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GRAZIELE CRISTINA ALVIM DA SILVA

ANÁLISE DAS PROPRIEDADES BIOLÓGICAS E MECÂNICAS DE UM ADESIVO PARA PRÓTESE DENTÁRIA MODIFICADO COM VANADATO DE PRATA NANOESTRUTURADO DECORADO COM NANOPARTÍCULAS DE PRATA

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Orientadora: Profa. Dra. Denise Tornavoi de Castro

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RESUMO

Este estudo *in vitro* explorou as propriedades biológicas e mecânicas de um adesivo protético incorporado com diferentes porcentagens de vanadato de prata nanoestruturado decorado com nanopartículas de prata ($AgVO_3$). Foi verificada a concentração inibitória mínima (CIM) do $AgVO_3$ frente à *Candida albicans*, *Candida glabrata* e *Streptococcus mutans*. A seguir, espécimes em resina acrílica a base de polimetilmetacrilato (PMMA) foram confeccionados e divididos em grupos: Grupo controle – PMMA; PMMA + Adesivo Ultra Corega Creme; PMMA + Adesivo Ultra Corega Creme + 2,5% $AgVO_3$; PMMA + Adesivo Ultra Corega Creme + 5% $AgVO_3$ e PMMA + Adesivo Ultra Corega Creme + 10% $AgVO_3$. Sobre os espécimes foram cultivados biofilmes e o número de células viáveis dos micro-organismos foi determinado pela contagem de unidades formadoras de colônias por mililitro (UFC/mL) (n=9). A microscopia de fluorescência foi utilizada para complementar a análise (n=2). A viabilidade da linhagem celular VERO, exposta ao adesivo com diferentes concentrações de $AgVO_3$, foi avaliada através do ensaio de resazurina (n=5). A força adesiva foi testada após 5 minutos, 3 horas, 6 horas, 12 horas e 24 horas da aplicação do adesivo em espécimes de PMMA (n=10). Para a análise microbiológica foi utilizado o Teste de Kruskal-Wallis, seguido pelo pós-teste de Dunn. Os dados da força adesiva e da biocompatibilidade foram submetidos à Análise de variância (ANOVA), seguido pelo pós teste de Bonferroni (α=0,05). A CIM do $AgVO_3$ frente ao *S.mutans* foi de 250 μg/mL, e frente à *C. glabrata* e C. *albicans* de 62,5 μg/mL. A formação de biofilme nos grupos PMMA e Adesivo Ultra Corega Creme foi semelhante ($P>0,05$). Todas as concentrações do $AgVO_3$ incorporadas ao adesivo reduziram a formação de biofilme das três espécies selecionadas ($P<0,05$), o que foi comprovado na microscopia de fluorescência pela redução de células viáveis (verde). As formulações do adesivo com 2,5% e 5% de $AgVO_3$ não apresentaram efeito citotóxico sobre as células VERO ($P>0,05$). Em 5 minutos, o Ultra Corega Creme com 5% de $AgVO_3$ apresentou maior força adesiva do que o Ultra Corega Creme ($P=0,020$), e em 24 horas, o Ultra Corega Creme com 10% apresentou melhor desempenho ($P=0,026$). A incorporação de $AgVO_3$ promoveu atividade antibiofilme ao adesivo protético, com efeito positivo na força adesiva, sendo biocompatível com células VERO.

Palavras-chave: Adesão; Adesivos para prótese; Biofilme; Nanotecnologia; Prótese dentária; Teste de biocompatibilidade.

ABSTRACT

This in vitro study explored the biological and mechanical properties of a prosthetic adhesive incorporated with different percentages of nanostructured silver vanadate decorated with silver nanoparticles ($AgVO_3$). The minimum inhibitory concentration (MIC) of $AgVO_3$ against *Candida albicans*, *Candida glabrata* and *Streptococcus mutans* was verified. Next, polymethylmethacrylate (PMMA)-based acrylic resin specimens were made and divided into groups: Control group - PMMA, PMMA + Ultra Corega Cream Adhesive; PMMA + Ultra Corega Cream Adhesive + 2.5% $AgVO_3$; PMMA + Ultra Corega Cream Adhesive + 5% $AgVO_3$ and PMMA + Ultra Corega Cream Adhesive + 10% $AgVO_3$. Biofilms were cultivated on the specimens and the number of viable cells of microorganisms was determined by counting colony forming units per milliliter (CFU/mL) (n=9). Fluorescence microscopy was used to complement the analysis (n=2). The viability of the VERO cell line, exposed to the adhesive with different concentrations of $AgVO_3$, was evaluated using the resazurin assay (n=5). Adhesive strength was tested after 5 minutes, 3 hours, 6 hours, 12 hours and 24 hours of adhesive application on PMMA specimens (n=10). For the microbiological analysis, the Kruskal-Wallis test was used, followed by the Dunn post-test. The adhesive strength and biocompatibility data were submitted to analysis of variance (ANOVA), followed by the Bonferroni post test (α=0.05). The MIC of $AgVO_3$ against *S. mutans* was 250 μg/mL, and against *C. glabrata* and *C. albicans*, 62.5 μg/mL. Biofilm formation in the PMMA and Ultra Corega Cream Adhesive groups was similar ($P>0.05$). All concentrations of $AgVO_3$ incorporated into the adhesive reduced the biofilm formation of the three selected species ($P<0.05$), which was confirmed in fluorescence microscopy by the reduction of viable cells (green). The adhesives formulations with 2.5% and 5% $AgVO_3$ showed no cytotoxic effect on VERO cells ($P>0.05$). In 5 minutes, Ultra Corega Cream with 5% $AgVO_3$ showed greater adhesive strength than Ultra Corega Cream ($P=0.020$), and in 24 hours, Ultra Corega Cream with 10% showed better performance ($P=0.026$). The incorporation of $AgVO_3$ promoted antibiofilm activity to the prosthetic adhesive, with positive effect on the adhesive strength, being biocompatible with VERO cells.

Keywords: Adesion; Adhesives for prostheses; Biofilm; Biocompatibility test; Dental prosthesis; Nanotechnology.

SUMÁRIO

1. INTRODUÇÃO

Segundo a Organização das Nações Unidas (ONU), mundialmente, 700 milhões de pessoas possuem 60 anos ou mais, o que é projetado para dois bilhões em 2050. Com uma população supra envelhecida, inevitavelmente haverá maiores desafios para a prestação de cuidados voltados à saúde bucal (ONU 2014; CARDOSO *et al*., 2016; BO *et al.,* 2020).

A saúde geral dos idosos que possuem dentição natural é significativamente melhor em comparação com aqueles desdentados. Apesar das inúmeras campanhas de prevenção, ainda é grande a quantidade de pessoas com perda dentária (FELTON *et al.,* 2011; BAKKER *et al.,* 2021). Portanto, os profissionais da Odontologia devem estar capacitados a reabilitar um número crescente de idosos que não possuem os dentes naturais na cavidade bucal. Além disso, as políticas públicas de saúde devem considerar as necessidades relacionadas aos cuidados odontológicos voltados a essa população (DOUGLASS; SHIH; OSTRY, 2002; HATANO *et al*., 2021; SALIH; ALI; NASIR 2022).

Com o avanço da tecnologia e a evolução do conceito de osseointegração, a implantodontia proporcionou para as reabilitações orais uma modalidade de tratamento com alta taxa de sucesso e melhor manutenção do osso no local edêntulo (GUPTA; GUPTA; WEBER, 2022; MOLINERO-MOURELLE *et al*., 2022). Apesar dos benefícios ofertados por essa especialidade, ainda existem limitações por parte dos pacientes, sejam relacionadas à saúde sistêmica, ao custo ou a preferências pessoais (NOGUEIRA *et al.,* 2021; GHIASI *et al.,* 2022) que fazem com que as próteses totais removíveis convencionais ainda representem a principal opção de tratamento para pacientes edêntulos (MOYNIHAN; VARGHESE, 2022). Porém, muitas vezes essas próteses não satisfazem totalmente os usuários devido a falta de conforto e de estabilidade (SOBOLEVA; ROGOVSKA, 2022).

