Denture Adhesives Associated with Silver Vanadate: Antimicrobial Approach Against Multi-Species Biofilms on Acrylic Resin Surfaces

Keywords

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Authors

Denise T. de Castro *
(PhD)

Izabela Ferreira §
(MSc)

Viviane de C. Oliveira ǂ
(PhD)

Marco A. Schiavon ^
(PhD)

Andrea C. dos Reis Δ
(PhD)

Address for Correspondence

Andrea C. dos Reis Δ

Email: andreare73@yahoo.com.br

* *Postdoctorate, Department of Dental Materials and Prosthodontics, Ribeirao Preto School of Dentistry, University of Sao Paulo, Ribeirao Preto-SP, Brazil.*

§ *Graduate Student, Department of Dental Materials and Prosthodontics, Ribeirao Preto School of Dentistry, University of Sao Paulo, Ribeirao Preto-SP, Brazil*

ǂ *Specialized Laboratory Technician, Department of Dental Materials and Prosthodontics, Ribeirao Preto School of Dentistry, University of Sao Paulo, Ribeirao Preto-SP, Brazil*

^ *Titular Professor, Department of Natural Sciences, Federal University of São Joao Del-Rei (UFSJ), São Joao Del-Rei (MG), Brazil*

Δ *Associated Professor, Department of Dental Materials and Prosthodontics, Ribeirao Preto School of Dentistry, University of Sao Paulo, Ribeirao Preto-SP, Brazil.*

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ABSTRACT

Alternatives have been sought to add an antimicrobial property to denture adhesives. This study evaluated the antimicrobial potential of adhesives associated with nanostructured silver vanadate decorated with silver nanoparticles (β-$AgVO_3$). Specimens in acrylic resin were treated with the adhesives associated with β-$AgVO_3$ (1%, 2.5%, 5% and 10%). As control, specimens treated only with Ultra Corega Cream (UCC) or Ultra Corega Powder (UCP) adhesive were used. Multispecies biofilm of Candida albicans, Candida glabrata, Streptococcus mutans and Staphylococcus aureus was evaluated by counting colony forming units per milliliter (CFU/mL), colorimetric assay and fluorescence microscopy. The data were analyzed using the two-way analysis of variance (ANOVA) and Bonferroni multiple comparisons test (α=0.05). For both adhesives, a small amount of β-$AgVO_3$ (1%) completely inhibited S. mutans (P<0.05). For the other microorganisms, there was a reduction in metabolic activity and complete inhibition in the groups with intermediate or greater amounts of nanomaterial (P<0.05), except for C. albicans, which was reduced (P<0.05) but not completely inhibited in UCP. Microscopy that showed less biofilm in the groups with β-$AgVO_3$ and in the UCC than UCP. Denture adhesives in powder and cream form with β-$AgVO_3$ showed potential antimicrobial activity against multispecies biofilm. Powder adhesive showed higher biofilm formation.

INTRODUCTION

The success of a complete denture depends not only on the correct technique of fabrication, but also on other factors such as previous experience with the use of prosthesis, the type of ridge and the psychological profile of the patient.[1] In addition, aging is often accompanied by systemic diseases and oral disorders that make it difficult to keep dentures comfortably in the oral cavity.[2,3] Lack of retention, instability and functional problems are commonly reported by users of removable prosthesis, leading clinicians to prescribe adhesives.[4]

Denture adhesives act as intermediary substances between the internal surface of the prosthesis and the mucosa and have a positive effect on retention and chewing, providing safety, comfort and well-being for users.[5]

These materials also reduce the accumulation of residues under the denture base and the pressure on the mucosa.[4,6]

COREGA® denture adhesive is widely used and although it does not contain a specific antimicrobial agent, it does contain polyethylene glycol (PEG), a polymeric compound which, according to the literature, forms a physical barrier that makes it difficult for microorganisms to adhere and prevents infection.[7-9]

Despite this, studies have shown that the use of this adhesive can favour the accumulation of biofilm.[10,11] Thus, a porous and rough polymethylmethacrylate denture base associated with residues of adhesive could serve as a reservoir of infectious pathogens capable of leading to the development of denture stomatitis (DS).[10-12]

DS is a disease that affects about 70% of users of removable dental prosthesis, and was associated with infection by *Candida* spp.[13] Although *Candida albicans* is extensively studied, a polymicrobial profile marked by interactions between fungus and bacteria is involved in DS.[14] In addition, the relationship between the proliferation of oral bacteria with cardiovascular diseases and generalized respiratory tract infections has been proven.[15,16]

Since adhesive materials are widely used and there is a high frequency of local and systemic problems associated with the use of complete dentures, studies have sought alternatives to add an effective antimicrobial property to denture adhesives.[4,9,17-18] Almeida *et al*. (2018)[9] found that the COREGA® powder adhesive, in combination with herbal medicines, exhibited an antibiofilm effect against *C. albicans*. The same was observed by Peralta *et al*. (2023)[4] by associating this adhesive with silver nanoparticles (AgNPs). Therefore, given the limitations of conventional treatment with nystatin and azole-derived drugs, new therapies for the prevention and treatment of DS are being investigated.

