Energy Dispersive X-Ray Spectroscopy Evaluation of Demineralized Human Enamel after Titanium Tetrafluoride Application

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Objective**: Evaluate the presence of a titanium dioxide layer after application of titanium tetrafluoride* on human permanent tooth enamel. ***Study design: The sample consisted of unerupted *third molars. After the removal of the roots, each tooth was mesiodistally divided into 2 fragments, one reserved for the experimental group and the other for the control group. Before the treatments the fragments were artificially demineralized. The experimental group (n=5) received an application of 4% titanium tetrafluoride, for one minute and the control group (n=5) did not receive any treatment. The samples were sputter-coated with a 20-30nm gold layer as the energy dispersive x-ray spectrometer analysis was carried out in a scanning electron microscope and the results were descriptively analyzed. **Results**: The titanium dioxide layer was present on all experimental samples with a titanium peak varying between 6.82 and 26.37%. This layer was not found in the control group. Fluoride and calcium fluoride precipitates were present in the samples treated with titanium tetrafluoride. **Conclusion**: Titanium dioxide layer was formed after one titanium tetrafluoride application, but it was not uniform. Further studies should be carried out so that both the morphology and thickness of such layers can be better understood.*

***Key words:** Titanium; Dental caries; Dental Enamel; Calcium Fluoride*

INTRODUCTION

Fluoride has been widely used in the prevention and control of caries[1 2]. It has been proven to be an effective anticaries agent when delivered using various vehicles and concentrations, including the professionally applied F [3,4]. The fluoride mode of action is mainly due to its influence on the de- and remineralization kinetics of dental hard tissues [3]. The formation of calcium fluoride (CaF_2-like) precipitates induced by applying topical fluorides is regarded as decisive for the caries-inhibiting effect, because these precipitates act as a reservoir of free fluoride ions available during cariogenic challenge [3,5].

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Various fluoride vehicles have been described in the literature [1,2,6]. When compared with other topical fluoride solutions, titanium tetrafluoride (TiF_4) has shown some interesting properties. Since 1972, when studies of TiF_4 were first carried out, this solution has been indicated as another ally in the prevention and control of dental caries [7,8].

Titanium (Ti) itself is a non-toxic element, and no adverse side effects have been reported to date [9]. TiF_4 is a fluoridated compound which causes both chemical and mechanical actions on the tooth surface [7,10]. The hydrolysis processes of this substance forms fluoride complexes such as: calcium fluoride, fluorapatite, and other fluoridated compounds [8,11]. The mechanical protection is an additional effect of TiF_4 which is due to the formation of an acid-stable surface titanium dioxide (TiO_2) layer resulting from the reaction of titanium with the structural oxygen (O_2) [12,13]. However, very little is known about the characteristics of this TiO_2 layer.

Although the topical fluoride treatment has been used for caries control for many years, there remains further interest to find better products for this purpose. Therefore this present study aimed to evaluate using energy dispersive x-ray spectrometer (EDS) the effect of a single application of TiF_4 on a demineralized human permanent tooth enamel surface.

MATERIALS AND METHOD

In the present investigation, the sample consisted of five extracted human permanent unerupted third molars. The teeth were stored in a sealed container with a 0.1% thymol crystal aqueous solution (pH 7.0) until use. All enamel surfaces were carefully examined and only specimens with intact surfaces were used. After the roots had been removed, each tooth was then sectioned mesiodistally with a diamond saw into 2 fragments, one half being reserved for the experimental group (treated with 4% TiF_4 solution) and the other serving as control (no treatment).

Before the treatments all of the fragments were subjected to the formation of artificial caries lesion by immersion in 2% hydroxyethyl cellulose, 1M lactic acid, pH 4.5, for 24 hours at 37 ºC [14]. After that, a 4 % TiF_4 solution (pH 1.0) was applied on the surfaces of the specimens in the experimental group for 1 minute, after which the specimens were rinsed with tri distilled water for the same period of time. The enamel samples in the control group did not receive any treatment on their surfaces.

