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Influence of incorporation of nanostructured silver vanadate decorated with silver nanoparticles on roughness, microhardness, and color change of pit and fissure sealants

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ABSTRACT

Objective: The aim of this study was to evaluate the roughness, hardness, and color change of pit and fissure sealants of two commercial brands (Fluroshield TM and Ultraseal XT TM) incorporated with nanostructured silver vanadate nanomaterial decorated with silver nanoparticles (β -AgVO₃) in concentrations (0% - control, 2.5% and 5%). *Material and methods:* Two commercial brands Fluroshield TM and Ultraseal XT TM were used to

make the samples with dimensions of $6 \times 6 \times 4$ mm. The control group was made according to the manufacturer's instructions and in the groups with the addition of β -AgVO₃, the nanomaterial was added proportionally by mass at percentages of 2.5% and 5%. Roughness properties were evaluated using a 3D Laser Confocal Microscope (n = 10), Knoop microhardness by Microdurometer (n = 10), and color change by Portable Color Spectrophotometer on the CIEDE2000 system (n = 10). Data were evaluated by one-way ANOVA with Bonferroni adjustment and Tukey's mean comparison test at a 5% significance level.

Results: Ultraseal XT TM sealant roughness showed a significant difference between concentrations with the highest mean for the 5% group (P = 0.010). Regarding the hardness, both sealants showed no significant difference between the groups. Fluroshield TM sealant showed a significant difference in Δ E00 between the control-2.5% 24.93 (3.49) and control-5% 28.41 (2.58).

Conclusion: It may be concluded that the incorporation of β -AgVO₃ influenced the increase in roughness for Ultraseal XT TM pit and fissure sealant, did not interfere with the microhardness of both sealants, and promoted a change in the color of Fluroshield TM sealant within clinically acceptable limits.

1. Introduction

The poor hygiene associated with a high carbohydrate diet promotes a favorable scenario for the development of tooth decay, which, although it has been reduced over the years, is still the most prevalent chronic disease in the world [1-3]. The occlusal surface

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of the posterior teeth is the most affected by caries, mainly due to dental morphology (shape, depth, and narrowing of the fissures), which facilitates the accumulation of biofilm and prevents self-cleaning by food, tongue, and cheeks, and also hampers the mechanical hygiene and the action of fluorides compared to smooth surfaces [2–5].

Non-invasive treatments including fluoride varnish, fluoride toothpaste, and pit and fissure sealant treatment have been suggested as effective measures for preventing caries disease [6]. Fluoride varnish and toothpaste are applied to all teeth in the oral cavity, while sealants are selectively applied to posterior teeth with complex anatomy. Sealants are recommended for patients at high risk for caries as they function as a physical barrier that interrupts or inhibits the entry of bacteria and nutrients, resulting in a decrease of around 90% of posterior caries after one year of application and approximately 50% after five years [2–4,6–11]. Behroozian et al. used Feigal's criterion [12] to evaluate the treatment failure rate by analyzing three factors: marginal adaptation, marginal discoloration, and anatomical shape of permanent first molars treated with pit and fissure sealants. After 12 months, they observed that the success rate was 74.3% and with this, the authors emphasized that pit sealants are a valid therapy for the prevention of tooth decay [12,13].

To achieve treatment efficacy, some care must be taken, such as application technique, which must be careful to avoid humidity, because the presence of saliva can prevent the penetration of resinous fluid into the microporosities, decrease shear strength, and increase microleakage, these being the main causes of failure and failure of preventive treatment [5,11,13–15].

