UNIVERSIDADE DE UBERABA
MESTRADO ACADÊMICO EM ODONTOLOGIA

MARIANA PARDI

CIMENTO DE IONÔMERO DE VIDRO MODIFICADO COM NANOMATERIAL À BASE DE PRATA E VANÁDIO: ANÁLISE QUÍMICA E MORFOLÓGICA

UBERABA – MG
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Orientadora: Profa. Dra. Denise Tornavoi de Castro

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RESUMO

Este estudo incorporou o vanadato de prata nanoestruturado ($AgVO_3$) decorado com nanopartículas de prata (AgNPs) em um cimento de ionômero de vidro (CIV) e avaliou as características de superfície e a liberação de íons. Foram obtidos espécimes de CIV nas dimensões de Ø 6mm x 3 mm de espessura, de acordo com o grupo: Riva Self Cure, Riva Self Cure + 1% de $AgVO_3$, Riva Self Cure + 2,5% de $AgVO_3$ e Riva Self Cure + 5% de $AgVO_3$. A caracterização dos espécimes quanto à dispersão da carga utilizada foi realizada por microscopia eletrônica de varredura (MEV) (n=2). Foi realizada a análise química qualitativa por espectroscopia por energia dispersiva de raios X (EDS). Para a análise da liberação de íons de prata (Ag^+) e vanádio (V^{4+} /V^{5+}) por espectrometria de massas com plasma indutivamente acoplado (ICP-MS), os espécimes (n=5) foram suspensos por um fio de náilon em tubos de polipropileno com 9 mL de água deionizada e incubados a 37°C por 28 dias. Após esse período o líquido foi analisado quantitativamente por curvas de calibração construídas no equipamento NexIon 300X. Para a análise da liberação de íons fluoreto, os espécimes (n=4) foram suspensos por um fio de náilon em tubos de polipropileno com 4 mL de água deionizada. As amostras foram incubadas a 37°C e a água de cada frasco foi substituída após o 1º, 7º, 14º, 21º e 28º dias. Foi utilizado um eletrodo seletivo de íons flúor (ISE 4010-C00). As fotomicrografias obtidas por MEV mostram uma superfície com partículas maiores nos grupos modificados, sugerindo a presença de aglomerados de $AgVO_3$, o que foi comprovado pela análise química. Os grupos Riva Self Cure + 2,5% de $AgVO_3$ (0,16 ± 0,04 mg/L) e Riva Self Cure + 5% de $AgVO_3$ (0,18 ± 0,06 mg/L) apresentaram maior liberação de íons Ag^+ com diferença significativa em relação aos demais grupos ($p<0,05$). Maior liberação de V^{4+} /V^{5+} foi observada no grupo Riva Self Cure + 5% de $AgVO_3$ (70 ± 30 mg/L) ($p<0,05$). Nota-se maior liberação de íons V^{4+} /V^{5+} do que de íons Ag^+ nos grupos Riva Self Cure + 2,5% ($p=0,006$) e Riva Self Cure + 5% ($p<0,001$). Para a liberação de íons fluoreto, houve diferença significativa nas interações tempo x grupo ($p=0,004$). De maneira geral, todos os grupos apresentaram maior liberação no tempo de 7 dias e um declínio progressivo até o 28º dia. No 7º dia, houve diferença significativa entre os grupos, sendo que o Riva Self Cure (15±1 ppm) apresentou menor liberação de fluoretos em comparação com o Riva Self Cure + 1% (20±2 ppm) ($p=0,036$) e o Riva Self Cure + 2,5% (20±2 ppm) ($p=0,004$). Os resultados permitem concluir que as amostras de CIV modificadas apresentam alteração das características de superfície devido a presença do nanomaterial,

com liberação de íons Ag^{+} e V^{4+} /V^{5+} proporcional à quantidade de $AgVO_3$ incorporada. De forma geral a incorporação do nanomaterial não influenciou na propriedade de liberação de íons fluoreto, exceto em 7 dias em que os grupos com 1% e 2,5% apresentarem maior liberação em comparação com o controle.

Palavras-chave: Cimentos de Ionômero de Vidro; Flúor; Nanotecnologia; Prata; Vanádio.

