

Soft Liner with antimicrobial activity

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ABSTRACT | *Objective:* To develop a material for denture relining and assess the microbiological and mechanical properties. The proposed liner was obtained through the incorporation of nanostructured silver vanadate (AgVO_3) at 0, 1, 2.5, 5, and 10% polyethyl methacrylate (PEMA) containing plasticizer. *Methods:* Antimicrobial efficacy was evaluated by the Kirby Bauer agar method against *Enterococcus faecalis*, *Pseudomonas aeruginosa*, *Candida albicans* and *Staphylococcus aureus* ($n = 5$); and mechanical properties were also assessed through roughness, such as Shore A hardness and tensile test. The results were analyzed by ANOVA and Tukey's Multiple Comparison test ($\alpha = 0.05$). *Results:* The material with AgVO_3 at concentrations of 1% and 2.5% showed antimicrobial activity for *E. faecalis*, and 5% and 10% groups were effective for *E. faecalis*, *P. aeruginosa* and *C. albicans*. In the 5% group, hardness remained unchanged ($p < 0.001$). None of the tested concentrations significantly changed the roughness and the tensile strength ($P > 0.05$). *Conclusion:* Obtaining the material with antimicrobial potential promoted efficacy against *E. faecalis*, *P. aeruginosa* and *C. albicans*, kept the roughness property unchanged, did not change the adhesion property of the material to polymethyl methacrylate, and it maintained the hardness values compatible with resilient denture liners.

DESCRIPTORS | Denture Liners; Nanotechnology; Mechanical Properties; Polymers.

RESUMO | **Reembasadores resilientes com atividade antimicrobiana** • *Objetivo:* Desenvolver um material para refinação de próteses e avaliar suas propriedades microbiológicas e mecânicas. O revestimento proposto foi obtido por meio da incorporação de vanadato de prata nanoestruturado (AgVO_3) em 0, 1, 2,5, 5 e 10% de polimetilmetacrilato de metila (PEMA) contendo plastificante. *Métodos:* A eficácia antimicrobiana foi avaliada pelo método de Kirby-Bauer contra *Enterococcus faecalis*, *Pseudomonas aeruginosa*, *Candida albicans* e *Staphylococcus aureus* ($n = 5$); e propriedades mecânicas como rugosidade também foram avaliadas por meio do teste de dureza Shore A e teste de tração. Os resultados foram analisados por ANOVA e teste de Tukey ($\alpha = 0,05$). *Resultados:* O material com AgVO_3 nas concentrações de 1% e 2,5% apresentou atividade antimicrobiana para *E. faecalis*, e 5% e 10% foram eficazes para *E. faecalis*, *P. aeruginosa* e *C. albicans*. No grupo de 5%, a dureza permaneceu inalterada ($p < 0,001$). Nenhuma das concentrações testadas alterou significativamente a rugosidade e a resistência à tração ($P > 0,05$). *Conclusão:* A obtenção do material com potencial antimicrobiano promoveu eficácia contra *E. faecalis*, *P. aeruginosa* e *C. albicans*, manteve a propriedade de rugosidade inalterada, não mudou a propriedade de adesão do material ao polimetilmetacrilato e manteve os valores de dureza compatível com revestimentos de reembasadores de dentadura resilientes.

DESCRITORES | Reembasadores de Dentadura; Nanotecnologia; Propriedades Mecânicas; Polímeros.

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INTRODUCTION

Denture liners are used to modify the entire or partial denture base or implant supported prosthesis, and they function as a shock absorber,¹ distributing functional loads, preventing bone resorption,^{2,3} and allowing tissue remodeling during the healing phase,¹ transforming old prostheses into transitional ones,⁴ leaving them more stable until the complete surgical healing.⁵

Although the transition phase is necessary, adhesive failure,⁶ accumulation of microorganisms,^{2,3} unpleasant odor,⁷ and stiffening of the material⁸ can hinder long-term treatment and increase the risk of illness in older and debilitated patients under treatment for systemic diseases.^{9,10}

One proposal to solve such problems would be the development of a resilient line material with antimicrobial properties, which could result in better healing, reduction in bacterial proliferation and odor, stronger adhesiveness, and longer durability.