A scanning electron microscope (SEM) (JEOL-JSM; 5800LV, Tokyo, Japan) at 20 kV with a maximal 10,000x magnification and equipped with an Oxford analytical system was used for the morphological evaluation of the surfaces. For the EDS and SEM analyses, the samples were fixed in stubs with the enamel surface turned upward, after which the specimens were sputter-coated with a 20-30 nm gold layer. The EDS analysis was made to assess the mineral content of enamel, and to identify the chemical elements existing in the surface enamel. The analyzed elements were: Titanium (Ti), Fluoride (F), Oxygen (O), Carbon (C), Sodium (Na), Calcium (Ca) and Phosphorus (P). This analysis was performed with the link and automatic image analyzer system Kontron. One measurement was made in each sample to evaluate the chemical elements in the total area of surface. The results were analyzed descriptively and represented by the mean of the measured values for all samples in each group.

RESULTS

The percentage mean of chemical elements found on the enamel surfaces for the experimental and control group is described in Table 1. An irregular TiO_2 layer was found on all samples in the experimental group, with the titanium peak varying from 6.82 to 26.37%. This layer on the titanium samples was not uniform in quantity and was found in different areas on the same tooth. Considering different teeth, the application did not provide the same amount of titanium to each tooth. Besides this layer, a calcium fluoride-like layer as a reaction product was observed on all samples. There was no titanium peak, no titanium dioxide layer or calcium fluoride-like on the control group samples. Figure 1 illustrates the EDS map, exemplifying one sample in the experimental and control groups.

DISCUSSION

Many studies have been conducted to determine the best F therapy for dental caries treatment and prevention [3,15]. However, a high frequency application of products with a low F^- concentration has been considered the best treatment for caries prevention [1]. Nevertheless, in situations of high caries risk, professional topical fluoride is considered an excellent alternative because of its high F^- concentration [6,16].

In the present study, in vitro demineralization of the enamel was opted for in order to produce standardized artificial carious lesions for the application of TiF_4 as proposed by Hubbard [17] and White [18]. However some authors argue that in vitro tests can be rather limited, particularly in terms of their incapacity of offering a faithful reproduction of the complex biological process that takes place in carious lesions. Nevertheless, Arends and Christoffersen [19] concluded that when acids are used for demineralization, the artificial lesions produced have similar morphology and exhibit the same developmental stages as those lesions newly formed in the mouth.

The samples selected for the present study consisted of unerupted third molars, extracted for clinical or orthodontic reasons. Because these teeth had had no contact with the oral environment, an important criteria, there was no enamel mineral loss, which occurs on normal contact with the oral cavity [20].

The chemical analysis revealed the presence of titanium (Ti) in all samples treated with TiF_4. In the control group samples this element was not found in the EDS analysis. The elements oxygen, sodium, phosphorus, magnesium and calcium had almost the same percentages in all samples (Figure 1). The presence of a titanium-rich layer on the teeth surface following the application of TiF_4 solution was previously described [7,14,21]. EDS analysis showed that this layer is very rich in titanium thus reinforcing the idea that titanium dioxide is formed on the enamel surface irrespective of the penetration of the titanium.

Other authors have observed that the glaze-like surface layer formed after the application of the TiF_4 offers a protective effect against demineralization [7,8,22]. The reaction mechanisms of TiF_4 with the tooth surfaces and therefore the reason for the stability of the glaze and the protective effect have not been fully elucidated yet but it has been proposed that when TiF_4 is applied on dental surfaces the titanium breaks its connections with the fluoride ions and quickly links itself to the oxygen existing in the tooth enamel, thus forming a layer of TiO_2 [11,23]. Titanium ions play a decisive role since they can be linked to sound or decayed enamel surfaces [7,14].

