhardness and increasing the roughness of the material,[26] as observed under SEM (Fig. 3C). Therefore, the second null hypothesis was also rejected, because the microhardness and surface roughness of the tested materials were significantly influenced by the erosive/abrasive challenges.

The representative SEM images corroborated the findings, as the erosive/abrasive challenges damaged the material's topography, and these alterations were material dependent. The resin-based materials showed matrix degradation and filler particles exposure, while the ceramic surface exhibited vitreous degradation resembling an etching surface. For all materials, the contact with the acid solution combined with mechanical abrasion promoted surface degradation and the presence of irregularities, pores, and defects, leading to a non-uniform and uneven surface compared to the control group.

The erosive/abrasive challenges did not influence the multispecies biofilm (*S. Mutans, S. Sanguinis and C. Albicans*) adhesion on the surface of each CAD/CAM material, thus the third null hypothesis was accepted. However, ZLS, FE, and PICN displayed higher biofilm adhesion than RK and RG, regardless of the challenge to which these materials were submitted. Although these results are not in accordance with the surface roughness behavior showing significantly higher roughness for the FE ceramic submitted to E+A, it has been previously reported that some ceramics may be more hydrophilic than others, a circumstance that influences the contact angle between water and ceramic.[33] Besides, Shirtcliffe et al., reported that the surface with a contact angle between 0 and 180^{o} can be partially hydrophilic, so the surface still has a hydrophobic tendency that could affect the wettability, favoring bacterial retention.[34] However, it is important to emphasize that the CAD/CAM indirect materials tested, irrespective of the type, exhibited mean surface roughness values lower than (or up to) 0.2 μm, which is the minimum roughness value acknowledged to uphold clinically relevant biofilm accumulation.[20,35] Therefore, although the erosive/abrasive challenges partially damaged the indirect material's surface, compromising its mechanical behavior and possibly long-term predictability, the risk of biofilm accumulation on CAD/CAM seems clinically minor.

This study upholds the inherent limitations of an *in vitro* investigation, thus the results cannot be directly extrapolated to the clinical performance, but could predict the behavior of the CAD/CAM materials under similar erosive-abrasive challenges. It should bear in mind that saliva plays a role in buffering and regulating pH during acidic challenges. Since saliva was not used herein, the in vitro simulation may be more hazardous than the in vivo condition. In addition, neither the ceramics tested were glazed-coated, as it was not a manufacturer's requirement. This aspect alone could protect the ceramic-based materials against simulated abrasive-erosive degradation.

Other methods to determine surface topography, such as atomic force microscopy (AFM), could be used to evaluate the material's surface following erosive/abrasive challenges, as the method could offer a more detailed surface definition than SEM. Besides, since surface topography is three-dimensional in nature, 3-D surface measurements could provide more realistic data than those obtained from 2-D profiles, such as the surface tester used in this study.[36]

This study simulated three years of aggressive challenges related to gastroesophageal disorder behavior combined with toothbrushing abrasion. Although the materials have shown the ability to overcome such challenges, the evaluation period is limited, and long-term challenges, and randomized clinical analyses, are essential to assess the risks and benefits of using these materials in peculiar and clinically serious condition, that requires extra attention and care.

5. Conclusion

Based on this study's results, it can be concluded that:

1. The erosive/abrasive challenges affected the flexural strength of the tested materials. The feldspathic ceramic displayed the lowest flexure strength after erosion (E) and erosion and abrasion (E+A) simulations.
2. The acid-erosive challenges decreased the microhardness of the ceramic-based or hybrid material (ZLS, FE, and PICN).
3. The biofilm formation was material-dependent and the ceramic-based or hybrid material (ZLS, FE, and PICN) showed higher biofilm adhesion than the resin-based restoratives.

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2.2 Artigo 2

Surface roughness, color evaluation, and antibiofilm activity analyses of TiO_2 nanotubes incorporated into a glaze-coating ceramic.

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Abstract

Purpose: This study evaluated color change, the surface roughness and antibacterial effect, and of a ceramic glaze doped with different concentrations of TiO_2 nanotube (n-TiO_2).

Materials and Methods: The ceramic glaze powder (VITA Akzent Plus Powder mixed with VITA Akzent Plus Powder Fluid) with the addition of 1, 2.5, and 5 wt% n-TiO_2 or 0% (control) was applied with a flat brush on the surface of forty feldspathic ceramic specimens (FE, Vita Blocks Mark II, Vita Zahnfabrik) and sintered according to the manufacturer's recommendations. Color change of the doped glaze was determined using CIEDE2000 (ΔE_{00}) and CIELab (ΔEab) systems, and the whiteness index for dentistry (ΔW_{ID}). A contact profilometer determined the surface roughness average (Ra) of the feldspathic glass-ceramic before glaze application (T0) and after glaze crystallization (T1). The antibacterial effect against *S. Mutans* and *S. Sanguinis* was evaluated by colony-forming units (CFU/mL). Ra, ΔEab, ΔE_{00}, and CFU/mL were analyzed by two-way repeated-measures ANOVA, followed by the Bonferroni test ($\alpha = 0.05$)

Results: No statistical differences in ΔE_{ab} and ΔE_{00} parameters were observed among groups ($p>0.05$), and W_{ID} was only affected with the addition of 5% n-TiO_2. All groups, including the control, exhibited mean values higher than 1.8 (ΔE_{00}) and 2.3 (ΔE_{ab}), indicating that regardless of n-TiO_2 concentration, the glaze application surpassed the perception thresholds. Before glaze application, no surface roughness differences were detected among groups ($p>0.05$), but after glazing, Ra decreased for most groups tested (p<005). Contrary to these findings, the surface roughness of the ceramic glaze containing 5% n-TiO_2 increased in comparison with the control group (0 wt% n-TiO_2). The addition of 0%, 1%, and 2.5% of n-TiO_2 into the glaze powder did not impair the bacteria formation and growth, and no differences were found among the concentrations ($p<0.05$). However, the addition of 5% n-TiO_2 into the glaze reduced the mean number of CFUs. TiO_2

Conclusion: The experimental glaze containing 5% n-TiO_2 caused minimal interference in the color and roughness of the ceramic, but the tested concentrations did not inhibit biofilm formation.

