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ALTERAÇÕES DE RUGOSIDADE E COR DE UMA RESINA PARA BASE DE PRÓTESE SUBMETIDA A UM PROTOCOLO DE IMERSÃO DE LONGA DURAÇÃO EM CINAMALDEÍDO

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RESUMO

Introdução: A má higienização das próteses em resina acrílica pode levar ao desequilíbrio microbiológico e ao desenvolvimento da estomatite protética. O cinamaldeído é um fitoconstituinte que tem se colocado como uma alternativa para a desinfecção de próteses devido sua promissora ação antimicrobiana. Entretanto, seus possíveis efeitos deletérios sobre os materiais de base de prótese em longo prazo não são conhecidos. **Objetivo:** O objetivo do presente estudo foi de avaliar os efeitos da imersão simulada por até cinco anos nas alterações superficiais de rugosidade e cor de uma resina termicamente ativada por energia de micro-ondas imersa em soluções de limpeza de prótese. **Materiais e métodos:** Oitenta espécimes em forma de disco (10 x 5 mm) foram confeccionados em polimetacrilato de metila (PMMA) ativado termicamente por energia de micro-ondas (Vipi Wave) e foram imersos em quatro soluções (n=20): GTW –Água de torneira (Controle); GSH – Hipoclorito de sódio 0,5%; GPX – Peróxido alcalino (Polident Overnight Whitening); GCA – Cinamaldeído 27µg/mL, simulando até cinco anos de higienização. Análises dos parâmetros de rugosidade superficial (Sa) e de cor CIELab (L* a* b*, ΔEab), CIEDE2000 (ΔE00) e National Bureau of Standards (NBS) foram analisados, respectivamente, através de perfilometria óptica (CCI-MP, Taylor Hobson, UK) e espectrofotometria (Vita EasyShade, Vita, Germany) em quatro intervalos de tempo: *baseline* (t=0), após 104, 913 e 1825 ciclos de imersão. A diferença entre as médias dos grupos foi verificada pela análise de variância Two-Way ANOVA para medidas repetidas e teste de Tukey, com nível de significância de 1% (α=0,01). **Resultados:** A rugosidade superficial foi influenciada pelo fator tempo, porém não pelo fator solução. Verificou-se um aumento da rugosidade Sa para todos os grupos, após 1825 ciclos, com diferença estatisticamente significante ($p<0,01$) quando comparado ao *baseline,* independentemente da solução estudada. Em relação às alterações de ΔEab, ΔE00 e NBS, apenas o fator tempo foi relevante na alteração da cor das amostras, com todos os valores classificados abaixo dos limites de perceptibilidade e aceitabilidade. O grupo GCA não apresentou valores de rugosidade e cor estatisticamente diferentes com as demais soluções testadas após cinco anos de imersão simulada ($p>0,01$). **Conclusões:** A solução de cinamaldeído (27µg/mL) causou pequenas alterações nos parâmetros de rugosidade superficial e cor avaliados, semelhantes ao hipoclorito de sódio 0,5% e peróxido alcalino após 5 anos de imersão simulada.

Palavras-chave: Polimetil Metacrilato; Biofilme; *Candida*; Cinamaldeído; Propriedades de Superfície; Cor.

ABSTRACT

Introduction: Poor hygiene of acrylic resin dentures can lead to microbiological imbalance and the development of prosthetic stomatitis. Cinnamaldehyde is a phytoconstituent that has been placed as an alternative for denture disinfection due to its promising antimicrobial action. However, its possible deleterious effects on the long-term denture base materials are not known. **Objective:** The aim of the present study was to evaluate the effects of simulated immersion for up to five years on surface roughness and color changes of a resin thermally activated by microwave energy immersed in denture cleaning solutions. **Materials and methods:** Eighty disc-shaped specimens (10 x 5 mm) were made in polymethyl methacrylate (PMMA) thermally activated by microwave energy (Vipi Wave) and were immersed in four solutions (n=20): GTW –Tap water (Control); GSH - 0.5% sodium hypochlorite; GPX - Alkaline peroxide (Polish nighttime whitening); GCA - Cinnamaldehyde 27µg/mL, simulating up to five years of cleaning. Analyzes of surface roughness (Sa) and color parameters CIELab (L* a* b*, ΔEab), CIEDE2000 (ΔE00) and National Bureau of Standards (NBS) were deviated, respectively, by optical profilometry (CCI-MP, Taylor Hobson, UK) and spectrophotometry (Vita EasyShade, Vita, Germany) at four time intervals: baseline (t=0), after 104, 913 and 1825 immersion cycles. The difference between the means of the groups was verified by analysis of variance ANOVA bidirectional for repeated measures and Tukey test, with a significance level of 1% (α=0.01). **Results:** The surface roughness was influenced by the time factor, but not by the solution factor. There was an increase in roughness Sa for all groups after 1825 cycles, with a statistically significant difference ($p<0.01$) when compared to the baseline, regardless of the solution studied. Regarding changes in ΔEab, ΔE00 and NBS, only the time factor was relevant in changing the color of the previous ones, with all values classified below the perceptibility and acceptability limits. The GCA group did not show roughness and color values statistically different from the other solutions tested after five years of simulated immersion ($p>0.01$). **Conclusions:** Cinnamaldehyde solution (27µg/mL) caused small changes in surface roughness and relative color parameters, similar to 0.5% sodium hypochlorite and alkaline peroxide after 5 years of simulated immersion.

Keywords: Polymethyl Methacrylate; Biofilms; *Candida*; Cinnamaldehyde; Surface Properties; Color.

SUMÁRIO

1. INTRODUÇÃO

O polimetilmetacrilato (PMMA) é o material de escolha mais comumente utilizado na confecção de próteses totais e parciais, estando introduzido para tal finalidade desde o final dos anos 1930 por Walter H. Write (1–3). A predileção pelo PMMA ocorre em virtude de suas características e vantagens, apresentando boa estabilidade dimensional no ambiente bucal, compatibilidade com os tecidos orais, além de proporcionar um resultado estético satisfatório, ser de baixo custo e de fácil manipulação (4–7).

No entanto, como a cavidade oral é um ambiente constituído por uma rica e diversificada microbiota composta por vírus, bactérias e fungos patogênicos e não patogênicos que habitam em total comensalismo (8,9), alterações locais promovidas pelo uso contínuo de próteses e a má higiene oral, e sistêmicas como xerostomia e desordens imunológicas, podem acarretar em mudanças significativas nesse ambiente, principalmente em pacientes imunologicamente comprometidos e em idosos dependentes, proporcionando a ocorrência de infecções oportunistas por microorganismos (8–13). Vale salientar que implicações sistêmicas graves ocasionadas pela invasão tecidual e pela alta patogenicidade do biofilme oral, como pneumonia aspirativa, fungemia e endocardite bacteriana, podem ser desencadeadas caso a higienização das próteses não seja realizada de maneira adequada (1,14,15).