Nanomaterials have become an important tool in the field of health due to their physical, chemical and biological properties. Nanostructured silver vanadate decorated with AgNPs (β-$AgVO_3$), is a nanomaterial that has attracted interest in dentistry,[19-27] since it interacts directly with the cell membranes of fungi and bacteria causing cell death.[28]

Considering the relevance of nanotechnology and the growing use of denture adhesives, this study evaluated the antimicrobial potential of cream and powder adhesives associated with β-$AgVO_3$.

MATERIAL AND METHODS

EXPERIMENTAL DESIGN

The factors under study were the commercial form of denture adhesive (cream and powder) and the percentage of β-$AgVO_3$ incorporated (0% - control, 1%, 2.5%, 5%, and 10%). The quantitative response variables were the amount of biofilm on the specimens, evaluated by colony forming units count (in $\log_{10}$ CFU/mL) and the metabolic activity, evaluated by the colorimetric XTT assay (in absorbance). The qualitative variable was the distribution of the biofilm on the specimens, evaluated by fluorescence microscopy.

To assess the biofilm by UFC and XTT, the research was carried out using three technical replicates, with the aim of explaining the variability that may occur due to small differences in the performance of the experiment, human error or the precision of the equipment, and three biological replicates, which are related to the natural variation of the microorganisms being tested, ensuring a representative response from a wider population. Therefore, assays were performed in triplicate on 3 different days (n=9).[29] However, microscopic images were only taken on one day to illustrate the findings (n=2).

SYNTHESIS AND CHARACTERIZATION OF THE NANOMATERIAL

The nanomaterial was synthesized using silver nitrate (Merck 99.8%) and ammonium metavanadate (Merck 99%) reagents[19-22,24-28] and analyzed by transmission electron microscopy with a JEOL JEM-100CX II microscope.

SAMPLE PREPARATION

Rectangular specimens (6 x 10 x 3 mm) of heat-cured acrylic resin based on polymethylmethacrylate - PMMA (Classical; Classical Dental Articles) were made in metal flasks. Polymerization was performed by conventional heating. The surface roughness was standardized using a Rugosimeter (Surftest SJ 201P, Mitutoyo Corporation) (3.0 µm ± 0.3), with water sandpaper.[11,30]

Specimens were divided into groups consisting of acrylic resin specimens with cream (Ultra Corega Cream - UCC, GlaxoSmithKline) and powder (Ultra Corega Powder - UCP, GlaxoSmithKline) denture adhesive containing or not containing the nanomaterial: UCC; UCC + 1% (w/w) β-$AgVO_3$; UCC + 2.5% (w/w) β-$AgVO_3$; UCC + 5% (w/w) β-$AgVO_3$; UCC + 10% (w/w) β-$AgVO_3$; UCP; UCP + 1% (w/w) β-$AgVO_3$; UCP + 2.5% (w/w) β-$AgVO_3$; UCP + 5% (w/w) β-$AgVO_3$ and UCP + 10% (w/w) β-$AgVO_3$.

Sterilization of the specimens was performed with low temperature hydrogen peroxide with a cycle duration of 35 minutes.[31] An amount of 0.025 g of adhesive with or without the nanomaterial was spread evenly over the surface of the specimens with a spatula. The UCP was moistened with sterilised deionised water and applied to the specimens. This process was performed in a laminar flow chamber (Pachane; Pa 400-ECO) and then, the specimens remained under the action of ultraviolet light for 20 minutes for the disinfection of the applied adhesives.[10,11] Only the adhesives were applied to the control groups (UCC and UCP). The nanomaterial-incorporated groups were prepared after weighing the β-$AgVO_3$ on a precision analytical balance according to the indicated percentages, which was added proportionally by mass to the adhesive.

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BIOFILM FORMATION

A multispecies biofilm composed of *C. albicans* (ATCC 10231), *C. glabrata* (ATCC 2001), *S. mutans* (ATCC 25175) and *S. aureus* (ATCC 25923) was used to contaminate the specimens.

The microorganisms were thawed and incubated for 48 hours in the selective culture media (CHROMagar Candida - *C. albicans* and *C. glabrata*; Mitis Salivarius Agar - *S. mutans*; Salt Mannitol Agar - *S. aureus*). After reactivation, one colony of each microorganism was transferred to Brain Heart Infusion Broth (BHI) and incubated at 37ºC for 24 hours. Next, the culture was centrifuged at 4200 g for 5 minutes. The resulting pellet was washed and suspended again in the same buffer.

The cell concentration of bacteria (10^7 CFU/mL) was evaluated according to the optical density of the suspension verified in a spectrophotometer (Multiskan GO, Thermo Scientific, Thermo Scientific Multiskan Spectrum) with a wavelength of 625 nm (O_{D600} nm). The yeast cell concentration (10^6 CFU/mL) was counting in a Neubauer chamber (Kasvi Import and Distribution of Products for Laboratories Ltd).

The specimens were placed in a cell culture plate with 0.5 mL of BHI inoculated from each microorganism, totaling a volume of 2 mL. Then, the plates were incubated at 37°C for 48 hours in microaerophilic conditions.

