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Cyclodextrin and TiF_4 Nanocomplex on Enamel Demineralization

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The aim of this study was to assess the effect of a newly developed nanocomplex formed of hydroxypropyl-β-cyclodextrin and 1% titanium tetrafluoride (TiF_4) after distinct complexation periods (12/72 h) on demineralization of bovine enamel in vitro. Enamel blocks (n=60) were allocated in different groups: Mili-Q water, hydroxypropyl-β-cyclodextrin, 1% TiF_4, hydroxypropyl-β-cyclodextrin + 1% TiF_4 after 12 h of complexation and hydroxypropyl-β-cyclodextrin + 1% TiF_4 after 72 h of complexation. The samples were evaluated by surface microhardness, cross-sectional microhardness and micro-CT. Scanning electron microscopy and energy dispersive X-ray spectrometry (SEM/EDX) were also obtained. Hydroxypropyl-β-cyclodextrin + 1% TiF_4 after 12 h complexation resulted in lower percentage of surface microhardness loss compared to Mili-Q water, hydroxypropyl-β-cyclodextrin, 1% TiF_4 and hydroxypropyl-β-cyclodextrin + 1% TiF_4 after 72 h of complexation group, with a large effect size (from 1.307 to 2.943) and high power (84.9 to 99%). All groups resulted in similar integrated mineral loss (ΔZ) obtained by both internal microhardness and micro-CT techniques. Enamel treated with TiF_4 and TiF_4 + hydroxypropyl-β-cyclodextrin groups showed a TiO_2 glaze-layer, while EDX evaluation identified Ti. The solution containing the inclusion complex of hydroxypropyl-β-cyclodextrin + TiF_4 with 12 h of complexation period demonstrated a significant ability to reduce surface demineralization of sound enamel under an artificial cariogenic challenge.

Key Words: dental caries, cyclodextrins, tooth demineralization, enamel, fluorides.

Introduction

Dental caries is still the most prevalent oral disease in the world, and for this reason, a worldwide public health problem. Moreover, Latin America presents the higher occurrence of untreated caries in permanent dentition compared to global indicators (1). The current understanding on the establishment and development of carious lesions are directly linked to undisturbed biofilm accumulation over the tooth surfaces (2). In this context, sucrose or other fermentable carbohydrates increase, while fluoride reduces the speed in which the lesions will progress (3,4).

In fact, fluoride effectively inhibits enamel dissolution by the formation of CaF_2 reservoirs, which slowly release fluoride into the plaque fluid in order to interact during enamel dissolution (5). For this reason, frequent contacts with fluoride should be emphasized in order to provide a balance on the de- and remineralization kinetics of dental hard tissues.

Titanium tetrafluoride (TiF_4) has been, for many years a promising caries preventive agent due to its capacity of inducing the formation of calcium fluoride and a relatively insoluble layer of titanium dioxide on the tooth surface (6). The majority of laboratorial studies agree that a single application of this product is enough to provide a significant decrease in enamel dissolution (7-9). However, its hydrolysis (10) and very acidic pH has always limited its clinical use (11). To overcome these problems and to improve the stability of this agent, research has been focusing on the development of new titanium tetrafluoride delivery systems, such as the association with cyclodextrin complexes (12,13).

Cyclodextrin inclusion complexes formed with certain drugs may have physical, chemical, and biological properties which are dramatically different from those of either the parent drug or the cyclodextrin molecule (14). These complexes can be used to increase solubility and dissolution rate, decrease volatility, alter release rates, modify local inflammatory potential, and increase the stability of drugs. Cyclodextrins with six to eight dextrose units have been named α-, β-, and γ-cyclodextrins, respectively (15). Among the natural cyclodextrins, β-cyclodextrin is the most widely used, because of its high availability, low cost and dimensions of its cavity, which is able to accommodate a large number of drug molecules (16).