O sucesso de uma prótese total convencional depende de vários fatores, e não apenas da experiência prévia do profissional que está confeccionando. Fatores como a experiência anterior do paciente com o uso de próteses, o tipo de rebordo e os aspectos psicológicos podem influenciar no resultado final (OWEIS *et al*., 2022). Com o envelhecimento, muitas vezes é difícil manter as próteses confortavelmente na boca devido a fatores sistêmicos e orais, como múltiplas doenças sistêmicas, distúrbios do movimento oral, xerostomia causada pelos efeitos colaterais dos medicamentos, reabsorção do rebordo e alterações mandibulares desencadeadas (BO *et al.,* 2020; NOMURA *et al.,* 2020).

Nos últimos anos, o *American College of Prosthodontists* e a *American Dental Association* expressaram consenso sobre o uso dos adesivos para próteses. Por muito tempo cirurgiões-dentistas evitaram a indicação desses materiais aos seus pacientes, correlacionando sua utilização a falhas técnicas e ao aparecimento de lesões como hiperplasia dos tecidos moles e reabsorção do rebordo alveolar (ELLIS; PELEKIS; THOMASON, 2007; EMAMI *et al.*, 2009; FELTON *et al.*, 2011). Porém, como já foi dito, sabe-se atualmente que mesmo profissionais experientes muitas vezes não conseguem satisfazer as expectativas dos pacientes em relação a retenção e estabilidade por limitações inerentes ao caso clínico (OWEIS *et al.*, 2022).

Diante desse cenário, os adesivos protéticos são reconhecidos como agentes auxiliares na retenção e na estabilidade das próteses aumentando o desempenho mastigatório e melhorando a qualidade de vida dos portadores de próteses totais (NOMURA *et al.*, 2019; FLORÊNCIO COSTA *et al.*, 2020; ITO *et al.*, 2021). Em relação a forma comercial, são classificados como pó, creme ou fita e atuam na tensão interfacial entre as bases de próteses e os tecidos moles. Quando bem indicados também podem ser usados para estabilizar bases de prótese durante o registro das relações maxilo mandibulares, aumentar o conforto e a função de próteses imediatas, além de servir como via de entrega de fármacos (ADISMAN, 1989; KORE *et al.*, 2013; FIGUEREDO *et al.*, 2021; LEMOS *et al.*, 2022).

Em contrapartida, os adesivos são difíceis de serem removidos da mucosa oral e da superfície da prótese após o uso. Uma base de prótese porosa e rugosa de polimetilmetacrilato (PMMA) associada a resíduos de adesivos protéticos pode servir como um substrato favorável para adesão microbiana e formação de biofilme (DE OLIVEIRA JUNIOR *et al.*, 2018; NAMANGKALAKUL *et al.*, 2020; COSTA *et al*., 2022).

As espécies de *Candida* fazem parte da flora comum da cavidade bucal, no entanto, podem causar infecções oportunistas leves em pacientes saudáveis e afetar sistemicamente pacientes imunocomprometidos, causando risco de perder a vida. A incidência de candidíase aumentou recentemente, em parte devido ao número crescente de pacientes imunocomprometidos e ao envelhecimento da população (SARDI *et al.*, 2013).

Atualmente, as diretrizes de tratamento para candidíase orofaríngea recomendam clotrimazol tópico , miconazol ou nistatina para doença leve e fluconazol oral para lesões moderadas a graves (NAMANGKALAKUL *et al.*, 2020). Além disso, é necessária a realização de uma higiene oral adequada e a desinfecção ou substituição das próteses infectadas (PAPPAS *et*

al., 2016). No entanto, esta abordagem apresenta limitações relacionadas ao comprometimento motor dos pacientes e aos aspectos financeiros, além disso os antifúngicos podem ter uma ação terapêutica dificultada não agindo efetivamente sobre a superfície interna das próteses dentárias devido a formação do biofilme, presença do fluxo salivar e da língua e movimentos de deglutição (BUENO *et al.*, 2015; ALMEIDA *et al.,* 2018). Dessa forma, manter a concentração eficaz da droga topicamente se torna um desafio, por isso, o uso de materiais adesivos biocompatíveis associados a compostos antimicrobianos pode ser muito benéfico para evitar problemas locais, e até sistêmicos.

Com o advento da nanotecnologia, houve uma revolução na área do desenvolvimento de novos materiais antimicrobianos possibilitando a formulação de diversos produtos (WADY *et al.,* 2012). Isso foi possível devido as características intrínsecas dos próprios materiais nanométricos, que por possuírem um tamanho reduzido entre 1 a 100 nanômetros, apresentam uma interação mais efetiva com as membranas microbianas devido a sua maior proporção superfície-volume, quando comparados com materiais não nanométricos (BORZABADI-FARAHANI; BORZABADI; LYNCH, 2014).

Holtz *et al*., 2010 desenvolveram o vanadato de prata decorado com nanopartículas de prata ($AgVO_3$). Esse material atua como suporte para nanopartículas de prata (AgNPs), que em contato com as moléculas desencadeia um distúrbio nas paredes celulares, capaz de inibir o crescimento das bactérias. As AgNPs podem ancorar e penetrar nas membranas celulares e liberar íons Ag+ por meio da dissociação oxidativa, apresentando atividade antimicrobiana. O vanádio, quando em seu estado oxidativo V^{5+}, pode se ligar a grupos tiol de proteínas celulares e formar complexos estáveis. Quando ocorrem oxidação e redução entre V^{4+} e V^{5+} as bactérias sofrem um estresse oxidativo, responsável pela atividade antimicrobiana do composto. Portanto, o $AgVO_3$ trata-se de um composto híbrido capaz de interagir fortemente com as paredes celulares bacterianas interferindo no metabolismo celular (HOLTZ *et al.,* 2010; HOLTZ *et al.,* 2012; DE CAMPOS.; BOTELHO; DOS REIS., 2021).

O $AgVO_3$ é um composto recente e inovador, que apresenta potencial para ser utilizado em diferentes materiais odontológicos como por exemplo em vitrocerâmicas (BAPTISTA *et al.,* 2022), materiais de moldagem (CASTRO *et al.,* 2019) cimentos endodônticos (TEIXEIRA *et al.,* 2021), cimentos resinosos (KREVE *et al.,* 2022) e até como tratamento de superfícies de implantes (OLISCOVICZ *et al.,* 2018) com o intuito de reduzir a probabilidade de infecções devido ao

acúmulo de biofilme. O uso do $AgVO_3$ associado a adesivos para prótese dentária representa uma ideia inovadora, ainda não explorada, que pode proporcionar benefícios aos pacientes.

O objetivo desta pesquisa foi investigar o efeito da incorporação do $AgVO_3$ nas propriedades biológicas e mecânicas de um adesivo protético comercialmente disponível. A hipótese nula testada foi a de que a incorporação de $AgVO_3$ não influenciaria nas propriedades deste material.

2. OBJETIVO

2.1 OBJETIVO GERAL

Avaliar a influência da incorporação de diferentes porcentagens do vanadato de prata nanoestruturado decorado com nanopartículas de prata ($AgVO_3$) nas propriedades biológicas e mecânicas de um adesivo protético.

2.2 OBJETIVOS ESPECÍFICOS

- Sintetizar e caracterizar o $AgVO_3$;
- Determinar a concentração inibitória mínima do $AgVO_3$ frente ao *Streptococcus mutans*, *Candida albicans* e *Candida glabrata*;
- Incorporar diferentes concentrações do $AgVO_3$ em um adesivo protético comercialmente disponível;
- Avaliar a atividade antibiofilme do adesivo protético modificado com $AgVO_3$ frente ao *Streptococcus mutans*, *Candida albicans* e *Candida glabrata* através da contagem de unidades formadoras de colônias (UFC/mL) e da microscopia de fluorescência;
- Avaliar a biocompatibilidade de um adesivo protético modificado com $AgVO_3$ frente a célula VERO pelo ensaio de resazurina;
- Avaliar a força adesiva do adesivo protético modificado com $AgVO_3$ após 5 minutos, 3 horas, 6 horas, 12 horas e 24 horas da aplicação na superfície do PMMA

3. CAPÍTULO 1

Evaluation of the antibiofilm effect, biocompatibility and adhesive strength of an adhesive for dental prosthesis modified with nanomaterial

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ABSTRACT

Statement of Problem. Prosthetic adhesives are difficult to remove from the oral mucosa after use and residue on the surface of dentures can become complementary substrates for the accumulation of microorganisms.