Maintaining the integrity of pit and fissure sealants on the tooth surface is critical to treatment success. The primary cause of loss of integrity is material wear, especially when applied to the occlusal surface [16,17]. The wear resistance can be evaluated by mechanical tests, among them microhardness, which can provide a good indication of wear resistance, in addition, pit and fissure sealants wear out over time and therefore need to be reapplied, which increases the cost of treatment [16,18–20]. Microleakage is one of the main disadvantages of pit and fissure sealants and can be caused by defects in the mechanical properties and oral environmental challenges, such as the chewing cycle, temperature changes, and pH [14,21]. Microleakage may allow cariogenic substances to enter, reducing the retention rate and resulting in caries initiation under sealed surfaces [21,22]. Because of the deficiencies of conventional sealants, it is better to work on improving their physical and mechanical problems, especially in children with a higher risk of caries [21,23].

In this context, investigations have been carried out in order to improve pit and fissure sealants by incorporating materials into their composition [1,24–28]. The study proposed by Silva et al. was to add amorphous calcium phosphate to the sealant to provide better remineralization of the compromised dental elements [27]. Shanmugaavel et al. and Garcia et al. in their studies, incorporated the antimicrobials poly hexamethylene guanidine hydrochloride (PHMGA) and chlorhexidine, respectively, in order to reduce the number of microorganisms at the sealant-tooth interface, and the results were promising with the reduction in the number of microorganisms near the material [1,28].

Following this line of materials incorporation, nanostructured silver vanadate decorated with silver nanoparticles (β -AgVO₃), was developed and patented by the research group and its application and effectiveness have been investigated in dental materials such as acrylic resin [29,30], resin cement [31], endodontic sealers [32,33], porcelain [34–36] and soft liners [37]. β -AgVO₃ stabilizes the silver nanoparticles (AgNPs) by reducing particle agglomeration due to deposition on the vanadium nanowires, which allows the maintenance of a larger contact area and continuous release of silver ions (Ag+), which acts in the antimicrobial action [38,39]. The synergy between AgNPs and vanadium (4V+) favors increased antimicrobial activity by altering cell membrane integrity through oxidative stress and ROS formation [29,30,40]. Studies [32,34,37,41] have revealed antimicrobial efficacy against gram-positive and gram-negative bacteria, including *Staphylococcus aureus* and *Streptococcus mutans*. Besides antimicrobial capacity, the incorporation of β -AgVO₃ can improve or not alter the mechanical properties of dental materials, such as acrylic resins, endodontic cement, soft liner, and dental ceramics [32,34,37,41].

The use of nanotechnology has enabled the advancement of dentistry through the innovation of modified dental materials with the possibility of acquiring better properties and antimicrobial activity, as well as being able to promote intelligent materials that can respond to specific stimuli and overcome the limitations of current materials [21,42–44]. Given the effective application of nanomaterial β -AgVO₃ in other dental materials and the possibility of improving pit and fissure sealants, this study aimed to evaluate the roughness, microhardness, and color change of pit and fissure sealants incorporated with different percentages of nanostructured silver vanadate decorated with silver nanoparticles (β -AgVO₃). It presents a null hypothesis that the incorporation of the nanomaterial will not promote changes to the sealant.

2. Material and methods

2.1. Synthesis of nanostructured silver vanadate

Nanostructured silver vanadate was synthesized via a precipitation reaction between silver nitrate (AgNO3, Merck 99.8%) and ammonium vanadate (NH4VO3, Merck 99%). Initially, 0.9736 g of NH4VO3 and 1.3569 g of AgNO3 were solubilized in 200 mL of distilled water, respectively. The solutions were stirred separately on a heated surface at 65 °C for 10 min. Next, the silver nitrate solution was added dropwise with the use of a burette to the ammonium vanadate solution under constant stirring at 65 °C. The precipitate obtained was washed with distilled water and absolute alcohol several times, then filtered and dried in a vacuum oven for 10 h [38]. Nanostructured silver vanadate is obtained, with particles of size 100 nm and the silver particles that decorate the vanadium nanowires with a size in the range of 1-10 nm [38,39].

2.2. Fabrication of the specimens

Two commercial brands of resin sealants were used, Fluroshield (Dentsply) TM and Ultraseal XT (Ultradent) TM (Table 1). The

sealants were divided into three groups, control and those modified with the incorporation of 2.5% and 5% of β -AgVO₃.

