Objective: Titanium tetrafluoride (TiF₄) is a topical agent used in the control of dental caries; however, it is highly acidic. To minimize this effect, cyclodextrins (CDs) are used. This study evaluated the in vitro potential of TiF₄ and β-CD on remineralization. Methods: Forty bovine enamel blocks were selected by microhardness and randomly assigned to four groups (n = 10 per group): control (distilled and deionized water), 1% β-CD solution, 1% TiF₄ solution, and TiF₄: β-CD solution. The blocks were subjected to a pH cycling regimen for 8 days. After that, samples were evaluated by cross-sectional microhardness (CSMH), scanning electron microscopy (SEM), and energy dispersive spectrometry (EDS). Data were assessed for normality and analyzed using ANOVA and Tukey’s tests (α = 0.05). Results: Regarding CSMH, TiF₄: β-CD was statistically superior to the control (P = 0.033), β-CD (P = 0.022), and TiF₄ (P = 0.006). SEM photomicrography revealed the titanium dioxide coating on slabs treated with TiF₄ and TiF₄: β-CD. EDS assessment demonstrated the presence of titanium on the surface of slabs treated with TiF₄ and TiF₄: β-CD. Conclusion: The solution containing the inclusion nanocomplex formed of TiF₄ and β-CD was able to reharden the enamel subsurface.

Keywords: Beta-cyclodextrins, dental caries, fluoride, microhardness, tooth remineralization

Introduction

The use of fluoride (F⁻) is known as a major factor in reducing the occurrence of caries. Among the main products, titanium tetrafluoride (TiF₄) has been widely used as a minimally invasive treatment for the disease and in vitro or in situ studies. While fluoride topical applications (especially sodium fluoride) result in the formation of globular deposits of calcium fluoride (CaF₂) and CaF₂-like materials that serve as fluoride reservoir that release fluoride and reduce enamel dissolution, the mechanism of action of TiF₄ consists of a physicochemical barrier based on an acid-resistant coating formed of titanium dioxide referred as a glaze-like layer over tooth surface associated to fluoride materials formation that protects enamel from mineral loss. Despite the positive effects of TiF₄ on the rehardening of the previous artificial carious lesions, it has never found broad application in the clinical dental field. On the other hand, literature indicates that remineralization is also inhibited by TiF₄. The very low pH (1.0) of the pure solution is one of the major disadvantages of TiF₄. Although promising, its instability represents a limitation to clinical use; therefore, to obtain a therapeutic improvement using TiF₄, inclusion complexes should be adopted.

Molecular complexation is an area of great interest in biotechnology because it enables the selection, separation, solubilization, and stabilization of various biomolecules. For the purpose of achieving...
improvements in stability and treatment efficacy of certain chemical compounds, cyclodextrins (CDs) have been the subject of many studies in different areas. β-CD is the most accessible, the lowest-priced and generally, the most useful. It is structurally organized in a truncated cone form with the ends exposing hydrophilic sites due to the presence of hydroxyl groups, and the cavity presents hydrogen atoms and glycosidic oxygen bridges, assigning a highly hydrophobic character.[16] In the inclusion complex formation with drug molecules, no covalent bonds are created between the carrier and its guest. The drug molecules included within the CDs cavity may therefore be dissociated on dilution or displaced by a more suitable guest.[17] They are used to increase the solubility of liposoluble drugs in water, stabilizers, and as host-guest delivery carriers for a variety of drugs.[18]

Owing to the paucity of in vitro studies that analyze the effect of this compound incorporated into solution on remineralization, the present study was performed as a laboratory rehearsal. The null hypothesis was that TiF₄ associated with β-CD would not remineralize the enamel surface and subsurface. Taking into account these considerations, the aim of this study was to evaluate the in vitro effect of TiF₄ with a β-CD inclusion complex on remineralizing predemineralized artificial enamel blocks.