COUNTING COLONY FORMING UNITS PER MILLILITER (CFU/ML)

The specimens were washed and inserted into propylene tubes with 10 mL of PBS. They were sonicated in a 200 W, 40 KHZ ultrasonic cleaner (Altsonic, Clean 9CA) for 20 minutes to loosen the adhered cells. 10-fold-dilution aliquots (10^0-10^{-3}) were prepared and seeded in Petri plates containing the selective agar medium. The plates were incubated at 37°C for 48 h and the number of colonies was recorded.[32]

XTT-COLORIMETRIC ASSAY

The colorimetric XTT [2,3-bis-(2-methoxy-4-nitro-5-sulfophenyl)-2H-tetrazolium-5-carboxanilide] assay was used according to the protocol described.[33] XTT (Sigma-Aldrich, Saint Louis, Missouri, USA) was dissolved in PBS buffer to a final concentration of 1 mg/mL. Menadione solution (Sigma-Aldrich, Saint Louis, Missouri, USA) was prepared at a concentration of 0.4 mM in acetone (Sigma-Aldrich, Saint Louis, Missouri, USA) immediately prior to use. For each assay, the XTT solution was mixed with the menadione at a ratio of 20:1.

After the biofilm formation period, the specimens were washed with PBS and transferred to a sterile cell culture plate with 24 wells, each containing 1.2 mL of solution composed of 948 µL of PBS supplemented with 100 mM glucose (Sigma Aldrich), 252 µL of previously prepared XTT and menadione solution. The plates were covered with aluminum foil and incubated in the dark at 37°C for 2h. After the incubation time, the plates were shaken and 100 µL of the solution from each well containing one specimen were transferred to 96-well plates in triplicate and readings were obtained at 492 nm. The mean of the readings was recorded and expressed in absorbance units.

FLUORESCENCE MICROSCOPY

The biofilms formed in the samples were stained with the LIVE / DEAD BacLight kit (Molecular Probes) and evaluated by fluorescence microscopy in a microscope (Axio Observer A1; Carl Zeiss®) with 20x magnification. Next, the images were processed in the ZEN 2.3 Lite software (Carl Zeiss® Microscopy Ltd.).[11,19]

STATISTICAL ANALYSIS

The data were analyzed for distribution (Levene) and normality (Kolmogorov-Smirnov) and evaluated by two-way Analysis of Variance (ANOVA) and Bonferroni multiple comparisons test (α=0.05), using SPSS version 22.0 software (SPSS Inc. Chicago, IL, USA).

RESULTS

CHARACTERIZATION OF THE NANOMATERIAL

The β-$AgVO_3$ consists of vanadium nanowires with micrometric length and diameter of approximately 150 nm covered by spherical nanoparticles *(Figure 1)*.

MICROBIAL LOAD

The β-$AgVO_3$ promoted antimicrobial activity to the tested denture adhesives (P<0.05).

Specimens treated with UCC + 5% and 10% of β-$AgVO_3$ showed significantly lower CFU/mL values of *C. albicans* (P<0.05), with complete inhibition in the more concentrated. For *S. aureus* there was a reduction in CFU count starting at 2.5% of β-$AgVO_3$ (P<0.05), with complete inhibition in the 5% and 10% groups. For *C. glabrata* and *S. mutans* there was a reduction in CFU count starting at 1% of β-$AgVO_3$ (P<0.05), and for *S. mutans*, this percentage already promoted a complete inhibition of the biofilm, whereas for *C. glabrata* the complete inhibition was verified from 2.5% *(Figure 2)*.

All percentages of β-$AgVO_3$ associated with UCP, reduced the values of CFU/mL against the microorganisms tested (P<0.05), except for *S. aureus* whose reduction was observed from 5% of the nanomaterial. A complete inhibition of *C. glabrata* and *S. aureus* was observed in the groups with 10% and of *S. mutans* with 1%. Although complete inhibition of *C. albicans* was not observed, UCP + 5% (3.23 $\log_{10}$) and UCP + 10% (1.8 $\log_{10}$) produced Δ values ($\log_{10}$ CFU/mL) of -1.97 and -3.4, respectively, compared to the group without incorporation (5.2 $\log_{10}$) *(Figure 3)*.