Keywords: ceramic, CAD-CAM, glaze, titanium dioxide, nanotubes, biofilm

1 Introduction

A large variety of monolithic ceramic-based indirect restorative materials (vitreous or reinforced) are available in pre-processed blocks for machining in CAD-CAM systems (Computer Aided Design/Computer Aided Manufacturing)[1], a technique that allows for automated preparation of restorations, with high standard parameters and excellent marginal adaptation after scanning and milling.[2]

Based on the glass-ceramics composition, the surface of the restoration after milling exhibits differences in particle size, porosities, and impurities that can affect the physical characteristics and performance of the material against the surrounding periodontal tissues.[3] To minimize this problem and provide surface smoothness, finishing and polishing procedures are mandatory to inhibit biofilm formation.[4]

The finishing and polishing procedures of glass ceramics comprise the use of rubbers, discs, and diamond pastes.[5] Glaze coating consists of the application of a thermally compatible low-melting amorphous glass layer followed by sintering and may also be applied to the milled glass ceramics after finishing and polishing.[6] According to investigations, glazing could decrease the surface roughness and stain in comparison to the mechanical finishing and polishing systems, and it is reported to increase the mechanical properties of glass-ceramic after milling.[7,8]

Though clinical long-term evaluations display high survival rates of indirect glass-ceramics restorations and full-contour crowns, [9,10] the oral condition provides a challenging environment for dental materials as it implicates constant pH-changes, microorganism colonization, thermal variations, physiological and pathological masticatory loading.[11] This scenario leads to ceramic restoration failures and the most frequent clinical occurrences are chipping, and marginal sealing failures that will culminate in biofilm formation, gingival tissue inflammation, and finally, recurrent caries lesions at the restoration interface.[12] Therefore, the development of materials presenting biological and biocompatibility features, able to resist long-term clinical challenges is essential to enhance the material's clinical performance.[13] In this context, several investigations have focused on the incorporation of antibacterial agents capable of preventing, decreasing, or controlling biofilm accumulation.[14,15,16]

The manipulation and incorporation of particles on a nanometric scale became more popular in materials science, as nanotechnology enhances the material's effects and allows structural recombination, increasing the applicability of known materials.[17] The titanium dioxide (TiO_2) nanosized molecule is an example of an agent that presents great versatility, such as hydrophilicity, chemical stability, biocompatibility properties, and antimicrobial potential.[18]

Titanium dioxide nanotubes (n-TiO_2), which are tubular and hollow nanostructures, present a high active surface area and antibacterial potential since titanium dioxide displays photocatalytic activity under ultraviolet irradiation or electrostatic interactions between metallic ions and bacteria.[19] The n-TiO_2 has been incorporated into dental materials, such as glass ionomer cement and polymer-based materials, and studies have shown the bioactive potential of these nanostructures. TiO_2 nanotubes are effective in providing either an antibacterial effect or enhanced mechanical properties to glass ionomer cement.[20,21] Other studies showed that the incorporation of n-TiO_2 in polymethylmethacrylate-based materials showed great antimicrobial activity potential.[22]

According to observations, n-TiO_2 releases reactive oxygen species (ROS) through photocatalytic processes when exposed to ultraviolet light, disrupting the bacterial cell membrane.[23] In a previous study, Kantovitz KR et al., 2020 incorporated n-TiO_2 into glass ionomer cement (GIC) and concluded that 5% of n-TiO2 improved the mechanical properties of GIC without affecting the material dentin adhesiveness.[21] In another report, Araujo IJS et al., 2021 incorporated 3 and 5% n-TiO_2 into GIC and observed that the antibacterial properties of GIC against Streptococcus mutans, increased.[24] Another investigation showed that conventional polymethylmethacrylate (PMMA) modified by 5% n-TiO_2 lead to promising antimicrobial potential against S. mutans, Lactobacillus acidophilus, and Candida albicans, regardless of UV light exposure.[22]

Considering that the ceramic glaze is the outmost layer applied on the ceramic restoration and will be submitted to clinical long-lasting challenges, the incorporation of n-TiO_2 may modulate biofilm formation and prevent caries lesions at the adhesive interface. Therefore, this in vitro study aimed to incorporate different concentrations of n-TiO_2 into a ceramic glaze and evaluate its effect on color stability, surface roughness and morphology and biofilm accumulation, after application and synthetization on a CAD/CAD feldspar ceramic. The null hypotheses tested stated that the incorporation of n-TiO_2 into the glaze coating would not (1) change the color of glazed-coating ceramic and (2) would not change the surface roughness of the glaze coating surface (3) would not inhibit biofilm formation.

2 Materials and methods

2.1 Experimental Design

The experimental units of this *in vitro* study were CAD-CAM blocks (FE, Vita Blocks Mark II, Vita Zahnfabrik) covered with glaze coating (VITA Akzent Plus Powder) with or without the addition of titanium dioxide nanotubes (n-TiO_2). The material composition is described in Table 1. Analysis of color change and surface roughness (Ra) were performed before (baseline: T0) and after glazing (T1). At T1, biofilm growth was also evaluated by viable colony counting units (CFU/mL). Representative surface images were obtained using scanning electron microscopy (SEM).

2.2 Specimen preparation

Forty blocks (7.0 mm length x 7.0 mm width x 1.5 mm thickness) of feldspar ceramic were sectioned using a low-speed diamond disc coupled with a precise cutting machine (Isomet 1000, BUEHLER Ltd. Lake Buff, IL, USA) under water cooling. The samples were wet-polished (Arotec Ind. Com., São Paulo, Brazil) with silicon carbide abrasive discs (Carbimet Paper Discs; Buehler, IL, USA) for 60 s on each sandpaper grit (#600, #800 and #1200). The specimens were cleaned in an ultrasonic bath for 10 min to remove the polishing debris and randomly distributed into four groups: **(GL0%)** no n-TiO_2 addition; **(GL1%)** glaze doped with 1wt% n-TiO_2; **(GL2.5%)** glaze doped with 2.5wt% n-TiO_2; **(GL5%)** glaze doped with 5wt% n-TiO_2. The titanium dioxide nanotubes (20nm long and 10nm in diameter) were synthesized by the alkaline method and formed by a single TiO2 leaf spirally rolled as described by Arruda LB et al. 2015. Briefly, TiO_2 in the anatase phase, was mixed with sodium hydroxide (NaOH) in an open Teflon container under heating. After 24h the precursor reagents were subjected to alkaline treatment and washed with hydrochloric acid (HCl) to remove the sodium ions.[25] The same glaze and solvent proportion were used for all groups (VITA Akzent Plus Powder + Fluid). However, for the experimental groups, the amount of n-TiO_2 was weighted for each concentration based on the amount of glaze powder previous determined and homogeneously incorporated into the powder (VITA Akzent Plus Powder), before dilution in the solvent (VITA Akzent Plus Powder Fluid). The mixing process of the powder dopped with each concentration of the nanotube and the solvent was performed in a Speed Mixer for 2 minutes under 2000 rpm. The glaze was manually applied on top of the samples, in a single coat, using a flat brush. Then, the samples were sintered according to the manufacturer's recommendations.