Purpose. This in vitro study explored the biological and mechanical properties of a prosthetic adhesive incorporated with nanostructured silver vanadate decorated with silver nanoparticles ($AgVO_3$).

Material and Methods. The minimum inhibitory concentration (MIC) of $AgVO_3$ against *Candida albicans, Candida glabrata* and *Streptococcus mutans* was verified. Next, specimens in acrylic resin (PMMA) were divided into groups: Control group – PMMA; PMMA + Ultra Corega Cream Adhesive (UCCA); PMMA + UCCA + 2.5% $AgVO_3$; PMMA + UCCA + 5% $AgVO_3$ and PMMA + UCCA + 10% $AgVO_3$. Biofilm of *C. albicans, C. glabrata* and *S.mutans* were grown on the specimens. After maturation of the biofilm, the number of viable cells of microorganisms was determined by counting colony forming units. Fluorescence microscopy was used to complement the analysis, evaluating the distribution of the biofilm on the specimens. The viability of VERO cell line, exposed to the adhesive with different concentrations of $AgVO_3$, was evaluated by the resazurin assay. Adhesive strength was tested up to 24 hours of adhesive application on PMMA specimens.

Results. The MIC of $AgVO_3$ against *S. mutans* was 250 μg/mL, and against *C. glabrata* and *C. albicans* was 62.5 μg/mL. Biofilm formation in PMMA and UCCA groups was similar. All concentrations of $AgVO_3$ incorporated into the adhesive reduced the biofilm formation of the selected species. Adhesive formulations with $AgVO_3$ showed no cytotoxic effect on VERO cells.

In 5 minutes, UCCA with 5% of $AgVO_3$ showed greater adhesive strength than UCCA, and in 24 hours, UCCA with 10% presented better performance.

Conclusions. The incorporation of $AgVO_3$ promoted antibiofilm activity to the prosthetic adhesive, with positive effect on the adhesive strength, being biocompatible with VERO cells.

CLINICAL IMPLICATION

The combination of prosthetic adhesives and $AgVO_3$ may provide clinical benefit by allowing an antimicrobial agent to remain close to the inner surface of the contaminated denture base and the oral mucosa, enabling the prevention and/or therapy of local and systemic diseases.

3.1 INTRODUCTION

Conventional removable complete dentures still represent the main treatment option for edentulous patients. However, as aging progresses, it is often difficult to keep them comfortably in the mouth, due to systemic and oral factors such as multiple systemic diseases, oral movement disorders, dry mouth caused by medication side effects, ridge resorption and mandibular changes triggered by aging.[1,2]

In recent years, the American College of Prosthodontists and the American Dental Association have expressed consensus on the use of denture adhesives.[3,4] These materials are classified according to commercial form in powder, cream and strip, and can assist in the retention and stability of dentures, increasing masticatory performance and improving the quality of life of users of removable prosthesis.[5,6]

In contrast, the adhesives are difficult to remove from the oral mucosa after use. In addition, residues on the surface of dentures can become complementary substrates for the accumulation of

microorganisms.[7,8] Candida species are part of the common flora of the oral cavity, but can cause mild infections in healthy people and severe infections in people with compromised immunity. The incidence of candidiasis is increasing mainly due to an aging population and consequently a higher number of patients with compromised immunity.[9-11] A polymethylmethacrylate (PMMA) prosthesis whose internal surface is rough and porous, associated with prosthetic adhesive residues can serve as a good substrate for the accumulation of microorganisms and consequently for biofilm formation.[12]

The treatment of oropharyngeal candidiasis includes the use of antifungals such as miconazole and nystatin for mild cases, and oral fluconazole for moderate to severe cases, associated with proper oral hygiene and replacement of the infected denture.[11,12] However, this approach has limitations, since the presence of oral fluids as well as tongue movement and swallowing can minimize the action of antifungals on the inner surface of dentures.[13,14] Thus, maintaining the effective concentration of the drug topically becomes a challenge, so the use of biocompatible denture adhesive associated with antimicrobial agent can be very beneficial to avoid local and even systemic problems.

Holtz et al[15] developed silver vanadate decorated with silver nanoparticles ($AgVO_3$). This is a hybrid material, in which silver vanadate nanowires support silver nanoparticles (AgNPs) that release Ag+ ions inhibiting the growth of bacteria by triggering a disruption in the cell walls. Associated with this, vanadium in the oxidative state V5+ binds to cellular proteins and may lead the bacteria to oxidative stress. Therefore, $AgVO_3$ is a compound able to interfere with bacterial cellular metabolism.[16,17]

$AgVO_3$ is a recent and innovative compound, capable of being used in different dental materials such as acrylic resins,[18] glass ceramics,[19,20] molding materials,[21] endodontic cements,[22]

resin cements,[23] and even as a treatment of implant surfaces[24] in order to reduce the infections due to biofilm. Despite this, its use in denture adhesives has not yet been investigated. The objective of this study was to investigate the effect of $AgVO_3$ incorporation on the biological and mechanical properties of a prosthetic adhesive. The null hypothesis tested was that the incorporation of $AgVO_3$ would not influence the biological and mechanical properties of the studied material.

3.2 MATERIALS AND METHODS

The factor under study was the concentration of nanostructured silver vanadate decorated with silver nanoparticles ($AgVO_3$) incorporated into a prosthetic adhesive (0%, 2.5%, 5% and 10%). For microbiological analyses, the quantitative response variable was the amount of biofilm on the surface of the specimens, evaluated by colony forming units count (in $\log_{10}$ CFU/mL) and the qualitative response variable was the distribution of the biofilm on the specimens, evaluated by fluorescence microscopy. For the analysis of biocompatibility, the quantitative response variable was cell viability (%). For the mechanical analysis, the quantitative response variable was the adhesive force (N), at 5 minutes, 3 hours, 6 hours, 12 hours and 24 hours. Figure 1 shows the study flowchart (Fig. 1).

The nanomaterial was obtained by a reaction between silver nitrate ($AgNO_3$; Merck 99.8%) and ammonium vanadate (NH_4VO_3; Merck 99%), and characterized by transmission electron microscopy (STEM) according to the methodology described in the literature.[15,16,21,25]

The values of the minimum inhibitory concentration (MIC) of $AgVO_3$ against *C.albicans* (ATCC 10231), *C.glabrata* (ATCC 2001) and *S.mutans* (ATCC 25175) were determined based on successive dilutions described by the Clinical and Laboratory Standards Institute (CLSI) (Clinical

and Laboratorial Standards Institute, 2003) in cell culture plates (TPP tissue culture; TPP) containing 96 wells according to the study of Castro et al 2014.[26]

A heat-cured polymethyl methacrylate acrylic resin (Classical; Classical Dental Articles) was used. Initially, the inclusion of dies in metallic flasks (OGP; Dental Products) was performed to prepare the molds where the acrylic resin was inserted during the plastic phase. The metallic flasks were positioned in hydraulic presses (Hydraulic presses Protecni; Protécni Equipment) with a load of 1000 Kgf for 60 minutes. The samples were polymerized by conventional heating, according to the manufacturer's instructions (immersion in water at 73°C for 90 minutes and boiling for 30 minutes),[27] in an electric thermocycler (Thermocycler T100). After disinclusion, the specimens were finished and stored in distilled water for 24 hours at 37ºC to eliminate residual monomers.[8]

The specimens prepared for microbiological analysis and for the analysis of biocompatibility measured 6 mm wide x 10 mm long and 3 mm thick, and for the analysis of adhesive strength they measured 25 mm in diameter x 35 mm in height.[8]

The surface roughness of the specimens was standardized (3.0 µm ±0.3), with sandpaper grit 150 (Norton) simulating the internal region of a complete denture.[28]

The biofilm formation on substrates was evaluated. The substrates consisted of acrylic resin (PMMA) specimens and specimens with denture adhesives with and without the nanomaterial: PMMA + UCCA (Corega; GlaxoSmithKline) (Table 1); PMMA + UCCA + 2.5% $AgVO_3$; PMMA + UCCA + 5% $AgVO_3$ and PMMA + UCCA + 10% $AgVO_3$.

