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Cytotoxicity and Elemental Release of Dental Acrylic Resin Modified with Silver and Vanadium Based Antimicrobial Nanomaterial

Citotoxicidade e Liberação Elementar de Resina Acrílica Odontológica Modificada com Nanomaterial Antimicrobiano a Base de Prata e Vanádio

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Abstract

The acrylic resin used for the prosthesis base accumulates biofilm, causing diseases such as stomatitis. The addition of some nanoparticles promotes antimicrobial action. This study incorporated the nanostructured silver vanadate decorated with silver nanoparticles ($AgVO_3$) to the acrylic resin by two methods and evaluated the cytotoxicity for human gingival fibroblasts (HGF) and the released silver and vanadium ions. The concentrations of 0.5, 1, 2.5, and 5% of $AgVO_3$ was incorporated by vacuum spatulation and polymeric film. The vacuum spatulation was performed for 60 s using the Turbomix equipment, and the polymeric film was obtained from the polymer solubilization in chloroform, the film was subjected to a cryogenic grinding, and the powder obtained was manually mixed at the monomer. HGF cell viability was assessed after 24 hours, 7 and 14 days by the MTT assay. The release of silver (Ag) and vanadium (V) ions were quantified by inductively coupled plasma mass spectrometry after 30 days. Kruskal-Wallis and Dunn's test were applied ($\alpha = 0.05$). The HGF viability was inversely proportional to the incubation time. Both incorporation techniques and the negative and positive control groups presented significant statistical differences ($p<0.05$). The experimental groups presented no statistical difference compared to the negative control ($p>0.05$), except the vacuum spatulation group with 5% of $AgVO_3$ that showed greater viability than the negative control ($p=0.013$) in 24 hours. The release of Ag and V ions was proportional to the concentration of $AgVO_3$ The 5% group presented a significant difference compared to the other groups ($p<0.05$). In conclusion, the acrylic resin with and without the $AgVO_3$ incorporation had a small cytotoxic potential for HGF in 24 hours, with a lower viability in longer contact times; the release of Ag and V ions was proportional to the concentration of $AgVO_3$, not influencing cell viability.

Keywords: Acrylic Resins. Cell Survival. Nanotechnology. Ions.

Resumo

A resina acrílica utilizada para a base da prótese acumula biofilme, causando doenças como a estomatite. A adição de algumas nanopartículas promove ação antimicrobiana. Este estudo incorporou o vanadato de prata nanoestruturado decorado com nanopartículas de prata ($AgVO_3$) à resina acrílica por dois métodos e avaliou a citotoxicidade para fibroblastos gengivais humanos (HGF) e os íons prata e vanádio liberados. As concentrações de 0,5%, 1%, 2,5% e 5% de $AgVO_3$ foram incorporadas por espatulação a vácuo e filme polimérico. A espatulação a vácuo foi realizada por 60 s no equipamento Turbomix, e o filme polimérico foi obtido a partir da solubilização do polímero em clorofórmio, o filme foi submetido a uma moagem criogênica e o pó obtido foi misturado manualmente ao monômero. A viabilidade celular de HGF foi avaliada após 24 horas, 7 e 14 dias pelo ensaio de MTT. A liberação de íons prata (Ag) e vanádio (V) foi quantificada por espectrometria de massa com plasma indutivamente acoplado após 30 dias. Os testes de Kruskal-Wallis e Dunn foram aplicados ($\alpha=0,05$). A viabilidade de HGF foi inversamente proporcional ao tempo de incubação. As técnicas de incorporação e os grupos controle negativo e positivo apresentaram diferença estatisticamente significante ($p<0,05$). Os grupos experimentais não apresentaram diferença estatística em relação ao controle negativo ($p>0,05$), exceto o grupo de espatulação a vácuo com 5% de $AgVO_3$ que apresentou maior viabilidade que o controle negativo ($p = 0,013$) em 24 horas. A liberação de íons Ag e V foi proporcional à concentração de $AgVO_3$. O grupo 5% apresentou diferença significativa em relação aos demais grupos ($p <0,05$). Em conclusão, a resina acrílica com e sem a incorporação de $AgVO_3$ apresentou um pequeno potencial citotóxico para o HGF em 24 horas, com menor viabilidade nos tempos de maior contato, e a liberação de íons Ag e V foi proporcional à concentração de $AgVO_3$, não influenciando na viabilidade celular.