METHODS

Experimental design
This randomized, blind study evaluated the enamel remineralization capacity of a TiF₄: β-CD solution in vitro adopting a previously validated protocol.[19] This study assessed the remineralizing ability of a new inclusion complex (TiF₄: β-CD) on previously demineralized bovine enamel blocks. Four groups with ten predemineralized bovine enamel blocks in each were randomly chosen to evaluate the following four treatment groups: Control (distilled deionized water), a solution containing 1% β-CD, a solution carrying 1% TiF₄ and the experimental formulation containing TiF₄: β-CD.

The response variables investigated were cross-sectional microhardness (CSMH) and energy dispersive X-ray spectrometry (EDS) analysis. A qualitative analysis of the blocks was also applied by scanning electron microscopy (SEM) after the pH-cycling regimens and treatments with the formulations.

Preparation of cyclodextrin complexes
The inclusion complexes of TiF₄: β-CD were made by kneading, solubilization, and freeze drying at molar ratios of 1:4. Physical mixtures were prepared by mixing β-CD and TiF₄ in a mortar at the same molar ratios and blending them in a mortar for 5 min. An ethanol: water (70:30; v/v) solution was added and mixed for 30 min to obtain a homogeneous paste, which was dried under reduced pressure and the granulometry adjusted using a 40-mesh sieve.[6]

Regarding the solution preparation method, the appropriate proportions of TiF₄ and β-CD were blended in 20 mL of distilled water with a magnetic stirrer for 72 h. The samples remained frozen in liquid nitrogen and were lyophilized. The particle size was calibrated with a 40-mesh sieve. The inclusion yield was calculated by ultraviolet spectroscopy.

Preparation of bovine enamel blocks
Forty bovine sound enamel blocks (4 mm × 4 mm × 3 mm), which had been stored in 2% formaldehyde solution, were selected from 100 bovine teeth, incorporated into acrylic devices, and polished with 600- and 1200-grit silicon carbide paper, followed by 3- and 1-μm diamond abrasive slurry (Buehler Ltd., Lake Bluff, Illinois, USA).[6] Each enamel slab was prepared from a separate bovine tooth, and blocks were selected on the basis of baseline surface microhardness (SMH) (mean 321.35 ± 32.13 kg/mm²). SMH was measured using a microhardness tester (Buehler, Micromet 5104, 679-MIT4-00335, Japan) with a Knoop diamond under a 50 g load for 5 s, by making five indentations spaced 100 μm from each other at the center of the enamel surface.[20]

Enamel demineralization
Forty enamel blocks were immersed a demineralizing solution (0.05 M acetate buffer, pH 5.0, 1.28 mM Ca, 0.74 mM P, 0.03 μg/mL F; 2 mL/mm² of enamel area) for 16 h, and mineral loss was evaluated. The time of 16 h was chosen to induce caries-like lesions on the enamel because the enamel blocks presented measurable caries-like subsurface lesions without surface erosion, allowing the evaluation of mineral loss or gain by determining SMH.[19] The slabs with known SMH (sound enamel) were exposed to the demineralizing solution for 16 h, the SMH was again determined (demineralized enamel), and the blocks with caries-like lesions were used for the further evaluation.

Enamel surface treatment and pH-cycling regimen
The solutions were applied only once on the surface of the blocks with a Microbrush® and left for 1 min.[6] After this, the blocks were rinsed with deionized and distilled water and dried with soft paper. Subsequently, the pH cycling started. The investigators were blinded to the solutions used.

The pH-cycling regimen occurred over 8 days, and the blocks were kept at 37°C for 2 h in the demineralizing solution and 22 h in the remineralizing solution. The demineralizing solution contained 0.05 M acetate buffer,
RESULTS

Cross-sectional microhardness

As seen in Table 1 and Figure 1, as the depth increased, the mean CSMH values in the TiF$_4$; β-CD group increased relative to the other treatment groups. TiF$_4$; β-CD was able to increase internal enamel microhardness when compared with the control (P = 0.033), β-CD (P = 0.033) and TiF$_4$ group (P = 0.006).

Scanning electron microscopy and energy dispersive spectrometry evaluation

The surface images demonstrated the formation of an acid-resistant titanium dioxide coating on the slabs treated with TiF$_4$ and TiF$_4$; β-CD, as can be seen in Figure 2. Chemical analysis (EDS) revealed the presence of titanium element in all slabs treated with TiF$_4$ and TiF$_4$; β-CD on the surface, in accordance with Figure 2. The presence of titanium element was not evident at the subsurface of the blocks treated with TiF$_4$ and TiF$_4$; β-CD at the surface, as shown in Figure 3.