The acrylic resin specimens were sterilized with hydrogen peroxide (Multilav Sterilization)[29,30] and then the adhesives were applied in a laminar flow chamber (Pachane; Pa 400-ECO). The adhesive samples were standardized at 0.025 g and applied evenly with a spatula on

the surface of the specimens. After application, all specimens remained in ultraviolet light for 20 minutes to disinfect.[8]

The evaluation of biofilm growth on the specimens (n=9) was performed against the 3 species mentioned above. The assay was performed in triplicate at three different times.

For the preparation of the inoculum, the microorganisms were thawed, seeded in Petri plates on specific culture medium [*S. mutans*: Brain Heart Infusion (BHI; Kasvi); *C. albicans* and *C. glabrata*: Sabouraud Dextrose (SD; Kasvi)] and incubated at 37ºC for 48 hours. Next, one colony was transferred to the respective culture broth and incubated at 37ºC until they reached the exponential growth phase. The evaluation of the cell concentration of *S. mutans* was obtained by evaluating the optical density in a PCB 687 spectrophotometer (BYK GardnerThermo Scientific), at a wavelength of 625 nm. For yeasts, the cell concentration was determined by counting in a Neubauer chamber (HBG; Giessen). The inoculum was prepared at a concentration of 10^6 CFU/mL in BHI (*S. mutans*) or SD (*C. albicans* and *C. glabrata*) culture broth.

Aseptically, in a laminar flow chamber (Pachane; pa 400 – ECO), specimens were distributed in 24-well culture plates and received 1 mL of the culture medium with microbial inoculum.

The plates were incubated at 37ºC for 1 h and 30 minutes under agitation at 750 rpm in a bacteriological oven (Shaker Incubator; Mod. – CE-320) for adherence of microorganisms to the specimens. Then, 2 mL of sterile culture medium was added to each well. The plates were incubated at 37ºC under agitation at 75 rpm for 48 h for biofilm maturation. After this period, the specimens were removed from the plates, separately inserted into test tubes containing 3 mL of PBS, and placed in an ultrasonic vat (Altsonic; Clean 9CA) at 200 watts/40Hz for 20 minutes.

Viable cells were evaluated by counting the number of colony forming units per milliliter (CFU/mL). 25 µL aliquots of decimal dilutions (10^0 to 10^{-3}) were seeded on Agar SD (*C. albicans* and *C. glabrata*) and Agar BHI (*S.mutans*). The plates were incubated at 37°C for 48 hours. The incubation of *S. mutans* was performed in microaerophilic.

After the incubation period, the number of colonies from each dilution was counted, the CFU/mL value was obtained and presented in $\log_{10}$.

After biofilm formation, the specimens (n=2) were stained with the FilmTracer LIVE/DEAD cell viability kit (Thermo Fisher Scientific) according to the manufacturer's instructions. Specimens were evaluated at 63x magnification in an inverted microscope with filters at wavelengths of 490 nm and 546 nm (Axio Observer A1; Carl Zeiss). For image acquisition ZEN 2.3 lite software (Carl Zeiss Microscopy Ltd.) was used.

Considering the clinical applications of the materials tested here, it is essential to demonstrate their biocompatibility. In this study, the cell viability of VERO cell lines, a classic cell line, was evaluated to determine the biocompatibility.[31-33]

VERO cells were grown in T-25 (Nunc) culture vessels, at 37°C, in a 5% CO_2 atmosphere, with RPMI medium (Sigma-Aldrich) enriched with 10% fetal bovine serum. The RPMI medium was renewed 2-3 times a week until VERO reached 80-90% confluence. Then, cells were removed using 0.25% trypsin, 0.53 mM EDTA solution and seeded at 1 x 10^5 cells/mL in 24-well culture plates.

Appropriate amounts of denture adhesive from each experimental group were added to the RPMI culture medium to produce a 1% weight/volume eluate. The adhesive and the nanomaterial were weighed on a precision balance, manipulated and inserted into tubes, and then the culture

medium was added to the tubes with the aid of a serological pipette. The tubes were closed and stored for 24 hours at 37ºC.

VERO cells were then treated with the eluates of UCCA, UCCA + 2.5%, UCCA + 5% and UCCA + 10%. The treatment was also carried out with the extracts obtained only with the amount (mg) of the pure nanomaterial, referring to each group, being 1.25 mg of $AgVO_3$, 2.5 mg of $AgVO_3$ and 5 mg of $AgVO_3$, and only with the medium RPMI (positive control). After 24 hours of incubation at 37°C, 5% CO_2, the cells were mechanically removed and evaluated by the resazurin assay based on the fluorescence emitted by the redox indicator (resazurin) as it is reduced by viable cells.[34] Results were expressed as percentage of viable cells.

For the analysis of the adhesive force, ten cylindrical pairs of acrylic resin (25 mm in diameter × 35 mm in height) were used.[8,35] One of the cylinders of the pair was humidified with water and then coated with 0.3 g of the adhesive with or without the nanomaterial, according to the established groups. The amount of adhesive was determined based on Chew's[36] study as the amount needed to hold a maxillary prosthesis in position. Then, the set (cylinder + adhesive) was inserted into a sealed container with 100% hydration and kept at time intervals of 5 minutes, 3 hours, 6 hours, 12 hours and 24 hours. After this period, a thin layer of artificial saliva was applied on the other cylinder, and the pair was aligned in the Universal Mechanical Testing Machine (EMIC, DL 3000). A compressive force of 12 N (1.2 kg weight) was applied for 30 seconds, simulating a slight occlusion force.[35] Finally, the cylinders were separated by a pulling force at a speed of 1 mm/min and the maximum force (N) was calculated. The tests were repeated 10 times for each group. After each test, the specimen was sanitized using a sponge, detergent and dried with paper.[36]

Statistical analysis was performed using SPSS version 22.0 software. Once the distribution (Levene test) and homogeneity (Shapiro-Wilks test) of the data were verified, the statistical tests were performed. For microbiological analysis, the Kruskal-Wallis test was used, followed by Dunn's post-test. The adhesive strength and biocompatibility data were submitted to ANOVA analysis of variance, followed by the Bonferroni post test (α=0.05).

3.3 RESULTS

The silver vanadate nanowires have an average diameter of 150 nm and length in the order of micrometers and are coated with hemispherical metallic silver nanoparticles (Fig. 2).

The value obtained through the MIC against the strain of *S. mutans* was 250 μg/mL, and for both yeasts it was 62.5 μg/mL. The expressed results show the antimicrobial efficacy of the studied nanomaterial against colonizing microorganisms of complete dentures.

The CFU/mL count of each microorganism varied according to the $AgVO_3$ concentration (*P*<.05) however, no significant difference was observed when comparing the control group (PMMA) with the UCCA group (*P*>.05).

Overall, there was a dose-dependent effect on antibiofilm activity. No biofilm formation of *C. albicans* was observed in the groups incorporated with the different concentrations of the nanomaterial (*P*<.05). There was a significant reduction in the number of CFU/mL of *S. mutans* with the association of the nanomaterial at 2.5% compared to PMMA (*P*=.028), resulting in a complete inhibition in the groups with 5% (*P*<.001) and 10% (P<0.001). For *C. glabrata*, a significant reduction was observed with the incorporation of 5% and 10% of the nanomaterial compared to PMMA and UCCA (*P*<.05) (Table 2).

Fluorescence microscopy confirmed the results obtained by counting CFU/mL, since it was possible to observe a smaller amount of viable cells (in green) in the groups incorporated with the nanomaterial, demonstrating that this association is capable of providing antimicrobial activity to the prosthetic adhesive (Fig. 3).