A β-cyclodextrin inclusion complex formed with TiF_4 has shown some potential to reduce enamel demineralization

(12) and to increase the hardness of enamel subsurface (17). The nanocomplexes of hydroxypropryl-β-cyclodextrin with TiF_4 (12 and 72 h of complexation) also prevented enamel dissolution from a cariogenic challenge caused by a monospecies-biofilm (13). However, the effect of these nanocomplexes under a pH-cycling regimen, which provides a more controlled ion exchange environment, has not been studied yet. Thus, the aim of this study was to investigate the effect of a water-soluble polymer cyclodextrin inclusion nanocomplex (hydroxypropyl-β-cyclodextrin) formed with 1% TiF_4 after distinct complexation periods (12 or 72 h) on demineralization of bovine enamel in vitro.

Material and Methods

Preparation and Characterization of β-Cds and TiF_4 Nanocomplexes

To produce the 1% TiF_4-β-cyclodextrin nanocomplexes, 0.1974g of TiF_4 (Sigma Aldrich®, St Louis, MO, USA; purity 37 to 39% of titanium) and 2.0112 g of hydroxypropyl-β-cyclodextrin (Sigma Aldrich®) were mixed in 20 mL of distilled water using a magnetic stirrer for 12 or 72 h. The mean pH value and total fluoride concentration (µg F/mL) from the nanocomplexed solutions were 1.78±0.07 and 6123.67±343.56, respectively (18). After that, the solutions were frozen in liquid nitrogen and lyophilized. Particle size was calibrated using a 40-mesh sieve and the inclusion yield was calculated by UV spectroscopy. Further, these nanosystems were characterized by X-ray powder diffraction, Fourier transform infrared spectroscopy and differential scanning calorimetry, as described previously (12).

Preparation of Bovine Enamel Blocks

To determine the sample size, Hedge's G value of 1.97 was used from a previous study (unpublished data), a two-tailed significance level of 5% and a power test of 97.45%, resulting in a sample size of 9. Twelve blocks per group were used to compensate for sample loss (30%).

Sixty enamel blocks (4 mm x 4 mm) were obtained from sterile bovine incisors by slicing the buccal surfaces with a diamond disk mounted in a low speed cutting machine (Isomet, Lake Bluff, IL, USA). The blocks were embedded in acrylic resin and ground sequentially using 600 and 1200 grit Al_2O_3 paper in a semi-automatic polisher (model PLF, Fortel, São Paulo, SP, Brazil) in order to obtain a 'window' of flattened and polished enamel in each specimen.

Enamel Blocks Selection and Allocation

Enamel specimens were selected by surface microhardness. Knoop hardness values were obtained by means of a microhardness tester (Buehler, Micromet 5104, 679-MIT4-00335, Japan) with a load of 50 g for 5 s (12). The first set of measurements was obtained in order to select enamel specimens presenting microhardness values within ±10% around the mean (341±10%KHN) to achieve homogeneity of the samples. The selected blocks were randomly allocated to each treatment group (5 groups / 12 enamel blocks per group): Mili-Q water, hydroxypropyl-β-cyclodextrin, 1% TiF_4, hydroxypropyl-β-cyclodextrin + 1% TiF_4 after 12 h of complexation and hydroxypropyl-β-cyclodextrin + TiF_4 after 72 h of complexation.

Enamel Surface Treatment and pH-Cycling Regimen

Immediately after preparation, the solutions were applied on the enamel surface for 1 min by a blinded operator, before the start of the pH-cycling regimen. After that, the specimens were washed with deionized water and dried with a soft absorbent paper.

The pH-cycling regimen followed a methodology previously described (19). Briefly, the blocks remained during 4 h in the demineralizing solution and 20 h in remineralizing solution at 37 °C during 8 days. The demineralizing solution consisted of 0.05 M acetate buffer (pH 5.0), 1.28 mM calcium, 0.74 mM phosphorus, 0.03 mg/mL fluoride. The remineralizing solution consisted of 1.5 mmol/L calcium, 0.9 mmol/L of phosphorus, 150 mmol/L potassium chloride, 0.05 mg of fluoride/ml in 0.1 mol/L Tris buffer (pH 7.0). On the fourth day of pH cycling, all solutions were replaced with freshly prepared ones. After the eighth day of the cycle, the blocks remained in remineralizing solution for an additional 24 h before starting further analysis. The proportion of demineralizing and remineralizing solutions per area of block was 6.25 mL/mm^2 and 3.12 mL/mm^2, respectively.

