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ORIGINAL ARTICLE

Incorporation of $AgVO_3$ into Glass Ionomer Cement: Ionic Release

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ABSTRACT
Objective: To evaluate the surface properties and ion release of a glass ionomer cement (GIC) incorporated with nanostructured silver vanadate ($AgVO_3$). **Material and Methods:** Specimens were obtained with $AgVO_3$ (1%, 2.5%, and 5%) and without nanomaterial. Charge dispersion was assessed by scanning electron microscopy (SEM) and energy dispersive X-ray spectroscopy (EDS). The release of silver (Ag^+) and vanadium (V^{4+}/V^{5+}) was determined using inductively coupled plasma mass spectrometry (ICP-MS). The release of fluoride was determined using an ion-selective electrode. Data were analyzed by ANOVA and Bonferroni post-test ($\alpha=0.05$). **Results:** Photomicrographs and EDS suggested the presence of $AgVO_3$. The 2.5% and 5% groups showed a greater release of Ag^+ ($p<0.05$). A greater release of V^{4+}/V^{5+} was observed with 5% ($p<0.05$). There was a greater release of V^{4+}/V^{5+} than Ag^+ in the 2.5% ($p=0.006$) and 5% ($p<0.001$) groups. All groups showed a greater fluoride release on day 7 and a progressive decrease ($p=0.004$). On day 7, groups with 1% ($p=0.036$) and 2.5% ($p=0.004$) showed greater release than control. **Conclusion:** All concentration test altered the surface properties of GIC, with greater release of Ag^+ and V^{4+}/V^{5+} in the group with 5%. In all groups, there was a greater release of fluoride on day 7 with a subsequent decrease. $AgVO_3$ at concentrations of 1% and 2.5% favored fluoride release on day 7.

Keywords: Glass Ionomer Cements; Fluorides; Nanotechnology; Silver; Vanadium.

Introduction

Glass ionomer cements (GICs), developed by Wilson and Kent, are widely used in dentistry and have undergone developments and new discoveries over the years to make them more suitable for different clinical situations [1,2].

The most common applications of GICs are dental restorations, deep cavity liners and fissure sealants [3-6]. In addition, they show good clinical results when used in Atraumatic Restorative Treatment (ART) [7], which is primarily targeted at underprivileged children in developing countries, as well as the elderly, specifically for the treatment of root caries [8]. By providing a simple and cost-effective treatment modality, ART using GIC is a viable approach for use in outreach dental services to restore carious root surface lesions where dental services are not readily available, as well as for the elderly and special needs groups, compared to treatments using conventional techniques and composite resins [9]. These materials also has potential medical applications, such as ear ossicles and bone grafting plates for craniofacial reconstruction [10]. Therefore, the field of GICs is of interest to the healthcare community.

GICs have unique properties, particularly in terms of chemical adhesion, reduced thermal expansion and fluoride release [11-13]. Several reports suggest that GICs may have an anticaries effect, mainly due to their significant fluoride release [14,15]. The battery effect, i.e., the recharging of GICs by repeated use of fluoridated dentifrices, has also been reported [16]. However, other studies suggest that the amount of fluoride leached is less than that required for antibacterial activity and that antibacterial activity is absent after full cure [17]. Biofilm growth has been reported on the tooth and GIC surfaces due to the wide variety of microbial species in the oral cavity and the complexity of the surface and roughness of GICs [18-22].

In addition, GICs show sensitivity to water during the initial setting period, and low resistance to wear and abrasion, which can lead to the formation of cracks and fissures, increasing the possibility of bacterial proliferation and secondary caries lesions and/or fracture of restorations [23]. Therefore, modifications that can promote greater resistance and antimicrobial efficacy are required for a material such as GIC [18,19].

For centuries, silver has been used throughout the world to prevent microbial infections [24]. With the development of nanoscience and the excellent antimicrobial properties of nanostructured silver-based formulations, interest in this topic has increased. The antimicrobial activity of silver nanoparticles (AgNPs) appears to be a function of surface area [25,26]. To improve the antimicrobial properties of silver, it has been combined with various metal oxides, such as vanadate (VO_3^-) [27]. The nanostructured silver vanadate compound ($AgVO_3$) decorated with silver nanoparticles (AgNPs) has been shown to be effective in controlling infections transmitted by microorganisms [28,29], with low cytotoxicity [27].