Além disso, a consequência desse desequilíbrio microbiológico pode ser observada clinicamente através do desenvolvimento da candidíase atrófica crônica (CAC), mais conhecida como estomatite protética (EP) (16). Apesar de apresentar uma etiologia multifatorial, a EP é frequentemente associada ao uso de próteses, tendendo a comprometer a qualidade de vida desses pacientes com o desencadeamento de sintomas como queimação (ardor), sensação dolorosa, alterações no paladar e dificuldade na deglutição. Clinicamente apresenta-se como uma lesão eritematosa inflamatória caracterizada por exibir uma mucosa difusamente avermelhada logo abaixo da prótese. Apesar do desconhecimento da maioria dos pacientes, estima-se que cerca de 65% a 75% dos usuários apresentem esta condição (10,16–19).

Mesmo sendo constituído por uma variedade altamente colonizada de microorganismos, o biofilme responsável pelas alterações provocadas pela EP tem como espécie causadora mais comum a *Candida albicans* (8,20–22). Esses microorganismos possuem a capacidade de adesão tanto a superfície do PMMA quanto aos tecidos orais subjacentes, habitat com fluxo de oxigênio e saliva reduzidos (17,19). Essa queda do fluxo salivar impede o acesso de lisozimas, lactoferrinas, histatinas, catelicidinas, calprotectinas e defensinas à interface mucosa/prótese, fator crucial para o controle do desenvolvimento e disseminação de *Candida albicans* (8,23). Além disso, superfícies protéticas mais porosas e hidrofóbicas favorecem a colonização de *Candida albicans* em sua forma mais patogênica (15). Outro fator inerente a própria resina acrílica, a hidrofobicidade, mensurada pelo ângulo de contato da superfície com um líquido, também parece estar diretamente relacionada com a aderência de microorganismos à superfície da prótese. Tem sido sugerido que quanto mais hidrofóbica a resina se torna, mais fácil fica a sua colonização (15,24).

Para contornar o acometimento da EP, além de uma adequada higiene oral, a desinfecção eficaz da prótese é essencial (2,3,21)**,** podendo ser realizada através de métodos químicos (soluções desinfetantes, comprimidos e agentes químicos), mecânicos (escovação, ultrassom ou energia por micro-ondas) ou por uma associação dos dois métodos (7,25–27). Apesar da maior facilidade de acesso proporcionado pelo método mecânico através da escovação, essa técnica possui desvantagens, como sua inviabilidade à usuários com desordens motoras ou visuais (28,29). Além disso, caso seja associada a um dentifrício abrasivo, danos irreversíveis (aumento da rugosidade) podem ser gerados na superfície da prótese (10,17,21). É importante mencionar que valores da rugosidade média (Ra) superiores a 0,2μm contribuem para a formação do biofilme e para a adesão microbiana ao PMMA (30,31), justificada também pela não atuação de forças de cisalhamento nessas regiões (32).

Uma alternativa eficaz consiste na imersão dos dispositivos protéticos em soluções químicas capazes de promover a desinfecção e eliminação do biofilme, atingindo áreas obstruídas de escala microscópica e anteriormente inacessíveis ao método mecânico (3,21,33). Contudo, as características ideais para uma solução química (demonstrar atividade antibiofilme, ser antibacteriano e antifúngico, não ser tóxico, apresentar compatibilidade com o material da prótese, não modificar ou degradar a superfície do acrílico, ser de fácil manuseio, ter um sabor agradável e não possuir alto custo) ainda não foram demonstradas em um único produto (10).

Independentemente da solução utilizada, algumas recomendações aos usuários também são importantes, tendo em vista que mesmo com uma higienização adequada da prótese e da cavidade oral, não é recomendado o uso contínuo destes dispositivos (24 horas por dia), afim de se evitar o acometimento pela EP. Ainda assim, devem ser higienizadas diariamente com um produto não abrasivo e pelo tempo adequado (10), entretanto, vários protocolos são recomendados para cada tipo e para uma mesma solução, gerando dúvidas entre usuários e dentistas.

Diversas soluções químicas de limpeza de próteses estão disponíveis no mercado, como hipocloritos, peróxidos, enzimas, ácidos e enxaguatórios (2,10). Como mencionado anteriormente, o principal objetivo dessas soluções é de promover sua ação antimicrobiana e detergente sem danificar as propriedades físico-mecânicas do PMMA, contudo, danos também podem ser gerados por algumas soluções durante as imersões (1,34). O hipoclorito de sódio (NaOCl) e os produtos químicos geradores de peróxidos compõem as soluções de limpeza de próteses mais comumente utilizados para tal finalidade (30,34). O hipoclorito de sódio é apresentado na forma de uma solução com capacidade bactericida e fungicida, atuando na matriz orgânica do biofilme, além de apresentar a capacidade de remover manchas. De acordo com diretrizes do *American College of Prosthodontics*, seu uso não deve ser superior ao limite de 10 minutos por imersão, evitando danos as próteses (10). Os peróxidos alcalinos são compostos por diversos agentes e são apresentados na forma de pó ou tabletes. Quando em contato com água, tornam-se soluções de peróxido de hidrogênio, capazes de promover a limpeza mecânica pela liberação de oxigênio ocorrida na reação (7,26,30).

Kiesow et al. (2016) (3) avaliaram a compatibilidade dos materiais utilizados na confecção próteses dentárias e os efeitos de regimes de limpeza típicos, simulando dois anos de higienização semanal ou três meses e meio de imersão diária. Os resultados revelaram que após 104 ciclos de 10 minutos, o hipoclorito de sódio 6% causou danos limitados ao PMMA, mas corroeu a liga de Cobalto Cromo e a solda. As pastilhas efervescentes à base de peróxido causaram poucos danos a quaisquer materiais após 104 ciclos de 15 minutos, exceto a formação da camada na solda de prata. Os autores concluíram que o hipoclorito de sódio foi incompatível com os componentes metálicos das próteses e que os comprimidos de limpeza à base de peróxido proporcionaram uma compatibilidade razoável com os materiais testados.

Arruda et al., (2015) (34) avaliaram a estabilidade de cor, rugosidade superficial e resistência à flexão de uma resina acrílica termopolimerizável após imersão em soluções de peróxido alcalino e hipoclorito alcalino, simulando um período de uso de cinco anos de imersão diária. Os autores concluíram que 1825 ciclos de 20 minutos em soluções de peróxido alcalino e NaOCl não causaram efeitos adversos clinicamente significativos na resina acrílica termopolimerizável.