Tabela 1. Material, composition, and manufacturer of all tested materials

Material	**Manufacturer**	**Composition**
Vita Blocks Mark II	Vita Zahnfabrik, Bad Säckingen, Alemanha)	>56% SiO_2; >20% Al_2O_3; >6% Na_2O; >6%K_2O; >0.3%CaO; 0.1%TiO_2
VITA Akzent Plus Powder	Vita Zahnfabrik, Bad Säckingen, Alemanha)	Alkaline aluminosilicate glass
VITA Akzent Plus Powder Fluid	Vita Zahnfabrik, Bad Säckingen, Alemanha	Solvent

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2.3 Surface roughness

Mean surface roughness (Ra) was determined by a contact profilometer (Mitutoyo Surftest SJ-410, São Paulo, SP, Brazil) in T0 and T1 times (n = 10). The mean Ra was obtained by three measurements on each sample, rotating the specimen 45°, with a cut-off of 0.8mm and 0.2mm/s of speed.

2.4 Colorimetric evaluation.

Color analyses (n=10) were performed with a digital spectrophotometer (Vita Easyshade Advance, VITA Zahnfabrik H. Rauter GmbH & Co. KG, Bad Sackingen, Germany), initially (T0) and after glaze sinterization (T1), the equipment was inside of natural light chamber simulator (MiniMatcher GTI Graphic Technology Inc) and fixed in platform with the tip positioned perpendicular pointing to the center of the sample which was placed in a standardized background (white opaque tile). The mean value of each sample were obtained according to the CIE system (ΔE_{ab}): L*(black to white), a*(red to green), b*(yellow to blue), and CIEDE2000 system, which uses h (hue) and C (chroma) values: $\Delta E_{00} = [(\Delta L'/K_L S_L)^2 + (\Delta C'/K_C S_C)^2 + (\Delta H'/K_H S_H)^2 + R_T*(\Delta C'/K_C S_C)*(\Delta H'/K_H S_H)]^{1/2}$. The parameters L*, a*, b*, C*, and whiteness index for dentistry (W_{ID}) were also evaluated. Thresholds were adopted as 50:50% perceptibility (PT) and acceptability (AT), considering ΔEab values of 1.2 and 2.7, respectively, and the corresponding ΔE00 values were 0.8 and 1.8, respectively. ΔW_{ID} thresholds were 0.72 (PT) and 2.60 (AT).[26]

2.5 Biofilm assay

Streptococcus mutans (ATCC 700610) and *Streptococcus sanguinis* (ATCC 10556) were growth overnight in 10 ml of Brain Heart Infusion Broth (BHI, Difco) supplemented with 1% sucrose at 37°C and 5% CO_2. All ceramic samples (n = 5) were decontaminated in UV-radiation inside a flow chamber for 30 min (15 min each side). Then, each sample was placed at the bottom of one well on a 24-well plate (Costar Corning, NY, USA) with the glazed surface facing upward. The ceramics were inoculated with approximately 2 x 10^6 CFU/mL in BHI with 1% sucrose (2 mL in each well), at 37 °C for 24h and CO_2. The samples were washed three times in sterile saline solution (0.9% NaCl) to remove non-adhered cells and were individually transferred to falcon tubes containing 1ml of sterile saline solution. Biofilm was scraped with a sterile spatula and sonicated (Sonoplus HD 2200, 50 W, Bandelin Eletronic) for 20 s (7W output) to collect the biofilm. The biofilm was then serially diluted in 96 well plates and plated in triplicate in BHI agar (from 10^{-1} to 10^{-9}).

2.6 Surface morphology

Three specimens of each group previously cleaned with isopropyl alcohol and left to dry for 24h were sputtered coated with gold (Blazers-SCD 050 Sputter Coater, Liechtenstein), and SEM (SEM, JSM 5600V, JEOL, Tokyo, Japan) images at 1000x magnification were obtained with work distance of 10mm at 15 kV.

2.7 Statistical analysis

Surface roughness and color parameters (ΔE_{ab}, ΔE_{00}, L*, a*, b*, C*, and W_{ID}) presented normally and homoscedastic distribution. Surface roughness data were analyzed by two-way for repeated measures ANOVA (between-subject factor: group; within-subject factor: glaze) followed by the Bonferroni test, while color parameters were analyzed by one-way ANOVA followed by the Bonferroni test. Biofilm data were transformed in log and analyzed by one-way ANOVA. Surface morphology was qualitatively analyzed.

3 Results

3.1 Surface roughness

Table 2 shows the mean surface roughness before and after the glaze application for each n-TiO_2 concentration tested. The “glaze” ($p < 0.001$) factor significantly influenced Ra, decreasing surface roughness results. Before glaze application, no differences were found among the groups.

After glaze application, the surface roughness was reduced for all groups tested. Moreover, the addition of 5wt% of n-TiO_2 increased the surface roughness in comparison with the control group (GL0%).

Table 2. Mean (SD) of surface roughness (Ra, in µm).

Group	Before glaze application (T0)	After glaze application(T1)
GL0%	0.146 (0.063) aA	0.037 (0.015) bB
GL1%	0.137 (0.055) aA	0.041 (0.014) abB
GL2.5%	0.138 (0.063) aA	0.051 (0.010) abB
GL5%	0.161 (0.074) aA	0.057 (0.020) aB

Means followed by different letters indicate significant differences among groups ($p<0.05$). Upper case letters compare before and after glaze application within the same n-TiO_2 concentration. Lowercase letters compare different n-TiO_2 concentrations within the same glaze application.