Palavras-chave: *Resinas Acrílicas. Sobrevivência Celular. Nanotecnologia. Íons.*

1 Introduction

Polymethylmethacrylate (PMMA) is the most used material for the manufacturing of dental prostheses, and although it meets the required characteristics, surface roughness and microporosities favor the biofilm formation.[1-4] In addition, the majority of complete denture users have advanced age, physical and mental disabilities, systemic diseases, immunosuppression, and impaired manual dexterity for a proper hygiene,[1-3] thus increasing the risk of local problems, such as prosthetic stomatitis,[5,6] and systemic infections due to inhalation and ingestion of microorganisms that detach from the mucosa and the denture base.[7]

The methods recommended for biofilm removal from the denture can damage the acrylic resin.[8] A suggested alternative was the addition of biocidal agents to the acrylic resin, such as silver nanoparticles, quaternary ammonium, nanosilicon dioxide, nanotitanium dioxide, 2-tert-butylaminoethyl methacrylate.[1,3,5,9-11] Among these agents, nanomaterials have a higher chemical reactivity and antimicrobial potential due to their greater surface area/mass ratio.[12]

Nanostructured silver vanadate decorated with silver nanoparticles ($AgVO_3$) is a hybrid nanomaterial, composed of vanadium and silver, which synergistically interact with the cell membrane of microorganisms. In addition to the antimicrobial effect of silver nanoparticles, this compound solves the limitation of dispersion of these particles due to the vanadate nanowires.[13] When incorporated into the acrylic resin, $AgVO_3$ inhibited the growth of pathogens such as *Candida albicans*, *Streptococcus mutans*, *Staphylococcus aureus* and *Pseudomonas aeruginosa,*[14,15] and when incorporated into soft denture liner also presented antimicrobial effect against *C. albicans*, *P. aeruginosa* and *Enterococcus faecalis*.[16]

Modifying the composition of restorative materials can cause the release of toxic substances, leading to irritation or allergic reactions in the oral mucosa.[1,2,11] The toxicity of silver nanoparticles, which depends on the concentration,[12] may induce necrosis or cellular apoptosis. In contrast, released ions are essential for the antimicrobial efficacy.[5]

The objective of this study was to evaluate the cell viability of human gingival fibroblasts (HGF) in contact with a heat-cured acrylic resin incorporated with $AgVO_3$ by two different methods, and the concentration of silver and vanadium ions released. The hypothesis tested was that the concentration and incorporation method would influence HGF viability and the concentrations of ions.

2 Material and Methods

2.1 Synthesis of $AgVO_3$ and sample preparation

The nanostructured silver vanadate was synthesized through a precipitation reaction between silver nitrate ($AgNO_3$, 99.8%, Merck KGaA, Darmstadt, Germany) and ammonium vanadate (NH_4VO_3, 99%, Merck KGaA, Darmstadt, Germany).[13] The morphology of the nanomaterial obtained was analyzed by scanning transmission electron microscopy (Magellan 400L; FEI Company, Hillsboro, OR, EUA).

The samples of heat-cured acrylic resin (0, 1, 2.5, and 5%) were obtained by two different methods using the 3:1 powder/liquid ratio.[17] In the first method, vacuum spatulation was performed for 60 s using the Turbomix equipment (EDG, São Carlos, SP, Brazil). In the second method, a polymeric film was obtained from the polymer solubilization in chloroform (Synth, Diadema, SP, Brazil), since the solubility parameter (δH = 19.0) is similar to PMMA (δH = 18.8). First, 10 g of the polymer was solubilized in 100 mL of chloroform and $AgVO_3$ in the tested concentrations were dissolved in 5 mL of the solvent. The $AgVO_3$ suspension was added to the polymer solution, which was then stirred for 15 min on a magnetic stirring plate. Then, the solution remained in an exhaust hood until the solvent evaporated completely and a film was obtained, which was then subjected to cryogenic grinding in a Mikro-Bantam mill (Model CF, Micron Powder Systems, New Jersey, USA). The powder obtained was manually mixed. Both methods had a control group without $AgVO_3$.[17]

For the specimens preparation, impression trays (Ø9 mm x 2 mm) were made by embedding cylindrical metal matrices in dental plaster type III (Gesso Rio, Rio Claro, Brazil) and condensation silicone (Zetalabor, Zermack, Badia Polesine, Italy). While still in the gelation stage, the resin was inserted into the impression trays and polymerized by conventional heating (immersion in water at 73 °C for 90 min and boiling for 30 min) in a thermocycler (Thermocycler T100, Ribeirão Preto, SP, Brazil).[17] After finishing and polishing, the samples of 9 mm diameter x 2 mm thickness were sterilized with ethylene oxide. The specimens surface characterization was performed by scanning electron microscopy (SEM - ZEISS model EVO 50, Cambridge, United Kingdom) with a 20 kV electron beam, using SE detector.