Studies indicate that dental materials incorporating $AgVO_3$ have antimicrobial activity against important microorganisms colonizing the oral cavity, including the major cariogenic agent *Streptococcus mutans*, both in monospecies and in multi-species biofilms [30-34]. This activity is associated with the binding of silver (Ag^+) and vanadium (V^{5+}) ions to the thiol (-SH) groups of bacterial enzymes, causing oxidative stress and cell death [28,29,31,32,35-40].

This nanomaterial has potential applications in the medical and dental fields and could be a proposal to avoid the need for infectious therapies with social and economic implications in the face of preventive and infection control measures. The use of $AgVO_3$ to modify GIC is innovative. This study was designed to evaluate the hypothesis that the addition of $AgVO_3$ to GIC would affect the structure of the material as well as its ability to release ions with potential antimicrobial activity. In this study, different proportions of $AgVO_3$ were added to

GIC and their effect on the morphology, composition and release of silver, vanadium and fluoride ions was investigated.

Material and Methods

Synthesis and Characterization of the Nanomaterial

Nanostructured silver vanadate ($AgVO_3$) decorated with AgNPs was synthesized by reacting a solution of silver nitrate ($AgNO_3$, Merck 99.8%) with a solution of ammonium metavanadate (NH_4VO^3, Merck 99%) (Figure 1), and characterized by transmission electron microscopy using a JEOL JEM-100CX II microscope [31,32,35-38,40].

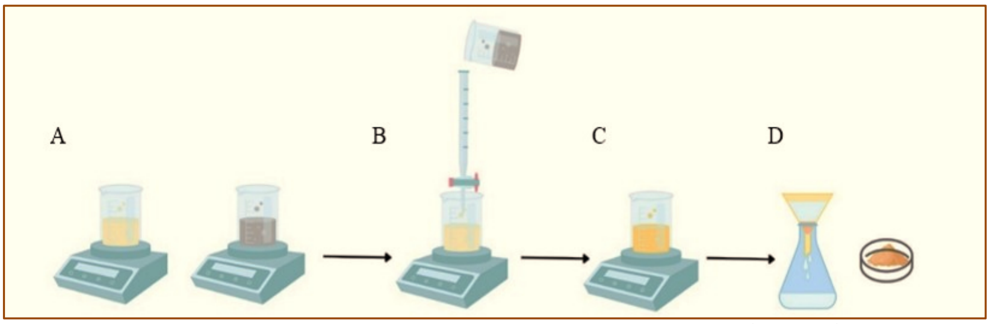


Figure 1. Diagram of the $AgVO_3$ production flow. A - Silver nitrate ($AgNO_3$) and ammonium metavanadate (NH_4VO_3) reagents; B - $AgNO_3$ solution added drop by drop to the NH_4VO_3 solution; C- $AgVO_3$ solution; D- Filtering the $AgVO_3$ solution; and obtaining the powder.

Preparation of Specimens

Forty-four specimens (Ø6 mm x 3 mm) of glass ionomer cement (Riva Self Cure) were made using a matrix (Table 1).

Table 1. Glass ionomer cement used in the study.

Brand/Manufacturer	Powder	Liquid	Batch Number
Riva Self Cure/SDI, Vitoria, Australia	Fluoro-aluminum-silicate + Polyacrylic acid	Polyacrylic acid + Tartaric Acid + Water	1150639

For the control group, the GIC was handled according to the manufacturer's instructions. For the nanomaterial incorporated groups, the samples were prepared by mixing the percentages of 1%, 2.5% and 5% $AgVO_3$ which were added proportionally by mass to the GIC powder. These percentages were based on previous studies [31,32,35-38,40]. The mass of the GIC powder was considered to be 100%, and from this total mass, the above percentage by mass of $AgVO_3$ was subtracted and then the $AgVO_3$ powder was added. The proportions of cement and AgVO3 were weighed on a precision analytical balance. They were then manipulated using a spatula on an unpolished glass plate according to the manufacturer's instructions and placed in the matrix for molding to the dimensions described. Excess material was removed by pressing an acrylic sheet against the molds to obtain a flat surface. After the polymerization time, the samples were finished and polished with #400, #600, #1200, #2000, #2500 and #5000 grit paper. All samples were prepared by a single operator to avoid performance bias.