AgVO₃, a mixture of ammonium vanadate and silver nitrate was incorporated to the polyethyl methacrylate with plasticizer (Trusoft – Bosworth Company, Skokie, IL, USA) with the aim of proposing a soft liner material with satisfactory antimicrobial properties, with the possibility of treating oral infections and reducing the risk of systemic infections.

MATERIALS AND METHODS

Synthesis of nanostructure silver vanadate and preparation of the liner resin containing AgVO₃

Nanostructured silver vanadate (b-AgVO₃) is synthesized through a precipitation reaction between silver nitrate (AgNO₃, Merck 99.8%) and ammonium metavanadate (NH₄VO₃, Merck 99%) according to the methodology previously described.^{11,12} After the solubilization of 0.9736 g of NH₄VO₃ and 1.3569 g of AgNO₃ in 200 mL of distilled water, the AgNO₃ solution was added dropwise to the NH₄VO₃ solution under

constant stirring at 65°C. A precipitate was obtained and then washed with distilled water and absolute alcohol, filtered and dried in a vacuum line for 10 hours.

The Trusoft (Bosworth Company, Skokie, IL, USA) relining material was used in this study. The powder was weighed to calculate the amount of AgVO₃ to be incorporated; the concentrations of 0, 1, 2.5, 5, and 10% of mass were evaluated.

Preparation of specimens

The specimens were prepared following the manufacturer's recommendations. For the bond test, a matrix of 10 mm diameter and 20 mm length was used to obtain specimens in wax, which were invested in plaster in a metal frame. A duplicated matrix was then created for flasking and packing with self-curing acrylic resin (Clássico Produtos Odontológicos, SP, Brazil). One hundred cylinders of each acrylic resin were obtained and polished with 320 grit sanding paper. The cylinders were joined two by two with the lining resin using a 5-mm spacer, producing 50 specimens of each resin (Ø10 mm × 43 mm, n = 10).

The AgVO₃+relining resin compound was mixed for 30 to 40 seconds on a non-polished glass slab, inserted in a metal matrix (Ø 8 x 3 mm), and pressed between two glass slabs. The procedure was done according to the manufacturer's recommendations.

Hardness and roughness tests

Surface hardness analysis was performed using a micro-hardness tester (Shimadzu HMV 2000 Kyoto, Japan), with five random equidistant measurements in each specimen using a Shore A indenter. Roughness was measured in three spots in each specimen (one in the center and 2 at 1-mm from the central measure) with an analogue rugosimeter (Surftest SJ – 210 P, Mytutoyo, Japan).

Tensile Test

For the tensile test, specimens were placed under tension until failure in a universal testing machine

(EMIC, São José dos Pinhais, Brazil) at a crosshead speed of 5 mm/min, and data were collected by a PC using the Tesc software v.3.01 (EMIC, São José dos Pinhais, Brazil).

The failure types were observed visually and classified as Adhesive failure: total separation at the interface between the resilient liner material and the acrylic resin; Cohesive failure: tearing within the resilient liner material; and Mixed failure: both adhesive and cohesive failure.

Tensile bond strength was calculated with the equation below:

$$\sigma = F/A$$

where, σ = stress (MPa), F = maximum recorded force at failure (N), and A = original cross-sectional area (mm²).

Antimicrobial activity analysis

Strains of *Staphylococcus aureus* (ATCC 6538), *Pseudomonas aeruginosa* (ATCC 27853), *Candida albicans* (ATCC 90028), and *Enterococcus faecalis* (ATCC 29212) were thawed and cultured in a microbiological oven at 37°C for 24 h. Cultures were centrifuged at 6,000 g for 5 min, the supernatant was discarded, and the pellet washed twice with 10 mL of PBS (Phosphate Buffered Saline, containing NaCl, KCl, Na₂HPO₄, and KH₂PO₄). The suspensions were diluted in BHI medium, and the concentration confirmed by optical density using a spectrophotometer; cells were counted in a Neubauer chamber. After standardization, the culture was diluted in the concentration of 10⁶/CFU ml⁻¹. The culture media were heated at 50°C to add the microorganisms at the set concentration and poured onto the plates.