3.2 Colorimetric evaluation

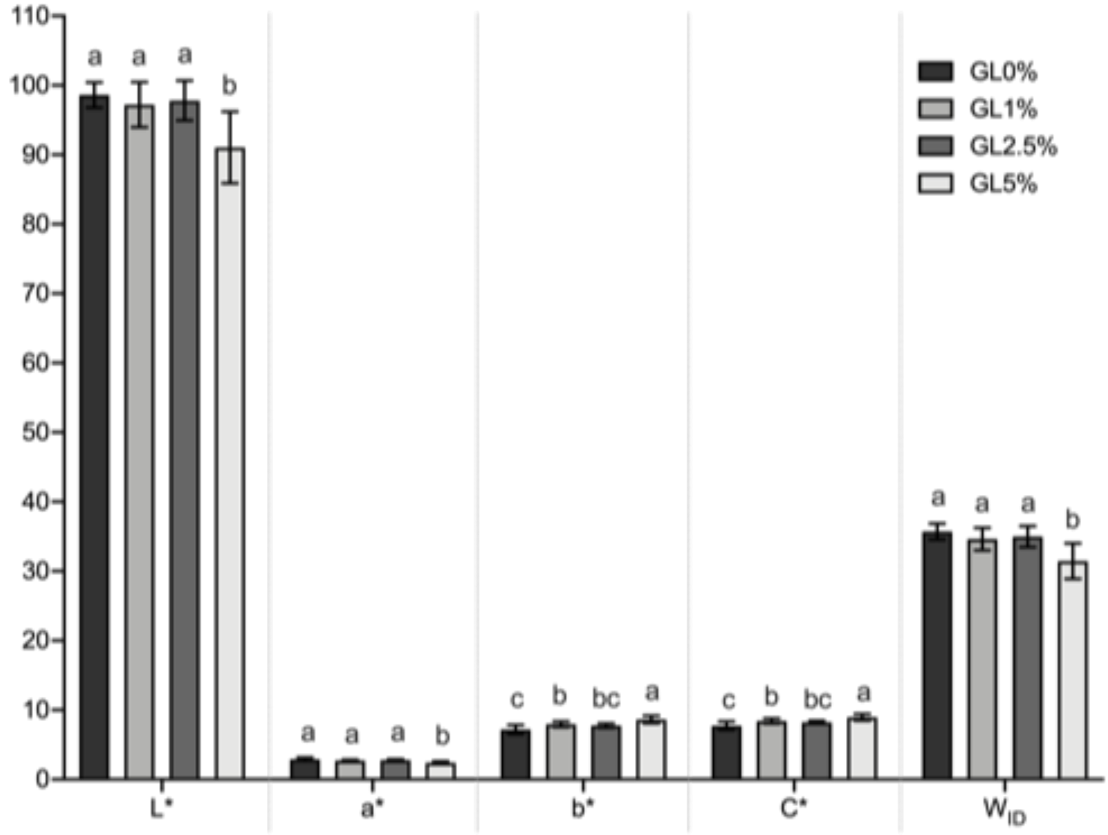
Table 3 depicts ΔE_{ab} and ΔE_{00} while Figure 1 shows L*, a*, b*, C*, and W_{ID} values. No statistical difference is observed for both ΔE_{ab} and ΔE_{00} parameters. However, the addition of 5wt% of n-TiO_2 statistically reduced the L*, a*, and W_{ID} values; meanwhile increased the b* and C* values compared to the other groups as shown in Figure 1. All the groups presented values above PT and AT thresholds, but the higher concentration of n-TiO_2 decreased L* values, indicating that this experimental glaze lost the translucency.

Table 3. Mean (SD) of ΔE_{ab} and ΔE_{00}, according to n-TiO_2-glaze concentrations.

Group	ΔE_{ab}	ΔE_{00}
GL0%	3.11 (1.25) a	3.20 (0.89) a
GL1%	3.57 (2.85) a	3.15 (1.51) a
GL2.5%	2.95 (1.36) a	2.79 (0.74) a
GL5%	6.75 (4.31) a	4.46 (2.50) a

Means followed by lower case letters indicate no significant differences among groups ($p<0.05$).

Figure 1. Means and standard deviation of L*, a*, b*, C* and W_{ID}, according to n-TiO_2-glaze concentrations

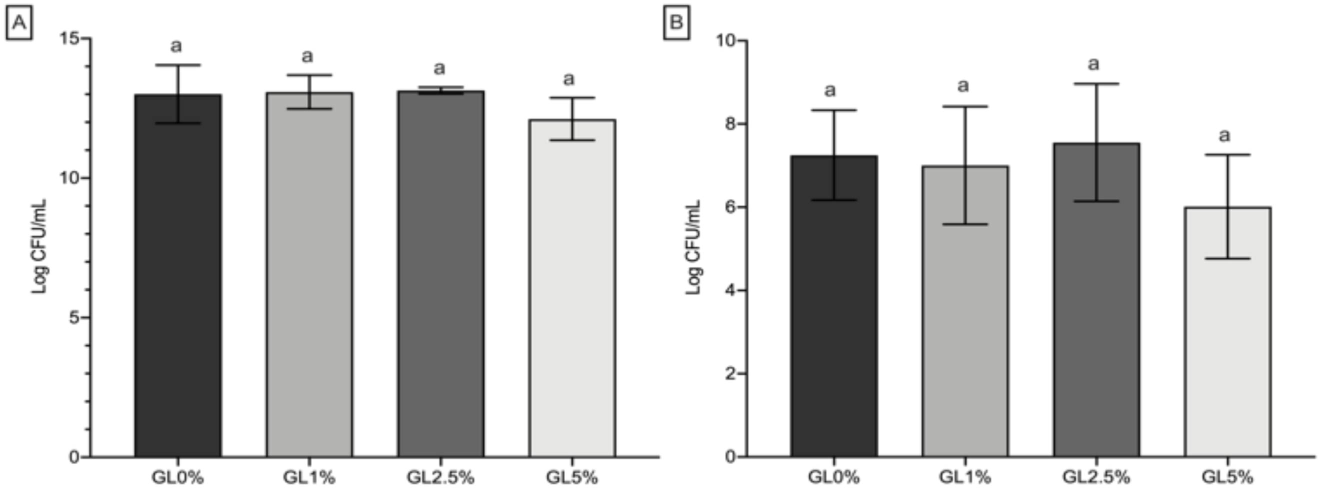


Different letters indicate significant differences among groups (p<0.05).

3.3 Biofilm assay

The bacterial viability (log CFU/ml) growth on top of the glazed ceramic surface is shown in Figure 2A (*S. mutans*) and Figure 2B (*S. sanguinis*). None of the tested concentrations of n-TiO_2 into the glaze material was capable of impairing the bacteria adhesion and growth and no differences were observed among the tested groups (p>0.05).

Figure 2. Means and standard deviation of CFU/mL for A: S. mutans and B: *S. sanguinis*, according to n-TiO_2-glaze concentrations.



Letters does not indicate the presence of significant differences among groups ($p<0.05$).

3.4 Surface morphology

Figure 3 shows representative SEM images of the ceramics after the application of the experimental glaze. Control group, with no n-TiO_2 addition, exhibited a smooth surface, while the incorporation of n-TiO_2, promoted areas that may correspond to clusters of n-TiO_2.