Morphological and Chemical Analysis of the Samples

The samples were characterized by scanning electron microscopy (SEM) (n=2) in terms of charge dispersion. For this purpose, the samples were coated by evaporating a thin layer of gold, making the surface conductive for electrons, and then analyzed in a scanning electron microscope - Prisma E (Thermo Fisher Scientific) at 400X magnification. Qualitative chemical analysis was carried out using energy-dispersive X-ray spectroscopy (EDS).

Analysis of Silver and Vanadium Ion Release

To analyze the release of silver (Ag^{+}) and vanadium (V^{4+}/V^{5+}) ions by inductively coupled plasma mass spectrometry (ICP-MS), samples (n=5) were suspended by a nylon thread in polypropylene tubes (BD Falcon) containing 9 mL of deionized water and incubated at 37°C for 28 days. After this period, they were removed from the tubes and the liquid was analyzed quantitatively using calibration curves generated on a NexIon 300X instrument [41,42].

Fluoride Release Analysis

For fluoride release analysis, the samples removed from the molds were suspended in polypropylene tubes (BD Falcon) with 4 mL of deionized water using a nylon thread. The samples were then incubated at 37°C. The deionized water in each vial was replaced after 1, 7, 14, 21 and 28 days [43]. To obtain the release profile as a function of time for each group, an ion-selective electrode (ISE) for fluoride (ISE 4010-C00), pre-calibrated from the linear regression curve E(mV) versus log [F-], was used. Potential measurements were made against an Ag/AgCl reference electrode using a potentiometer. To determine the calibration curve, nine standard solutions were prepared by diluting a 1000 ppm fluoride stock solution (ISE 4010-C00). The solutions were prepared in 25 mL flasks, with 2.5 mL of total ionic strength adjustment buffer (TISAB) added to each flask, and volumes of stock solution ranging from 10 µL to 5000 µL (5 mL). TISAB consisted of a solution composed of sodium chloride (NaCl, CRQ Produtos Químicos) 1 mol/L and acetic acid (CH_3CO_2H, CRQ Produtos Químicos) 1 mol/L, with pH adjusted to 5.5 with sodium hydroxide (NaOH) 1 mol/L. The flasks were then filled with deionized water. After preparation, these solutions were transferred to polyethylene bottles and stored in a refrigerator for the duration of the study. All measurements were carried out over three days at room temperature and a new calibration curve was plotted for each day of analysis. Values were expressed as ppm F-. In this way, data on the total amount of fluoride released at each interval was recorded.

Data Analysis

Silver and vanadium ion release data were statistically analyzed using two-way ANOVA. Fluoride release data were analyzed by two-way repeated measures ANOVA. Bonferroni's post-test ($\alpha = 0.05$) was used. The software used for the analyses was SPSS version 22.0 (IBM Corp., Armonk, NY, USA).

Results

Characterization of the Nanomaterial

$AgVO_3$ consists of vanadium nanowires with a length of a few micrometers and a diameter of approximately 150 nm coated with spherical nanoparticles (Figure 2).

Morphology and Chemical Composition of the Samples

The micrographs show a surface with larger particles in the modified groups, suggesting the presence of $AgVO_3$ agglomerates (Figure 3).