2.2 Cell viability assay

Human Gingival Fibroblasts (HGF) were cultured in Dulbecco's modified Eagle medium (DMEM, Gibco, Thermo Fisher Scientific, Waltham, MA, USA) supplemented with 10% fetal bovine serum (FBS, Cultilab, Campinas, SP, Brazil) and 1% penicillin and streptomycin (Sigma, St. Louis, MO, USA) at 37 °C, 95% oxygen and 5% CO_2 (Series, Shell Lab, Cornelius, OR, USA). Cells (3 x 10^3/well) were seeded in a 24-well plate on the surface of each specimen and in the negative (HGF + DMEM, no treatment) and positive (distilled water, control cytotoxic) control wells (n = 3). Plates were incubated at 37°C and 5% CO_2 for 24 hours, 7, and 14 days. Afterwards, the culture medium was removed and 500 µL of MTT (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide) solution of the proliferation quantification kit was added (5 mg of MTT/mL of DMEM without phenol red), and incubated for 4 hours at 37 °C. The solution was then removed and dimethyl sulfoxide solvent (DMSO, Synth, Diadema, SP, Brazil) added for 20 min at room temperature. The contents of each well were transferred to a 96-well plate and the absorbance read in a spectrophotometer at 550 nm (SYNERGY MX Monochromator-based reader, Biotek, Winooski, VT, USA). The analysis was performed in triplicate (n=3). Cell viability was reported as a percentage of the negative control (100% viable).

2.3 Analysis of metal ions release

The release of silver (Ag) and vanadium (V) ions was evaluated by inductively coupled plasma mass spectrometry (ICP-MS). Samples in polypropylene tubes (Becton Dickinson, Franklin Lakes, NJ, USA) with 9 mL of deionized water were suspended by a nylon thread, and incubated at 37°C for 30 days (n = 3). Then, the samples were removed from the tubes and the water was analyzed on NexIon 300X (PerkinElmer, Waltham, MA, USA), by the equipament calibration curves. The analysis was performed in triplicate (n=3). The results obtained in parts per billion (ppb) were converted to concentration (mg/L).

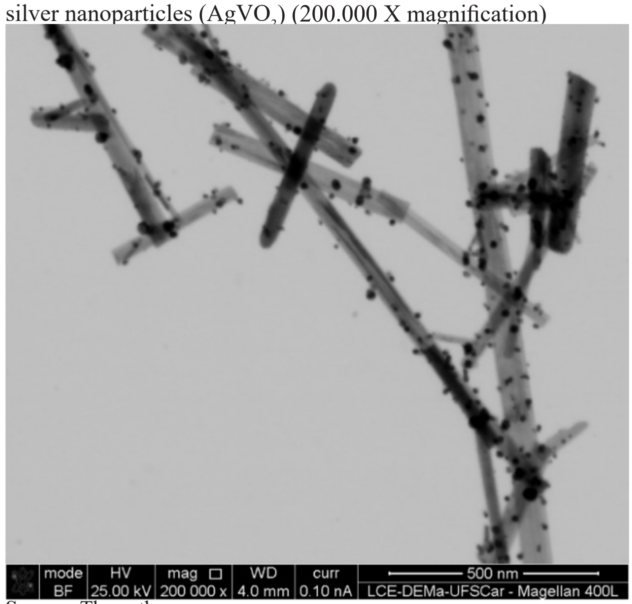
2.4 Statistical analysis

For the data analysis, the Kruskal-Wallis test and Dunn's post-hoc (α = 0.05) were applied using the software SPSS v 20.0 (SPSS Inc., Chicago, IL, USA).