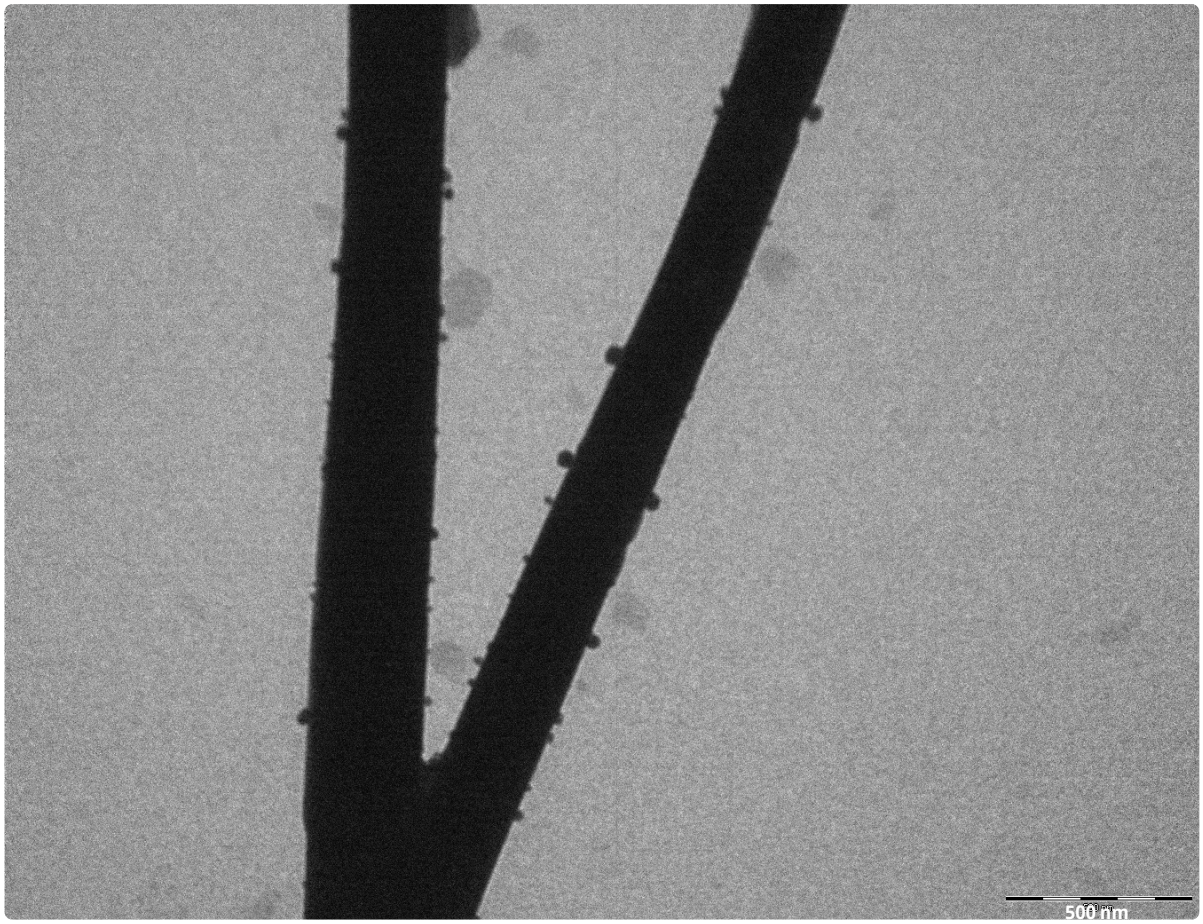

Figure 1: Photomicrograph of the nanomaterial.

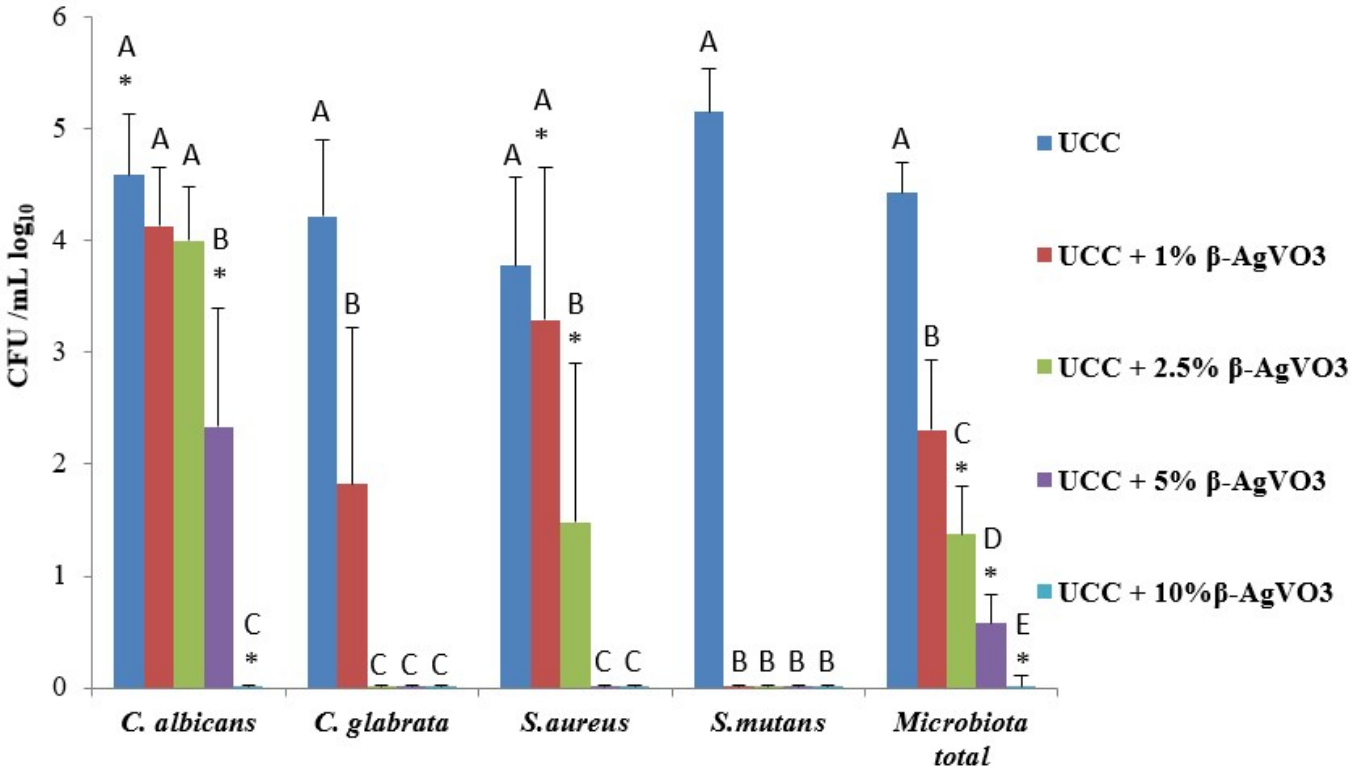

Figure 2: Colony forming units per milliliter (CFU/mL) of multispecies biofilm on the surface of PMMA samples treated with Ultra Corega Cream adhesive (UCC) associated with β-$AgVO_3$ or not. Different letters represent a statistically significant difference between different groups for the same microorganism, while the symbols represent a statistically significant difference between two commercial forms of the denture adhesive ($P<0.05$; two-way ANOVA test and Bonferroni post hoc test).