ABSTRACT

This study incorporated nanostructured silver vanadate ($AgVO_3$) decorated with silver nanoparticles (AgNPs) into a glass ionomer cement (GIC) and evaluated the surface characteristics and ion release. GIC specimens of Ø 6mm x 3mm thick were obtained according to the group: Riva Self Cure, Riva Self Cure + 1% $AgVO_3$, Riva Self Cure + 2.5% $AgVO_3$ and Riva Self Cure + 5% $AgVO_3$. The specimens were characterised in terms of the dispersion of the filler used using scanning electron microscopy (SEM) (n=2). A qualitative chemical analysis was carried out using energy dispersive X-ray spectroscopy (EDS). To analyse the release of silver (Ag^+) and vanadium (V^{4+} /V^{5+}) ions by inductively coupled plasma mass spectrometry (ICP-MS), the specimens (n=5) were suspended by a nylon thread in polypropylene tubes with 9 mL of deionised water and incubated at 37°C for 28 days. After this period, the liquid was analysed quantitatively using calibration curves constructed on NexIon 300X equipment. To analyse the release of fluoride ions, the specimens (n=4) were suspended by a nylon thread in polypropylene tubes with 4 mL of deionised water. The samples were incubated at 37°C and the water in each vial was replaced after the 1st, 7th, 14th, 21st and 28th days. A fluoride ion selective electrode (ISE 4010-C00) was used. SEM photomicrographs show a surface with larger particles in the modified groups, suggesting the presence of $AgVO_3$ agglomerates, which was confirmed by chemical analysis. The Riva Self Cure + 2.5% $AgVO_3$ (0.16 ± 0.04 mg/L) and Riva Self Cure + 5% $AgVO_3$ (0.18 ± 0.06 mg/L) groups showed greater release of Ag^+ ions with a significant difference compared to the other groups ($p<0.05$). Greater release of V^{4+} /V^{5+} was observed in the Riva Self Cure + 5% $AgVO_3$ group (70 ± 30 mg/L) ($p<0.05$). 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. For the release of fluoride ions, there was a significant difference in the time x group interaction ($p=0.004$). In general, all groups showed greater release at 7 days and a progressive decline up to the 28th day. On the 7th day, there was a significant difference between the groups, with Riva Self Cure (15±1 ppm) showing lower fluoride release compared to Riva Self Cure + 1% (20±2 ppm) ($p=0.036$) and Riva Self Cure + 2.5% (20±2 ppm) ($p=0.004$). The results allow us to conclude that the modified GIC samples have altered surface characteristics due to the presence of the nanomaterial with release of Ag^+ and V^{4+} /V^{5+} ions proportional to the amount of $AgVO_3$ incorporated. In general,

the incorporation of the nanomaterial did not influence the release of fluoride ions, except at 7 days when the 1% and 2.5% groups showed greater release compared to the control.

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

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1 INTRODUÇÃO

Os cimentos de ionômero de vidro (CIVs), idealizados por Wilson e Kent(WILSON e KENT, 1972) são amplamente empregados na odontologia e ao longo dos anos vem sofrendo evoluções e novas descobertas a fim de se tornarem mais adequados para as diferentes situações clínicas (AMIN *et al.*, 2021).

As aplicações mais comuns dos CIVs envolvem restaurações dentárias (UZEL *et al*., 2022), forramentos de cavidades profundas (RIBEIRO *et al*., 2020), selantes de fissuras (SCHRAVERUS *et al*., 2021), cimentação de coroa e aparelhos ortodônticos (FRIKER, 2022). Além disso, apresentam bons resultados clínicos quando usados no Tratamento Restaurador Atraumático (ART) (KAVERIKANA, VOJJALA e SUBRAMANIAM, 2022). Estes materiais têm potencial uso na área médica, como ossículos do ouvido e placas substitutas ósseas para reconstrução craniofacial (GU *et al*., 2005). Portanto, o campo dos CIVs é um tema de interesse na área da saúde.