Surface Microhardness Determination

After specimen selection, five indentations, spaced by 100 µm were performed at the surface of each enamel block before and after the pH cycling regimen. Surface microhardness indentations performed after the pH cycling regimen were taken far from those already carried out at baseline. The operator was also blinded regarding the groups in this phase.

The percentage of enamel surface microhardness loss (%SML) was obtained by the following formula (8):

$$100\, x\, \frac{(SM_{before} - SM_{after})}{SM_{before}}$$

where, SM=surface microhardness

Internal Microhardness Determination

After surface microhardness determination, the enamel blocks were longitudinally sectioned with a diamond

disk mounted on a low-speed cutting machine (Isomet, Buehler). One of the halves was embedded in acrylic resin and the exposed surface was polished with 600 and 1200 grit Al_2O_3 paper, as described previously. Two sequences of 15 indentations were taken: the first started at 10 µm from the enamel surface up to 100 µm depth at 10 µm intervals; the second set started at 100 µm from the enamel surface to 200 µm depth at 20 µm intervals (12). The integrated mineral loss (ΔZ) was calculated as the integrated area (% vol. min. x µm) of post-pH cycling treatments (Z1), with projection to normal enamel (Z) using the formula (20):

$$\Delta Z = 100\ \frac{(Z1 - Z)}{Z}$$

Micro-CT Analysis

The blocks in each group were scanned in a high energy micro-CT device (Skyscan 1173, Bruker micro-CT, Kontich, Belgium) after the pH cycling using the following acquisition parameters: 70 kV, 114 µA, 7.12 µm image pixel size, 1 mm Al filter, 1 s exposure time per frame, 0.5° rotation step over 360°, frame averaging of 5 and random movement parameter set to 20. The projections were reconstructed into cross-section slices by means of a proprietary software (Nrecon v.1.6.9.4, Bruker micro-CT) using standardized parameters to minimize artefacts: 50% beam hardening correction, ring artefact correction of 5, and input of similar contrast limits (0-0.08).

The enamel blocks cross-section slices were imported in a open-source image analysis software (ImageJ, FIJI implementation) (21) and resliced into longitudinal slices. An average density projection was obtained from each sample stack and gray value density profiles were taken from 2-line profiles in each image stack. The line profiles started from the outer enamel surface up to 200 µm deep into the enamel layer. Eight-bit gray values were taken at each pixel unit (7.12 µm) and the plots were exported to a separate worksheet. An integrated density profile, represented by the area under the plots (Z) was calculated for each profile (n=4/group), as described previously (22). Data from each group were averaged and presented in a graphic form and submitted to descriptive analysis.

Scanning Electron Microscopy (SEM) and Energy Dispersive X-Ray Spectrometry (EDX) Assessment

Two specimens from each group were mounted on aluminum stubs and evaluated using an environmental scanning electron microscope (Jeol-JSM 6460LV, Tokyo, Japan). The topography of the enamel surfaces was analyzed in secondary electrons at 20 kV voltage, low vacuum mode (45 Pa) to obtain images with a 1000x magnification.

Assessment of mineral content was carried out in EDX mode with link and using Kontron automatic image analyzer system to identify chemical elements and its percentage in weight at the enamel surfaces after the pH cycling.

Data Analysis

Data were considered normally distributed whether kurtosis and skewness presented variations of ± 2 (23). Data were considered homogeneous whether a ratio until 3 between the largest and the lowest variances were observed (24). One-way ANOVA was used to analyze the %SML. Unpaired t-test was used for homogeneous and non-homogeneous variances (a two-tailed significance level of 5%) for comparison between pairs of groups in the post hoc test.

Mann-Whitney post hoc test was adopted to analyze ΔZ microhardness. Statistical significance, effect size, its 95% CI, and power for ANOVA and the each pairwise comparison were included for parametric data. Statistical significance, effect size and power were included for non-parametric data (25). ΔZ micro-CT was descriptively assessed. SEM micrographs and EDX analysis were qualitatively evaluated. All analysis was carried out by SPSS (Statistical Package for Social Sciences – version 21.0, Chicago, IL, USA) and Microsoft® Office Excel (Microsoft Corporation).