3 Results and Discussion

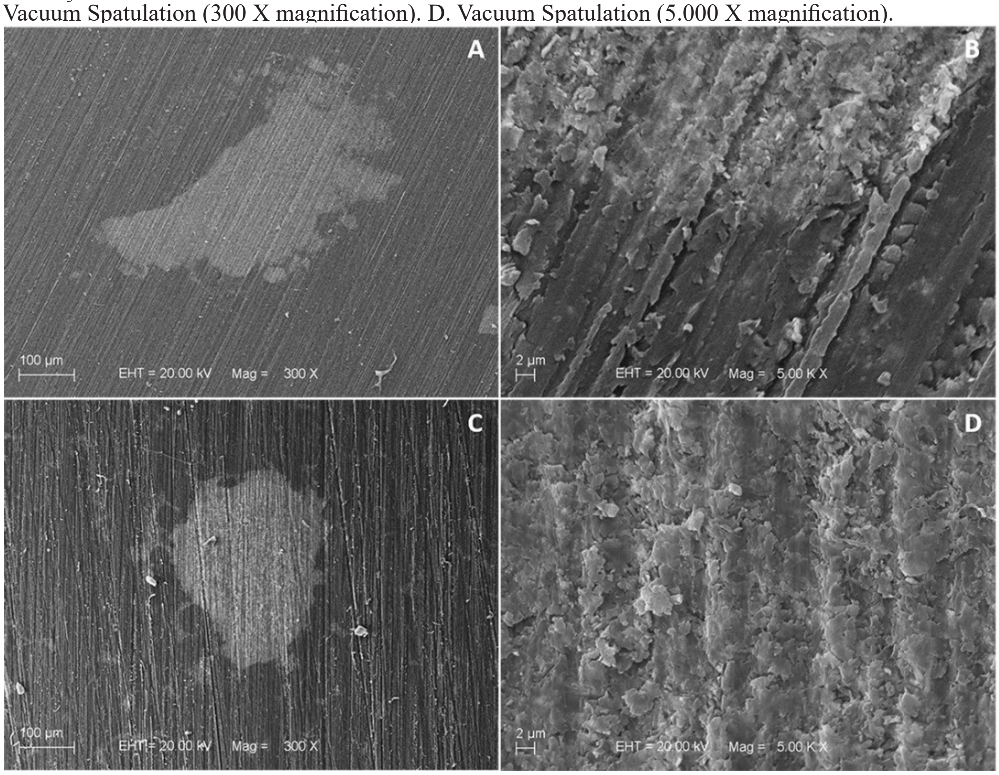
The powder of $AgVO_3$ photomicrograph demonstrated nanowires of silver vanadate with nano and micrometric dimensions, with an average diameter of 150 nm, coated with semi-spherical silver nanoparticles with 25 nm (Figure 1). And through the specimens surface photomicrograph obtained by SEM, it was observed that the $AgVO_3$ powder formed clusters around the pre-polymerized polymer particles, for both methods of incorporation (Figure 2).

Figure 1 - Photomicrograph by scanning transmission electron microscopy of the nanostructured silver vanadate decorated with silver nanoparticles ($AgVO_3$) (200.000 X magnification)



Source: The authors.

Figure 2 - Photomicrograph by scanning electron microscopy of the acrylic resin containing 5% of $AgVO_3$. A. Polymeric Film (300 X magnification). B. Polymeric Film (5.000 X magnification). C. Vacuum Spatulation (300 X magnification). D. Vacuum Spatulation (5.000 X magnification).



Source: The authors.

The viability of HGF in contact with the acrylic resin incorporated with $AgVO_3$ was inversely proportional to the incubation time. Both incorporation techniques (Table 1), negative and positive (cytotoxic) control groups were significantly different (p=0.008). The experimental groups presented intermediate values, with no statistical difference compared to the negative control (p>0.050), except for samples in the vacuum spatulation technique, which after 24 hours in contact with the cells, the 5% $AgVO_3$-modified group showed greater viability than the negative control (p=0.013). In addition, all the experimental groups exhibited a significant reduction in the viability of HGFs after 14 days compared to 24 hours (p=0.027), except for the 0% $AgVO_3$ obtained by vacuum spatulation, which did not change with time (p=0.066). Regardless the concentration and incubation time, cell viability did not differ between the two incorporation methods (p>0.050).

Table 1 - Human Gingival Fibroblasts Cell Viability (%) in contact with acrylic resins modified with different concentrations of $AgVO_3$ by two different techniques, determined by MTT assay, for three periods of time (n=3). Negative control: HGF + culture medium; Positive control: distilled water, cytotoxic