Os CIVs apresentam propriedades únicas, especialmente relacionadas à adesão química, expansão térmica reduzida e liberação de flúor (BOLLU; HARI; THUMU, *et al.*, 2016; SIDHU; NICHOLSON, 2016; SILVA; PEREIRA; MOTA, *et al.*, 2016). Porém, estudos mostram o crescimento de biofilme nas superfícies dentárias e dos CIVs devido à grande variedade de espécies microbianas na cavidade oral, bem como à complexidade da química da superfície do CIV e à rugosidade (TEUGHELS *et al*., 2006; ELSHENAWY *et al*., 2023) portanto, o potencial de inibição bacteriano pode não ser forte o suficiente para evitar lesões de cárie secundárias ao fazer uso de restaurações de CIV (FORSS; WIDSTRÖM, 2004; XIE *et al*., 2011; HAFSHEJANI *et al*., 2017).

Além disso, os CIVs apresentam sensibilidade à água durante o período inicial de presa, baixa resistência ao desgaste e à abrasão podendo levar à formação de fissuras e fendas o que aumenta a possibilidade de proliferação bacteriana e lesões de cárie secundárias e/ou fratura das restaurações (KANTOVITZ *et al*., 2020). Assim, modificações que possam promover maior resistência e efeito antimicrobiano são necessárias para um material como o CIV (TEUGHELS *et al*., 2006; ELSHENAWY *et al*., 2023).

Há séculos a prata vem sendo usada no mundo todo para a prevenção de infecções microbianas (BRUNA *et al*., 2021). Com a evolução da nanociência e as excelentes propriedades antimicrobianas de formulações à base de prata nanoestruturada, o interesse no assunto foi aprofundado. A atividade antimicrobiana das nanopartículas de prata (AgNPs) parece ser em função da área de superfície (TANG; ZHENG, 2018; FERNANDEZ *et al*., 2021).

A fim de melhorar as propriedades antimicrobianas da prata, esta tem sido combinada com diferentes óxidos metálicos, tais como o vanadato (VO_3) (PIMENTEL *et al*., 2023). O composto vanadato de prata nanoestruturado ($AgVO_3$) decorado com AgNPs tem se mostrado eficiente no controle de infecções bacterianas transmitidas por micro-organismos altamente patogênicos e resistentes (HOLTZ *et al*., 2010; HOLTZ *et al*., 2012), com baixa citotoxicidade (PIMENTEL *et al*., 2023).

Estudos indicam atividade antimicrobiana de materiais odontológicos incorporados com $AgVO_3$ frente a importantes micro-organismos colonizadores da cavidade bucal, dentre eles o principal agente relacionado à formação de cárie (LIMA *et al*., 2020), o *Streptococcus mutans,* seja em biofilme monoespécie (DE CASTRO *et al*., 2016; VIDAL *et al*., 2021; KREVE *et al*., 2022) ou multiespécies (TEIXEIRA *et al*., 2023) sendo este desempenho associado à ligação dos íons prata (Ag^{+}) e vanádio ($V^{5+)}$ aos grupos SH (tióis) das enzimas bacterianas, causando estresse oxidativo e morte celular (HOLTZ *et al*., 2010; HOLTZ *et al*., 2012, DE CASTRO *et al*., 2014; DE CASTRO *et al*., 2016; VILELA TEIXEIRA *et al*., 2019; DE CASTRO *et al*., 2019; DE CASTRO *et al*., 2021; VIDAL *et al*., 2021; DE CAMPOS *et al*., 2021; UEHARA *et al*., 2022).

Esse nanomaterial tem potencial aplicação na área médica e odontológica podendo envolver uma proposta para evitar a necessidade de terapias de infeção com efeitos sociais e econômicos, frente a ações preventivas e de controle de infecção. A utilização de $AgVO_3$ para modificar o CIV é inovadora. Este estudo foi desenvolvido para avaliar a hipótese de que a adição de $AgVO_3$ ao CIV teria um efeito na estrutura do material, bem como na capacidade de liberação íons com potencial ação antimicrobiana. No presente estudo, diferentes proporções de $AgVO_3$ foram adicionadas ao CIV e seu efeito na composição, morfologia e na liberação de prata, vanádio e flúor foi estudado.

2 OBJETIVOS

2.1 OBJETIVO GERAL

Avaliar a influência da incorporação do vanadato de prata nanoestruturado ($AgVO_3$) decorado com nanopartículas de prata (AgNPs) nas características de superfície e na liberação de íons de um cimento de ionômero de vidro.