Although the formation of this layer is an indisputable fact, there were a large number of irregularities on this layer among the different teeth and different areas of the same tooth. This fact can be explained by the lack of uniformity of the enamel structure added to the high porosity of the demineralized enamel that is due its inorganic nature, which makes it particularly vulnerable to ionic exchanges [24,25]. Also, the low water content found in demineralized enamel may prevent the link between titanium and oxygen and thus prevent the formation of a TiO_2 layer in these areas [14].

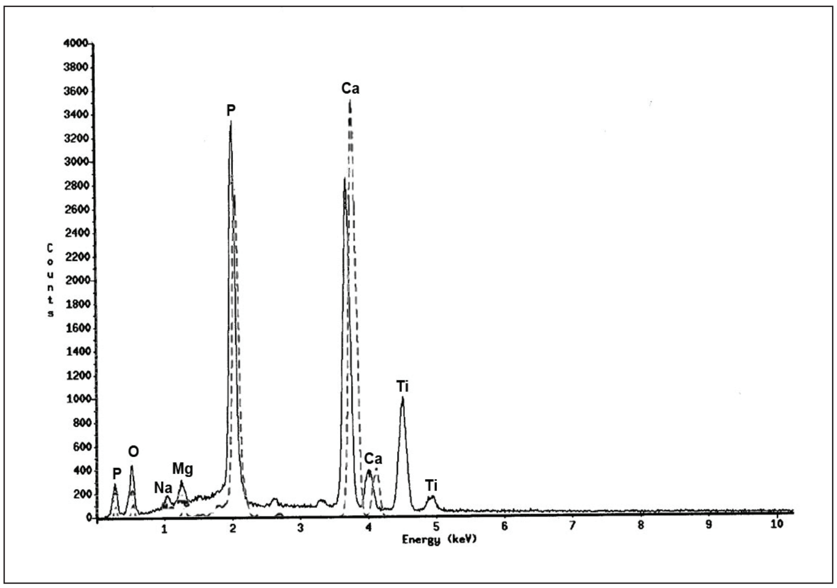
The results of the present study showed that the application of a 4% TiF_4 solution formed a glaze-like surface layer, and suggested that this layer can offer additional mechanical protection. However, further studies should be conducted for a better understanding of the morphology and thickness of this layer.

Table 1. Mean differences between experimental and control group in percentage of chemical elements found on the enamel surfaces.

Element	Experimental Group		Control Group	
	Element Wt %		Element Wt %	
	Mean	± SD	Mean	± SD
Sodium (Na)	0,81	0,52 - 1,18	0,62	0,41 - 1,21
Phosphorus (P	26,24	24,87 - 28,55	23,46	22,32 - 27,05
Oxygen (O)	1,12	0,56 - 1,85	1,12	0,32 - 1,17
Calcium (Ca)	54,20	44,05 - 65,19	62,14	52,31 - 70,91
Magnesium (Mg)	0,76	0,32 - 1,22	0,82	0,46 - 1,25
Titanium (Ti)	14,32	6,82 - 26,37	-	-
Others	3,36	2,01 - 4,10	11,84	8,52 - 14,30

The symbol (-) indicate that the element titanium was absent in the control group.

Figure 1 – EDS analysis of enamel samples after treatment with TiF_4, presence of the elements: oxygen, sodium, phosphorus, magnesium and calcium and titanium.