Group	24 hours	7 days	14 days
Control negative	100 [97.52;100.82]^{A}	100 [98.82;115.68]^{A}	100 [96.17;106.73]^{A}
Control positive	31.40 [30.57;33.88]^{Ba}	8.01 [6.13;8.84]^{Bab}	1.59 [1.45;1.59]^{Bb}
Polymeric film 0%	67.76 [66.11;82.64]^{Aba}	30.77 [30.42;37.38]^{ABab}	19.18 [11.09;26.59]^{ABb}
Polymeric film 0.5%	66.11 [65.28;68.59]^{Aba}	17.80 [16.15;18.75]^{ABab}	2.85 [2.76;3.43]^{ABb}
Polymeric film 1%	64.46 [61.15;75.20]^{Aba}	14.97 [13.67; 15.09]^{ABab}	2.66 [2.51; 10.80]^{ABb}
Polymeric film 2.5%	76.85 [76.03;80.16]^{Aba}	19.45 [18.86;20.04]^{ABab}	3.73 [3.73;3.77]^{ABb}
Polymeric film 5%	78.51 [72.72;81.81]^{Aba}	25.23 [23.70;26.17]^{ABab}	5.32 [5.32;6.73]^{ABb}
Vacuum spatulation 0%	58.67 [53.71; 61.15]^{Aba}	33.25 [14.74; 48.70]^{ABa}	35.70 [29.94; 42.78]^{ABa}
Vacuum spatulation 0.5%	101.65 [79.33;120.66]^{Aba}	19.33 [18.39;23.70]^{ABab}	4.60 [4.40;5.13]^{ABb}
Vacuum spatulation 1%	120.66 [85.95;133.05]^{Aba}	29.36 [20.16;32.66]^{ABab}	4.69[4.26;4.84]^{ABb}
Vacuum spatulation 2.5%	97.52 [76.03;136.36]^{Aba}	42.80 [38.79;50.70]^{ABab}	9.68 [9.10;27.71]^{ABb}
Vacuum spatulation 5%	195.04[124.79;209.09]^{Ba}	49.41 [40.33;51.53]^{ABab}	12.74 [10.07;15.40]^{ABb}

Median [minimum and maximum]. ^{AB} Equal capital letters indicate statistical similarities in the column (p<0,05). ^{ab} Equal lowercase letters indicate statistical similarities in the line (p<0,05). Kruskal-Wallis test and Dunn's post-hoc.

Source: Research data.

The modification of acrylic resin with different concentrations of $AgVO_3$ influenced the amount of leached Ag and V ions. The addition of 5% promoted the release of higher concentrations of Ag and V in both techniques. No statistical difference was observed between the two incorporation methods (p>0.050) (Table 2).

Table 2 - Concentration of Ag and V ions (mg/L) released from acrylic resins modified with different concentrations of $AgVO_3$ and obtained by two different techniques, in distilled water by 30 days, determined by ICP-MS (n = 3)

	Polymeric film		Vacuum spatulation	
[$AgVO_3$]	**Ag**	**V**	**Ag**	**V**
0%	0.006 [0.004;0.013]^{A}	0.016 [0.009;0.019]^{A}	0.006 [0.004;0.013]^{A}	0.016 [0.009;0.019]^{A}
0.5%	0.190 [0.140;0.190]^{AB}	0.140 [0.120;0.160]^{AB}	0.190 [0.140;0.190]^{AB}	0.140 [0.120;0.160]^{AB}
1%	0.280 [0.280;0.300]^{AB}	0.190 [0.170;0.190]^{AB}	0.280 [0.280;0.300]^{AB}	0.190 [0.170;0.190]^{AB}
2.5%	0.700 [0.550;0.780]^{AB}	0.390 [0.330;0.410]^{AB}	0.700 [0.550;0.780]^{AB}	0.390 [0.330;0.410]^{AB}
5%	2.360 [2.080;2.360]^{B}	1.150 [0.980;1.300]^{B}	2.360 [2.080;2.360]^{B}	1.150 [0.980;1.300]^{B}

Median [minimum and maximum]. ^{AB} Equal capital letters indicate statistical similarities in the column (p<0,05). Kruskal-Wallis test and Dunn's post-hoc.

Source: Research data.

The modification of dental materials to achieve antimicrobial effects should result in materials with long-term stability, preserved physico-chemical and mechanical properties, and biocompatibility. After confirming the antimicrobial effectiveness of the $AgVO_3$ incorporation into an acrylic resin by two different methods,[17] in the present study, the biocompatibility and release of the metal ions of the nanomaterial were investigated.

The hypothesis tested was partially accepted. The incorporation of different concentrations of $AgVO_3$ did not promote differences in HGF viability compared to the group without the nanomaterial (0%). A reduction in viability was observed with increased time of exposure. The concentration of released metal ions (Ag and V) was proportional to the concentration of $AgVO_3$ incorporated. The hypothesis that the incorporation methods would influence the results was rejected.