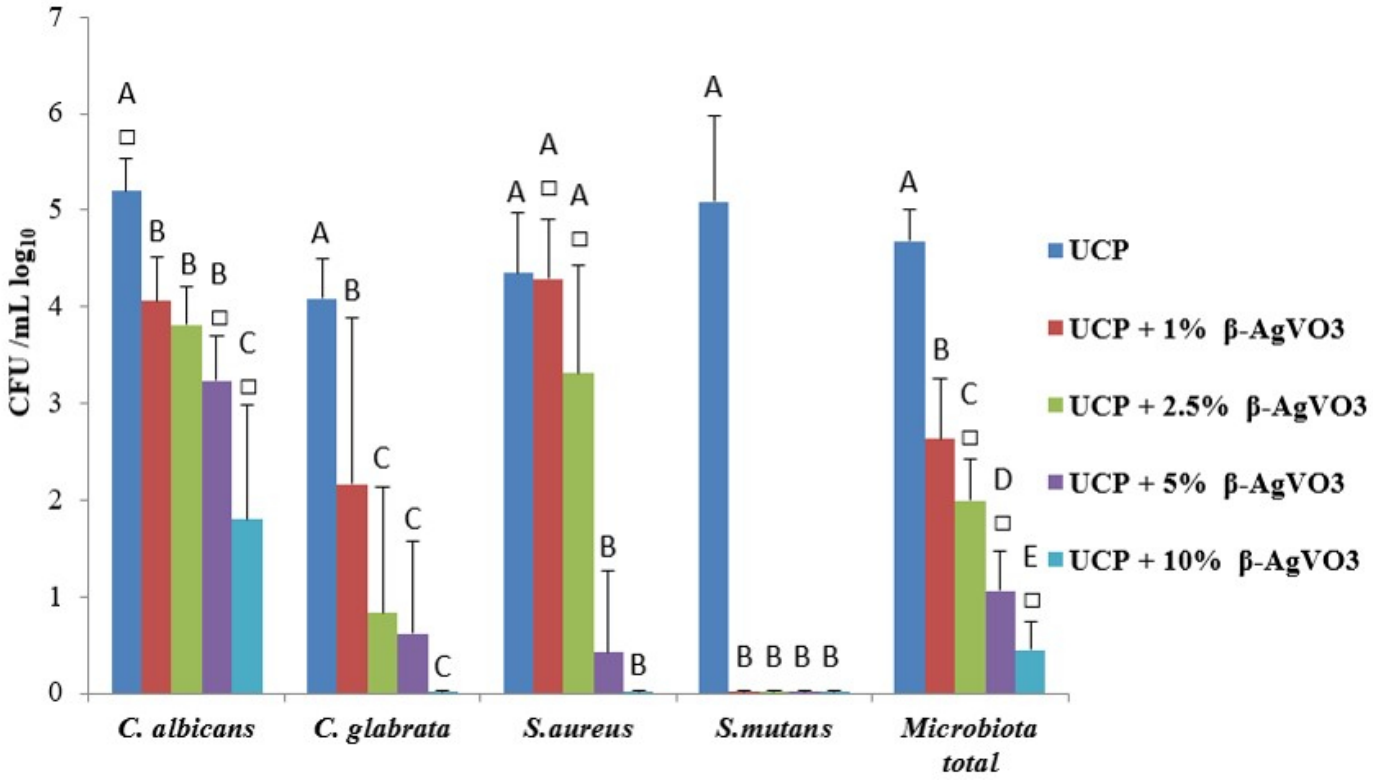

Figure 3: Colony forming units per milliliter (CFU/mL) of multispecies biofilm on the surface of PMMA samples treated with Ultra Corega Powder adhesive (UCP) associated with β-$AgVO_3$ or not. Different letters represent a statistically significant difference between different groups for the same microorganism, while the symbols represent a statistically significant difference between two commercial forms of the denture adhesive (P<0.05; two-way ANOVA test and Bonferroni post hoc test).

When comparing the two commercial forms, higher CFU/mL values were observed for powder adhesives than for cream (P<0.05) in the following groups: *C. albicans*: Control, 5% and 10% of β-$AgVO_3$; *S. aureus*: 1% and 2.5% of β-$AgVO_3$ and total microbiota: 2.5%, 5% and 10% of β-$AgVO_3$ *(Figures 2 and 3)*.

METABOLIC ACTIVITY

For both commercial forms of the denture adhesive, the XTT showed a reduction in biofilm metabolism starting at 2.5% of β-$AgVO_3$ (P<0.05).

The biofilm formed in the UCP + 2.5% and UCP + 5% groups showed greater metabolic activity compared to the biofilm formed in the same groups of the cream form (P<0.05) *(Figures 4 and 5)*.