Vários autores também têm apresentado novas alternativas para a desinfecção de próteses totais e parciais (12,21,35–37). Dentre essas alternativas encontra-se o cinamaldeído, fitoconstituinte obtido do óleo essencial presente em folhas e cascas de *Cinnamomum zeylanicum* (canela) e considerado um excelente agente antimicrobiano com potencial ação fungicida (12,21). O cinamaldeído promove o acúmulo de Espécies Reativas de Oxigênio (EROs) intracelular, além de ocasionar o aumento da concentração de cálcio no citoplasma e na mitocôndria. Sua ação frente à *Candida albicans* decorre também em virtude da liberação do citocromo c da mitocôndria para o citoplasma, assim como a externalização de fosfatidilserina, sinais claros de apoptose celular. Aliado a isso, o cinamaldeído provoca a diminuição do potencial de membrana mitocondrial ($\Delta\Psi m$), componente essencial no processo de armazenamento de energia (38). Além disso, vale mencionar que em estudos recentes foi demonstrado que o cinamaldeído apresentou citocompatibilidade e baixa toxidade celular, ratificando sua promissora capacidade no tratamento da EP (12).

Poucos estudos têm sido realizados acerca dos potenciais efeitos do cinamaldeído sobre os materiais protéticos (12,39,40). Entre eles, Almeida et al. (2020) (21) avaliaram o efeito do cinamaldeído na desinfecção de próteses totais removíveis assim como seu efeito nas propriedades físicas e mecânicas da resina acrílica em um estudo clínico *in situ.* Trinta e três voluntários portadores de *C. albicans,* porém sem a presença de estomatite protética, verificado previamente por uma triagem inicial, passaram por duas etapas de 14 dias: 7 dias envolvendo a formação de biofilme oral em espécimes de disco de resina acrílica inseridos na área basal das dentaduras, e 7 dias de imersão das próteses nos produtos avaliados – hipoclorito de sódio 0,5% e cinamaldeído 27 µg/mL, em um desenho cruzado, onde cada participante utilizou as duas substâncias. Os resultados revelaram que houve uma redução significativa ($p<0,05$) no número de microrganismos para cada espécie (microrganismos totais, *Streptococcus mutans* e *Candida* spp.), não havendo diferenças significativas ($p> 0,05$) entre hipoclorito e cinamaldeído. Houve aumento da rugosidade e diminuição da dureza dos corpos de prova, não havendo diferença entre as duas substâncias desinfetantes ($p> 0,05$). Tanto o hipoclorito quanto o cinamaldeído também causaram alteração na cor, mas sem diferença significativa entre as substâncias desinfetantes ($p >0,05$). Os autores concluíram que a solução de cinamaldeído com concentração de 27 µg/mL foi eficaz contra todos os microrganismos avaliados e causou pequenas alterações nos parâmetros de dureza, rugosidade superficial e cor, sem relevância clínica. Entretanto, o estudo avaliou o efeito do regime de higienização por um curto intervalo de tempo, sendo necessários estudos adicionais para verificar o comportamento da solução em longo prazo.

Apesar da promissora ação antimicrobiana evidenciada pelo cinamaldeído (12,21,39,41–43), há uma escassez na literatura a respeito da imersão a longo prazo de componentes protéticos nessa solução e suas possíveis alterações nas propriedades do PMMA. Assim, o objetivo do presente estudo foi de avaliar os efeitos da imersão simulada de cinco anos nas alterações superficiais de rugosidade e cor de uma resina termicamente ativada por energia de micro-ondas imersa em cinamaldeído, justificado pelo benefício potencial que apresenta o uso dessa substância contra microorganismos na prevenção da estomatite protética.

2. CAPÍTULO 1

O manuscrito a seguir foi submetido para publicação no periódico “The Journal of Prosthetic Dentistry”.

Effect of a long-term immersion protocol in cinnamaldehyde on the properties of a heat-cured denture resin

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ABSTRACT

Background: Poor hygiene of acrylic resin-based dentures can lead to microbial colonization and the development of denture stomatitis. While cinnamaldehyde has been successfully used for the short-term disinfection of dentures, its long-term effects on the surface and color properties of denture base materials remain largely unknown.

Objective: This study evaluated the effects of simulated immersion in cinnamaldehyde for up to five years on the surface roughness and color parameters of a heat-cured denture base resin. **Materials and methods:** Eighty disc-shaped specimens (10 x 5 mm) were prepared by microwave heat-cured polymethylmethacrylate (PMMA), and immersed in four solutions ($n = 20$), namely: TW – Tap water (control); SH – 0.5% sodium hypochlorite; PX – Alkaline peroxide; and CA – Cinnamaldehyde (27 µg/mL). The immersion protocol simulated up to five years of cleaning. Surface roughness (Sa) and the color parameters CIELab (L* a* b*, ΔEab), CIEDE2000 (ΔE00) and the National Bureau of Standards (NBS) were analyzed by optical profilometry and spectrophotometry, respectively, at baseline ($t = 0$) and after 104, 913 and 1825 immersion cycles. Differences in the means between the groups were determined by two-way analysis of variance (ANOVA) for repeated measures and Tukey's posthoc test, considering significance level at 1% ($\alpha = 0.01$).

Results: The surface roughness of treated specimens was influenced by the time factor, but not by the solution factor. Sa roughness was significantly increased in all groups after 1825 cycles compared to baseline ($P < 0.01$), regardless of the solution. Only the time factor affected significantly ΔEab, ΔE00, and NBS color parameters, which were below the perceptibility and acceptability thresholds. After simulated 5-year immersion, the surface roughness and color values of CA-treated specimens were not statistically different from those of the other groups ($P > 0.01$).

Conclusion: Cinnamaldehyde solution (27 μg/mL) produced minor effects on the surface roughness and color parameters of a heat-cure denture base resin, similar to 0.5% sodium hypochlorite and alkaline peroxide, after 5 years of simulated immersion.

CLINICAL IMPLICATIONS

Cinnamaldehyde is an alternative cleaning solution for PMMA-based dentures that causes only minor changes in color and surface roughness after long-term immersion. Overall, cinnamaldehyde can be considered advantageous in terms of odor, taste, and cost-effectiveness.