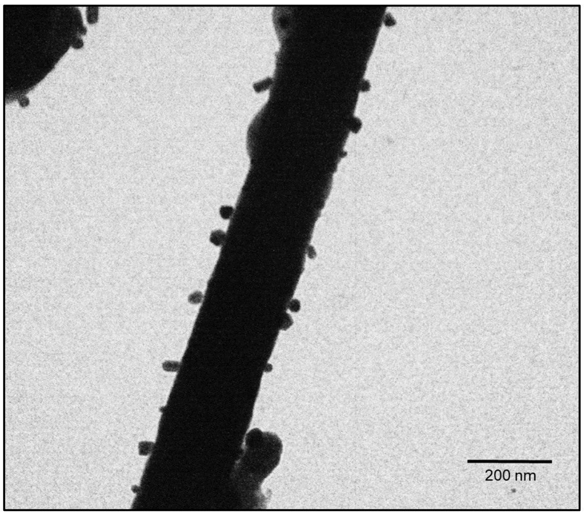
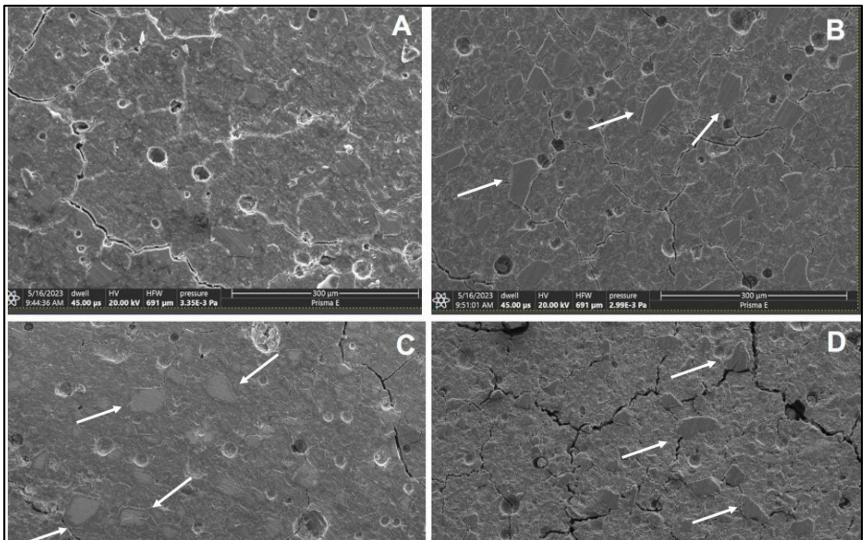


Figure 2. Photomicrograph of nanostructured silver vanadate decorated with silver nanoparticles.



Arrows indicate $AgVO_3$ particles.

Figure 3. Photomicrographs of commercial glass ionomer cement incorporated with different percentages of nanostructured silver vanadate decorated with silver nanoparticles: (A) Riva Self Cure, (B) Riva Self Cure + 1% $AgVO_3$, (C) Riva Self Cure + 2.5% AgVO3, (D) Riva Self Cure + 5% $AgVO_3$ (magnification × 400).

A comparison of the samples with different $AgVO_3$ contents shows an increase in the silver (Ag) and vanadium (V) components in proportion to the amount incorporated. EDS analysis showed the absence of Ag and V peaks in the control group (Riva Self Cure) and the presence of Ag and V peaks in the other groups. For Ag, the peaks represented 0.10%, 0.51% and 0.68% w/w for Riva Self Cure + 1% $AgVO_3$, Riva Self Cure + 2.5% $AgVO_3$ and Riva Self Cure + 5% $AgVO_3$, respectively. For V, the peaks represented 0.11%, 0.43% and 0.90%

w/w for Riva Self Cure + 1% $AgVO_3$, Riva Self Cure + 2.5% $AgVO_3$ and Riva Self Cure + 5% $AgVO_3$, respectively (Figure 4).