The sealants were applied in a Teflon matrix in the dimensions $6 \times 6 \times 4$ mm to prepare the specimens and photoactivated with an Optilux 501 curing unit (Kerr; Orange, CA, USA) for 20 s at 800 mW/cm² of energy. The control group was made according to the manufacturer's instructions. For the groups containing β -AgVO₃, the sealants were placed on glass plates on the rough side and weighed. Using a precision balance, the β -AgVO₃ was added proportionally to the incorporated concentration of 2.5% and 5%. To promote the homogenization of the sealants with β -AgVO₃ the materials were mixed until a homogeneous mass was obtained and placed in the matrix. After insertion into the Teflon matrix, light-curing was carried out for 20 s [31]. After their confection, the samples (Fig. 1) were polished with water sandpaper of different grits 100, 320, 640, 1000, 1200, 1500 and 2000. The sample size calculation tests were carried out by Power Test using the RStudio software, version 2023.09.0 + 463 (R Foundation for Statistical Computing, Vienna, Austria).

2.3. Roughness

A 3D Laser Confocal Microscope (LEXT 4000; Olympus, Hamburg, Germany) was used to evaluate the surface roughness of the sealants before and after laser irradiation. This test evaluates Ra, Rz, and Sa. The parameter Ra, or arithmetic mean roughness, is the average of the absolute values of the surface deviations from the mean line. Rz, or maximum profile height, measures the vertical distance between the highest peak and the lowest valley over a sampling length. Sa, or arithmetic mean of the absolute values of the surface deviations, is another parameter that represents the average surface roughness and was used in this study [46,47]. Scans will be performed at $10 \times$ magnification and 1024×1024 pixels resolution. Two measurements per sample were performed in different and random positions with n = 10 for each analysis group.

2.4. Microhardness

A microdurometer (Shimadzu HMV-2, Japan) was used for the evaluation of Knoop hardness (KHN). The surface of the samples (n = 10) was marked with a diamond indenter with a load of 25 g and a time of 15 s at least 1 mm from the edge of each upper and lower surface. The microhardness value of each sample consisted of the arithmetic mean of five markings. After removing the load, the diagonals of the notch were measured with an optical microscope. The microhardness value was defined as the ratio between the indentation load and the residual impression area [31].

2.5. Color change

The measurement of the color change of the specimens (n = 10) of each group was performed using a Portable Color Spectrophotometer - Model SP 62S (Spherical Geometry Spectrophotometer, D/8°, XRITE Incorporated, USA, 11/2012), with 3 measurements for each, with white background, in order to verify if the different percentages of the nanomaterial (2.5% and 5%) differ from the control group. This was done using the CIEDE2000 system that allows the evaluation of color attributes, identifying inconsistencies, and communicating its results numerically and accurately. The L*C*h system uses the same diagram as the L*a*b* color space but with cylindrical coordinates instead of rectangular coordinates. The Δ E00 was calculated according to the formula [48,49]:

$$\Delta E_{00} = \sqrt{\left(\frac{\Delta L^*}{K_L S_L}\right)^2} + \left(\frac{\Delta C^*}{K_C S_C}\right)^2 + \left(\frac{\Delta H^*}{K_H S_H}\right)^2 + R_T \left(\frac{\Delta C^*}{K_C S_C}\right) \left(\frac{\Delta H^*}{K_H S_H}\right)$$

where ΔL indicates the difference in luminosity, ΔC the difference in chroma and ΔH ' the difference in hue. The RT interaction function takes into account the interaction between the chroma and hue differences in the blue region. To adjust the total color difference, weighting functions are applied: SL (for luminosity), SC (for chroma), and SH (for hue), which help to take into account the differences in color space. KL, KC, and KH are empirical terms that correct or weight the metric differences calculated by the formula

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Table	

Commercial brands,	classification, and	l characteristics of	f the pit and	fissure sealants used.