The results indicate that the treatment of VERO cells with extracts obtained from the UCCA incorporated with $AgVO_3$ at 2.5% (*P*=.686) and 5% (*P*=.998) was biocompatible. Soluble $AgVO_3$ was biocompatible at 1.25mg/mL (*P*=.127). It is important to note that the reduction in cell viability of UCCA+10% $AgVO_3$ was less than 15%, and for 2.5 mg/mL and 5mg/mL of $AgVO_3$ they were less than 10% and 20%, respectively (Fig. 4).

There was a significant difference in adhesive strength when considering the factor "concentration of the nanomaterial" (*P*=.017) and the factor "time" (*P*<.001) independently, as well as in the interaction between the two factors (*P*=.001) (Table 3). No significant difference was observed in the adhesive strength of the groups at 3, 6 and 12 hours (*P*>.05). In 5 minutes, the UCCA + 5% showed greater adhesive strength, being statistically different from the UCCA (*P*=.020) and similar to the groups with 2.5% (*P*=.073) and 10% (*P*=1.000). In 24 hours, UCCA + 10% showed higher adhesive strength, being statistically different from UCCA (*P*=.026) and similar to the 2.5% (*P*=1.000) and 5% (*P*=1.000) groups (Fig. 5).

The adhesive strength of the UCCA + 5% group was not influenced by time (*P*>.05). On the other hand, UCCA + 2.5% and UCCA + 10% groups showed lower adhesive strength 5 minutes after application (*P*<.05). UCCA showed lower adhesive strength 5 minutes and 24 hours after application (*P*<.05) (Fig. 6).

3.4 DISCUSSION

The results of this study rejected the null hypothesis as significant differences were found in biofilm formation, biocompatibility and adhesive strength of the materials tested.

Adhesives are effective aids in the retention and stability of removable dentures, improving masticatory performance and patient satisfaction.[6] Among them, the COREGA prosthetic adhesive stands out, available in different formulations, being soluble (creams and powders) or insoluble (strip).[7]

The publicity strongly affects patients' choices of the type of prosthetic adhesive, and the cream formulation has been widely used.[37] These materials are mainly composed of sodium carboxymethyl cellulose and expand along the interface between the prosthesis surface and the mucosa, adhering to both sides without influencing the occlusal relationship. However, COREGA does not contain any antifungal component in the composition (Table 1), so that an ineffective removal of the surface of the prosthesis or the mucosa can cause health problems.

In the present study, there was a similarity in biofilm formation between the groups in which the commercial adhesive was applied and the control (PMMA). Oliveira Junior et al[7] and Costa et al,[8] on the other hand, report that the use of these adhesives can further favor the adhesion of the biofilm on the surface of the prostheses and recommend the inclusion of an antimicrobial agent in the composition in order to prevent local and systemic problems.

The present results revealed that an antibiofilm effect can be promoted when the acrylic resin is treated with $AgVO_3$ incorporated into the adhesive. Statistically significant differences in CFU values were found when the nanomaterial was used, so that all concentrations tested were able to promote antimicrobial activity to the selected species, with a general dose-dependent effect. Microscopy confirmed the results obtained, as it was possible to observe the reduction of viable

cells (green) as the concentration of the nanomaterial increases in the formulation of the prosthetic adhesive, in comparison with the PMMA group and with the groups treated with the adhesives in the commercial form (Fig.3).

These results can be explained by the promising antimicrobial capacity of $AgVO_3$, which, when in contact with microbial membranes, is able to promote changes, preventing DNA replication capacity,[15,16] and are consistent with studies that incorporated this nanomaterial into acrylic resins,[18] dental ceramics,[19,20] endodontic cements,[22] and irreversible hydrocolloid,[21] reporting the antimicrobial effect.

According to the results of the study, it seems reasonable to conclude that $AgVO_3$ promoted antibiofilm activity to the prosthetic adhesive. However, to enable clinical application, biocompatibility must be evaluated. VERO cells are African monkey epithelial cell lines commonly used as a research model for the manufacture of immunobiologicals for human use, assessment of the cytotoxicity of biomaterials, toxin detection, efficacy testing, media and mycoplasma testing.[31-33]

The International Standard Organization (ISO 10993-5:1992)[38] classifies materials according to the cytotoxic effect as non-cytotoxic (cellular viability above 75% in relation to the control group), discretely cytotoxic (cellular viability between 50% and 75% in relation to the control group), moderately cytotoxic (cell viability between 25% and 50% in relation to the control group) and severely cytotoxic (cellular viability below 25% in relation to the control group). In this study, the results obtained by the resazurin assay showed that concentrations of 2.5% and 5% were biocompatible with the VERO cell and that the reduction in cell viability of UCCA+10% $AgVO_3$ was less than 15%.[39]

Considering the functions of the denture adhesive, it is essential to carry out mechanical tests to verify if the association of COREGA with the nanomaterial can change its properties. If, on the one hand, these materials must have high adhesion to the mucosa for fixation, on the other hand, they must have low adhesion to facilitate removal, which is contradictory. It is expected that fixing agents will provide removable prostheses with retention and stability for a period of time.[8]

The test used in the present study to assess the adhesive strength was performed as suggested in the literature.[8,35,36] Studies report a peak in adhesive strength after 3 to 6 hours of application, followed by a loss of effectiveness over time[35] due to the action of oral fluids that promote adhesive breakdown and degradation.[40] In the present study, in general, the prosthetic adhesive modified with the nanomaterial presented similar or superior values of adhesive strength compared to the groups without modification, being able to provide safety and comfort to the patient for longer than expected (12 to 16 hours).[41]

The combination of prosthetic adhesives and $AgVO_3$ has clinical relevance and should be studied further. Nystatin is an antifungal agent widely used in the treatment of denture stomatitis, however, it may cause some side effects and recurrent inflammation due to the resistance of *C. albicans*.[42] This work does not propose the replacement of this antifungal agent, it suggests the addition of a nanomaterial with antimicrobial properties, aiming to increase the benefits promoted by the use of adhesive by users of removable prostheses. Moreover, this proposal will allow more effective preventive and therapeutic actions due to the presence of the antimicrobial agent near the inner surface of the contaminated denture base and oral mucosa.[14]

Among the limitations of the study, we can mention the use of only one commercial form of the prosthetic adhesive, as well as the analysis of the antibiofilm action against a mono species biofilm and the use of only one cell line for biocompatibility analysis. Also, in the adhesive

strength test, the presence of the mucosa was not considered. Therefore, as future studies, the analysis of other commercial forms of the prosthetic adhesive are suggested, as well as the study of the antibiofilm action against a multispecies biofilm and the analysis of the biocompatibility against epithelial cells of the human palate. Still, studies are suggested considering the presence of the mucosa, quantity and quality of saliva for the adhesive strength.

3.5 CONCLUSION

Based on the findings of this *in vitro* study, the following conclusions were drawn:

1. The addition of all tested concentrations of $AgVO_3$ (2.5%, 5% and 10%) promoted antibiofilm activity to the prosthetic adhesive against *Streptococcus mutans, Candida albicans* and *Candida glabrata*.
2. Adhesive and $AgVO_3$ formulations were biocompatible with VERO cells.
3. Adhesive and $AgVO_3$ formulations showed satisfactory adhesive strength within 24 hours of application.

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3.7 TABLES

Table 1. Composition and manufacturer of Ultra Corega Cream Adhesive (UCCA).