INTRODUCTION

Candida albicans is the most common species causing biofilm-related denture stomatitis (DS).[1–4] Yeast cells can adhere both to the surface of polymethylmethacrylate (PMMA) materials and underlying oral tissues, on which there is reduced oxygen availability and a low salivary flow. [5–7] Hence, thorough oral hygiene and denture disinfection are critical to prevent the onset of DS.[2,8–10] The latter can be performed through chemical and mechanical methods or an association of both.[11–14] While the mechanical method (brushing) can be readily applied by most users, it has important disadvantages to consider, particularly for individuals with motor or visual disorders.[14–17] Furthermore, if brushing is associated with an abrasive dentifrice, the surface of the denture can be susceptible to irreversible damage, such as increased roughness and loss of matter.[2,5,18] Surface roughness values greater than 0.2 μm were shown to favor microbial adhesion and biofilm formation on PMMA materials,[19,20] which is likely due to the absence of shear forces in these areas.[21] More importantly, serious systemic complications caused by tissue invasion and the high pathogenicity of oral biofilms, such as aspiration pneumonia, fungemia, and infective endocarditis, may occur in susceptible individuals if dentures are not properly cleaned.[17,22–25]

To overcome this issue, an effective alternative consists of immersing the denture in chemical solutions, [17,26] thereby reaching obstructed areas on a microscopic scale that are often inaccessible to mechanical cleaning. [2,9,27] Several chemical solutions for cleaning dentures are available on the market, such as hypochlorite, peroxides, enzymes, acids, and mouthwashes. [8,18,28] The main purpose of these solutions is to have antimicrobial and detergent action without damaging the physical-mechanical properties of PMMA materials.[22,28–31] Sodium hypochlorite (NaOCl) and alkaline peroxides are the most commonly used solutions for dentures.[19,29] Yet, the corrosive power of NaOCl causes damages in denture base materials whereas the high cost of alkaline peroxides renders them unaffordable to a large part of the population, specially in developing countries.[6,32]

Over the years, biotechnological advances worldwide have contributed to natural product research and development, including the use of essential oils (EOs) and their phytoconstituents. To overcome microbial resistance issues, EOs have been the subject of several studies due to their biological and pharmacological properties.[2,33–36]

Cinnamaldehyde is a phytochemical obtained from the leaves and bark of *Cinnamomum zeylanicum* (cinnamon). It is considered an excellent antimicrobial agent with a potential fungicidal action through the inhibition of ATPase activity in the plasma membrane and by dropping the intracellular pH[2,33]. In a previous study, cinnamaldehyde showed cytocompatibility when exposed to fibroblasts, macrophages, and keratinocyte cell lines.[33] In a previous *in situ* clinical study in denture users, a cinnamaldehyde solution at the concentration of 27 μg/mL was effective against all microbial strains and caused only minor, clinically irrelevant changes in the parameters of hardness, surface roughness, and color. Users preferred this solution because of its features, such as pleasant taste and odor. However, only the short-term effects of the cleaning regimen were examined, requiring additional research to determine the long-term behavior of the experimental solution.[2]

In this study, we analyzed the use of products commonly used for the disinfection of dentures compared to an experimental cinnamaldehyde solution. The hypothesis was that cinnamaldehyde produces only minor changes in the color and surface roughness of PMMA-based dentures, compatible with sodium hypochlorite and alkaline peroxide, during simulated long-term immersion up to 5 years.

MATERIAL AND METHODS

The experimental methodology of this study was adapted from previous reports.[2,9,29] Eighty medium-pink PMMA discs heat-cured by microwave energy (Vipi Wave, Vipi, São Paulo, Brazil) were prepared according to the manufacturer's recommendations.

Cylindrical stainless-steel dies (10 x 5 mm) were used for the impression and subsequent preparation of the specimens. A medium-pink microwave-cured acrylic resin (Vipi Wave, Vipi, São Paulo, Brazil) was selected. The resin was dosed, manipulated, included in the spaces created by the stainless-steel dies, placed on a glass plate (to provide a smooth surface), and heat-cured by microwave energy according to the manufacturer's instructions. Due to the surface smoothness provided by the glass plate, no polishing was needed.[2] Excess resin was finished with a tungsten bur (Maxicut, Labordental, São Paulo, Brazil), and the specimens were submitted to an ultrasonic bath (BioWash STD, Bio Art, São Paulo, Brazil) in distilled water for 15 minutes to remove debris. **Table 1** presents the specifications of the materials used.

The sample size ($n = 20$/group) was based on similar reports published elsewhere.[19,29] The specimens were randomly allocated into four groups according to the cleaning solution through an online random number generation tool (www.randomizer.org): TW – Tap water; SH – 0.5% sodium hypochlorite; PX – Alkaline peroxide; and CA - Cinnamaldehyde (27µg/mL).

The tap water used for the TW and PX solutions was stored in recipients to avoid variations from daily collections. The preparation of the PX solution was carried out according to the manufacturer's instructions, that is, 200 mL of water at 37ºC for one alkaline peroxide effervescent tablet (GSK - GlaxoSmithKline, Philadelphia, PA, USA). The 0.5% SH solution was purchased from a compounding pharmacy (Dilecta, João Pessoa, PB, Brazil). The CA solution (Sigma Aldrich, St. Louis, MO, USA) was prepared at a concentration of 27 µg/mL according to the study by Almeida et al. (2020).[2] To determine the hydrogenionic potential of the solutions, baseline pH values were measured in a digital pH meter (Quimis, Model Q400 AS Diadema, SP, Brazil). The acidic pH can promote the degradation of the acrylic resin surface and favor biofilm development, leading to the onset of denture stomatitis.[6] Hence, the pH values of the solutions used in this study were measured and are described as follows: TW: 7.7; SH: 11.8; PX: 7.4; and CA: 6.9.

As there is no consensus in the literature regarding the most suitable cycle duration, two immersion protocols were used to simulate up to five years of cleaning.[9,29] A daily regimen (representative of 3.5 months) or a weekly regimen (representative of 2 years) of 10-minute immersion in TW, SH, and CA and 15-minute immersion in PX was considered, according to the instructions for use.[9] A 20-minute immersion time per cycle was also analyzed for all groups.[29] To simulate a 5-year immersion time in this regimen, 1825 cycles were required. The solutions were renewed every 8 hours and kept at room temperature.[9,29]

The study parameters were analyzed at baseline ($t = 0$), and after 104, 913, and 1825 immersion cycles (**Table 2**). Surface roughness was analyzed using a non-contact optical profiler (CCI MP, Taylor Hobson, England) associated with the Talysurf CCI software (Taylor Hobson, England). The parameters used were as follows: 0.25-mm cutoff with a 20x lens, a numerical aperture of 0.4, and 1x scan speed in *xyz* mode. Three measurements with 3 mm in between were performed on each specimen and averaged to obtain the final Sa value (µm).[2,20] The roughness parameter (Sa) is the three-dimensional representation of the height parameter Ra. It is considered an indicator of amplitude and corresponds to the arithmetic mean between peaks and valleys of an entire area, that is, the mean roughness of the analyzed surface.[37,38] Amplitude is considered the most important and used parameter of surface topography.[39]

The parameters were recorded in the L* a* b* color space, as established by the Commission Internationale de L'Eclairage (CIE) in 1978, using a portable spectrophotometer for dental use (Vita Easy Shade, Vita ZahnFabrick, Germany). After calibration, the color of the specimens was measured on a white surface, following the manufacturer's instructions.[20] Three readings were taken for each specimen and averaged. The L* coordinate represents the value; a* represents green on the positive axis and red on the negative axis, and b* represents yellow on the positive axis and blue on the negative axis. The a* and b* coordinates are also indicative of the specimen's chromaticity.[40] The calculation of the CIELab color variation (ΔEab) was performed based on possible color changes according to the following equation: $\Delta Eab = [(\Delta L^*)^2 + (\Delta a^*)^2 + (\Delta b^*)^2]^{½}$.[40] To better reflect the color differences perceived by the human eye, the ΔE00 (CIEDE2000) calculation was performed according to the equation: $[(\Delta L'/K_LS_L)^2 + (\Delta C'/K_CS_C)^2 + (\Delta H'/K_HS_H)^2 + R_T(\Delta C'/K_CS_C)(\Delta H/K_HS_H)]^{½}$.[41]

Color variation was also quantified following the National Bureau of Standards (NBS) guidelines, which determine how the human eye assesses color change. The formula used for this conversion was: NBS unit = ΔEab x 0.92. Mean ΔEab values were converted into NBS units to reveal whether the color variation of the study groups was clinically detectable.[42] The acceptability and perceptibility criteria for ΔEab, ΔE00, and NBS units[42,43] are described in **Table 3**.