The agar diffusion method was performed (n = 5) to evaluate the antimicrobial activity. Samples of the relining resin (Ø 8 mm x 3 mm) were first exposed to UV light in a laminar flow hood for 30 min on each side for disinfection and then placed in petri dishes containing culture media.

After determining the data were normally distributed (Kolmogorov-Smirnov test), ANOVA was applied followed by Tukey's multiple comparison test ($\alpha = 0.05$) and the two proportions equality test to analyze differences in proportions (percentages). The software used was SPSS v 20.0.

RESULTS AND DISCUSSION

The nanostructured silver vanadate decorated with AgNPs (AgVO₃) is a hybrid material made up of ammonium vanadate and silver nitrate in nanoscale¹² whose non-formation of clusters¹² presents advantages when compared with other types of nanoparticles used as antimicrobials.² Nanomaterials, in general, among them AgNPs, are thermodynamically unstable and have a natural tendency to cluster. AgNPs are also free of stabilizing agents, have a high antibacterial activity on a wide spectrum of gram-positive and gram-negative bacteria, including strains of multi-resistant bacteria and a high dispersion of silver nanoparticles on silver vanadate nanowires, maintaining a high contact surface.¹²

We opted for the evaluation of antimicrobial capacity of polyethylene methacrylate with plasticizer incorporated with AgVO₃ against *Staphylococcus aureus*, *Pseudomonas aeruginosa*, *Enterococcus faecalis* and *Candida albicans* by the association between these microorganisms and oral and systemic diseases.¹³ These microorganisms are opportunistic agents¹⁴ present in skin and mucous membranes¹⁵ and can cause many serious acute and chronic infections, such as pneumonia and diseases of the respiratory system, endocarditis, meningitis, urinary tract infections, and nosocomial infections that may be lethal to geriatric and immunosuppressed patients.^{10,13}

The test performed by the Kirby-Bauer test, evaluating the incorporation of 5% and 10% of AgVO₃ to the liner showed antimicrobial activity for *Pseudomonas aeruginosa* and *Candida albicans*, as for *Pseudomonas aeruginosa* the 10% incorporation showed a higher antimicrobial capacity compared with

the other concentrations, and as to *Candida albicans* both 5 and 10% groups showed similar antimicrobial effect (Figure 1). For *E. faecalis*, AgVO₃-incorporated resin showed a dose-dependent antimicrobial activity, with 10% being the most effective. The incorporation of 1 and 2.5% AgVO₃ to the liner resin did not result

in antimicrobial activity for *P. aeruginosa* and *C. albicans*. These results are beneficial because they reduce the risk of systemic bacterial contamination since the proximity of the oral cavity and airways is a risk factor for aspiration of these microorganisms to the lower respiratory tract.^{10,13}