2.2 OBJETIVOS ESPECÍFICOS

- Sintetizar e caracterizar o $AgVO_3$;
- Incorporar diferentes percentuais de $AgVO_3$ em um cimento de ionômero de vidro;
- Realizar a análise química e morfológica do cimento de ionômero de vidro modificado com $AgVO_3$ por microscopia eletrônica de varredura (MEV) e Espectroscopia de raios X por dispersão em energia (EDS);
- Avaliar a quantidade de íons de prata (Ag^{+}) e vanádio (V^{4+} /V^{5+}) liberada em 28 dias, por espectrometria de massas com plasma indutivamente acoplado (ICP-MS);
- Avaliar a quantidade de íons fluoreto liberada em 1, 7, 14, 21 e 28 dias utilizando um eletrodo seletivo para íons flúor.

3 CAPÍTULO 1

Morphological, elemental and release kinetics analysis of silver, vanadium and fluoride ions from a nanomaterial-modified glass ionomer cement

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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 two-way ANOVA and Bonferroni post-test (α=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 in the group 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. 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: The modification altered the surface properties of the GIC, with greater release of Ag^+ and V^{4+} /V^{5+} in the more concentrated groups. There was a greater release of fluoride on day 7 with a subsequent decrease. $AgVO_3$ favored fluoride release on day 7.

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

3.1 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, fissure sealants, crown cementation and orthodontic appliances [3-6]. In addition, they show good clinical results when used in Atraumatic Restorative Treatment (ART) [7]. These materials have potential medical applications, such as ear ossicles and bone grafting plates for craniofacial reconstruction [8]. 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 [9-11]. However, studies show that biofilm growth occurs on tooth and GIC surfaces due to the wide variety of microbial species in the oral cavity

and the complexity of GIC surface and roughness [12-13]. Therefore, the bacterial inhibition potential may not be strong enough to prevent secondary caries lesions when using GIC restorations [14-16].

In addition, GICs show sensitivity to water during the initial setting period, 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 [17]. Therefore, modifications that can promote greater resistance and antimicrobial efficacy are required for a material such as GIC [12,13].

For centuries, silver has been used throughout the world to prevent microbial infections [18]. 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 [19,20]. To improve the antimicrobial properties of silver, it has been combined with various metal oxides, such as vanadate (VO_3^-) [21]. The nanostructured silver vanadate compound ($AgVO_3$) decorated with silver nanoparticles (AgNPs) has been shown to be effective in controlling infections transmitted by microorganisms [22,23], with low cytotoxicity [21].

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 [24-28]. 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 [22,23,25,26,29-34].

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.

3.2 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 [25,26,29-32,34].

Preparation of specimens

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

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% $AgVO_3$ added proportionally by mass to the GIC powder. These percentages were based on previous studies [25,26,29-32,34]. 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 $AgVO_3$ 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 [35,36].

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 [37]. 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

Data were statistically analyzed by 2-factor ANOVA followed by Bonferroni's post-test ($\alpha = 0.05$) using SPSS software version 22.0.

3.3 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).

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).

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.

Fluoride release

The fluoride release profiles in deionized water of Riva Self Cure with and without $AgVO_3$ were recorded for 28 days at 5 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 the 28 days. At 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% ($p=0.004$).

3.4 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 [37]. Currently, there are several dental materials that release fluoride because of its anticariogenic effect [38]. 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 [2,10].

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 [37,39]. 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* [22,23,25,26,29-34].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 [35]. 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 [35] and endodontic cements [36] 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 [40]. 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.

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 [37,39].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 [39].

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 [37,39]. 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 [28].

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.

3.5 Conclusion

It can be concluded that the modification altered the surface properties of the GIC, with greater release of Ag^{+} and V^{4+} /V^{5+} in the more concentrated groups. There was a greater release of fluoride on day 7 with a subsequent decrease. $AgVO_3$ favored fluoride release on day 7.

3.6 Conflict of Interest

The authors declare no conflicts of interest.

3.7 References

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3.8 Tables

Table 1. Glass ionomer cement used in the study.

Material	Composition	Manufacturer
Riva Self Cure/SDI	Fluoroaluminosilicate glass, acrylic monomer, and polyacrylic acid + tartaric acid	SDI, Victoria, Australia

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% $AgVO_3$**	**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}

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

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

Groups	Day 1	Day 7	Day 14	Day 21	Day 28
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}

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

3.9 Figures

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.