Pit and fissure sealant (Manufacturer)	Classification	Characteristics	Composition
Fluroshield (Dentsply) ™	Light Cure Light cure for 20 s.	Fluoride release Viscosity Durability	NCO Monomer, Nupol Bis GMA, TEGDMA, Penta, N-methyl Diethylamine, BHT, 2_n Methacrylate, Camphorquinone, Cervit T 1000, Silanized Barium, Sodium Fluoride, Cabosil TS 720 and Titanox 3328.
Ultraseal XT (Ultradent) ™	Light Cure Light cure for 20 s.	Radiopaque Fluoride release Viscosity Wear resistance	>10%-≤25% TEGDMA, >2.5%-≤10% DUDMA, >2.5%-≤10% aluminum oxide, ≤2.5% HEMA, ≤2.5% amine methacrylate, ≤2.5% organophosphine oxide, 0.1% sodium monofluorophosphate [45]



Fig. 1. Image of the samples of Ultraseal XT TM and Fluroshield TM sealant at 5%, 2.5%, and control concentrations, taken by a camera. Legend: A Control group Ultraseal XT TM; B- 2.5% de β-AgVO₃ Ultraseal XT TM; C- 5% β-AgVO₃ Ultraseal XT TM; D- Control group Fluroshield TM, E- 2.5% β-AgVO₃ Fluroshield TM; F- 5% β-AgVO₃ Fluroshield TM.

[50]. Color changes with $\Delta E00 < 3.3$ were considered clinically acceptable in this study [51–53].

2.6. Statistical analysis

For statistical analysis, the distribution and homogeneity of the data were verified and thus, for the analysis of color change and microhardness, one-way ANOVA variance analysis with Bonferroni adjustment was used ($\alpha = 0.05$) and for roughness, ANOVA and Tukey tests were used for comparison between concentrations within the same group of sealants. Mann-Whitney's *U* test was applied to evaluate the difference between sealants. A significance level of 5% was adopted [54].

3. Results

3.1. Roughness

For surface roughness was observed that Ultraseal XT TM sealant showed a significant difference between concentrations (P = 0.010) with the highest mean for the 5% β -AgVO₃ group compared to 2.5% β -AgVO₃ (P = 0.006) and control (P = 0.002). For the Fluroshield TM sealant, no significant difference was observed between concentrations (P = 0.458). In the comparison between sealants, there was a significant difference for the control (P = 0.007) and 2.5% β -AgVO₃ (P = 0.028) groups, with higher roughness for Fluroshield TM; for the 5% β -AgVO₃ group there was no difference between sealants (P = 0.218) (Graph 1).

The images (Figs. 2–7) were obtained by laser confocal microscopy for the different groups. The control group of Fluroshield TM (Fig. 2) displayed a uniformly polished surface. In contrast, the 2.5% β -AgVO₃ Fluroshield TM group (Fig. 3) exhibited surface alterations with crater-like structures of β -AgVO₃, causing a loss of homogeneity relative to the control group. The 5% β -AgVO₃ Fluroshield TM group (Fig. 4) demonstrated heterogeneity in regions with visible agglomerated β -AgVO₃ particles. Surface observations indicate that the matrix surrounding the agglomerates remains unaffected.

The control group using the Ultraseal XTTM sealant (Fig. 5) shows a surface appearance distinct from the FluroshieldTM control, with crater-like regions and differing surface homogeneity. When 2.5% β -AgVO₃ Ultraseal XTTM (Fig. 6) is applied, no surface agglomerates

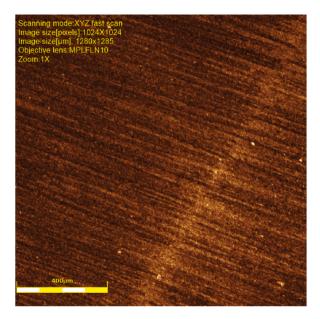


Fig. 2. Laser confocal microscope of the Fluroshield TM control.