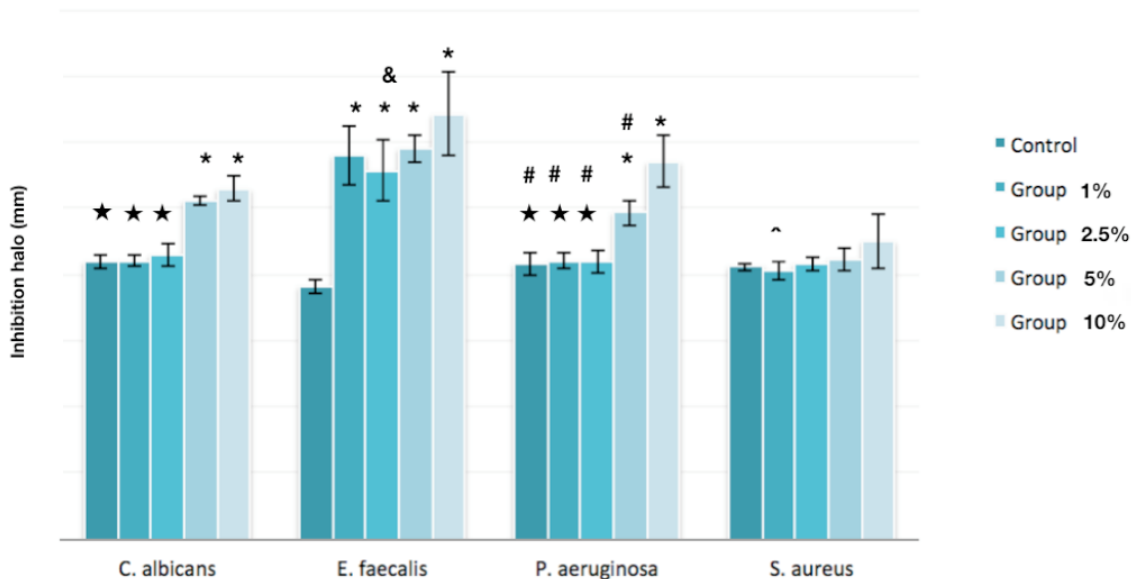


Figure 1 | Inhibition halo of AgVO₃ at different concentrations against different microorganisms.

* represents statistically significant difference between the control and other groups; & Represents statistically significant difference between 10% and 2.5% groups; # Represents statistically significant difference between 10% and all other groups; represents statistically significant difference between 5% and control group, 1% and 2.5% groups; ^ represents statistically significant difference between 10% and 1% groups.

It is difficult to wrestle *S. aureus*, especially with emergence of drug-resistant strains.¹⁶ Although, silver nanoparticles synthesized by different agents such as sodium citrate, hydrazine hydrate, citrus sinensis peel extract have shown effectiveness against *S. aureus*;¹⁷ the incorporation of AgVO₃ did not show effectiveness against this microorganism at any concentration.

The hardness property has remained unchanged when incorporated AgVO₃ at 5%, already with 1, 2.5, and 10% the Shore A hardness decreased ($p < 0.05$) (Table 1). The incorporation of 1% AgVO₃ produced specimens with the lowest Shore A hardness value (average of 43.77) in comparison with the other groups, which is compatible with the ISO recommendation of 40 (DIN 53505 and ASTM D2240 / 75) for soft reliners.

Table 1 | Hardness Shore A values for the control group and experimental groups with different concentrations of AgVO₃.

	Control	Group 1%	Group 2.5%	Group 5%
Group 1%	<0.001			
Group 2.5%	<0.001	0.002		
Group 5%	1.000	<0.001	<0.001	
Group 10%	0.003	<0.001	0.950	0.002