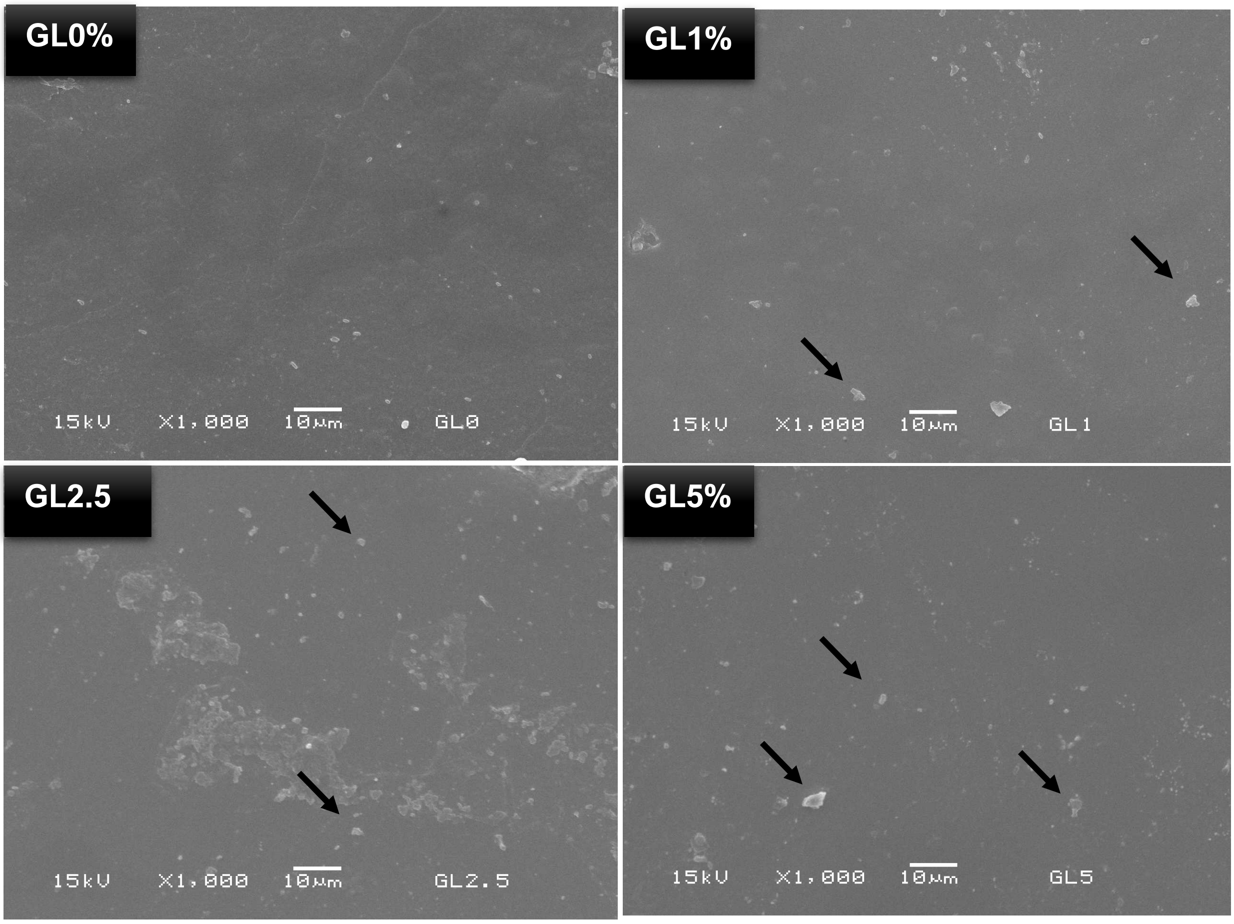


Figure 3. Representative images of the glazed ceramic surface with glaze containing different concentrations of TiO2 nanotubes (0 wt%, 1 wt%, 2.5 wt% and 5 wt%). Arrows indicate indicate the presence of nanotubes clusters on the surface.

4 Discussion

Titanium dioxide nanotubes (n-TiO_2) incorporated into the ceramic glaze coating are intended to provide antibacterial activity for the indirect restorative material. In a desirable scenario, the n-TiO_2 addition would control biofilm formation, without compromising the surface roughness, morphology, and color of the glaze-coating glass ceramics.

With regards to the roughness, the results indicated that the glaze-coating application reduced the surface roughness of the glass ceramics, independently of the n-TiO_2 incorporation. This result was expected since several investigations report the greater ability of glazing to decrease roughness in comparison to conventional finishing and polishing systems.[6,8,12] Furthermore, the lower n-TiO_2 concentrations (1 and 2.5%) exhibited intermediate surface roughness values, comparable to the group with no n-TiO_2 addition (control). However, the incorporation of 5% n-TiO_2 into the glaze promoted higher roughness mean values than the control group. The higher surface roughness observed for the uppermost n-TiO_2 concentration (5%) can be explained by the presence of clusters within the glaze material after homogenization. These results, reject the first hypothesis that the incorporation of n-TiO_2 into the glaze coating would not change the surface roughness of the glazed-coating ceramic surface.

The n-TiO_2 technology was previously added to glass ionomer cement (GIC), and the results showed that the incorporation of 3, 5, and 7% n-TiO_2 did not change the surface roughness of the GIC.[20,21] The authors suggest that the nanosized TiO2 tubes did not affect the distribution between particles and matrix and the interfacial particles bonding.[20] Although particle size directly influences the roughness of the material and the same n-TiO_2 was used, these findings are not in line with our study. The plausible explanation is the elementary characteristic of the ceramic glaze, an amorphous low temperature melting glass mas.[27] Even going through meticulous stages of n-TiO_2 incorporation into the glaze powder, the final glaze viscosity possibly impaired the dispersion and homogenization of the nanostructure. The lack of dispersion possibly formed clusters, increasing the surface roughness reported for the 5% n-TiO_2 concentration. This fact can be observed through SEM images (Figure 3.) that show the presence of nanotube clusters within the glaze applied on the glass ceramic surface. Although the incorporation of 5% n-TiO_2 into the glaze increased the surface roughness, according to Jones et al., 2004 patients are able to perceive roughness values of 0.5 μm,[28] it should be noted that the mean surface roughness value of this group (0.057μm +/- 0.02) was not critical for clinical perceptibility and is approximately 3.7 times lower than the surface roughness values considered critical for biofilm formation (<0.2 μm).