Results

Descriptive statistics are shown in Table 1. Table 2 shows one-way ANOVA assessment. The null hypothesis was rejected (p=0.0003). The potential of the nanocomplexed fluoride solutions was significant with an effect size of 0.312 and a statistical power of 98.9%. Hydroxypropyl-β-cyclodextrin + 1% TiF_4 after 12 h complexation resulted in lower percentage of surface microhardness loss compared to Mili-Q water, hydroxypropyl-β-cyclodextrin, 1% TiF_4 and hydroxypropyl-β-cyclodextrin + 1% TiF_4 after 72 h of complexation group, with a large effect size (from 1.307 to 2.943) and high power (84.9 to 99%) (Table 3).

All groups resulted in similar integrated mineral loss (ΔZ) obtained by both internal microhardness and micro-CT techniques (Table 1 and Table 4). Figure 1 discloses profiles of microhardness or mineral density obtained by micro-CT for the control and experimental groups.

SEM analysis indicated the formation of TiO_2 glaze-layer on the surface of the slabs treated with hydroxypropyl-β-cyclodextrin + TiF_4 and TiF_4 compared to a control solution without TiF_4 (Fig. 2). EDX assessment showed the presence of titanium on the surface of the blocks treated with TiF_4 and TiF_4 after cyclodextrin complexation (Table 5). Figure 3 discloses profile of demineralizing areas corresponding to artificial enamel carious lesions measured by micro-CT.

Discussion

pH-cycling studies are employed to assess the caries/demineralization protection or rehardening effects of several compounds (26) because they simulate dental caries in vivo (27). The main advantage of this protocol is related to its low cost compared with more rigorous and methodologically refined studies such as clinical research (28). However, the main shortfall is related to the impaired assessment of antibacterial effect from the fluoride agents tested (27).

The connection of TiF_4 to the enamel surface happens promptly and that coating is acid-insoluble and not readily removed by washing (29). Moreover, titanium fluoride complexes bind to the tooth surface by establishment of Ti-O (phosphate) bonds. It is expected that the titanium atom presents a high affinity for an oxygen atom that is part of a phosphate group in hydroxyapatite. The phosphate groups are disseminated on both enamel and root surfaces (30). Professional fluoride application compounds (solution, gel, foam and varnishes) have high fluoride concentrations and when applied locally to dental surfaces react either with enamel or dentin, resulting in reaction products. The main reaction product is called calcium fluoride-like (CaF_2-like) deposits. Over 90% of the reaction product is CaF_2, but there is also fluorapatite formation (31). And the fluoride released to biofilm fluid from CaF_2 formed on enamel reduces enamel demineralization (32). In the current study, the reaction products (CaF_2, fluorapatite, TiO_2 layer) were allowed in this in vitro context. Considering the above mentioned, the experimental conditions adopted in the present study allowed the fluorapatite formation as well as reaction products such as CaF_2 and TiO_2 layer.

TiF_4 is composed by a polyvalent metal ion in association with fluorides. This compound presents, in aqueous solution, antimicrobial effect against *Streptococcus mutans* and *Lactobacillus casei* (33). Some conflicting findings in relation to the effectiveness of TiF_4 in inhibiting tooth demineralization are reported. While some studies show mineral loss reduction (12,13), others reported that this phenomenon was not observed (33). In this sense, new studies are still needed to confirm the effects of TiF_4 in on enamel demineralization.

Cyclodextrins are cyclic oligosaccharides composed of dextrose units joined by a α-1,4 linkage. If composed of seven dextrose units, they have been named β-cyclodextrins, while hydroxypropyl-β-cyclodextrin is formed by addition of a water-soluble polymer ($(CH_2CH(OH)CH_3)$). Inclusion complexes formed between certain drugs and cyclodextrins may show extremely different properties from those of the parent drug, including the increase in solubility and dissolution rate of the parent compound, modification in local inflammatory properties, decrease in volatility and increase of the stability of the original drug (15).