The data were analyzed in the SPSS software version 24.0 (SPSS, Chicago, USA). Shapiro-Wilk test was used to check the normality of data distribution and the Levene test was used to determine the homogeneity of variances. The difference between the means was analyzed by two-way analysis of variance (ANOVA) for repeated measures followed by Tukey's posthoc test, considering significance level at 1% ($\alpha = 0.01$).

RESULTS

Surface roughness

The two-way ANOVA for repeated measures showed that only the number of cycles (time factor) significantly affected Sa values, regardless of the cleaning solution. Higher Sa values (mean of peaks and valleys) were observed in all experimental groups after the simulated 5-year immersion ($P < 0.01$) compared to baseline values, with no significant differences between the groups or control (TW) (**Table 4**).

Figure 1 shows the profilometric images of the experimental groups at baseline (t = 0) and the end of the immersion cycles (1825 cycles), demonstrating that the surface topography of the specimens was not significantly altered upon any of the experimental treatments.

Color variation

The data analysis indicated that both the solution factor and the time factor (number of immersion cycles) altered L* and b* color parameters. However, the interaction between the factors did not significantly affect the L* and a* parameters.

After the simulated 5-year immersion protocol, only b* values were significantly changed in PX-treated specimens compared to baseline and 3.5 months. CA-treated specimens showed statistically significant differences in the b* color parameter between 3.5 months and 5 years. The SH group exhibited significant differences in the b* parameter compared to the TW group at t = 0, 3.5 months, and 2.5 years. After the 5-year immersion protocol, TW-treated specimens were statistically different from those of the other groups (**Table 5**).

Our findings indicated that only the time factor significantly altered ΔEab, ΔE00, and NBS values, although there was a significant interaction between the two factors. Both ΔEab and NBS values were altered after 5 years of simulated immersion compared to 3.5 months and 2.5 years, with higher values observed in the PX group. Higher ΔE00 values were also found in the PX group after 5 years of simulated immersion, with significant differences compared to the other time points. Significant differences in all three color parameters were observed between the groups at 5 years of simulated immersion. Yet, CA-treated specimens were not statistically different from the other groups (**Table 6**).

DISCUSSION

In the present study, we confirmed the hypothesis that cinnamaldehyde only causes minor variations in the color and surface roughness of PMMA-based dentures, similar to sodium hypochlorite and alkaline peroxide, after simulated long-term immersion (up to 5 years).

Some mechanisms of action are proposed to explain the changes in PMMA properties after immersion in the cleaning solutions. Exposure to constant absorption and desorption cycles in water and hygiene solutions, as well as the release of residual monomers, can eventually cause irreversible damage to the material due to the formation of microcracks, increased roughness, and alterations in optical properties, interfering with the PMMA color.[2,10,14,17,25,26,28,30,31] In our study, despite the increase in surface roughness compared to baseline values, all treatments produced alterations below the clinically acceptable threshold for biofilm accumulation and bacterial adhesion (0.2μm)[22] and retention of *C. albicans* cells (> 1μm).[7] These findings are consistent with previous reports[2,9,29] showing that cleaning protocols for PMMA produced negligible and clinically irrelevant changes in the surface properties. However, values higher than these may increase surface roughness and improve microbial colonization.[5,9] Thus, low Sa values are directly correlated with reduced microbial adhesion to the polymer surface and a lower likelihood of biofilm formation.

In contrast to our data, other authors[19] observed an increase in the surface roughness of a heat-cured PMMA resin upon simulated 1.5-year immersion in 0.5% NaOCl, when compared to distilled water and alkaline peroxide. These conflicting results can be explained by methodological differences, such as the polymer brand and immersion time, which was 8 h daily (overnight) for 185 days[19]. Hence, the greater exposure of the specimens to the 0.5% NaOCl solution may have been responsible for their increased roughness in that study.

The variation in the surface roughness observed in PX-treated specimens, albeit non-significant, can be explained by the release of oxygen during the chemical reaction of the effervescent tablet with water. While this mechanism allows for mechanical cleaning of the denture, it may negatively increase its surface roughness.[22] Yet, the magnitude of the variation in the PX group was smaller compared to that of the SH group, which is in line with literature findings.[19,29]

As previously mentioned, cinnamaldehyde (27 μg/mL) presented a behavior similar to that of the other tested solutions, but it has additional attributes to be considered, such as cytocompatibility, low cell toxicity as well as antifungal activity, as demonstrated by our research group[2,33]. Thus, cinnamaldehyde seems to be a safe alternative solution for long-term use, similar to others currently available on the market, with good acceptability and cost-effectiveness.

In our study, baseline roughness values were below to the ones often observed clinically in the denture base, that is, 3 μm.[6] Generally, the denture base is never polished, which explains why surface roughness is commonly found to be greater in clinical situations.[18] Our study was primarily focused on examining the damaging effects of the cleaning solutions for comparative purposes. Hence, we reasoned to use the glass plate to standardize the initial surface smoothness of the specimens – which was below the clinical parameters of roughness. Moreover, heat-cured PMMA denture-base resins have a high rate of conversion of monomers into polymers, which reduces the number of residual monomers to be released and ultimately prevents the occurrence of material deformities, such as grooves and cracks.[6] Microorganisms colonizing these areas are less prone to the action of shear forces;[32] therefore, proper polymerization and polishing of PMMA-based dentures are critical to prevent the onset and aggravation of denture stomatitis.

The chemical solutions were further tested for their ability to change the color parameters of PMMA. Significant variations were observed in the b* coordinate in the PX group after the 5-year immersion protocol when compared to t = 0. This parameter reflects the measure of the yellow (b* positive) to bluish (b* negative) hue. Our findings showed that PX-treated specimens had a reduction in b* values, particularly in the yellowish hue.[44] However, changes in color parameters (a* and b*) are less important to the human visual perception than the dimension represented by lightness (L*),[45] which did not show significant variations at any time point.