Adhesive	**Manufacturer**	**Composition**
Ultra Corega Cream	STAFFORD-MILLER IRELAND Limited; GlaxoSmithKline	Sodium/calcium salts of poly (methylvinylether/maleic acid), carboxymethylcellulose, mineral oil, petroleum jelly

Table 2. Comparison of colony forming unit count (CFU / mL) in $\log_{10}$ under different experimental conditions

	C. albicans	*C. glabrata*	*S.mutans*
Control - PMMA	9.15[9.06;9.20]^{A}	8.20[8.00;8.41]^{A}	8.40[8.00;8.58]^{A}
PMMA+ UCCA	8.77[8.33;9.99]^{A}	7.90[7.19;8.30]^{A}	8.24[8.08;8.41]^{AB}
PMMA + UCCA + 2.5% $AgVO_3$	0.0^{B}	4.02[3.32;4.56]^{AB}	1.60 [0.28;3.28]^{BC}
PMMA + UCCA + 5% $AgVO_3$	0.0^{B}	2.30[1.32;2.80] ^{B}	0.0^{C}
PMMA + UCCA + 10% $AgVO_3$	0.0^{B}	0[-0.19;0.94] ^{B}	0.0^{C}

Data are expressed as median [Confidence Interval] (n=9). * Different letters indicate significant difference between groups for the same microorganism. Kruskal-Wallis followed by Dunn's post hoc test. $P<.05$. *UCCA = Ultra Corega Cream Adhesive

Table 3. ANOVA for different concentrations of $AgVO_3$ incorporated in denture adhesive on adhesive strength (N)

Cross-subject effect testing

Dependent variable: Adhesive strength

Source	Type III Sum of Squares	Df	Medium square	Z	Sig.
Corrected model	219.892[a]	24	9.162	5.004	<.001
Ordered at origin	27742.764	1	27742.764	15153.216	<.001
Concentration	22.559	4	5.640	3.081	.017
Time	121.392	4	30.348	16.576	<.001
Concentration * Time	75.940	16	4.746	2.592	.001
Error	411.934	225	1.831		
Total	28374.589	250			
Total corrected	631,825	249			

The. R squared = .348 (adjusted R squared = .278)

3.8 FIGURES

Fig.1. Study flowchart.

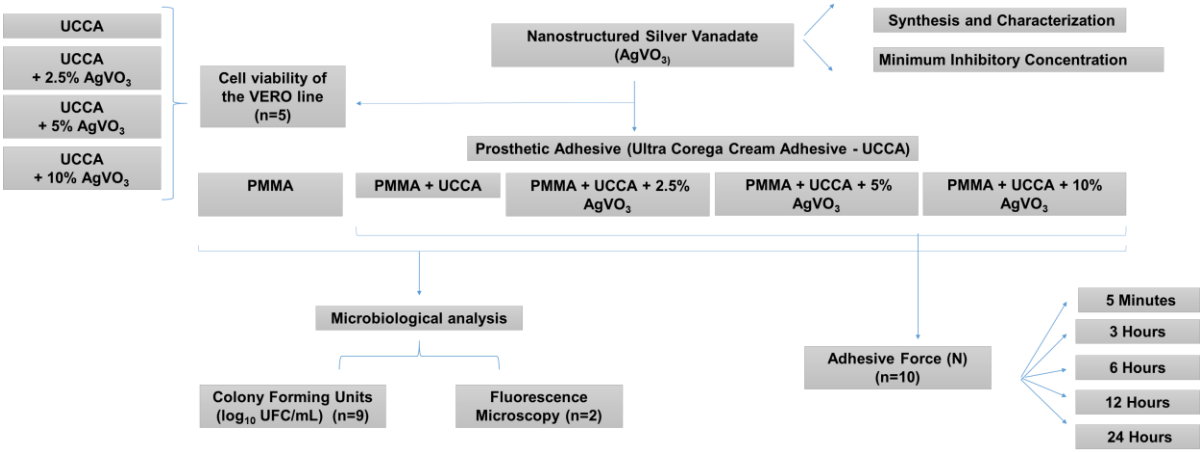


Fig.2. Transmission electron microscopy of nanostructured silver vanadate decorated with silver nanoparticles.

Fig.3. Fluorescence microscopy of *C. albicans, C. glabrata e S. mutans* biofilm (63x). Viable cells marked in green and dead cells marked in red.

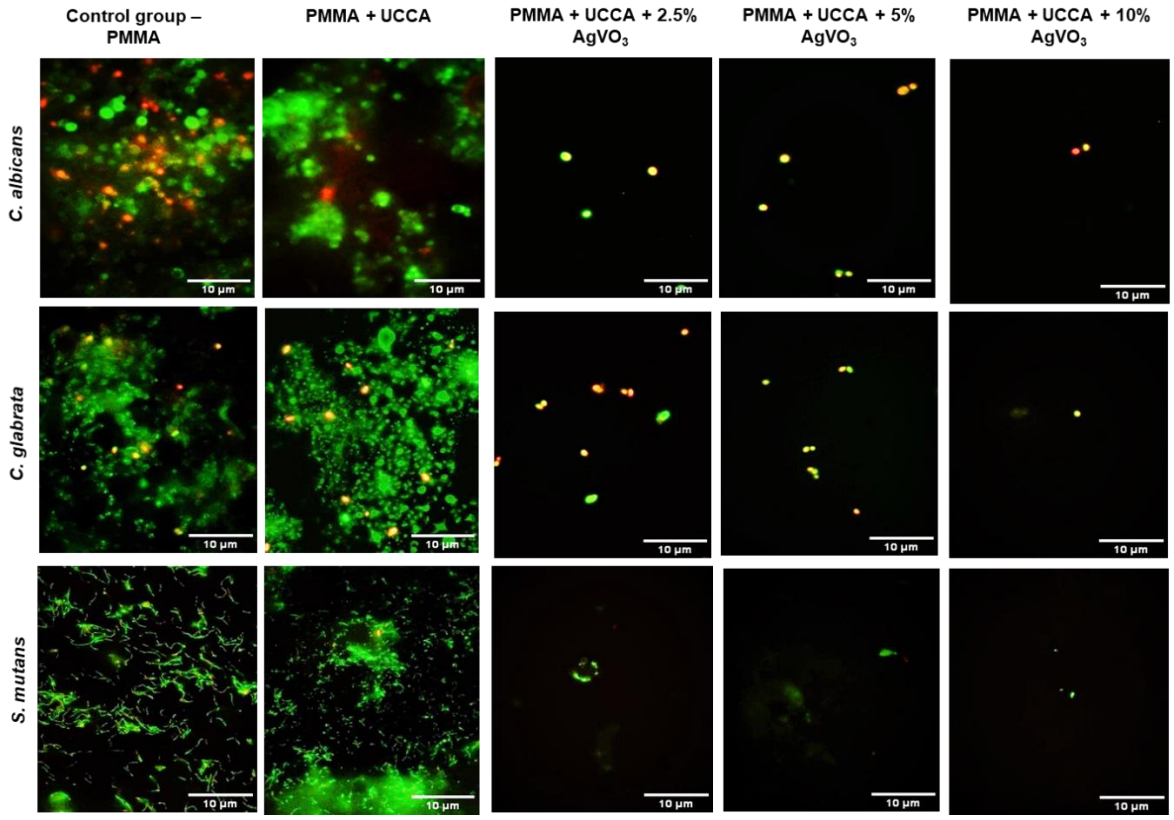


Fig.4. Cell viability of the VERO cell line for 24 hours. A. Cells treated with Ultra Corega Cream containing different concentrations of $AgVO_3$. B. Cells treated with different concentrations of $AgVO_3$. Cells maintained only in the presence of RPMI 1640 culture medium were used as controls. * indicates statistically significant difference compared to cells maintained in RPMI 1640 (*P*<.05; ANOVA test).

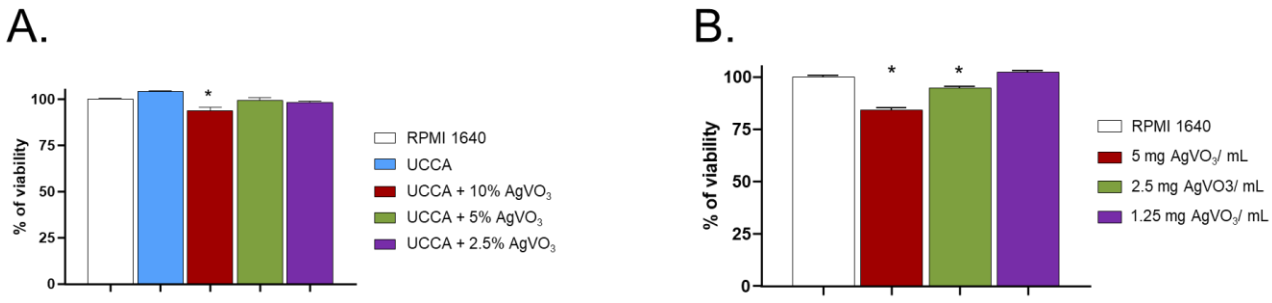


Fig.5. Comparison of adhesive strength (N) considering the interaction between experimental groups and time. The same letter indicates the statistical equality of the different groups at the same time (*P*>.05; two-way ANOVA and Bonferroni post hoc test).