Several studies have been undertaken in order to evaluate the impact of formulations containing cyclodextrins as inclusion complexes on the stability and bioavaliability of the complexed drug. This is due, among other things, to the ability of cyclodextrins to create inclusion complexes with a large number of molecules (34). For these reasons, cyclodextrins have been selected as TiF_4 carriers in the present study. Addition of water-soluble polymers enhances the complexation efficacy and bioavailability of the parent drug. The specific time of 72 h of complexation with β-cyclodextrin solely has been already examined in the literature (12). Taking into account these considerations, the present study was designed to evaluate

Table 2. One-way ANOVA test results for the effects of the nanocomplexed fluoride solutions on surface microhardness loss

Factor	Sum of squares	Degrees of freedom	Mean squares	F	p value	η^2	Power
%SML	1778.840	4	444.710	6.241	3.24×10^{-4}	0.312	0.989

Table 1. Descriptive analysis of %SML (mean±SD) and ΔZ microhardness and ΔZ micro-CT (median (minimum;maximum/interquatile range))

		% SML (Mean ±SD)	ΔZ microhardness (Me (Min;Max/interquatile range))	ΔZ micro-CT (Me (Min;Max/interquatile range))
Control groups	Mili-Q water	29.97±11.54	790.20(-8.00;1534.60/342.13)	3172.75(3065.00;3241.00/152.88)
	Hydroxypropyl-β-cyclodextrin	32.40±5.64	823.00(319.80;1628.00/718.13)	4702.50(4566.50;4801.50/206.75)
	1% TiF_4	26.45±6.73	533.10(214.40;1192.10/417.95)	4449.00(4263.50;4714.00/424.63)
Experimental groups	Hydroxypropyl-β-cyclodextrin + 1% TiF_4 12 h	16.49±5.16	414.95(268.30;1244.90/500.83)	4662.00(4636.50;4791.50/128.00)
	Hydroxypropyl-β-cyclodextrin + 1% TiF_4 72 h	27.64±10.90	447.20(-244.10;1112.30/412.05)	4562.50(4429.50;4709.50/272.25)

Table 3. Findings from post-hoc pairwise comparisons regarding %SML

Groups	p value	Hedge's G	Lower 95% CI	Upper 95% CI	Power
Mili-Q water X Hydroxypropyl-β-cyclodextrin	0.522	0.267	-0.606	1.141	0.080
Mili-Q water X 1% TiF_4	0.694	0.372	-0.481	1.226	0.129
Mili-Q water X Hydroxypropyl-β-cyclodextrin+ 1% TiF4 12 h	0.002	1.508	0.521	2.494	0.930
Mili-Q water X Hydroxypropyl-β-cyclodextrin+ 1% TiF_4 72 h	0.794	0.207	-0.641	1.056	0.065
Hydroxypropyl-β-cyclodextrinX 1% TiF_4	0.507	0.958	0.064	1.852	0.606
Hydroxypropyl-β-cyclodextrinX Hydroxypropyl-β-cyclodextrin+ 1% TiF_4 12 h	3.17 x 10^{-7}	2.943	1.721	4.165	0.999
Hydroxypropyl-β-cyclodextrinX Hydroxypropyl-β-cyclodextrin+ 1% TiF4 72 h	0.197	0.548	-0.333	1.430	0.224
1% TiF_4 X Hydroxypropyl-β-cyclodextrin+ 1% TiF_4 12 h	0.0005	1.660	0.679	2.642	0.970
1% TiF_4 X Hydroxypropyl-β-cyclodextrin+ 1% TiF_4 72 h	0.894	0.131	-0.716	0.978	0.046
Hydroxypropyl-β-cyclodextrin+ 1% TiF_4 12 h X Hydroxypropyl-β-cyclodextrin+ 1% TiF_4 72 h	0.005	1.307	0.348	2.266	0.849

one cyclodextrin carrier (hydroxypropyl-β-cyclodextrin) in two different complexation periods (12 or 72 h) in order to explore the effect of these nanocomplexes on bovine enamel demineralization.