The ΔEab and NBS color parameters have been extensively used in the scientific literature. Yet, to provide better indicators of human perceptibility and acceptability of color differences, we also analyzed the ΔE00 (CIEDE2000) parameter. The perceptibility and acceptability threshold can only be quantified by combining visual and instrumental measurement methods. The 50:50% perception threshold indicates that color differences between the test specimens can be perceived by 50% of the observers. Likewise, the acceptability threshold indicates that 50% of the observers accept color variations between two objects.[40]

All test solutions promoted color changes after the 5-year immersion protocol. Although there remains no consensus in the literature regarding acceptability and perceptibility threshold values for the ΔEab and ΔE00 parameters of heat-cured acrylic resins, all values found in our study were below the thresholds determined in a previous study (**Table 4**).[43] Most studies consider ΔEab = 3.7 as a reference limit for the acceptability of color variation,[2,20] which is beyond the ΔEab values reported herein. The data showed that the greatest color variations occurred in PX-treated specimens after 5 years of immersion, which is consistent with previous data.[9,19] Similarly, PX-treated specimens only had minor color changes in the NBS scale, a better outcome compared to the other groups, similar to the findings of Arruda et al. (2015).[29] However, these findings disagree with those reported by Almeida et al. (2020)[2], who observed "noticeable" changes in the NBS scale of treated specimens. Such differences can be explained by the study design, considering that Almeida et al. (2020)[2] carried out a blinded randomized crossover clinical trial. This was a more comprehensive analysis that considers the patient's history, dietary and hygiene habits, and alcohol and tobacco consumption.

Only a few studies have addressed color variations in acrylic resins using the ΔE00 formula, which renders inter-study comparisons a difficult task. However, as previously mentioned, ΔE00 values were below the predetermined acceptability and perceptibility thresholds.

Cleaning regimens determining the hydrogenic potential of test solutions are poorly studied. The acidic pH of chemical solutions can influence the surface degradation of PMMA materials by increasing their roughness and favoring biofilm development.[6] Our study demonstrated that TW, PX, and CA solutions had pH values in the neutral range, whereas the SH solution had an alkaline pH, which may have contributed to the negligible changes in Sa values (below the clinically acceptable limit).

Methyl methacrylate (MMA) monomers are more easily leached in acidic environments. This is important to minimize the release of residual monomers in the oral cavity.[46] Among the test solutions, cinnamaldehyde had the lowest pH values, which is an important advantage. Collectively, our findings and those previously published in the literature provide mounting evidence that cinnamaldehyde can be used as a denture cleaning solution with no long-term deleterious effects on PMMA properties. Furthermore, data from a previous study carried out by our research group indicated a preference among users for this solution due to its pleasant taste and ease of use.[2] Lastly, the present study has important limitations to consider, such as the analysis of just one type of acrylic resin and the study design, which does not reflect oral conditions. New studies are suggested to evaluate additional properties, such as the flexural strength and microhardness of PMMA materials in the proposed immersion protocol, as well as the combination of long-term cleaning regimens and their effects on other components used for the manufacture of dentures (*e.g.*, metallic frameworks and artificial teeth).

CONCLUSION

An experimental cinnamaldehyde solution (27 μg/mL) had minor effects on the surface roughness and color parameters of a heat-cure denture base resin, similar to 0.5% sodium hypochlorite and alkaline peroxide, after 5 years of simulated immersion.

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LIST OF TABLES

Table 1. Materials and control/experimental cleaning solutions.

Materials	Brand name	Manufacturer	Batch
Denture base acrylic resin (Powder)	VIPIWAVE	VIPI	0000138668
Denture base acrylic resin (Monomer)	VIPIWAVE	VIPI	0000144347
Laboratory Dental Condensation Silicone	LABOR MASS	VIPI	0000070111
Catalyst for Condensation Silicone	VIPI SIL	VIPI	000357722K

Solution	Brand name	Manufacturer	Batch
Tap water	N/A	-	-
0.5% Sodium hypochlorite	N/A	Dilecta	-
Alkaline peroxide	Polident Overnight Whitening	GSK	PP9T
Cinnamaldehyde (132.16 g/mol)	-	Sigma Aldrich	MKCF6295

N/A: not applicable.

Table 2. Immersion protocols, cycle duration, and daily/weekly regimens.

Group	Number of cycles	Cycle duration	Regimen	Simulated time
TW	104	10 minutes	Daily	3.5 months
			Weekly	2 years
	913	20 minutes	Daily	2.5 years
	1825	20 minutes	Daily	5 years
SH	104	10 minutes	Daily	3.5 months
			Weekly	2 years
	913	20 minutes	Daily	2.5 years
	1825	20 minutes	Daily	5 years
PX	104	15 minutes	Daily	3.5 months
			Weekly	2 years
	913	20 minutes	Daily	2.5 years
	1825	20 minutes	Daily	5 years
CA	104	10 minutes	Daily	3.5 months
			Weekly	2 years
	913	20 minutes	Daily	2.5 years
	1825	20 minutes	Daily	5 years

*10-minute daily or weekly immersion for the TW, SH, and CA groups, and 15-minute immersion for the PX group, according to Kiesow et al. (2016). [8]

**20-minute daily immersion for all groups, according to Arruda et al. (2015).[20]

Table 3. Perceptibility and acceptability thresholds for the ΔEab and ΔE00 parameters of heat-cured acrylic resins, and the NBS scale.

		CIELAB (ΔEab)	CIEDE2000 (ΔE00)
Ren et al. (2015)[32]	**Perceptibility**	2.1	1.7
	Acceptability	4.6	4.1

	Critical comments on color differences	**NBS units**
Hong et al. (2009)[31]	**Trace**	0.0 – 0.5
	Slight	0.5 – 1.5
	Noticeable	1.5 – 3.0
	Appreciable	3.0 – 6.0
	Much	6.0 – 12.0
	Very much	>12.0

Table 4. Mean Sa roughness (µm) and standard deviation of specimens treated with different cleaning solutions, and the number of immersion cycles.

	Solutions	Number of immersion cycles			
		Baseline	105 cycles* (3.5 months)	913 cycles** (2.5 years)	1825 cycles** (5 years)
Sa	TW	0.027 (±0.011)^{a} A	0.028 (±0.008)^{a} AB	0.034 (±0.005)^{a} C	0.034 (±0.005) ^{a} BC
	SH	0.023 (±0.007)^{a} A	0.029 (±0.009)^{a} B	0.030 (±0.005)^{a} B	0.035 (±0.004)^{a} B
	PX	0.026 (±0.011)^{a} A	0.033 (±0.006)^{a} B	0.035 (±0.004)^{a} B	0.036 (±0.004)^{a} B
	CA	0.028 (±0.009)^{a} A	0.029 (±0.004)^{a} AB	0.034 (±0.004)^{a} AB	0.034 (±0.004)^{a} B

Different uppercase letters in the same line indicate statistically significant differences between the time points within the same group ($P < 0.01$, Two-way ANOVA followed by Tukey's posthoc test).