The acrylic resin commonly used for denture bases can release residual monomers from the polymerization process that cause mucosal irritations and can inhibit cell proliferation.[2,18,19] The acrylic resin cytotoxicity is more influenced by differences in composition than by different polymerization methods.[2] In this study, modification of the composition with the addition of $AgVO_3$ presented results similar to acrylic resin without the compound, reducing HGF viability in relation to the negative control, without the influence of the incorporation methods.

The direct contact cytotoxicity test was performed according to ISO 10993-5,[20] for which the material is cytotoxic if cell viability is lower than 30%, and between 30% and 70%, it has only cytotoxic potential. In this study, samples obtained by the polymeric film method showed cytotoxic potential after 24 hours of contact with cells, including the 0% group. After this time, the groups incorporated with $AgVO_3$ by vacuum spatulation presented greater cell viability than the negative control, which may be due to the microporosities present on the acrylic resin surface that favor cell development.

The viability of HGF was inversely proportional to the contact time with the samples, since results of cytotoxic effects (viability <30%) were observed for the majority of groups at 7 and 14 days. This may be due to the applied methodology since the direct contact of the cells with the specimens can cause stress and reduction of viability. This reduction in HGF viability cannot be associated with the nanomaterial incorporation since similar results were also observed in the groups without $AgVO_3$ (0%). The free acrylic resin monomers might leach into the medium (water or saliva) together with ions and other substances, being considered as the cause of cytotoxic effects of such materials.[19]

Studies have reported that chemical solutions used to clean dentures , such as sodium hypochlorite and chlorhexidine digluconate, have a cytotoxic potential to oral mucosa cells.[21-24] Similarly, antimicrobial additives incorporated into the acrylic resin release potentially toxic substances, causing reactions in oral tissues.[1,2,11] Silver nanoparticles, of which toxicity is concentration-dependent,[5,12] may decrease the mitochondrial function in murine neuroblastoma cells, hepatic cells, germline stem cells, human skin carcinomas, human skin keratinocytes, and fibroblasts.[4] Therefore, the antimicrobial action of these products may be associated with deleterious effects in different cell lines.

The release of metal ions is essential for the antimicrobial activity of $AgVO_3$, allowing an electrostatic interaction between the nanoparticles and the bacterial cell membrane, causing damage and cell death.[4,5,9,16] The silver (Ag^+) and vanadium ions (V^{4+}/V^{5+}) are cations and can interact with negatively charged bacteria. Ag^+ and V^{5+} act in the thiol groups of the bacterial metabolism enzymes, forming stable complexes, prevent the DNA replications, and the V^{4+} to V^{5+} oxidation-reduction causes reactive oxygen species.[16,19,25,26] The ionic charge in the denture base also favors the adsorption of defense molecules in saliva, inhibiting biofilm formation.[4]

In this study, the release of Ag and V ions was proportional to the concentration of $AgVO_3$ incorporated, with a higher amount of Ag ions than V ions, which is in accordance with the nanomaterial synthesis, since 2 mmol of silver nitrate is added against 1 mmol of ammonium metavanadate.[13] The $AgVO_3$ cytotoxicity to *Daphnia similis*, an aquatic organism, was attributed to silver,[27] and when incorporated into endodontic sealers, the silver was considered the $AgVO_3$ component cytotoxic for HGF.[25] In addition, Yin et al.[28] reported that the silver concentration required to reduce HGF cell viability by 50% is approximately 0.04 µg/L. The amount of Ag released in this study was higher than the silver IC_{50} for HGF and the groups cytotoxicity with 5% of $AgVO_3$, for example, was not influenced by the ions released, but the toxicity of the acrylic resin itself.

With time, the leaching of these elements may cause the loss of the antimicrobial effect, as it occurs with antifungal and antiseptics incorporated into PMMA that are continuously released and their efficacy decreases over time.[29] Investigations on the release of Ag and V ions at distinct periods are recommended.

The results obtained in this study support the association of $AgVO_3$ with acrylic resin, since this innovative proposal exhibited a small cytotoxic potential to HGF after 24 hours of direct contact, and the reduction in cell viability could not be attributed to the nanomaterial. In addition, the release of the metal ions Ag and V assists in the antimicrobial effect without influencing toxicity. Further research on $AgVO_3$ genotoxic potential should be undertaken.