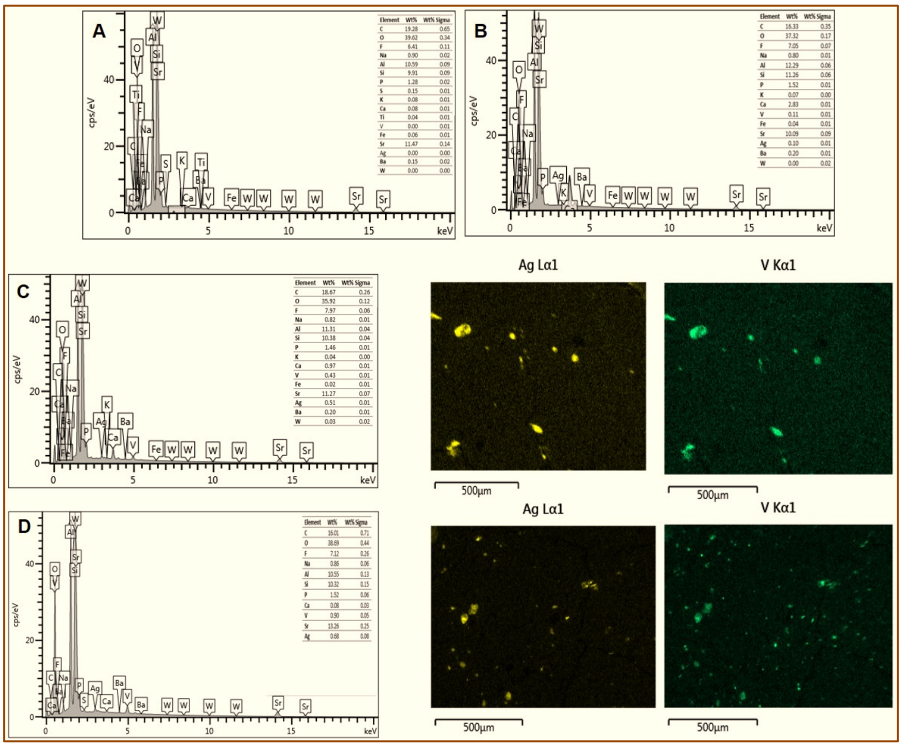


Figure 4. EDS spectra and elemental microanalysis showing the chemical elements present in the samples. Distribution map of the chemical elements Ag and V in the samples with 2.5% and 5% $AgVO_3$. (A) Riva Self Cure, (B) Riva Self Cure + 1% $AgVO_3$, (C) Riva Self Cure + 2.5% $AgVO_3$, (D) Riva Self Cure + 5% $AgVO_3$.

Release of Silver and Vanadium Ions

There was an effect of nanomaterial concentration on the release of Ag^+ and V^{4+}/V^{5+} ions ($p<0.001$). The Riva Self Cure + 2.5% $AgVO_3$ and Riva Self Cure + 5% $AgVO_3$ groups showed a greater release of Ag^+ ions with a significant difference compared to the other groups ($p<0.05$). A greater release of V^{4+}/V^{5+} was observed in the Riva Self Cure + 5% $AgVO_3$ group ($p<0.05$). There was a greater release of V^{4+}/V^{5+} ions than Ag^+ ions in the Riva Self Cure + 2.5% ($p=0.006$) and Riva Self Cure + 5% ($p<0.001$) groups (Table 2). Therefore, the release of ions was proportional to the amount of $AgVO_3$ incorporated into the glass ionomer cement, with a greater amount of V^{4+}/V^{5+} ions being released than Ag^+ ions.

Table 2. Release of Ag^+ and V^{4+} /V^{5+} ions (mg/L) from glass ionomer cement samples.

	Riva Self Cure	Riva Self Cure + 1% $AgVO_3$	Riva Self Cure + 2.5% AgVO3	Riva Self Cure + 5% $AgVO_3$
Ag^+	0.000 (0.000)^{Aa}	0.03 (0.03)^{Aa}	0.16 (0.04)^{Ba}	0.18 (0.06)^{Ba}
V^{4+} /V^{5+}	0.002 (0.001)^{Aa}	7 (4)^{Aa}	29 (8)^{Ab}	70 (30)^{Bb}

Similar uppercase letters indicate statistical similarity between columns. Similar lowercase letters indicate statistical similarity between rows.

Fluoride Release

The fluoride release profiles in deionized water of Riva Self Cure with and without $AgVO_3$ were recorded for 28 days at five specific intervals. The amount of fluoride released was documented in parts per million (ppm). There was no effect of the group factor considered individually on fluoride release ($p=0.178$). There was a significant difference in the time factor, considered individually ($p<0.001$) and in the time x group interactions ($p=0.004$). Table 3 and Figure 5 show the comparative evaluation of fluoride release considering the time x group interaction.