REFERENCES

1. Hicks J, Garcia-Godoy F, Flaitz C. Biological factors in dental caries: role of remineralization and fluoride in the dynamic process of demineralization and remineralization (part 3). J Clin Pediatr Dent; 28:203-214. 2004.
2. Seppa L. Fluoride varnishes in caries prevention. Med Princ Pract;13:307-311. 2004.
3. ten Cate JM. Current concepts on the theories of the mechanism of action of fluoride. Acta Odontol Scand;57:325-329. 1999.
4. Scheifele E, Studen-Pavlovich D, Markovic N. Practitioner's guide to fluoride. Dent Clin North Am;46:831-846. 2002.
5. Altenburger MJ, Schirrmeister JF, Wrbas KT, Klasser M, Hellwig E. Fluoride uptake and remineralisation of enamel lesions after weekly application of differently concentrated fluoride gels. Caries Res;42:312-318. 2008.
6. Maia LC, de Souza IP, Cury JA. Effect of a combination of fluoride dentifrice and varnish on enamel surface rehardening and fluoride uptake in vitro. Eur J Oral Sci;111:68-72. 2003.
7. Shrestha BM, Mundorff SA, Bibby BG. Enamel dissolution. I. Effects of various agents and titanium tetrafluoride. J Dent Res;51:1561-1566. 1972.
8. Mundorff SA, Little MF, Bibby BG. Enamel dissolution. II. Action of titanium tetrafluoride. J Dent Res;51:1567-1571. 1972.
9. Sen BH, Buyukyilmaz T. The effect of 4% titanium tetrafluoride solution on root canal walls--a preliminary investigation. J Endod;24:239-243. 1998.
10. Vieira A, Ruben JL, Huysmans MC. Effect of titanium tetrafluoride, amine fluoride and fluoride varnish on enamel erosion in vitro. Caries Res;39:371-379. 2005.
11. Nobrega CB, Fujiwara FY, Cury JA, Rosalen PL. TiF4 varnish - A 19 F-NMR Stability Study and Enamel Reactivity Evaluation. Chem Pharm Bull;56:139-141. 2008.
12. Wefel JS, Harless JD. The effect of topical fluoride agents on fluoride uptake and surface morphology. J Dent Res;60:1842-1848. 1981.
13. Wei SH, Soboroff DM, Wefel JS. Effects of titanium tetrafluoride on human enamel. J Dent Res;55:426-431. 1976.
14. Chevitarese AB, Chevitarese O, Chevitarese LM, Dutra PB. Titanium penetration in human enamel after TiF4 application. J Clin Pediatr Dent;28:253-256. 2004.
15. Petersson LG, Twetman S, Dahlgren H, Norlund A, Holm AK, Nordenram G et al. Professional fluoride varnish treatment for caries control: a systematic review of clinical trials. Acta Odontol Scand;62:170-176. 2004.
16. Marinho VC. Cochrane reviews of randomized trials of fluoride therapies for preventing dental caries. Eur Arch Paediatr Dent;10:183-191. 2009.
17. Hubbard MJ. Correlated light and scanning electron microscopy of artificial carious lesions. J Dent Res;61:14-19. 1982.
18. White DJ. The application of in vitro models to research on demineralization and remineralization of the teeth. Adv Dent Res;9:175-193.1995.
19. Arends J, Christoffersen J. The nature of early caries lesions in enamel. J Dent Res;65:2-11. 1986.
20. Moreno EC, Zahradnik RT. Chemistry of enamel subsurface demineralization in vitro. J Dent Res; 53:226-235. 1974.
21. Buyukyilmaz T, Ogaard B, Rolla G. The resistance of titanium tetrafluoride-treated human enamel to strong hydrochloric acid. Eur J Oral Sci;105:473-477. 1997.
22. Nassur C, Alexandria AK, Pomarico L, de Sousa VP, Cabral LM, Maia LC. Characterization of a new TiF(4) and beta-cyclodextrin inclusion complex and its in vitro evaluation on inhibiting enamel demineralization. Arch Oral Biol;58:239-247. 2013.
23. Gu Z, Li J, Soremark R. Influence of tooth surface conditions on enamel fluoride uptake after topical application of TiF4 in vitro. Acta Odontol Scand;54:279-281. 1996.
24. Ribeiro CC, Gibson I, Barbosa MA. The uptake of titanium ions by hydroxyapatite particles-structural changes and possible mechanisms. Biomaterials;27:1749-1761. 2006.
25. Buslaev Y. A. DDS, Ragsdale R. O. Hydrolysis of Titanium Tetrafluoride. lnorganic Chemistry;06:2208-2212 .1967.