4 Conclusion

It was concluded that the acrylic resin with and without the $AgVO_3$ incorporation by different methods showed a mild cytotoxic potential for HGF after 24 hours, with reduced viability for longer contact time. The release of Ag and V ions was proportional to the concentration of $AgVO_3$ incorporated into the resin, without influencing the cell viability.

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References

1. Mirizadeh A, Atai M, Ebrahimi S. Fabrication of denture base materials with antimicrobial properties. J Prosthet Dent 2018;119:292-8. doi: 10.1016/j.prosdent.2017.03.011. 2 . Masetti P, Arbeláez MIA, Pavarina AC, Sanitá PV, Jorge JH. Cytotoxic potential of denture base and reline acrylic resins after immersion in disinfectant solutions. J Prosthet Dent 2018;120:155.e1-7. doi: 10.1016/j.prosdent.2018.01.001.

3. Compagnoni MA, Pero AC, Ramos SMM, Marra J, Paleari AG, Rodriguez LS. Antimicrobial activity and surface properties of an acrylic resin containing a biocide polymer. Gerodontology 2014;31:220-6. doi: 10.1111/ger.12031.

4. Sivakumar I, Arunachalam KS, Sajjan S, Ramaraju AV, Rao B, Kamaraj B. Incorporation of Antimicrobial Macromolecules in Acrylic Denture Base Resins: A Research Composition and Update. J Prosthodont 2014;23:284-90. doi: 10.1111/jopr.12105.

5. Monteiro DR, Gorup LF, Takamiya AS, Camargo ER, Ruvolo-Filho AC, Barbosa DB. Silver distribution and release from an antimicrobial denture base resin containing silver colloidal nanoparticles. J Prosthodont 2012;21:7-15. doi: 10.1111/j.1532-849X.2011.00772.x.

6. Regis RR, Zanini AP, Vecchia MPD, Silva-Lovato CH, Paranhos HFO, Souza RF. Physical properties of an acrylic resin after incorporation of an antimicrobial monomer. J Prosthodont 2011;20:372-9. doi: 10.1111/j.1532-849X.2011.00719.x.

7. Hayran Y, Sarikaya I, Aydin A, Tekin YH. Determination of the effective anticandidal concentration of denture cleanser tablets on some denture base resins. J Appl Oral Sci 2018;26:e20170077. doi: 10.1590/1678-7757-2017-0077.

8. Procópio ALF, da Silva RA, Maciel JG, Sugio CYC, Soares S, Urban VM, Neppelenbroek KH. Antimicrobial and cytotoxic effects of denture base acrylic resin impregnated with cleaning agents after long-term immersion. Toxicol in Vitro 2018;52:8-13. doi: 10.1016/j.tiv.2018.05.012.

9. Şuhani MF, Băciuţ G, Băciuţ M, Şuhani R, Bran S. Current perspectives regarding the application and incorporation of silver nanoparticles into dental biomaterials. Clujul Med 2018;91:274-9. doi: 10.15386/cjmed-935.

10. Ghaffari T, Hamedirad F, Ezzati B. In vitro comparison of compressive and tensile strengths of acrylic resins reinforced by silver nanoparticles at 2% and 0.2% concentrations. J Dent Res Dent Clin Dent Prospects 2014;8:204-9. doi: 10.5681/joddd.2014.037.

11. Paleari AG, Marra J, Pero AC, Rodriguez LS, Ruvolo-Filho A, Compagnoni MA. Effect of incorporation of 2-tert-butylaminoethyl methacrylate on flexural strength of a denture base acrylic resin. J Appl Oral Sci 2011;19:195-9. doi: 10.1590/S1678-77572011000300003.

12. Kvitek L, Panacek A, Procek R, Soukupova J, Vanickova M, Kolar M, Zboril R. Antibacterial activity and toxicity of silver - nanosilver versus ionic silver. J Phys Conf Ser 2011;304:1-8. doi: 10.1088/1742-6596/304/1/012029.

13. Holtz RD, Lima BA, Souza-Filho AG, Brocchi M, Alves OL. Nanostructured silver vanadate as a promising antibacterial additive to water-based paints. Nanomed-Nanotechnol 2012;8:935-40. doi: 10.1016/j.nano.2011.11.012.

14. Castro DT, Valente MLC, Agnelli JAM, Silva CHL, Watanabe E, Siqueira RL, Alves OL, Holtz RD, Reis AC. In vitro study of the antibacterial properties and impact strength of dental acrylic resins modified with a nanomaterial. J Prosthet Dent 2016;115:238-46. doi: 10.1016/j.prosdent.2015.09.003.