DISCUSSION

There are significant differences in the inner layer, with increased CSMH in the blocks treated with a TiF$_4$; β-CD solution. TiF$_4$ is a topical fluoride agent that forms a titanium-rich glaze/coating following its application. Attributed to this coating is the anticaries effect observed in enamel treated with this agent in de- and re-mineralization studies. This layer offers a diffusion barrier and retains fluoride, leading to slow release onto the tooth and into its environment. The hydrolysis of TiF$_4$ has been known since 1967, and in light of these data, a huge effort has been made to create new compounds to establish and realize its clinical use.

The ability of β-CD to improve the pH of TiF$_4$ has already been examined in the literature. The remineralizing pH-cycling study was chosen because its potential to penetrate the demineralized enamel had not been fully investigated.

Table 1: Values (means and standard deviation) of surface microhardness analysis of enamel blocks before and after pH-cycling according to the groups and ΔZ (cross-sectional microhardness)

| Groups     | SMH sound | SMH demineralized | ΔZ (vol% × µm) |
|------------|-----------|-------------------|----------------|
| Control    | 321.61±13.14 | 30.69±6.49         | 1198.68±285.20a |
| β-CD       | 323.87±12.66 | 29.44±3.88        | 1055.89±262.19a |
| TiF$_4$    | 320.77±14.24 | 28.80±4.25        | 1230.7±285.51a  |
| TiF$_4$; β-CD | 325.11±10.27 | 30.88±4.54        | 850.4±381.80b   |

$^a$ Determined before pH cycling. $^b$ Determined after pH cycling. Different upper case letters indicate statistically significant differences within the same column (P<0.05). SMH: Surface microhardness, β-CD: β-cyclodextrin, TiF$_4$; Titanium tetrafluoride.
yet been studied. This is the first study to analyze the effect of TiF$_4$ associated with β-CD incorporated into solution in a remineralization study design.

As described in Table 1, the inclusion complex tested here was capable of rehardening the enamel subsurface after pH cycling. According to Figure 1 and Table 1, the TiF$_4$·β-CD inclusion complex solution was capable of remineralizing the predemineralized enamel subsurface up to a depth of 40 µm as mentioned previously. An in situ study evaluating the effect of sodium fluoride and TiF$_4$ varnish and solution on carious demineralization of enamel demonstrated that TiF$_4$ was not able to reharden the enamel subsurface.$^{[10]}$ We can hypothesize that the in situ conditions utilized in that study (presence of biofilm) did not allow the penetration of the titanium to the subsurface.

Some researchers agree with our results with respect to the enhancement of subsurface hardness. When conducting an in vitro study on demineralization and remineralization of bovine enamel by 4% TiF$_4$ varnish, it was observed that 4% TiF$_4$ varnish significantly enhanced the mineral content compared with the other treatments (Duraphat$^®$ and Duofluorid$^®$) up to a depth of 30 µm.$^{[15]}$ The inclusion complex tested here was able to improve the mineral content up to a depth of 40 µm, according to Figure 1. A difference concerning these two studies refers to the adopted concentration, which was 1% and the solution of a nanoinclusion complex of TiF$_4$·β-CD instead of a TiF$_4$ varnish. It is important to point out that that study$^{[3]}$ and the present paper adopted a single application of TiF$_4$ over the enamel blocks.