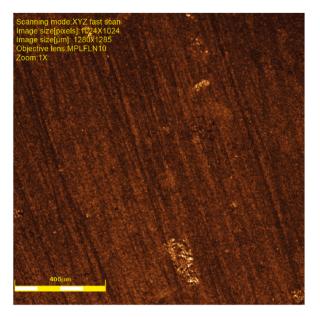


Fig. 3. Laser confocal microscope of the Fluroshield TM 2.5% of β-AgVO₃.

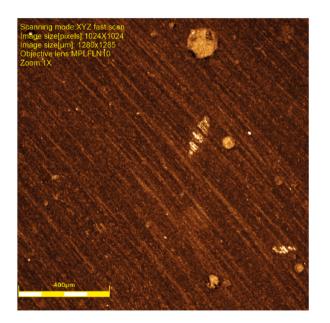


Fig. 4. Laser confocal microscope of the Fluroshield TM 5% of β-AgVO₃.

are detected, suggesting the sealant matrix exhibits greater encapsulation. This is similarly observed in the Ultraseal XTTM 5% β -AgVO₃ group (Fig. 7). These results support the roughness data presented earlier, as a result of the increased encapsulation of β -AgVO₃ within the Ultraseal XTTM sealant.

3.2. Microhardness

Regarding hardness, no significant difference was observed between the concentrations for each type of sealant, (P > 0,05) and Fluroshield TM (P > 0,05), that is, the incorporation of nanomaterial did not promote changes in the hardness of the different groups. However, when comparing the sealants, a difference was observed, with Ultraseal XT TM showing statistically different mean microhardness values than Fluroshield TM in the control group, 2.5% β -AgVO₃ 5% β -AgVO₃ (P < 0.001; P = 0.014; P < 0.001) (Graph 2).

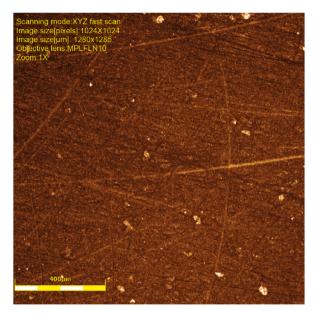


Fig. 5. Laser confocal microscope of the Ultraseal XT TM control.

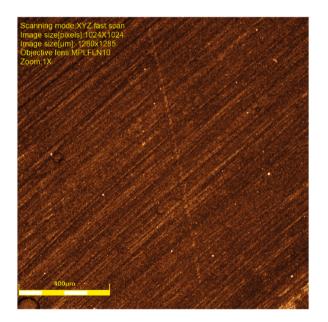


Fig. 6. Laser confocal microscope of the Ultraseal XT TM 2.5% of β-AgVO₃.

3.3. Color change

For Ultraseal XT TM sealant no significant difference in Δ E00 was observed between control-2.5% β -AgVO₃ and control-5% β -AgVO₃ (P = 0.790) groups. For the Fluroshield TM sealant, a significant difference in Δ E00 was observed between control-2.5% β -AgVO₃ and control-5% β -AgVO₃ groups (P = 0.004). When comparing sealants, there was a significant difference between brands (P < 0.001), with lower mean Δ E00 scores for Ultraseal XT TM in both the control-2.5% β -AgVO₃ and control-5% β -AgVO₃ groups (Table 2).