The surface roughness results of this evaluation agree with another investigation that incorporated n-TiO_2 into zirconia surfaces to improve the interaction between resin cement and Y-TZP ceramics.[29] In that study, n-TiO_2 was mixed with isopropyl alcohol at 50 wt% to obtain a paste that was actively applied by rubbing the surface of the zirconia specimen to form a thin layer. After alcohol evaporation and sinterization, surface roughness was observed in 3D confocal microscopy and SEM. Authors report surface roughness increase after n-TiO_2 incorporation to the zirconia surface, but not uniformly: some areas exhibited higher concentrations of nanoagglomerates while others were deprived of n-TiO_2.[29]

Colorimetric evaluation (CIElab and CIEDE2000 systems) showed that the n-TiO_2 incorporation into the glaze did not promote significant statistical differences for ΔE_{ab} and ΔE_{00}, regardless of the concentrations tested. However, it was possible to observe that the values exceed the color thresholds of perception (PT) and acceptability (AT), according to the limits previously proposed[25] for both the CIELAB (1.2:2.7) and CIEDE2000 systems (0.8:1.8). Furthermore, the incorporation of 5% of n-TiO_2 into the glaze promoted lower luminosity (L*), higher b* parameter (towards a yellow appearance), and lower whiteness index (W_{ID}) than the remaining groups. Thus, the second null hypothesis attesting that incorporation of n-TiO_2 into the glaze would not change the color of glazed-coating ceramic, could also be rejected. The white color of the nanotube particles, the high concentration (5% n-TiO_2), or the presence of clusters, possibly contributed to the decrease in the luminosity and changes in b* and W_{ID} parameters. But still, since no color differences were detected among groups, regardless of the parameters used (ΔE_{ab}, ΔE_{00}), or the presence/concentration of n-TiO_2, these variations may be clinically irrelevant.

No significant antibacterial activity of n-TiO_2 was found among groups, and this result accepts the third null hypothesis that the incorporation of n-TiO_2 into the glaze coating would not inhibit biofilm formation. However, the highest concentration tested (5% by weight) indicated a tendency to decrease biofilm formation. One important characteristic of TiO_2 nanostructure is the photocatalytic property a phenomenon that occurs when n-TiO_2 is exposed to light irradiation exhibiting higher energy than its band gap.[30] According to investigations, n-TiO_2 nanostructures activated by ultraviolet (UV) light may exhibit photocatalytic activity by generating reactive oxygen species (ROS), capable of disrupting the cell membrane of microorganisms, thus providing a material with antimicrobial potential.[22,31] In this study, before growing *S. mutans* and *S. sanguinis*, all groups were decontaminated in UV-radiation in a flow chamber for 30 min, which could provide a photocatalytic effect of the nanostructure. However, this antibacterial effect might not have been as significant as expected since the nanotubes were possibly trapped in the glaze's vitreous structure after sintering, avoiding the release of the reactive oxygen species. Moreover, cluster formation compromised the nanotube dispersion into the glazed ceramic, limiting the antibacterial potential of the 5% n-TiO_2 group.

Previous studies reported the antibacterial effect of n-TiO_2 against *S. mutans* and *S. sanguinis* and related its efficacy with the bacteria's resistance to ROS, which depends on cell membrane thickness, and on the nanotube modification, such as surface functionalization.[22,24] Based on the surface roughness and topography, our findings suggest that n-TiO_2 functionalization might impact the nanotube dispersion in the material tested.

Titanium dioxide nanostructures tend to agglomerate in their original state and a way of overcoming this limitation is the functionalization of the TiO_2.[32] TiO_2 nanostructures are mainly terminated by hydroxyl groups (-OH) and can be functionalized by organic bifunctional molecules, which allows the nanostructures to remain chemically stable, preventing agglomeration.[33,34] In a previous report, TiO_2 nanostructures (nanotubes or nanoparticles) were functionalized with organic bifunctional molecules and the resin composite properties were evaluated. As anticipated, the n-TiO_2 functionalized with 3-(trimethocysilyl) propyl methacrylate containing (TSMPM) enhanced the properties of the resin-based material tested.[34]

Rossi et al., 2022 evaluated the incorporation of 1 and 2.5% silver-coated silica nanoparticles (NPs) in a glaze and tested the minimum inhibitory concentration (MIC) for antibiofilm formation against *C. albicans*. According to the MIC results, the functionalized NPs presented superior dispersion and were more effective in reducing fungal growth than the non-functionalized NPs. Although the functionalized NPs triggered a 64% reduction in CFU/specimen count for the glaze, the authors reported that this reduction was not statistically significant and concluded that further investigations are necessary to boost silver release, improving nanoparticles' antifungal potential. Although the authors reported the antifungal potential of silver-coated NPs, which are essentially different from the n-TiO_2 in composition and morphology, the functionalization of the nanotubes could contribute to the dispersion and antibacterial activity against *S. mutans* and *S. sanguinis*.[35]

To the best of the author's knowledge, this is the first report on n-TiO_2 incorporation into a ceramic glaze. Therefore, several conjectures were raised, and methodology limitations were found. The most important limitation is reproducing an effective method of dispersing the nanotubes into the glaze. The n-TiO_2 powder was added to the glaze powder, following a series of tested concentrations ranging from 1 to 50 wt%. After homogenization with the liquid of the glaze, concentrations higher than 5 wt% n-TiO_2 were discarded because they did not allow adequate viscosity and uniform application over the glass ceramic. Additionally, obtaining homogeneity for the higher concentrations was challenging, indicating that the functionalization of the n-TiO_2 could facilitate the incorporation and dispersion, potentializing the antibacterial response of the modified glaze. Another concern was the possibility of n-TiO_2 melting during the glaze firing process, losing its tubular conformation, and agglomerating while cooling, which was a possibility raised by Dos Santos et al., 2018 during the sintering process of zirconia coated with n-TiO_2. However, this remains a possibility and should be confirmed under FT-Infrared spectroscopy to evaluate the band shift of n-TiO_2, and high-resolution microscopy analysis to evaluate the morphology of the nanostructures.

The overall scenario leads to the conclusion that 5 wt% n-TiO_2 incorporated into the ceramic glaze coating was inclined to provide antibacterial activity. Although this concentration promoted higher surface roughness than the control group, the mean values were still below the critical limit for biofilm accumulation, and morphology was not significantly changed. Besides, although 5wt% n-TiO_2 decreased L*, a*, W_{ID}, and increased b* parameters, no differences in color change were found among the groups. Considering the limitations described, future evaluations should be performed to validate if the incorporation of n-TiO_2 could be a viable and promising alternative to develop a glaze coating with antibacterial potential, without damaging the material's mechanical and optical properties.

5 Conclusion

Within the limitations of the current study, the following conclusions can be made:

1. The higher concentration (5%) of n-TiO_2 decreased L*, a*, W_{ID}, and increased b*, but caused no color change differences (ΔE_{ab}, ΔE_{00}).
2. The higher concentration (5%) of n-TiO_2 increased the ceramic surface roughness, and all concentrations presented some surface morphology alterations.
3. None of the tested concentrations were able to inhibit biofilm formation.