Regarding surface findings, previous studies demonstrated that solutions containing TiF_4/TiF are able to decrease microhardness loss (7,8,12,13,35). In the present study, the ability of hydroxypropyl-β-cyclodextrin + TiF_4 nanocomplex after 12 h of complexation to decrease enamel softening under adverse conditions (pH-cycling)

Table 4. Results of pairwise comparisons regarding ΔZ microhardness

Groups	p value	Hedge's G	Power
Mili-Q water X Hydroxypropyl-β-cyclodextrin	0.772	0.091	0.047
Mili-Q water X 1% TiF_4	0.043	1.081	0.524
Mili-Q water X Hydroxypropyl-β-cyclodextrin+ 1% TiF4 12 h	0.037	1.649	0.547
Mili-Q water X Hydroxypropyl-β-cyclodextrin+ 1% TiF_4 72 h	0.073	1.330	0.432
Hydroxypropyl-β-cyclodextrinX 1% TiF_4	0.056	0.771	0.478
Hydroxypropyl-β-cyclodextrinX Hydroxypropyl-β-cyclodextrin+ 1% TiF_4 12 h	0.009	1.104	0.738
Hydroxypropyl-β-cyclodextrinX Hydroxypropyl-β-cyclodextrin+ 1% TiF4 72 h	0.094	0.966	0.387
1% TiF_4 X Hydroxypropyl-β-cyclodextrin+ 1% TiF_4 12 h	0.644	0.463	0.067
1% TiF_4 X Hydroxypropyl-β-cyclodextrin+ 1% TiF_4 72 h	0.908	0.304	0.032
Hydroxypropyl-β-cyclodextrin+ 1% TiF_4 12 h X Hydroxypropyl-β-cyclodextrin+ 1% TiF_4 72 h	0.686	0.117	0.059

Table 5. Values (percentage in weight) of the analyzed elements

	Mineral content (weight %)									
	C	O	Na	Mg	Al	P	Cl	K	Ca	Ti
Mili-Q water	26.95	25.61	0.37	-	0.24	15.32	0.34	-	31.19	-
1% Hydroxypropyl-β-cyclodextrin	34.20	23.48	0.44	-	-	13.63	0.30	-	27.94	-
1% TiF_4	24.08	28.46	-	-	0.16	15.42	0.47	-	30.98	0.43
Hydroxypropyl-β-cyclodextrin: 1% TiF_4 12 h	31.42	25.38	0.49	-	-	13.85	0.33	-	28.26	0.28
Hydroxypropyl-β-cyclodextrin: 1% TiF_4 7 2h	30.17	25.74	0.45	-	-	14.05	0.47	0.32	28.02	0.77

was shown (Table 3). The improved effect of this solution compared to the other experimental or controls solutions could be attributed to the sustained release (36) of fluoride, keeping a constant concentration of fluoride available for interaction with the enamel surface during its application and decreasing surface demineralization.

In relation to subsurface data, internal integrated mineral loss data showed that all TiF_4 containing solutions were not effective in reducing overall mineral loss in enamel. Data from micro-CT also showed a non-protective effect for all groups tested. Micro-CT is a non-destructive technique which can be used to evaluate mineral changes in enamel after preventive or therapeutic treatments (22,37). Another aspect to be emphasized is the formation of a TiO_2 layer on the surface of the enamel treated with TiF_4 (6,7). After a topical application, this coating is formed by the reaction between titanium and the oxygen groups derived from water or phosphate-bound oxygen (38) and acts like a diffusion barrier. This barrier may block the transport of calcium and phosphate, protecting the subsurface enamel from further demineralization (35). The acid-resistant barrier of TiO_2 was observed on the surface of the blocks treated with TiF_4 and TiF_4 + hydroxypropyl-β-cyclodextrin (Fig. 2). One can speculate that the TiO_2 glaze-layer added by a sustained release of fluoride from the 1% TiF_4 + hydroxypropyl-β-cyclodextrin solutions hindered enamel dissolution. The sustained release of fluoride is related to the 1-min application from the nanocomplexed fluoride solutions in contrast with the plain TiF_4 solution in which