4. Discussion

The modification of pit and fissure sealants to promote better physicochemical and mechanical properties and antimicrobial activity aims to find a material that better combines structure-property correlation and its clinical application. In this study, the null hypothesis that the incorporation of β -AgVO₃ nanomaterial does not promote changes in the properties of the evaluated pit and fissure

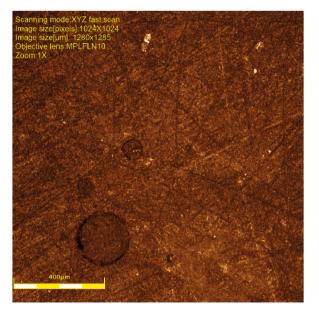
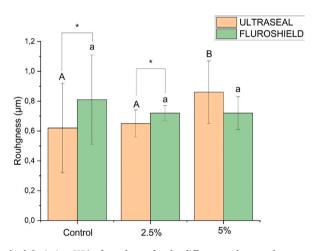


Fig. 7. Laser confocal microscope of the Ultraseal XT TM 5% of β-AgVO₃.

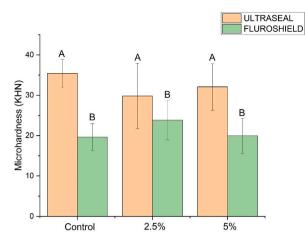


Graph 1. Graph with mean and standard deviation (SD) of roughness for the different sealants and concentrations. Legend: One-way ANOVA, Tukey test (P > 0.05). Different capital letters indicate statistical differences for Ultraseal TM. Different lowercase letters indicate statistical differences for Fluroshield TM. * Indicates difference between brands.

sealants was rejected.

The β -AgVO₃ was effectively incorporated and showed results compatible with acceptable clinical parameters for analyzing antimicrobial activity, compression, hardness, and roughness for acrylic resins, radiopacity and pH for endodontic cement, and promotion of antimicrobial activity for dental implants [33,40]. Thus, the incorporation of β -AgVO₃ in different dental materials, pit and fissure sealants are a class of materials that can benefit from the incorporation of the nanomaterial by promoting antimicrobial activity and improving mechanical properties that are still deficient and can hinder the preventive treatment of dental caries.

When a material is modified, its properties can be altered and in this study, the roughness, hardness, and color change of sealants prepared with β -AgVO₃ were evaluated. It was observed that the incorporation of nanomaterials resulted in increased roughness for the 5% Ultraseal XTTM group. This phenomenon can be explained by the non-uniform distribution of nanomaterial particles in the organic matrix, which promotes the formation of agglomerates and leads to the creation of surface irregularities. This result is consistent with the findings of Ferreira et al. and Vidal et al. who reported increased surface roughness in the 10% group of β -AgVO₃ incorporated into dental ceramics [34,35]. Increased roughness is frequently associated with the formation of biofilms and gingival inflammation, and it should be carefully monitored due to its potential to cause treatment failure [48]. In their study, Hesaraki et al. pointed out that the incorporation of nanoparticles results in a gradual increase in surface roughness, and despite proper nanoparticle dispersion in the polymer, nano-agglomerates may still be observed [52,55]. A difference was observed between the groups when comparing Ultraseal



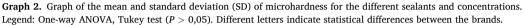


Table 2

Mean and standard deviation (SD) of the comparison between the control group and the 2.5% and 5% concentrations of β -AgVO3 in color change.

Groups	$\Delta E00$	p-value
Ultraseal XT control-2.5%	13.92 (2.25)A	P = 0.790
Ultraseal XT control-5%	13.62 (1.33)A	P = 0.790
Fluroshield control-2.5%	24.93 (3.49)B	P = 0.004
Fluroshield control-5%	28.41 (2.58)B	P = 0.004

Legend: One-way ANOVA, Tukey test (P > 0.05). * Indicates difference between brands.

XT[™] and Fluroshield[™], with Fluroshield[™] presenting a rougher surface even when compared to the control groups. This may be explained by the difference in formulation between the materials since roughness can be influenced by the size and distribution of the filler particles and the polymerization contraction of the different polymer particles.