Different lowercase letters in the same column indicate statistically significant differences between the groups within the same time point ($P < 0.01$, Two-way ANOVA followed by Tukey's posthoc test).

*10-minute daily or weekly immersion for the TW, SH, and CA groups, and 15-minute immersion for the PX group, according to Kiesow et al. (2016). [8]

**20-minute daily immersion for all groups, according to Arruda et al. (2015).[20]

Table 5. Mean (± standard deviation) L*, a* and b* values of specimens treated with different cleaning solutions, and the number of immersion cycles.

	Solution	Number of immersion cycles			
		Baseline	**105 cycles* (3.5 months)**	**913 cycles** (2.5 years)**	**1825 cycles** (5 years)**
L	TW	54.94 (±0.92)^{a} A	55.02 (±0.90)^{a} A	55.22 (±0.85)^{a} A	55.30 (±0.92)^{a} A
	SH	55.29 (±0.65)^{a} A	55.41 (±0.67)^{a} A	55.63 (±0.70)^{a} A	55.79 (±0.76)^{a} A
	PX	55.28 (±0.57)^{a} A	55.35 (±0.55)^{a} A	55.67 (±0.57)^{a} A	55.81 (±0.58)^{a} A
	CA	55.20 (±0.70)^{a} A	55.19 (±0.71)^{a} A	55.43 (±0.66)^{a} A	55.49 (±0.75)^{a} A
a	TW	25.08 (±1.32)^{a} A	24.99 (±1.14)^{a} A	25.02 (±1.11)^{a} A	24.68 (±1.23)^{a} A
	SH	24.61 (±0.87)^{a} A	24.67 (±0.93)^{a} A	24.51 (±0.92)^{a} A	24.27 (±0.99)^{a} A
	PX	25.15 (±0.82)^{a} A	25.09 (±0.82)^{a} A	24.94 (±0.78)^{a} A	24.62 (±0.77)^{a} A
	CA	24.91 (±0.74)^{a} A	24.97 (±0.90)^{a} A	24.85 (±0.82)^{a} A	24.40 (±0.90)^{a} A
b	TW	25.87 (±1.62)^{a} A	25.67 (±1.42)^{a} A	25.70 (±1.46)^{a} A	25.17 (±1.58)^{a} A
	SH	24.74 (±0.71)^{b} A	24.82 (±0.67)^{b} A	24.59 (±0.71)^{b} A	24.00 (±0.68)^{b} A
	PX	25.20 (±0.75)^{ab} A	25.11 (±0.80)^{ab} A	24.94 (±0.72)^{ab} AB	24.26 (±0.73)^{b} B
	CA	25.07 (±0.90)^{ab} AB	25.16 (±0.97)^{ab} A	25.00 (±0.83)^{ab} AB	24.30 (±0.93)^{b} B

Different uppercase letters in the same line indicate statistically significant differences between the time points within the same group ($P < 0.01$, Two-way ANOVA followed by Tukey's posthoc test).

Different lowercase letters in the same column indicate statistically significant differences between the groups within the same time point ($P < 0.01$, Two-way ANOVA followed by Tukey's posthoc test).

*10-minute daily or weekly immersion for the TW, SH, and CA groups, and 15-minute immersion for the PX group, according to Kiesow et al. (2016). [8]

**20-minute daily immersion for all groups, according to Arruda et al. (2015).[20]

Table 6. Mean (± standard deviation) color variation (ΔEab, ΔE00, and NBS) of specimens treated with different cleaning solutions.

	Solutions	Number of immersion cycles		
		105 cycles* (3.5 months)	913 cycles** (2.5 years)	1825 cycles** (5 years)
ΔEab	TW	0.45 (±0.34)[a] A	0.51 (±0.29)[a] A	0.93 (±0.45)[a] B
	SH	0.36 (±0.13)[a] A	0.47 (±0.20)[a] A	1.01 (±0.32)[a] B
	PX	0.30 (±0.13)[a] A	0.54 (±0.28)[a] A	1.32 (±0.44)[b] B
	CA	0.52 (±0.58)[a] A	0.64 (±0.58)[a] A	1.24 (±0.41)[ab] B
ΔE00	TW	0.30 (±0.20)[a] A	0.34 (±0.21)[a] AB	0.52 (±0.22)[a] B
	SH	0.25 (±0.10)[a] A	0.35 (±0.15)[a] A	0.62 (±0.19)[ab] B
	PX	0.19 (±0.08)[a] A	0.40 (±0.18)[a] B	0.77 (±0.31)[b] C
	CA	0.33 (±0.31)[a] A	0.43 (±0.34)[a] A	0.65 (±0.28)[ab] B
NBS	TW	0.41 (±0.31)[a] A	0.47 (±0.26)[a] A	0.86 (±0.41)[a] B
	SH	0.33 (±0.12)[a] A	0.43 (±0.18)[a] A	0.93 (±0.29)[ab] B
	PX	0.27 (±0.12)[a] A	0.50 (±0.26)[a] A	1.21 (±0.41)[b] B
	CA	0.48 (±0.54)[a] A	0.59 (±0.53)[a] A	1.14 (±0.38)[ab] B

Different uppercase letters in the same line indicate statistically significant differences between the time points within the same group ($P < 0.01$, Two-way ANOVA followed by Tukey's posthoc test).