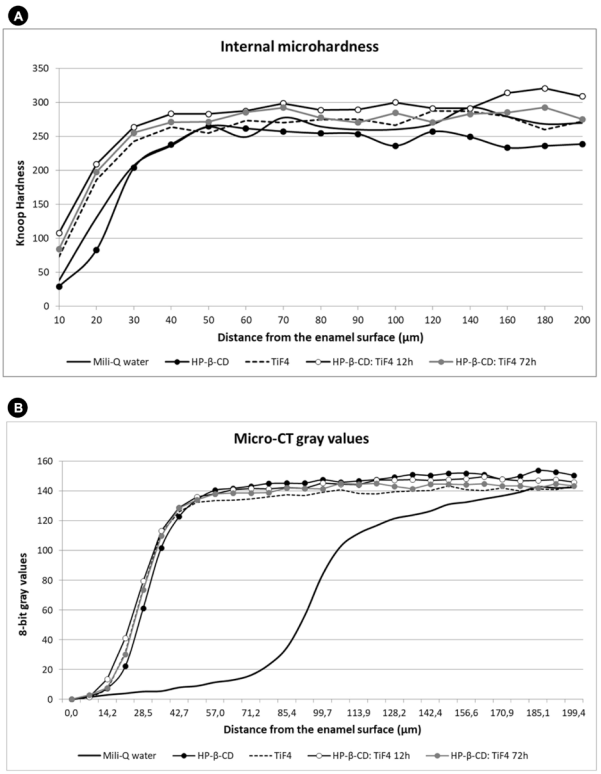

Figure 1. Internal microhardness values (KHN) (A) and enamel mineral density values (g/cm³ hydroxyapatite) (B) from the surface to the inner enamel after treatment with the control/experimental solutions and pH cycling. Note: HP-β-CD=hydroxypropyl-β-cyclodextrin; TiF_4=titanium tetrafluoride.

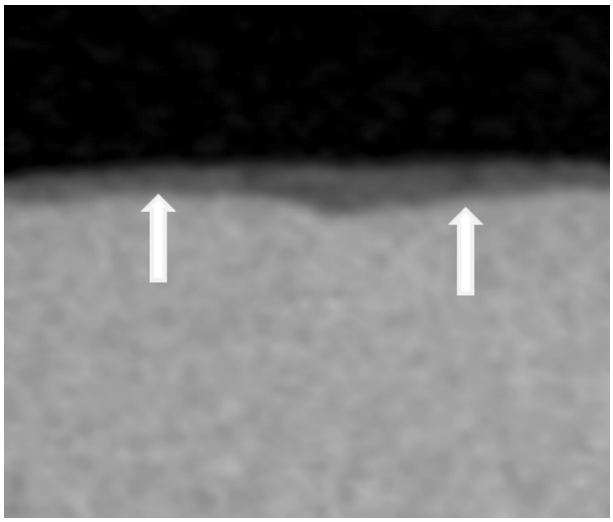

Figure 3. Representative qualitative micro-CT specimen showing demineralized areas such carious-like lesions (white arrows).

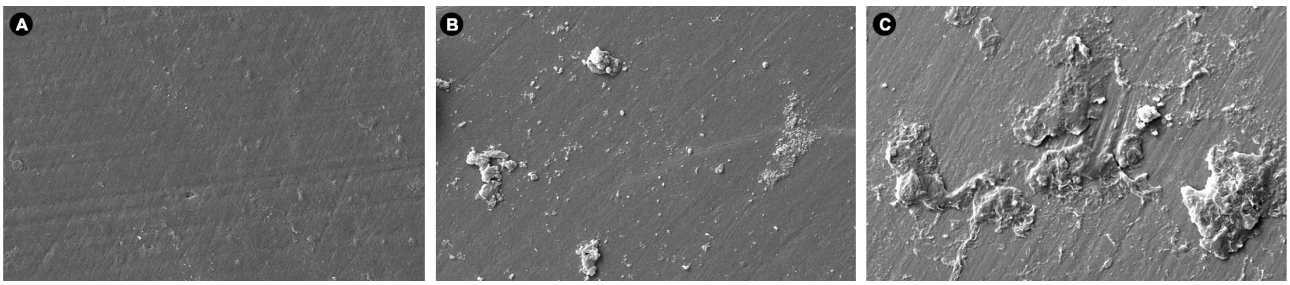

Figure 2. Representative surface SEM images from a sample after treatment with hydroxypropyl-β-cyclodextrin (A), plain TiF_4 solution (B) and hydroxypropyl-β-cyclodextrin + 1% TiF_4 after 12 h of complexation (C).

this behavior is not expected.