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3 DISCUSSÃO

Os avanços nas técnicas e materiais restauradores trouxeram novos conceitos para confecções de restaurações indiretas, através de abordagens minimamente invasivas e da Odontologia adesiva que foram consolidadas por meio de evidências e sucesso clínico a longo prazo (Bayne et al., 2019). Com isso, uma grande variedade de materiais CAD/CAM monolíticos surgiu com diferentes composições microestruturais visando suprir as demandas de restaurações com alto nível estético, biocompatíveis e duradouras (Silva et al., 2017).

No entanto, além de serem testados quanto as suas propriedades físicas e mecânicas, esses novos materiais precisam apresentar longevidade clínica adequada diante das adversidades presentes no meio oral, que podem ser tanto de origem exógena (alimentos e bebidas acidas) quanto endógenas (biofilme cariogênico, enzimas salivares, ácido gástrico) (Sagsoz et al., 2016), pois serão eleitos para reabilitação de destruições dentais severas causadas principalmente por esses fatores (Kulkarni et al, 2020).

Como consequência do aumento do número de pacientes portadores de bulimia nervosa e doença do refluxo gastresofágico (DRGE), observa-se cada vez mais desgastes dentais por meio de processos erosivos pelo excessivo contato do esmalte com o acido clorídrico proveniente do suco gástrico e associados com abrasão, pois geralmente os episódios de regurgitação são seguidos de escovação dental (Alnasserr et al., 2019, Kulkarni et al., 2020, da Cruz et al., 2022). Dessa forma, deve-se considerar que os materiais restauradores irão enfrentar degradação decorrente dos desafios ácidos que impactarão diretamente na performance.

Ainda poucos estudos presentes na literatura investigaram os efeitos do suco gástrico associado ou não a escovação em materiais monolíticos do sistema CAD/CAM, principalmente realizando testes microbiológicos como formação de biofilme multiespécies como complemento da investigação da performance mecânica (Backer et al., 2017, Alnasser et al., 2019, Willers et al., 2020, Kulkarni et al., 2020, da Cruz et al., 2022)

De maneira geral, todos os materiais estudados independente da composição, apresentaram alterações na resistência a flexão, microdureza e rugosidade de superfície. Porém a resina nanocerâmica Grandio Blocs, VOCO (RG) apresentou resultados positivos para esta classe de materiais, principalmente para resistência a flexão, a qual foi similar à cerâmica a base de silicato de lítio reforçada por zircônia (ZLS). Ainda, a microdureza da RG foi mantida mesmo após desafios erosivos e abrasivos. Até o presente momento RG não foi utilizado em outro estudo sob desafios semelhantes e que validem este material para restaurações que demandarão maior carga mastigatória.

Backer et al., 2017 avaliou a microdureza e rugosidade de superfície em resinas CAD/CAM (Lava Ultimate e Paradigm Z100), apenas simulando processos erosivos causados pelo suco gástrico e independente do tempo de imersão (6 e 24h). Os autores observaram que a microdureza das resinas não foi afetada, e a rugosidade de superfície diminuiu após 6h em contato com a solução acida para ambos os materiais, e após 24h apenas Lava Ultimate aumentou a rugosidade (Backer et al., 2017).

Embora o presente estudo tenha utilizado a mesma solução para simular a erosão decorrente do suco gástrico, a escovação mecânica simulada foi adicionada como desafio abrasivo e neste estudo, houve maior tempo de exposição aos desafios ácidos/abrasivos para simular o cenário clínico. Estes fatores influenciaram, em especial o parâmetro da rugosidade de superfície, pois houve aumento para todos os grupos, especialmente para as resinas nanocerâmicas e cerâmica feldispática. O aumento da rugosidade foi corroborado no estudo de Alnasser et al., 2019, que investigou diferentes tipos de materiais CAD/CAM expostos a pH extremamente ácidos durante 45 e 91h. Neste estudo, no entanto, a simulação de processos abrasivos não foi realizada, fato que pode não demonstrar todo o dano que o material pode sofrer uma vez presente no ambiente oral (Alnasser et al., 2019).

Em recente estudo, Cruz et al., 2022 mostrou que resina nanocerâmica e PICN apresentaram alteração na topografia de superfície, tanto pela imersão em saliva artificia seguida de escovação simulada, quanto pela ação do suco gástrico associada com a escovação (da Cruz et al., 2022), corroborando os resultados deste estudo. Estes resultados demonstram que materiais com rede polimérica são mais susceptíveis a degradação hidrolítica, e o declínio do pH acelera ainda mais esse processo, pois ácidos orgânicos penetram mais facilmente na rede polimérica provocando lixiviação dos monômeros.

Apesar dos desafios erosivos e/ou abrasivos afetarem as propriedades mecânicas e topografia de superfície dos materiais testados, eles influenciaram estatisticamente na adesão de biofilme multiespécie, porém observou-se aumento nas unidades formadoras de colônias para alguns materiais (ZLS, FE e PICN) independentemente se destes terem sido submetidos à erosão e/ou abrasão. Sabe-se que a formação de biofilme na superfície de materiais cerâmicos está diretamente relacionada com sua rugosidade de superfície, uma vez que valores de Ra acima 0,2 µm é estabelecido como limiar clínico relevante para adesão de microrganismos (Bollen et al., 1997). Os valores obtidos para os materiais CAD/CAM indiretos testados não ultrapassaram 0,2 µm. O ângulo de contato é outro fator que influencia a formação de biofilme, pois superfície com características hidrofóbica favorecem a formação e adesão bacteriana (Contreras et al., 2018).

Em estudo prévio, Willers et al., 2020 avaliou a rugosidade de superfície e formação de biofilme *(S. mutans)* na superfície de cerâmica de dissilicato de lítio e zircônia recoberta por glaze após processos erosivos (HCl 0.06M por 30h) e abrasivos (400.000 ciclos) e observou que a abrasão causada pelos ciclos de escovação mecânica simulada foi a principal causa do aumento de rugosidade na camada de glaze. Os autores indicaram que houve maior deposição de biofilme após 24h, confirmando que a rugosidade de superfície interfere diretamente na adesão bacteriana (Willers et al., 2020).

Os resultados obtidos no presente estudo também demonstraram que houve aumento da rugosidade de superfície após desafios, porém foi mais expressivo após erosão e associação de erosão e abrasão. Tal diferença, provavelmente se deve ao fato de terem sido utilizadas soluções ácidas e tempo de imersão diferentes, e não ter sido aplicado a camada de glaze, que foi o principal objetivo do estudo de Wilers et al., 2020. Os blocos CAD/CAM avaliados não precisam necessariamente da aplicação do glaze, pois de acordo com os fabricantes, apenas acabamento e polimento com sistemas apropriados podem ser realizados para finalização das restaurações. Porém independente do fator causador, o presente estudo corrobora com os achados de Willers et al., 2020, que mesmo um discreto aumento da rugosidade, por processos erosivos e/ou abrasivos, provocam irregularidades na superfície dos materiais cerâmicos e aumentam a área para adesão de biofilme.