BIOFILM MASS

Microscopic images show viable (green) and non-viable (red) cells in biofilms on the surface of samples *(Figure 6)*. Higher cell density and proportion of green cells were observed in the control and 1% β-$AgVO_3$ groups than in the other groups with higher nanomaterial percentages. Changes in cell viability (increase in the proportion of red/green fluorescence) and a reduction in the number of cells with more dark spaces between them were observed in samples containing β-$AgVO_3$. The images complement the previous results and show a higher cell density in the UCP groups compared to UCC.

DISCUSSION

In the present study, we added β-$AgVO_3$ in two commercial forms of the denture adhesive, a material commonly used by patients with removable prosthesis.[4,9-11] We chose β-$AgVO_3$ due to its proven antimicrobial effectiveness in studies in the dental field,[23] involving materials such as acrylic resin,[19,24] endodontic sealers,[20] resin cements,[25] porcelains,[22,26] and impression materials.[21] However, the effect of the association of β-$AgVO_3$ with denture adhesives has not been evaluated to date.

In the present study, a multispecies biofilm model was used because, although *Candida spp*. infection has been considered the main etiological factor in denture stomatitis, interactions between kingdoms are known to have important roles in maintaining the microbial community, which can affect host health and disease.[34]

In the oral cavity, the presence of *S. aureus* is associated with angular cheilitis, mucositis and periodontitis.[35] It is also suggested that complete dentures can be colonized by *S. aureus*, which in turn interacts with *C. albicans*, having its virulence and resistance increased.[36] Elderly individuals are generally more susceptible to heart and respiratory disease which can be further aggravated by staphylococcal infections.[37]

S. mutans is also involved in oral diseases and may interact with *C. albicans*, stimulating the virulence factors. There are reports of a coadhesion between these microorganisms, which favors the increased adhesion of *C. albicans* to the mucosa and resin, with effect on the progression of denture stomatitis.[35,38,39]

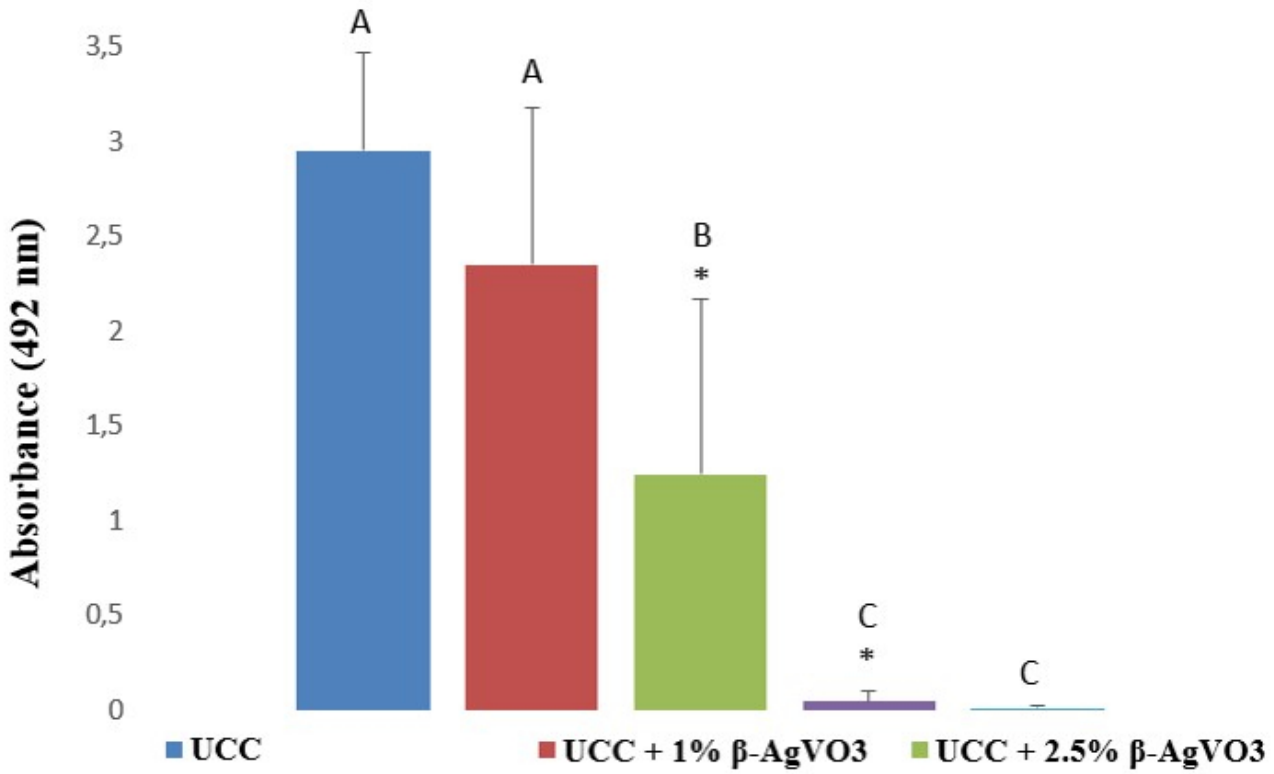

Figure 4: Metabolic activity of multispecies biofilm on the surface of PMMA samples treated with Ultra Corega Cream adhesive (UCC) associated with β-$AgVO_3$ or not. Different letters represent a statistically significant difference between different groups, while the symbols represent a statistically significant difference between two commercial forms of the denture adhesive ($P<0.05$; two-way ANOVA test and Bonferroni post hoc test).