The adsorption capacity and substantivity are directly related to mineral loss control, especially for TiF_4-solutions. Previous study had already demonstrated that one-minute application of 4% TiF_4 solution showed an irregular TiO_2 layer on human enamel blocks surface (39). This coating was not uniform in quantity and was found in distinct areas on the same tooth (39,40). One may suppose that this specific glaze was not uninterrupted enough to protect subsurface demineralization. SEM findings from the present study show that such properties were more pronounced in the enamel blocks treated with hydroxypropyl-β-cyclodextrin + 1% TiF_4 after 12 h of complexation. Future studies are necessary to better understand this phenomenon in this nanotechnology (cyclodextrin-based-products) context.

This is the first pH-cycling study which evaluated the potential of hydroxypropyl-β-cyclodextrin + TiF_4 on reducing bovine enamel dissolution under cariogenic challenge in vitro. Cytotoxic assessment revealed that these solutions did not induce critical cytotoxic effects at the experimental time points against L929 cells. Moreover, until the period of 30 min, all products showed cell viability above 70% indicating a non-cytotoxic effect (13). Future studies should be carried out to evaluate its performance in in situ conditions and to observe if the fluoride delivery from the solutions with cyclodextrins employed here demonstrates the same performance throughout time.

Although testing the acid value of the products was not the objective of the study, the pH of the products was evaluated and it was verified that both the TiF_4 and the nanocomplex solutions presented a pH lower than 2.0. The association of TiF_4 to the nanocarriers used here promoted a very small reduction of the acidity.

A limitation is the lack of free oral calcium which is expected to be found in saliva and biofilm fluid that could interact and form CaF_2. In the present work, only calcium extracted from enamel was available in order to simulate a professional fluoride application. This may not represent what happens in in vivo condition in which both salivary/biofilm fluid calcium and calcium extracted from enamel might be available for CaF_2 establishment (41).

Within the limitations of the present study, the current nanocompound represents a valid nanosystem for the prevention of dental caries especially in high risk patients, patients with impaired oral hygiene, individuals with exposed root surfaces and patients with reduced salivary flow.

A single application of hydroxypropyl-β-cyclodextrin + 1% TiF_4 with 12 h of complexation was significantly able to reduce the enamel surface demineralization while internal enamel demineralization was similar between the groups under cariogenic challenge.

Resumo

O objetivo deste estudo foi avaliar o efeito de um nanocomplexo recém-desenvolvido formado entre hidroxipropil-β-ciclodextrina e 1% de tetrafluoreto de titânio (TiF_4) após distintos períodos de complexação (12/72 h) na desmineralização do esmalte bovino in vitro. Blocos de esmalte (n=60) foram alocados para cada grupo: água Mili-Q, hidroxipropil-β-ciclodextrina, TiF_4 a 1%, hidroxipropil-β-ciclodextrina + TiF_4 1% após 12 h de complexação e hidroxipropil-β-ciclodextrina + TiF_4 1% após 72 h de complexação. As amostras foram avaliadas pela microdureza superficial, microdureza transversal e micro-CT. Microscopia eletrônica de varredura / espectrometria de raios X por dispersão de energia (MEV / EDX) foram obtidas. A hidroxipropil-β-ciclodextrina + TiF_4 1% após 12 h de complexação resultou em um menor percentual de perda de microdureza superficial em comparação com água Mili-Q, hidroxipropil-β-ciclodextrina, TiF_4 a 1% e hidroxipropil-β-ciclodextrina + TiF_4 1% após 72 h de complexação com uma ampla magnitude de efeito (1,307 a 2,943) e alto poder (84,9 a 99%). Todos os grupos resultaram em similar perda integrada de minerais (ΔZ) obtida por ambas as técnicas de microdureza e micro-CT. O esmalte tratado com os grupos TiF_4 e TiF_4 + hidroxipropil-β-ciclodextrina apresentou camada de esmalte TiO_2, enquanto a avaliação de EDX identificou Ti. A solução contendo o complexo de inclusão de hidroxipropil-β-ciclodextrina + TiF_4 com 12 h de período de complexação demonstrou uma capacidade significativa para reduzir a desmineralização superficial do esmalte hígido sob um desafio cariogênico artificial.

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