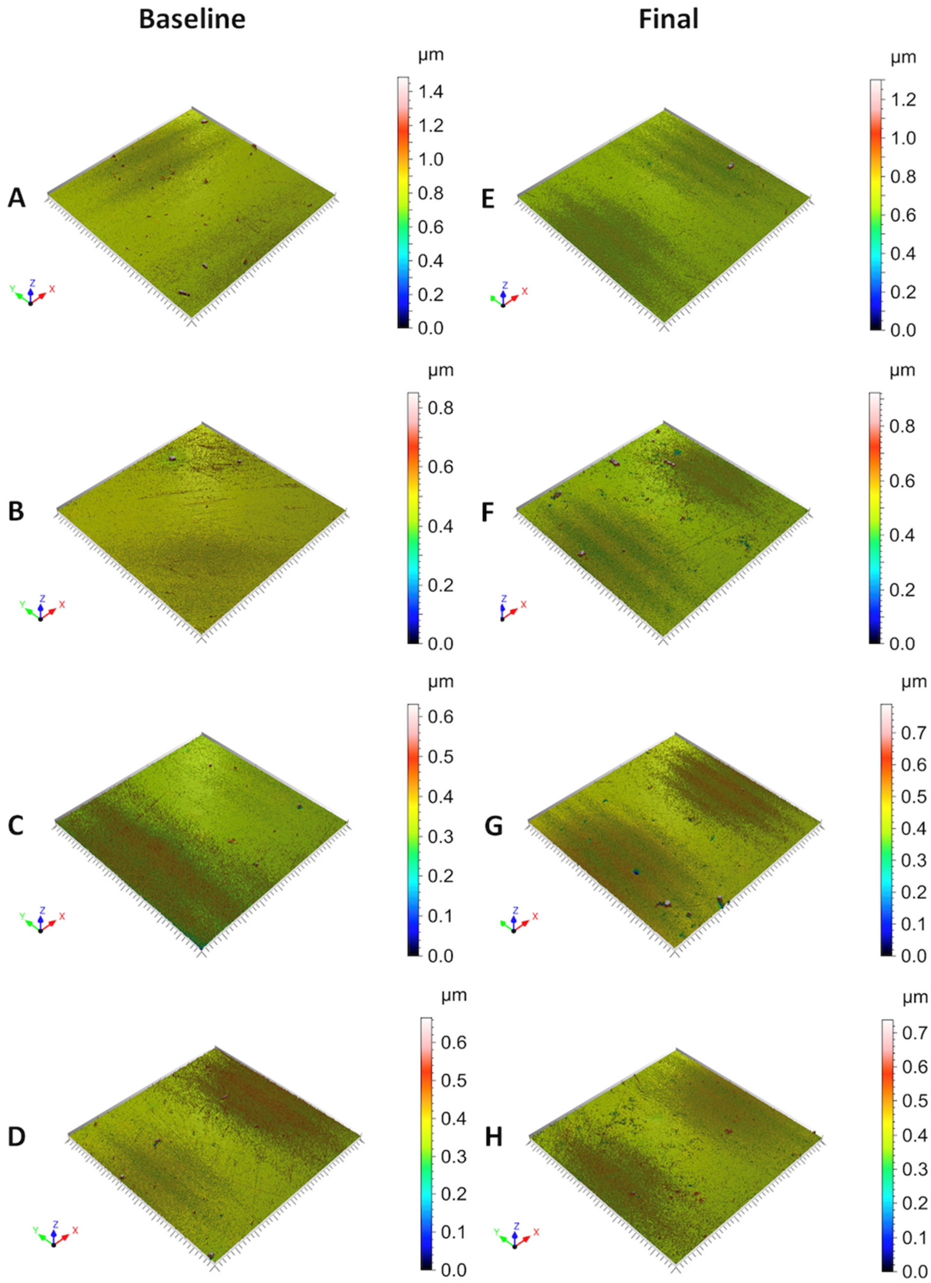
Different lowercase letters in the same column indicate statistically significant differences between the groups within the same time point ($P < 0.01$, Two-way ANOVA followed by Tukey's posthoc test).

*10-minute daily or weekly immersion for the TW, SH, and CA groups, and 15-minute immersion for the PX group, according to Kiesow et al. (2016). [8]

**20-minute daily immersion for all groups, according to Arruda et al. (2015).[20]

FIGURES

Fig 1. Profilometry surface topographies of specimens at baseline (t = 0, left column) and at the end of the immersion protocol (t = 1825 cycles, right column). Groups: Tap water (TW) - (A and E); 0.5% Sodium hypochlorite (SW) - (B and F); Alkaline peroxide (PX) – (C and G); and Cinnamaldehyde (CA) – (D and H).



3. CONSIDERAÇÕES GERAIS

Com o passar dos anos e o constante desenvolvimento biotecnológico mundial, diversos produtos de origem vegetal, mais precisamente os óleos essenciais, vêm sido alvo de diversos estudos devido as suas potenciais ações farmacológicas já demonstradas cientificamente, se colocando dessa forma como uma alternativa frente à resistência microbiana às diversas drogas convencionais já existentes (12,21,35–37). Um desses produtos de origem vegetal, o óleo essencial da canela, apresenta em sua composição altas concentrações de cinamaldeído, fitoconstituinte que demonstrou em diversos estudos elevado potencial antimicrobiano, baixa toxicidade, biocompatibilidade com os tecidos orais em baixas concentrações e odor agradável (12,21,44).

Recentemente seu uso passou a ser analisado como alternativa para higienização química de próteses orais no combate ao desenvolvimento da estomatite protética (12,21). Além da viabilidade para usuários com desordens motoras e visuais, esse método químico proporciona o acesso a áreas inacessíveis da prótese, permitindo a ação antimicrobiana das soluções, tendo em vista que, como demonstrado em estudos anteriores, as hifas da *Candida albicans* podem penetrar a uma profundidade de até 631 μm dentro do interior da resina acrílica de base de prótese, o que poderia ser uma das razões da frequente recidiva da EP nos usuários de prótese (3,21,28,29,33,45). Entretanto, a rugosidade superficial e alterações de cor também são essenciais para o sucesso clínico de novos agentes de limpeza no longo prazo.

O hipoclorito de sódio (NaOCl) e os peróxidos alcalinos compõem as soluções de limpeza de próteses mais comumente utilizadas para tal finalidade (30,34), porém, o poder corrosivo do hipoclorito de sódio, bem como o elevado custo dos peróxidos alcalinos, causam, respectivamente, prejuízos aos componentes protéticos e dificultam o acesso para grande parcela da população (19,46).

Os resultados do presente estudo demonstraram que a solução de cinamaldeído apresentou alterações de rugosidade superficial e cor no PMMA semelhante a outras substâncias já destacadas no mercado, sem causar alterações clinicamente relevantes nessas características no longo prazo, apresentando-se como uma promissora solução química para limpeza de próteses. Outras vantagens já demonstradas pelo cinamaldeído em estudos anteriores, compreendem a presença de muitas das características consideradas ideais para uma solução química de limpeza de próteses (10). Sua facilidade de uso e aceitável custo-benefício, aliado aos resultados encontrados no estudo atual, a colocam como uma excelente candidata ao posto de solução ideal.

Outro ponto interessante a ser colocado diz respeito a questão do protocolo de limpeza das soluções. Apesar das diferentes recomendações propostas em relação ao período de tempo de imersão por diversos autores e preconizadas por grande parcela dos cirurgiões-dentistas (3,10,34), o cinamaldeído apresentou-se de forma semelhante as demais soluções já preconizadas mesmo com períodos de imersão distintos.

Os resultados são promissores, entretanto o presente estudo apresenta algumas limitações, como a análise em apenas um tipo de resina acrílica e ausência de condições bucais. Novos estudos são sugeridos com o intuito de avaliar propriedades adicionais, tais como a resistência à flexão e microdureza do PMMA frente ao protocolo de imersão proposto, bem como a combinação de regimes de higienização a longo prazo e seus efeitos em outros componentes utilizados para confecção de próteses.

4. CONCLUSÃO

Dentro das limitações deste estudo, é possível concluir que a solução de cinamaldeído 27 μg/mL mostra-se como uma solução inovadora capaz de promover a limpeza de próteses semelhante a outros produtos de limpeza já utilizados internacionalmente sem causar alterações relevantes nas propriedades superficiais de rugosidade e de cor, em um período simulado de imersão de até 5 anos.

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