In general, all the groups showed a higher release at 7 days and a progressive decrease up to 28 days. On day 7, there was a significant difference between the groups, with Riva Self Cure showing lower fluoride release compared to Riva Self Cure + 1% ($p=0.036$) and Riva Self Cure + 2.5% ($p=0.004$).

Table 3. Comparison of fluoride release between groups over days (mean ± SD) ppm.

Groups	Day 1 Mean (SD)	Day 7 Mean (SD)	Day 14 Mean (SD)	Day 21 Mean (SD)	Day 28 Mean (SD)
Riva Self Cure	10 ± 1^{Ba}	15 ± 1^{Ca}	10 ± 3^{BCa}	5.9 ± 0.7^{Aa}	4 ± 1^{Aa}
Riva Self Cure + 1% $AgVO_3$	9.5 ± 0.9^{Ba}	20 ± 2^{Cb}	10 ± 1^{Ba}	6 ± 1^{ABa}	4.8 ± 0.3^{Aa}
Riva Self Cure + 2.5% $AgVO_3$	9.7 ± 0.7^{BCa}	20 ± 2^{Db}	10.4 ± 0.8^{BCa}	6.2 ± 0.8^{ABa}	4.9 ± 0.4^{Aa}
Riva Self Cure + 5% $AgVO_3$	9.2 ± 0.7^{BCa}	19 ± 2^{Dab}	12 ± 2^{Ca}	5.2 ± 0.7^{ABa}	4.9 ± 0.7^{Aa}

Similar uppercase letters indicate statistical similarity between columns; Similar lowercase letters indicate statistical similarity between rows.

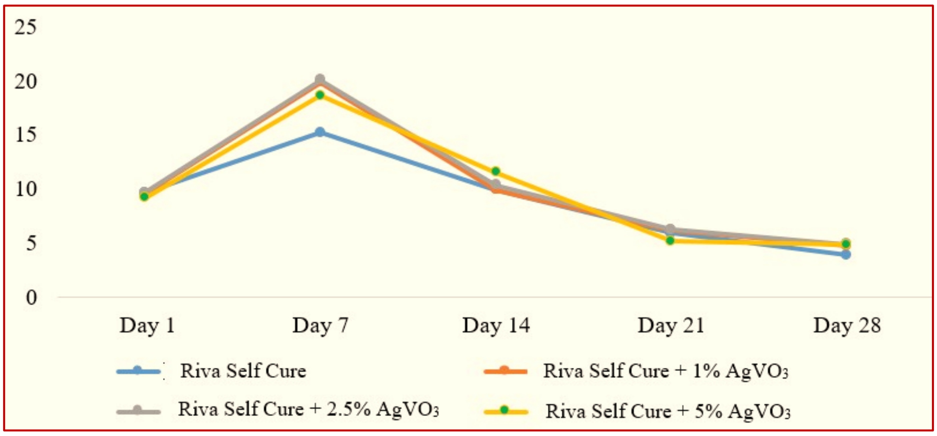


Figure 5. Fluoride release from glass ionomer cement modified or not by $AgVO_3$ over time.

Discussion

Dental caries is a non-communicable disease that affects more than 2.5 million people worldwide and impairs their health and quality of life [43]. Currently, there are several dental materials that release fluoride because of its anticariogenic effect [44]. These materials include glass ionomer cements (GICs), which release fluoride for periods of time, an attribute generally considered to be advantageous, although the evidence to support this is somewhat ambiguous, and also have the ability to absorb fluoride [2,12].

According to the literature, organic materials such as chitosan and inorganic materials such as titanium dioxide nanoparticles can be added to GICs to improve their properties [43,45]. Nanostructured silver vanadate ($AgVO_3$) is an antimicrobial agent that has been widely studied due to its important advantages, including the ability to stabilize AgNPs on silver vanadate nanowires and its effectiveness against various microorganisms colonizing the oral cavity, including *S. mutans* [28,29,31,32,35-40]. The use of $AgVO_3$ to modify GICs is an innovative strategy.

The modification of GICs to confer effective antimicrobial activity requires the continuous release of components. In this study, the release of ions from $AgVO_3$-modified GIC was investigated. The hypothesis tested was accepted as the nanomaterial modified the composition and morphology of the GIC and influenced the elemental release.