15. Castro DT, Valente MLC, Silva CHL, Watanabe E, Siqueira RL, Schiavon MA, Alves OL, Reis AC. Evaluation of antibiofilm and mechanical properties of new nanocomposites based on acrylic resins and silver vanadate nanoparticles. Arch Oral Biol 2016;67:46-53. doi: 10.1016/j.archoralbio.2016.03.002.

16. Kreve S, Oliveira VC, Bachmann L, Alves OL, Reis AC. Influence of $AgVO_3$ incorporation on antimicrobial properties, hardness, roughness and adhesion of a soft denture liner. Sci Rep 2019;9:1-9. doi: 10.1038/s41598-019-48228-8.

17. Castro DT, Nascimento C, Alves OL, Santos ES, Agnelli JAM, Reis AC. Analysis of the oral microbiome on the surface of modified dental polymers. Arch Oral Biol 2018;93:107-14. doi: 10.1016/j.archoralbio.2018.06.005.

18. Ghaffari T, Hamedi-rad F. Effect of silver nano-particles on tensile strength of acrylic resins. J Dent Res Dent Clin Dent Prospects 2015;9:40-3. doi: 10.15171/joddd.2015.008.

19. Bacali C, Baldea I, Moldovan M, Carpa R, Olteanu DE, Filip GA, et al. Flexural strength, biocompatibility, and antimicrobial activity of a polymethyl methacrylate denture resin enhanced with graphene and silver nanoparticles. Clin Oral Investig 2019;1-13. doi: 10.1007/s00784-019-03133-2.

20. International Standard Organization. ISO 10993-5:1992. Biological Evaluation of Medical Devices – Part 5: Tests for in vitro cytotoxicity. Geneva, 2009.

21. Eren K, Ozmeriç N, Sardaş S. Monitoring of buccal epithelial cells by alkaline comet assay (single cell gel electrophoresis technique) in cytogenetic evaluation of chlorhexidine. Clin Oral Investig 2002;6:150-4. doi: 10.1007/s00784-002-0168-1.

22. Hidalgo E, Bartolome R, Dominguez C. Cytotoxicity mechanisms of sodium hypochlorite in cultured human dermal fibroblasts and its bactericidal effectiveness. Chem-Biol Interact 2002;139:265-82. doi: 10.1016/S0009-2797(02)00003-0.

23. Arabaci T, Türkez H, Çanakçi CF, Özgöz M. Assessment of cytogenetic and cytotoxic effects of chlorhexidine digluconate on cultured human lymphocytes. Acta Odontol Scand 2013;71:1255-60. doi: 10.3109/00016357.2012.757646.

24. Hidalgo E, Dominguez C. Mechanisms underlying chlorhexidine-induced cytotoxicity. Toxicol in Vitro 2001;15:271-6. doi: 10.1016/S0887-2333(01)00020-0.

25. Teixeira ABV, Castro DT, Schiavon MA, Reis AC. Cytotoxicity and release ions of endodontic sealers incorporated with a silver and vanadium base nanomaterial. Odontology 2020;1-8. doi: 10.1007/s10266-020-00507-x.

26. De Matteis V, Cascione M, Toma CC, Albanese G, De Giorgi ML, Corsalini M, Rinaldi R. Silver nanoparticles addition in poly(methyl methacrylate) dental matrix: topographic and antimycotic studies. Int J Mol Sci 2019;20:1-14. doi:10.3390/ijms20194691.

27. Artal MC, Holtz RD, Kummrow F, Alves OL, Umbuzeiro GA. The role of silver and vanadium release in the toxicity of silver vanadate nanowires toward *Daphnia similis*. Environ Toxicol Chem 2013;32:908-12. doi: 10.1002/etc.2128.

28. Yin IX, Yu OY, Zhao IS, Mei ML, Li QL, Tang J, Chu CH. Developing biocompatible silver nanoparticles using Epigallocatechin Gallate for dental use. Arch Oral Biol 2019;102:106-12. doi: 10.1016/j.archoralbio.2019.03.022.

29. Rodriguez LS, Paleari AG, Giro G, Oliveira-Junior NM, Pero AC, Compagnoni MA. Chemical Characterization and Flexural Strength of a Denture Base Acrylic Resin with Monomer 2-Tert-Butylaminoethyl Methacrylate. J Prosthodont 2013;22:292-7. doi: 10.1111/j.1532-849X.2012.00942.x.