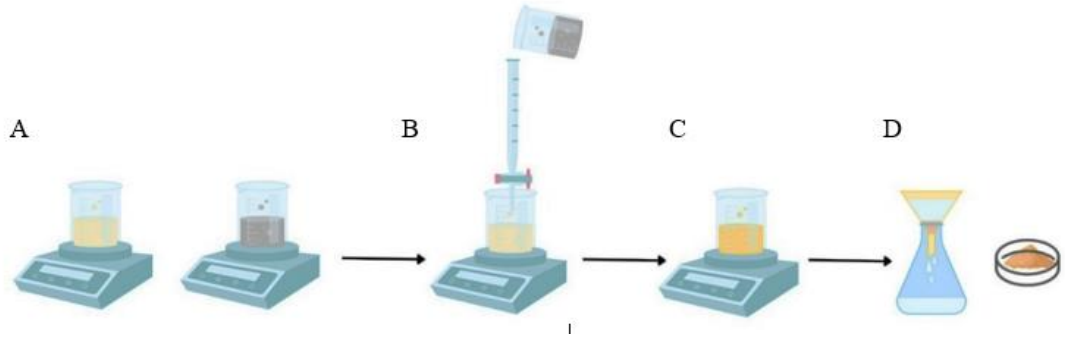


Figure 2. Photomicrograph of nanostructured silver vanadate decorated with silver nanoparticles.

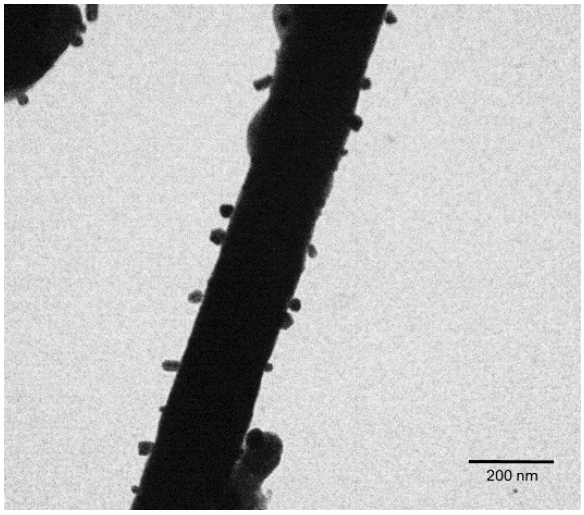


Figure 3. Photomicrographs of commercial glass ionomer cement incorporated with different percentages of nanostructured silver vanadate decorated with silver nanoparticles: (A) Riva Self Cure, (B) Riva Self Cure + 1% $AgVO_3$, (C) Riva Self Cure + 2.5% $AgVO_3$, (D) Riva Self Cure + 5% $AgVO_3$ (magnification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