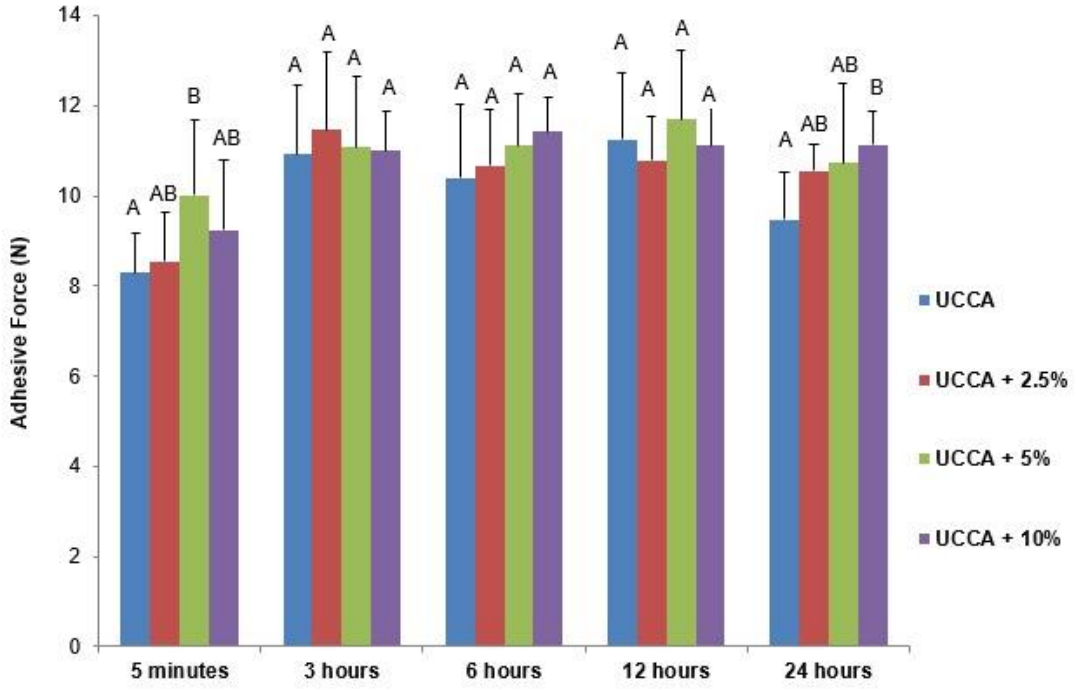
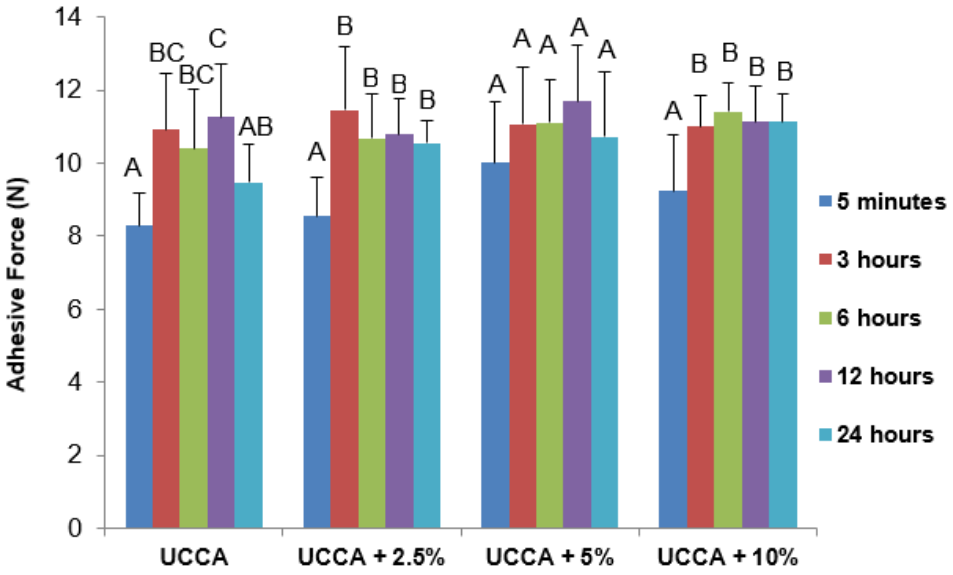


Fig.6. Comparison of adhesive strength (N) considering the interaction between experimental groups and different concentrations. The same letter indicates statistical equality of the same group at different time intervals (*P*>.05; two-way ANOVA and Bonferroni post hoc test).



4. CONCLUSÃO

Com base nos resultados deste estudo *in vitro*, foram tiradas as seguintes conclusões:

1. A incorporação de todas as concentrações testadas de $AgVO_3$ (2,5%, 5% e 10%) promoveu atividade antibiofilme ao adesivo protético frente ao *Streptococcus mutans*, *Candida albicans* e *Candida glabrata.*
2. As formulações de adesivo e $AgVO_3$ foram biocompatíveis com células VERO.
3. As formulações de adesivo e $AgVO_3$ apresentaram força adesiva satisfatória em até 24 horas após a aplicação.

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APÊNDICE

Figura 1. Forma comercial do adesivo para prótese dentária (Ultra Corega Creme)

Figura 2. Confecção dos espécimes em Resina Acrílica. A- Preparação dos moldes em cera, B- Resina Acrílica Utilizada C- Moldes dos espécimes, D- Prensagem em prensa hidráulica, E- Termocicladora elétrica, F- Acabamento e polimento dos espécimes.

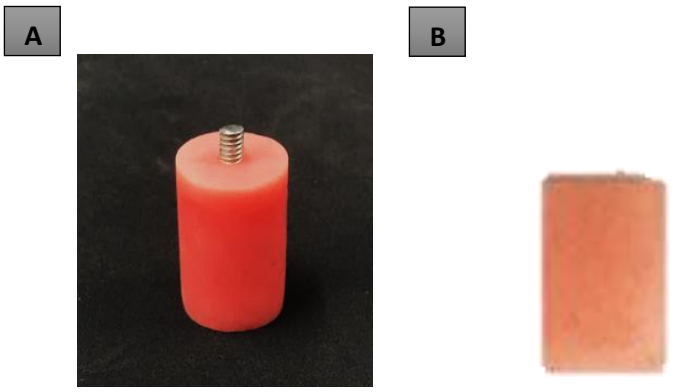

Figura 3. A- Espécime em resina acrílica em formato cilíndrico, B- Espécime em resina acrílica em formato retangular

Figura 4. Vanadato de Prata Nanoestruturado Decorado com Nanoparticulas de Prata

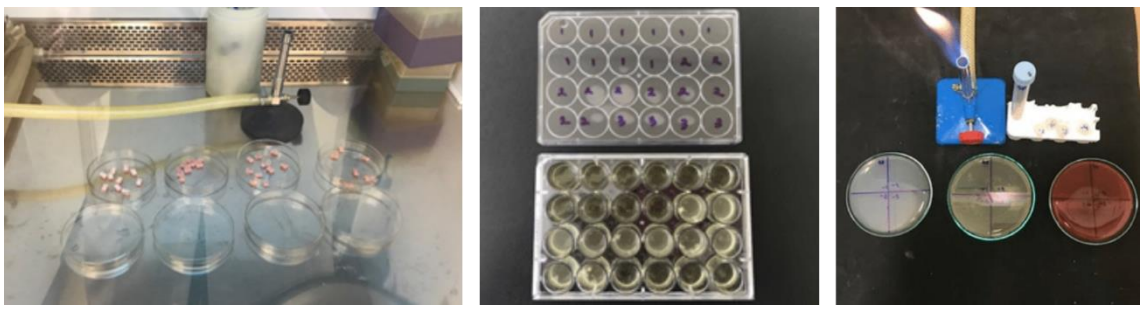

Figura 5. Formação do biofilme e semeadura em placas de Petri para contagem de UFC/ mL