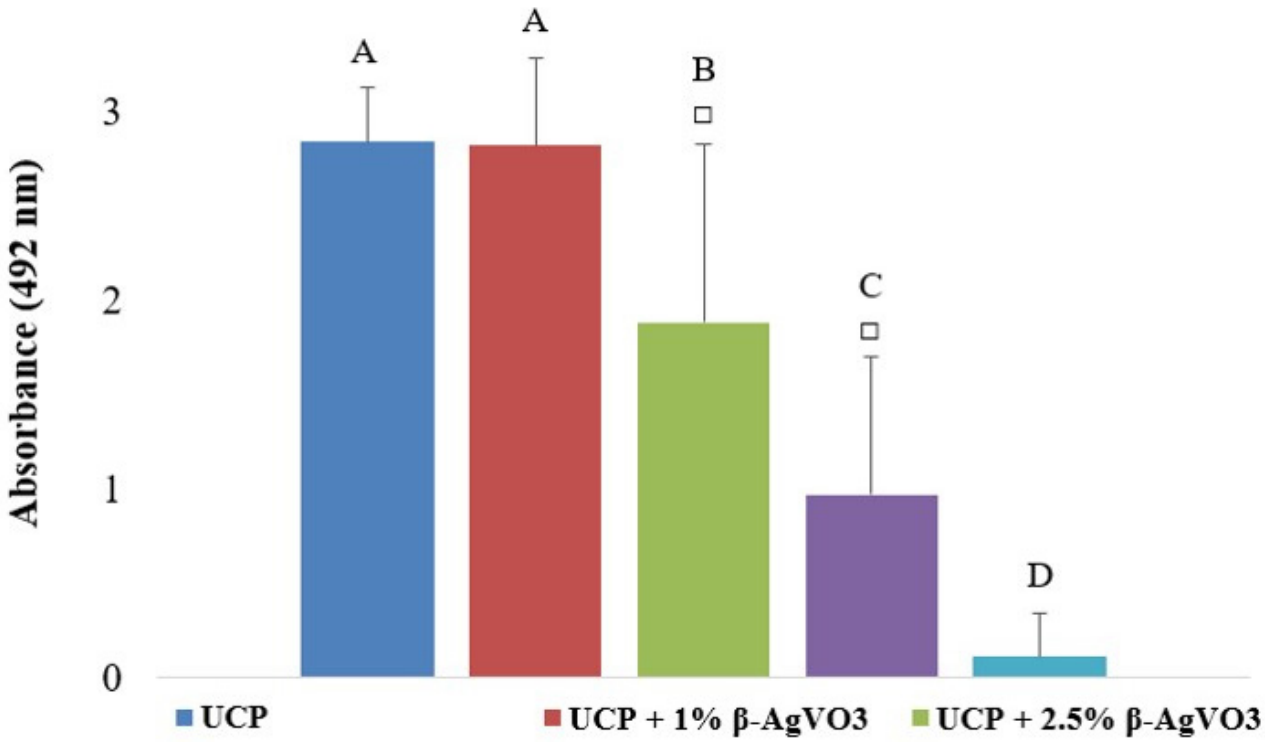

Figure 5: Metabolic activity of multispecies biofilm on the surface of PMMA samples treated with Ultra Corega Powder adhesive (UCP) associated with β-$AgVO_3$ or not. Different letters represent a statistically significant difference between different groups, while the symbols represent a statistically significant difference between two commercial forms of the denture adhesive ($P<0.05$; two-way ANOVA test and Bonferroni post hoc test).