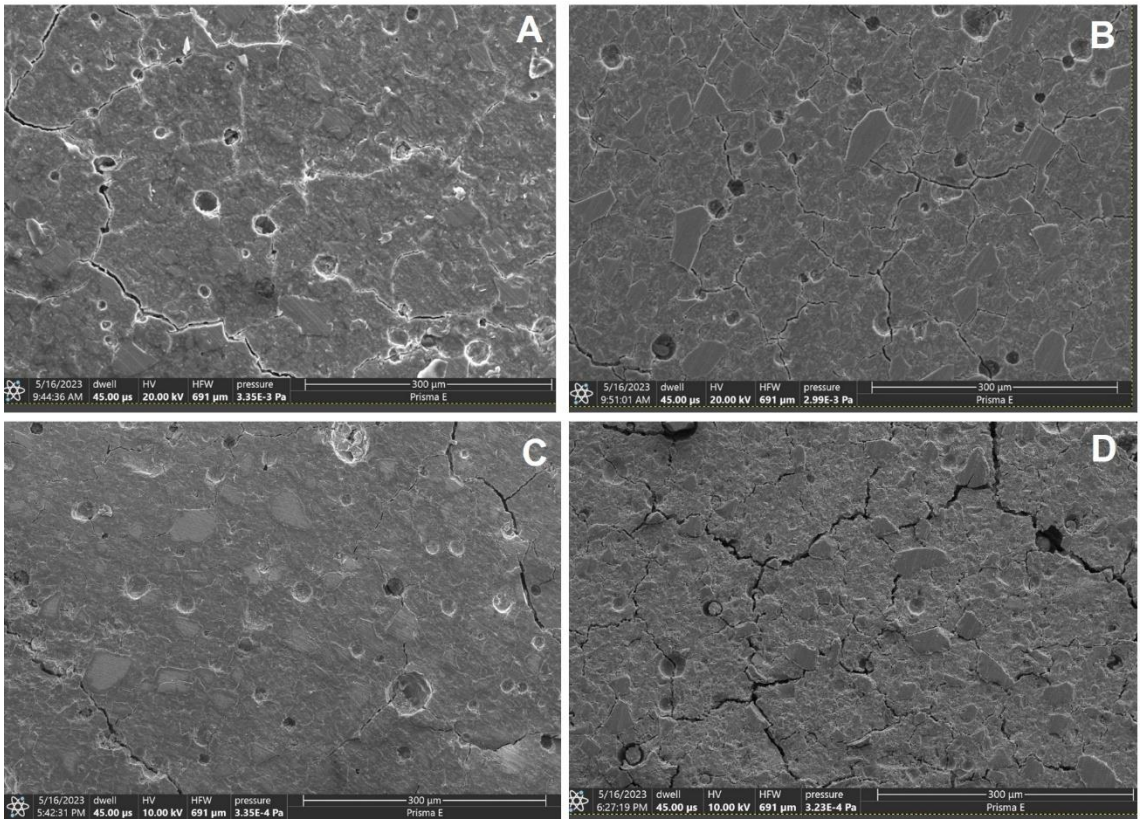


Figure 4. EDS spectra and elemental microanalysis showing the chemical elements present in the samples. Distribution map of the chemical elements Ag and V in the samples with 2.5% and 5% $AgVO_3$. (A) Riva Self Cure, (B) Riva Self Cure + 1% $AgVO_3$, (C) Riva Self Cure + 2.5% $AgVO_3$, (D) Riva Self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