The antimicrobial activity of $AgVO_3$-based composites is mainly due to the release of silver ions. Vanadium can also interact with thiol groups in the cell membranes of microorganisms, synergistically contributing to efficacy [41]. In the present study, the amount of Ag^+ and V^{4+}/V^{5+} ions released from GIC incorporated with $AgVO_3$ was measured using plasma coupled mass spectrometry (ICP-MS).

The concentration of Ag^+ and V^{4+}/V^{5+} ions released over the 28-day period was higher in the groups with the highest concentration of $AgVO_3$ incorporated, which corroborates studies in the literature evaluating the release of these ions in acrylic resins [41] and endodontic cements [42] and may suggest that the greater the amount of $AgVO_3$, the greater the availability of ions to interact with bacterial cells, promoting an antimicrobial effect. These results can be complemented by EDS analysis, which showed an increase in the peaks of the silver (Ag) and vanadium (V) components proportional to the amount incorporated. However, there are concerns about the cytotoxic effect of modifications to dental materials. Silver vanadate nanowires decorated with silver nanoparticles were toxic to *D. similis*, and in this case, the silver released into the medium seems to be responsible for the toxicity. The 48h EC50 was 1.1 μg/L when silver nitrate was used as the source of silver ions and 1400 μg/L for vanadium when vanadium pentoxide was used as the source of vanadium ions, indicating that a smaller amount of silver is capable of causing greater ecotoxicity [46]. In this study, in general, GIC incorporated with $AgVO_3$ generally released more V^{4+}/V^{5+} than Ag^+, which may have a more favorable effect on biocompatibility. By incorporating $AgVO_3$ into acrylic resins, the literature suggests that low concentrations could avoid the risk of cytotoxicity for patients using dental prosthesis [41]. Studies should be planned to assess the pulp response to the use of modified glass ionomer cement, considering that it could be used to treat deep carious lesions that are close to this tissue.

In addition to the release of Ag^+ and V^{4+}/V^{5+} ions, it is important to consider whether the incorporation of $AgVO_3$ affects the release of fluoride ions. It is believed that GIC releases fluoride in two phases, with an initial rapid release pattern and a decrease in fluoride release after the preliminary explosion, followed by a long-term sustained release [43,45]. This release pattern is attributed to the high instability and erosion of glass ionomers during the initial setting period. In view of this, studies have highlighted the importance of developing materials capable of maintaining a higher and constant level of fluoride release [45].

In this study, the maximum release, related to the slower dissolution of the glass particles through the pores of the material over time, was observed on day 7 for all groups, followed by a smaller, steady-state release [43,45]. It is interesting to note that on day 7, the $AgVO_3$-modified groups promoted a greater release of fluoride than the unmodified group, which may have a beneficial effect during this period, helping to inhibit dental demineralization. A possible explanation may be related to the presence of nanomaterial agglomerates. Studies have reported that when there are agglomerates of nanoparticles, some areas of the material may be left without reinforcement, increasing instability at this initial stage and, consequently, greater diffusion of fluoride [34].

The *in vitro* nature of this study is a limitation, as it is known that glass ionomer cement, when used clinically, is involved in the dynamic environment of the oral cavity, with variations in pH, salivary composition and biofilm, which are different from laboratory conditions. Future research should focus on analyzing the antimicrobial activity and cytotoxicity of $AgVO_3$-modified GIC to verify its therapeutic efficacy.

Conclusion

Modification of GIC with all concentration tests (1%, 2.5% and 5% of $AgVO_3$) altered the surface properties, with the greater release of Ag^+ and V^{4+} /V^{5+} in the group with 5%. In all groups, there was a greater release of fluoride on day 7, with a subsequent decrease. $AgVO_3$ at concentrations of 1% and 2.5% favored fluoride release on day 7 compared to the control.

Authors' Contributions

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All authors declare that they contributed to critical review of intellectual content and approval of the final version to be published.

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Conflict of Interest

The authors declare no conflicts of interest.

Data Availability

The data used to support the findings of this study can be made available upon request to the corresponding author.

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