Figura 6. Conjunto espécime e adesivo submerso em água destilada após o período pré-determinado na estufa

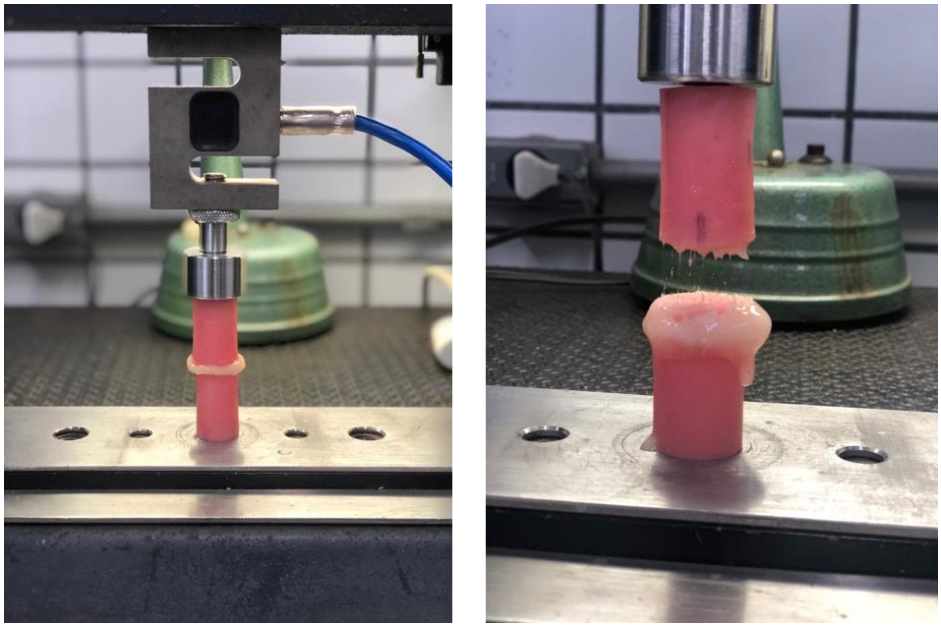

Figura 7. Ensaio da força adesiva em Máquina de Ensaios Universais

ANEXOS

Anexo 1 – Certificados

Figura 8. Certificado da Apresentação Oral na Jornada Odontológica de Ribeirão Preto (JORP)

Figura 9. Certificado de menção honrosa na Jornada Odontológica de Ribeirão Preto (JORP)

Certificado

DE APRESENTAÇÃO ORAL

Certificamos que **Graziele cristina alvim da silva**, apresentou o trabalho **"ANÁLISE DA ATIVIDADE ANTIBIOFILME E DA BIOCOMPATIBILIDADE DE UM ADESIVO PROTÉTICO MODIFICADO COM NANOMATERIAL HÍBRIDO"**, que possui como autores: **Graziele cristina alvim da silva, Marcelo Rodrigues Pinto, Andrea Candido dos Reis, Marcos Vinicius da Silva e Denise Tornavoi de Castro**, na modalidade de Apresentação Oral, durante a XXIV Jornada Odontológica da Universidade de Brasília, realizada pelo formato digital nos dias 24, 25, 26 e 27 de agosto de 2021.

Brasília, 28 de agosto de 2021.

Helhen Cristina da Luz Cardoso
Presidente Discente

Lucas Fernando Tabata
Presidente Docente

Universidade de Brasília

Figura 10. Certificado de apresentação oral na Jornada Odontológica na Universidade de Brasília

34º COB

CERTIFICADO

Certificamos que

Graziele cristina alvim da silva

Apresentou o trabalho intitulado **"Efeitos de diferentes formas de adesivos na formação de biofilme, resistência adesiva e higiene de próteses totais."**, de autoria de **Graziele cristina alvim da silva** e **Costa, M.S., Lepri, C. P.; Reis, A.C, Oliveira, V.C., Castro, D.T.**, na área temática de **Dentistica e materiais odontológicos**, modalidade **Pesquisa e Revisão Sistemática**, no **34º Congresso Odontológico de Bauru- Profª. Dra. Daniela Gamba Garib Carreira** intitulado "Ciência aliada à clínica: caminhos para a Odontologia de excelência no dia-a-dia".

O evento online foi realizado pela plataforma da Softaliza e organizado pela Faculdade de Odontologia de Bauru - Universidade de São Paulo, nos dias 12/5 a 15/5 de 2021.

Prof. Dr. Carlos Ferreira dos Santos
Diretor da Faculdade de Odontologia de Bauru - Universidade de São Paulo

Profª. Dra. Ana Carolina Magalhães
Coordenadora da Comissão Científica 34º COB

Flávia Cristina Costa
Presidente - 34º COB

Universidade de São Paulo
Faculdade de Odontologia de Bauru

Figura 11. Certificado de apresentação oral no Congresso Odontológico de Bauru

Figura 12. Matéria do site da UNIUBE sobre a Menção Honrosa na JORP

Anexo 2 - Comprovante de submissão do artigo

DESCRIPTION *The Journal of Prosthetic Dentistry* is the leading professional journal devoted exclusively to **prosthetic** and **restorative dentistry**. The *Journal* is the official publication for 24 leading U.S. international prosthodontic organizations. The monthly publication features timely, original peer-reviewed articles on the newest techniques, dental materials, and research findings. The *Journal* serves prosthodontists and dentists in advanced practice, and features color photos that illustrate many step-by-step procedures. *The Journal of Prosthetic Dentistry* is included in Index Medicus and CINAHL.

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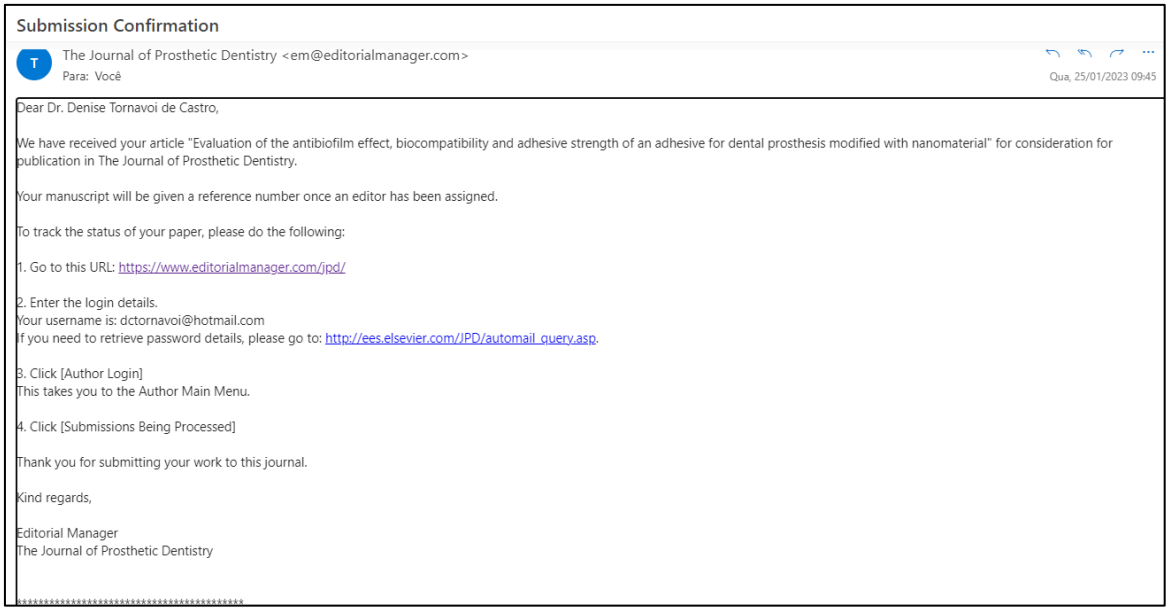

Submission Confirmation

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Dear Dr. Denise Tornavoi de Castro,

We have received your article "Evaluation of the antibiofilm effect, biocompatibility and adhesive strength of an adhesive for dental prosthesis modified with nanomaterial" for consideration for publication in The Journal of Prosthetic Dentistry.

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