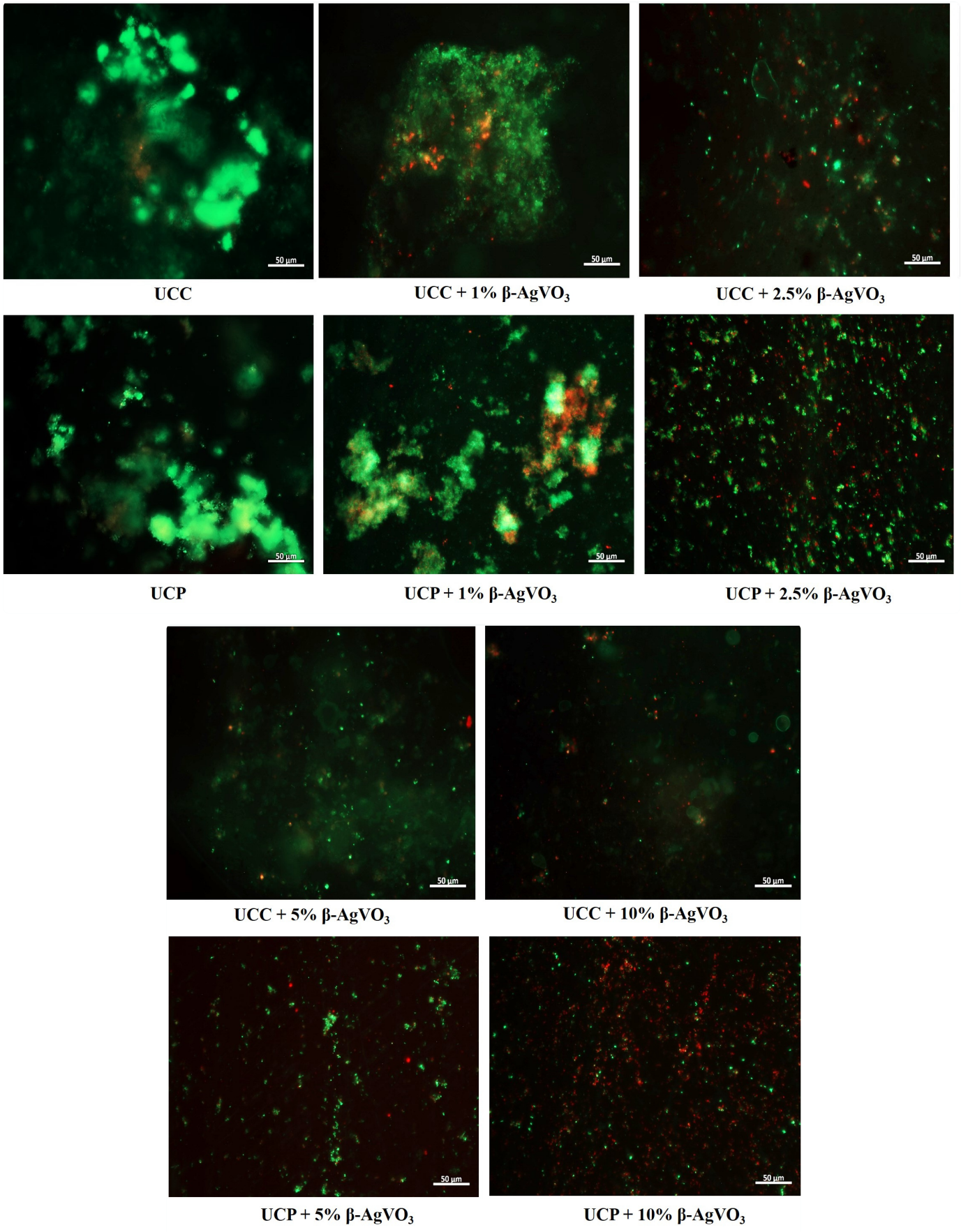

Figure 6: Fluorescence microscopy (×63) of viable (green) and non-viable (red) cells in multispecies biofilm formed on the specimens.

To study the association between adhesives and nanomaterial and the efficacy on the multispecies biofilm, an analysis with adhesives only was performed. In general, for both commercial forms, β-$AgVO_3$ promoted antimicrobial action in a dose-dependent manner; that is, as the percentage of β-$AgVO_3$ increased, the reduction of metabolic activity and of colony formation also increased, compared to the groups treated with adhesives only. This idea was also defended by de Castro *et al.* (2021),[24] who studied the incorporation of the same nanomaterial into acrylic resin and by Uehara *et al.* (2022)[26] who obtained a more evident antimicrobial performance of dental porcelain in the groups with higher percentages of β-$AgVO_3$.

β-$AgVO_3$ is a hybrid compound that dissociates into silver and vanadium ions. When in contact with the bacteria's DNA, silver prevent its replication and vanadium ions promote oxidative stress.[28] This mechanism of action may explain the results obtained. As in the present study, denture adhesives also showed better antimicrobial performance due to the incorporation of herbal compounds (*Equisetum giganteum* and *Punica granatum*),[9] miconazole,[40] peptides,[41] silver nanoparticles[4] and natural biopolymers (chitosan).[12]

When comparing the two forms of denture adhesives, some UCP groups showed higher microbial load and biofilm metabolic activity than the UCC. For example, for *C. albicans*, even with the reduction provided by the incorporation of β-$AgVO_3$, the UCP + 5% group showed an average of 3.23 log_{10} and the UCP + 10% group an average of 1.8 log_{10}. This was significantly different from the same UCC groups, which showed 2.4 log_{10} and complete inhibition of biofilm formation in the highest concentration group (10%). Oliveira Júnior *et al.* (2018)[10] also observed lower adhesion of *C. albicans*, both single and mixed species, to the UCC, but compared to the commercial strip form. However, clinically, the remaining colony count in the UCP + 5% and UCP + 10% groups may not be significant, as the Δ values (log_{10} CFU/mL) were -1.97 and -3.4, respectively, compared to the group without incorporation (5.2 log_{10}). According to the literature,[42] Δ (log_{10} CFU/mL) values of -1 (corresponding to 90% death), -2 (99% death) and -3 (99.9% death) are reported. So the results obtained show the antimicrobial potential of UCP with β-$AgVO_3$ also against *C. albicans*.

It can be inferred, therefore, that β-$AgVO_3$ was able to promote antimicrobial activity to both commercial forms of denture adhesives, which can be potentially innovative in the dental clinic since the use of local and systemic antifungal agents has been reported as the standard treatment for denture stomatitis. However, recurrent stomatitis is prevalent due to resistance to antifungal agents and prolonged and frequent use of these drugs can also lead to side effects.[43]

In this way, this study could contribute to future studies leading to technological innovations in the field of health sciences. Limitations include the fact that only ATCC strains were used, which limits the evaluation of results between different clinical strains. Therefore, future studies should be carried out to obtain more information on the proposed formulation, including comparison with the gold standard of practice, such as nystatin, and evaluations of cytotoxicity, mucoadhesion tests and drug release.

CONCLUSION

Denture adhesives in powder and cream form with β-$AgVO_3$ showed potential antimicrobial activity against multispecies biofilm. Powder adhesive showed higher biofilm formation than cream.

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