No significant differences in hardness were observed among the various modified sealant compositions, i.e. the incorporation of different percentages of β -AgVO₃ did not alter the hardness of the sealants, as can be seen in Graph 2, where the groups with added nanomaterial showed a hardness statistically similar to the control group, since β -AgVO₃ acts as a reinforcing structure for the sealants. The findings align with Monteiro et al. which noted no changes in sealant hardness when incorporating 1,3,5-triacrylonyl hexahydro-1,3,5-triazine (TAT) and α -tricalcium phosphate (α -TCP) in the composition. However, they found significant improvements in other properties, including increased tensile strength [56]. Hamilton et al. found that adding chitosan to the composition of pit and fissure sealants resulted in a significant increase in hardness. This could be attributed to the interfacial bonding between the matrix and the chitosan fibers [51].

Color stability, or the ability of a material to maintain its original color, can be influenced by factors such as the nature of the material, water absorption, and extrinsic influences like food and beverage coloring. Studies [52,53] have demonstrated that the incorporation of β -AgVO₃, a yellowish nanomaterial, into pit and fissure sealants can lead to significant alterations in coloration, potentially impacting its application. This is an important consideration given the current emphasis on esthetics in dentistry [48,52, 53]. Results of color change revealed that the Fluroshield TM brand demonstrated a significant difference when contrasted with the Ultraseal XT TM brand. A smooth surface enhances light reflection, thereby resulting in brightness, a property measured by spectro-photometers and colorimeters. Hence, one probable reason for the color change observed in Fluroshield TM sealant treated with β -AgVO₃ could be the rough surface that diminishes the light reflection capacity as read by the spectrophotometer [48,57].

The alteration in the FluroshieldTM sealant upon the inclusion of the nanomaterial, when compared to the control group, could be attributed to its interaction with a particular component of the material's chemical composition. Color change evaluation is typically employed to examine the correlation between the duration of material use inside the oral cavity and internal discoloration. Certain analyses may still impact the results of this study, specifically those concerned with evaluating the effects of time of use, exposure to acids, food coloring, and other cavity-related factors that could cause alterations to β -AgVO₃-modified sealants [57]. Nonetheless, it is important to note that a noticeable color change occurs when $\Delta E00 > 1$, and acceptable $\Delta E00$ values are restricted to $\Delta E00 < 3.3$ [51–53]. Therefore, the modified sealants tested in this study fall within the range of acceptable clinical values [48,52].

This study has limitations regarding the dispersal of β -AgVO₃ particles in fissure sealants. An alternative incorporation method is recommended. Additionally, an evaluation of the nanomaterial's potential antimicrobial activity, which could play a vital role in caries prevention, was absent. Further evaluation is also necessary for physical-mechanical properties such as degree of conversion, adhesion to dental structures, and changes in the color of extracted teeth. Further research is required to address these limitations. Nonetheless,

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the implementation of nanostructured silver vanadate adorned with silver nanoparticles exhibits prospective usefulness in pit and fissure sealants for preventing caries disease. However, additional in vitro assessments are required to assess the modified material's antimicrobial effectiveness, ion discharge, cytotoxicity, shear strength, and other physicochemical and mechanical traits, to determine its practicality.

5. Conclusion

We can infer from the data found in this study that the addition of 2.5% and 5% β -AgVO₃ in pit and fissure sealants:

- Promoted changes in the roughness of Ultraseal XT TM sealant;
- Did not promote changes in the microhardness of Ultraseal XT TM and Fluroshield TM sealants.
- Altered the color of Fluroshield TM sealant, but within clinically acceptable limits.

Data availability statement

Data will be made available on request.

CRediT authorship contribution statement

Izabela Ferreira: Writing – review & editing, Writing – original draft, Methodology, Investigation, Formal analysis, Data curation, Conceptualization. Oswaldo Luiz Alves: Validation, Resources, Methodology. Marco Antônio Schiavon: Validation, Software, Resources, Methodology. Andréa Cândido dos Reis: Writing – review & editing, Writing – original draft, Visualization, Validation, Supervision, Software, Resources, Project administration, Methodology, Investigation, Funding acquisition, Formal analysis, Data curation, Conceptualization.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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