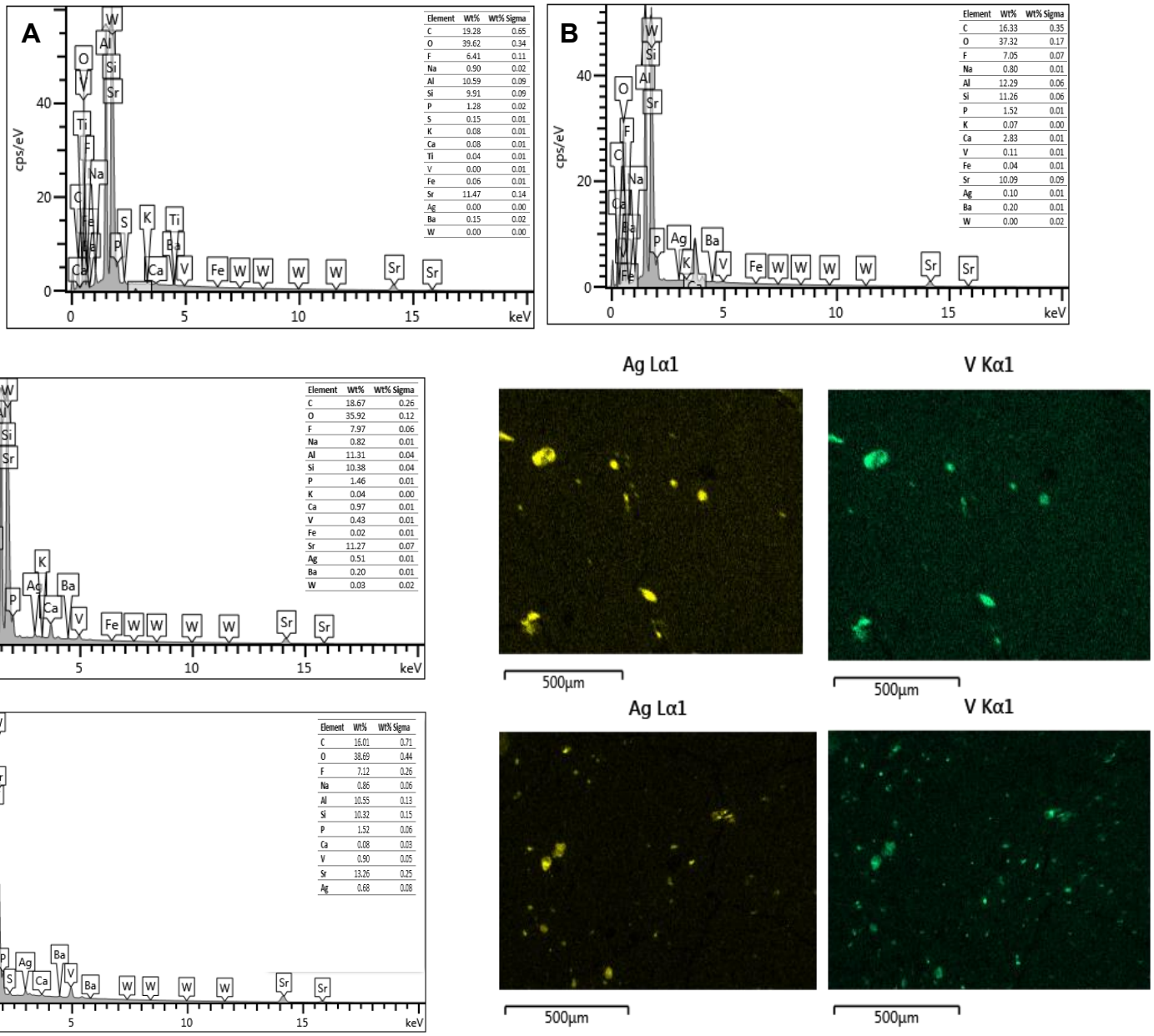
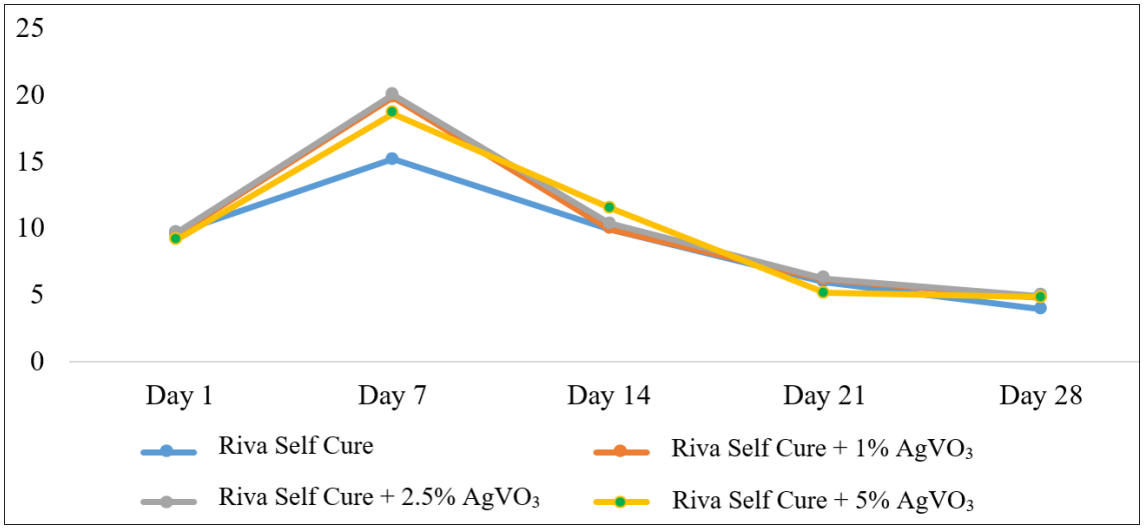


Figure 5. Fluoride release from glass ionomer cement modified or not by $AgVO_3$ over time.



4 CONCLUSÃO

Conclui-se que foi possível sintetizar o $AgVO_3$ por meio da reação de precipitação entre o nitrato de prata e vanadato de amônio e incorporá-lo ao CIV. As amostras de CIV modificadas apresentam alteração das características de superfície devido a presença do nanomaterial com liberação de íons Ag^+ e V^{4+} /V^{5+} proporcional a quantidade de $AgVO_3$ incorporada. De forma geral a incorporação do nanomaterial não influenciou na propriedade de liberação de íons fluoreto, exceto em 7 dias em que os grupos com 1% e 2,5% apresentaram maior liberação em comparação com o controle.