The SEM study of the predemineralized enamel blocks showed the formation of an acid-resistant TiO$_2$ coating changing the resistance of this surface to acid attack$^{[8,12,22]}$ as can be observed in Figure 2. From this coating, we can speculate that titanium could penetrate the enamel layers, resulting in increased mineral hardness. However, in spite of the presence of elements such as oxygen, chlorine, potassium, carbon, phosphorus and sodium, which were available in surface and subsurface enamel, according to Figures 2 and 3, the presence of titanium was only observed on the enamel surface in the TiF$_4$ and TiF$_4$·β-CD groups. Although the depth of titanium penetration could not be observed at subsurface cross-sectional slabs using an EDS analysis method, one can conclude that the titanium released from TiF$_4$·β-CD induced the rehardening of the enamel subsurface. We can suppose that a small amount of this compound was able to be incorporated into the enamel, favoring its mineral content. Another possibility that can explain the absence of titanium in the inner enamel is that its penetration seems to be greater in sound enamel than in predemineralized enamel.$^{[7,14]}$ This difference could be related to a larger quantity of water, carbonate, and oxygen, improving the reactivity between titanium and the oxygen available in the dental structure.$^{[6]}$

It is well known that fluoride uptake is higher in decalcified enamel when compared with sound enamel$^{[23]}$ due to the porosity of the decayed enamel.$^{[24]}$ However, titanium penetration occurred more deeply into sound enamel compared with artificially demineralized enamel.$^{[7]}$ This can be explained by the decrease in water and carbonate in the carious enamel, which decreased the titanium connection with oxygen in the dental structure. It is reasonable to suggest that the titanium applied to the predemineralized enamel blocked the surface used in this study, contributing to the enhancement of inner enamel microhardness by modifying the underlying enamel; however, this amount of titanium was not sufficient to be detected in the EDS analysis.

The cavity of the CD molecule is lipophilic (lined with skeletal carbons and ethereal oxygens of the glucose residues), resulting in a microenvironment into which suitably sized drug molecules may enter and be included, bestowing a useful property of these excipients, such as retarding or accelerating degradation. The CD molecule shields the drug molecule from attack by various reactive molecules. In others words, the CD can insulate a labile compound from a potentially corrosive environment, thereby reducing drug hydrolysis, oxidation, steric rearrangement, racemization, polymerization, and enzymatic decomposition.$^{[25]}$

Among all natural CDs, β-CD is the most accessible, the lowest-priced and generally the most useful.$^{[16]}$ The advantages to using β-CD as employed here have already
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been illuminated.[6] The use of β-CD can improve on the methods by increasing the pH of the TiF₄ nanosystems in solution, allowing professional use and increasing thermal stability. Further studies are necessary to assess the rehardening of other inclusion complexes to investigate if this phenomenon is similar to the findings presented in this paper.

Several studies were limited in scope to the potential of TiF₄ to reduce the demineralization of dental hard tissue.[2-4,6] The fact that remineralization is inhibited by TiF was explained by assessing the effect of a new TiF on enamel remineralization. It was observed that treatment with a TiF derivative resulted in strongly reduced calcium loss, but calcium uptake

Figure 2: Scanning electron microscope photomicrography (left) of surface enamel and energy dispersive spectrometry evaluation (right). (a) control group (distilled and deionized water), (b) β-cyclodextrin group, (c) titanium tetrafluoride group, (d) titanium tetrafluoride: β-cyclodextrin group
was also inhibited. This finding highlights that this compound may not be capable of improving lesion repair.

A previous in vivo study showed that a single application of 4% TiF₄ solution for 1 min produced an unexpected pigmented layer over the enamel surface. This phenomenon may have occurred probably because the interaction between TiF₄ and the proteins of the tooth that provoked changes in the glaze-like layer. The solutions reported in the current study are not 4% but 1%, and over the bovine enamel slabs, this undesirable pigmented layer was not observed in vitro.
In this in vitro study, the TiF₄-β-CD inclusion complex was effective in rehardening artificially predemineralized bovine enamel blocks. TiF₄ is also advantageous in protecting enamel from demineralization. Lesion arrest seemed to be the maximum achievable result because TiF₄ provides long-lasting fluoride delivery and leaves large amounts of titanium on the surface glaze layer.

**Conclusion**

A single application of the solution containing the inclusion nanocomplex formed of TiF₄ and β-CD over the enamel blocks was able to reharden the enamel subsurface. This paper highlights a new inclusion nanocomplex used for the treatment of demineralized enamel. Future studies should be carried out before the clinical application of this compound.

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**Conflicts of interest**

There are no conflicts of interest.

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