A manutenção da lisura, brilho e estabilidade de cor de um material restaurador é tão importante quanto as propriedades mecânicas para sucesso e longevidade no ambiente oral, pois além de manter as características estéticas, diminui o acúmulo e adesão de biofilme, e muitas vezes alterações de cor e textura podem ser motivo para substituição de restaurações sem que o material tenha falhado mecanicamente (Sagsoz et al., 2016). Neste contexto, o objetivo do segundo estudo, foi desenvolver um glaze contendo diferentes concentrações de n-TiO_2 (0, 1, 2.5 e 5%) aplicado à cerâmica. Neste estudo, foram avaliados a estabilidade de cor, rugosidade de superfície e atividade antibacteriana contra *S. mutans* e *S. sanguinis*.

Nanotubos de dióxido de titânio (n-TiO_2) são materiais de estruturas tubulares ocas medindo 20nm de comprimento por 10 nm de diâmetro, que apresentam alta reação superfície-volume e alta energia de superfície, além de apresentarem efeitos antibacterianos por meio de interações eletrostáticas entre o titânio nanoestruturado e a bactéria (Kantovitz et al., 2020).

Os n-TiO_2 foram incorporados em diversos materiais odontológicos, como o cimento de ionômero de vidro, géis clareadores e cerâmicas policristalinas como a zircônia (Y-TZP) com o propósito de melhora nas propriedades físico-químicas e mecânicas além de exercerem atividade antimicrobiana (Cibim et al., 2017, Dos Santos et al., 2017, Kantovitz et al., 2020). A incorporação de agentes antibacterianos já vem sendo realizada em diversos materiais restauradores, estudos recentes investigam o desenvolvimento de um glaze contendo nanopartículas a base de prata e fluoreto de sódio ou finas coberturas de carbeto de silício para finalização de cerâmicas afim de agregar potencial antibacteriano para esses materiais (Oh et al., 2019, Afonso Camargo et al., 2020).

A avaliação colorimétrica (sistemas CIElab e CIEDE2000) mostrou que a incorporação de n-TiO2 ao esmalte não promoveu diferenças estatisticamente significativas para ΔE_{ab} e ΔE_{00}, independente das concentrações testadas. No entanto, foi possível observar que os valores ultrapassam os limiares de percepção (PT) e aceitabilidade (AT) das cores, de acordo com os limites propostos anteriormente (Paravina et al., 2015) tanto para o CIELAB (1.2:2.7) quanto para o CIEDE2000 sistemas (0,8:1,8). Além disso, a incorporação de 5% de n-TiO2 no esmalte promoveu menor luminosidade (L*), maior valor de b* (para uma aparência amarela) e menor índice de alvura (W_{ID}) do que os demais grupos. A cor branca das partículas dos nanotubos, a alta concentração (5% n-TiO2), ou a presença de clusters, possivelmente contribuíram para a diminuição da luminosidade e mudanças nos parâmetros b* e W_{ID}. Ainda assim, como não foram detectadas diferenças de cor entre os grupos, independentemente dos parâmetros utilizados (ΔE_{ab}, ΔE_{00}), ou da presença/concentração de n-TiO2, essas variações podem ser clinicamente irrelevantes.

Com relação à rugosidade, os resultados indicaram que a aplicação do glaze reduziu a rugosidade superficial independentemente da incorporação de n-TiO2. Este resultado era esperado, uma vez que várias investigações relatam a maior capacidade do glaze em diminuir a rugosidade em comparação aos sistemas convencionais de acabamento e polimento (Motro et al., 2012, Sagsoz et al., 2016). No entanto, a incorporação de 5% de n-TiO2 promoveu maiores valores médios de rugosidade que o grupo controle, o que pode ser explicada pela presença de aglomerados no pó do glaze, mesmo após a homogeneização.

Possivelmente a dificuldade em homogeneizar o pó do glaze com a incorporação do n-TiO2 podem ter interferido no potencial antibacteriano, pois os resultados obtidos não demostraram diferenças estatísticas significativas na diminuição da contagem de colônias, embora a adição de 5% de n-TiO2 tenha promovido menores médias para ambos os microrganismos testados. As nanoestruturas podem ter aglomerado e não dispersado na superfície recoberta pelo glaze e durante o processo de queima as altas temperaturas podem ter alterado a estrutura, favorecendo a formação de aglomerados (Rong et al., 2005, Dafar et al., 2016, Dos Santos et al., 2018).

Até o presente momento e conhecimento dos autores, este foi o primeiro estudo que sugeriu a incorporação dos n-TiO2 em um glaze comercial. Por tratar-se de um estudo *in vitro*, há necessidade de aprimorando as técnicas de incorporação e homogeneização das nanoestruturas com o glaze para que um produto final possa atender aos requisitos ópticos desejados, e principalmente ser capaz de diminuir ou inibir a adesão e proliferação de microrganismos.

4 CONCLUSÃO

Com base nos resultados obtidos nos dois estudos conduzidos foi possível concluir que:

Artigo 1:

- Os desafios erosivos/abrasivos afetaram a resistência à flexão dos materiais testados. Todos os materiais apresentaram diminuição da resistência à flexão após simulações de erosão (E) e erosão e abrasão (E+A).
- Os desafios ácido-erosivos diminuíram a microdureza do material cerâmico ou híbrido (ZLS, FE e PICN). A formação do biofilme foi dependente do material e o material à base de cerâmica ou híbrido (ZLS, FE e PICN) apresentou maior adesão do biofilme do que os restauradores à base de resina.

Artigo 2:

- A maior concentração (5%) de n-TiO2 diminuiu L*, a*, W_{ID} e aumentou b*, mas sem diferenças na mudança de cor (ΔEab, ΔE00);
- A maior concentração (5%) de n-TiO2 aumentou a rugosidade da superfície da cerâmica, e todas as concentrações apresentaram algumas alterações na morfologia da superfície;
- Nenhuma das concentrações testadas foi capaz de inibir a formação de biofilme.

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Anexo 1- Relatório de verificação de originalidade e prevenção de plágio

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Effects of gastric acid and mechanical toothbrushing in CAD-CAM restorative materials: Mechanical properties, surface topography, and biofilm adhesion

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