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APÊNDICES

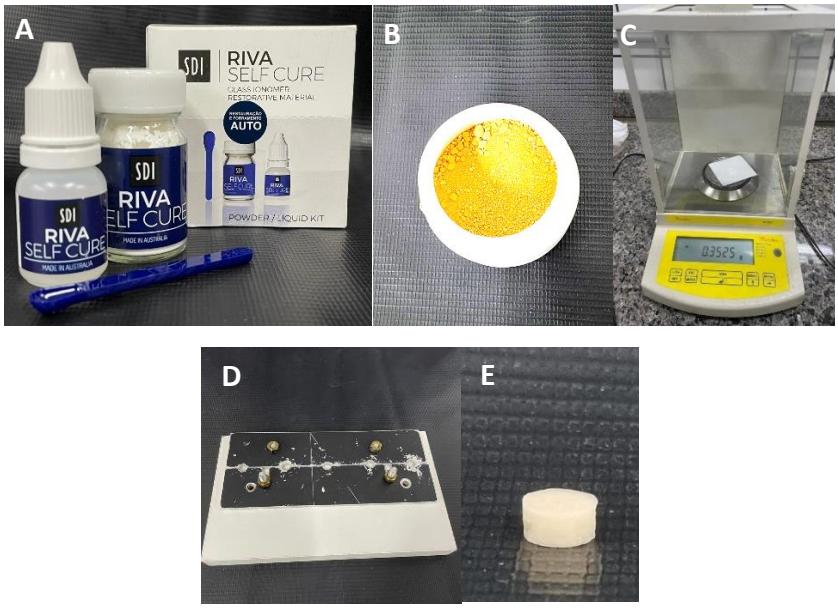

Figura 1. Confecção dos espécimes em CIV. A- Cimento de Ionômero de Vidro utilizado; B- $AgVO_3$sintetizado; C- Balança de precisão para pesagem; D- Matriz para confecção dos espécimes;E- Espécime de CIV – Fonte: Autoria própria.

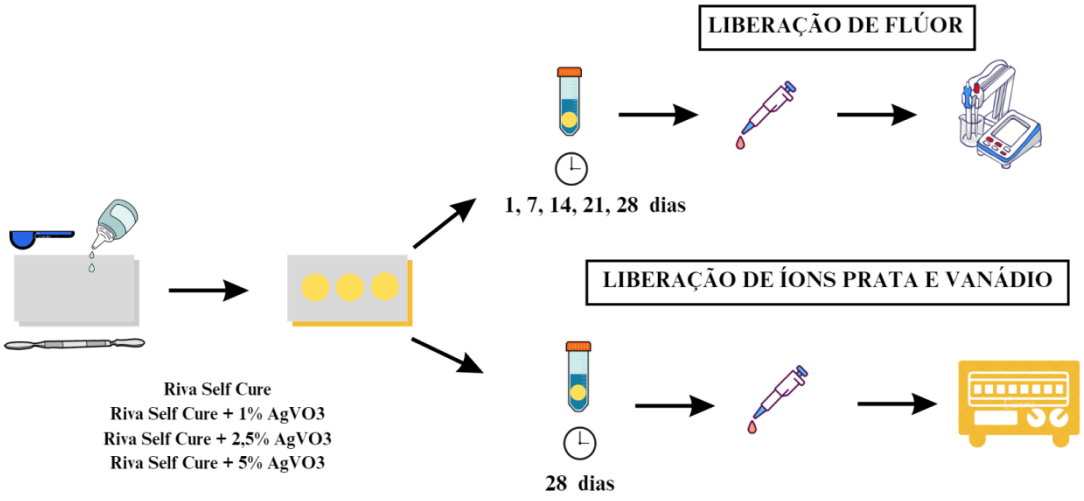

Figura 2. Esquema do fluxo das análises de liberação de fluoreto e de íons prata e vanádio – Fonte: Autoria própria.

ANEXO

Anexo 1- Comprovante de submissão do artigo

Submission Confirmation

Print

Thank you for your submission

Submitted to	Pesquisa Brasileira em Odontopediatria e Clínica Integrada
Manuscript ID	PBOCI-2024-0015
Title	Morphological, elemental and release kinetics analysis of silver, vanadium and fluoride ions from a nanomaterial-modified glass ionomer cement
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