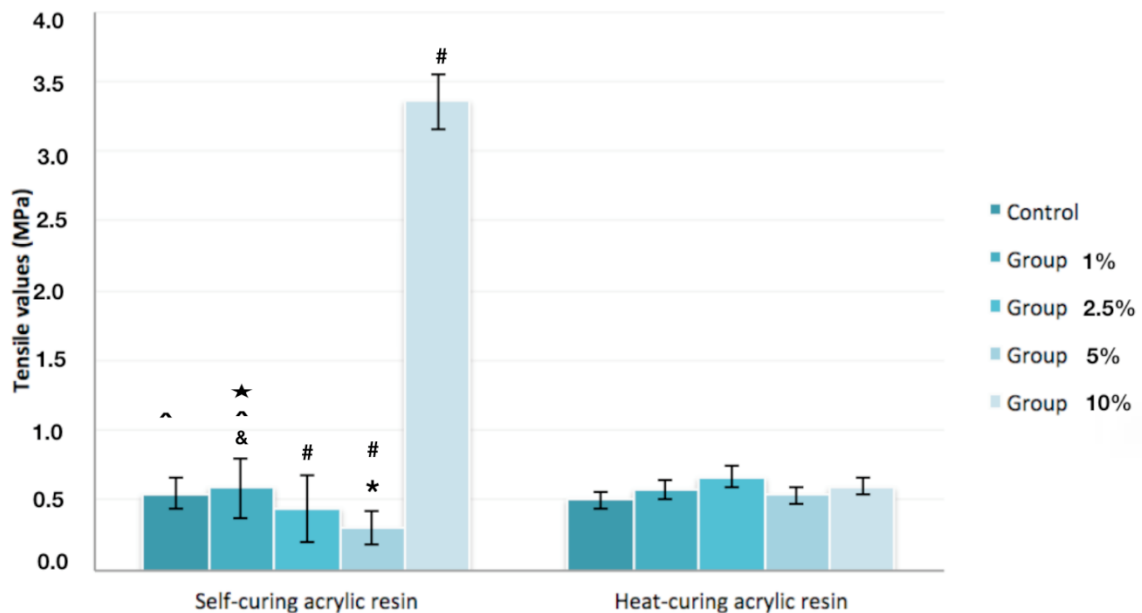
The addition of the nanomaterial at concentrations ranging from 0 to 10% did not significantly change roughness (Table 2), which is very beneficial because rough surfaces increase the susceptibility of soft liners to microbial colonization.¹⁰

Table 2 | Mean, median, and standard deviation for the roughness property of a denture liner incorporated with nanostructured silver vanadate at different concentrations.

Roughness	Mean	Median	SD	CV	Min	Max	N	IC	P-Value
Control	5.99	6.16	0.83	14%	4.18	7.48	20	0.36	0.144
Group 1%	6.24	6.21	0.82	13%	4.38	8.26	20	0.36	
Group 2.5%	6.56	6.55	0.85	13%	5.31	8.19	20	0.37	
Group 5%	6.17	6.03	0.71	11%	4.96	7.85	20	0.31	
Group 10%	5.84	6.07	1.28	22%	2.12	7.94	20	0.56	

The adhesion property of the obtained liner to the thermo-polymerizable polymethylmethacrylate base was tested by a tensile test, and we observed the incorporation of AgVO_3 did not promote changes in this property (Figure 2). However, statistical differences between groups were

observed when self-polymerizable resin ($P < 0.05$) was used, with a reduction in the adhesion property in the group of 5% compared with the control ($P = 0.005$). Regarding the types of failure, 2.5% group showed 50% of mixed failures, and 10% had 60% of adhesive failures.

**Figure 2** | Tensile strength values between a denture liner incorporated with nanostructured silver vanadate at different concentrations.

* represents statistically significant difference between control and 5% groups; # represents statistically significant difference of 1% and 2.5%, 5% and 10% groups; & represents statistically significant difference of 2.5% group compared to 1%; ^ represents statistically significant difference of 5% group compared to control and 1%; # represents statistically significant difference between 10% and 1% groups.

Previous studies on antimicrobial incorporation to lining materials differ and reports of highly efficient combinations are scarce. However, the incorporation of different antimicrobials, such as silver nanoparticles,¹⁸ antifungal drugs,^{7,19}

origanum oil,²⁰ silver zeolite,² and seed oils²¹ have found decreased bond strength,¹⁸⁻²⁰ changes in type of failure,¹⁸ decrease in viscoelasticity,^{2,8} roughness,^{7,20} hardness alterations,⁷ and tissue irritation.²⁰

The incorporation of AgVO₃ to the polyethyl methacrylate with plasticizer showed excellent results, helping when the lining is required and also in situations where it can assist in the treatment of infectious complications.

CONCLUSIONS

The results suggest the material proposed with 5% of AgVO₃ was efficient against *P. aeruginosa*, *E. faecalis*, and *C. albicans* and maintained the properties of hardness, adhesiveness and roughness unchanged.

CONFLICTS OF INTEREST

None.

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