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Original Article

Comparative *In Vitro* Study Regarding the Effect of 2% and 6% Titanium Tetrafluoride on Demineralized Human Enamel

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Abstract

Background: Dental caries is the most common disease of the childhood and adulthood where the mineral contents of the enamel of the teeth begin to lose due to acids attacks by demineralization process. It can be prevented by application of fluoride that increased the mineral content of the enamel and made it highly resistant to acid demineralization. **Objectives:** This study was conducted to investigate the effects of titanium tetrafluoride (TiF4) in different concentrations and frequencies on surface microstructure and chemical structure of artificially demineralized enamel. **Materials and Methods:** The sample consisted of 35 premolar teeth: seven teeth randomly selected remained sound (five for energy dispersive spectroscopy analysis and two for scan electronic microscopy, whereas the remaining 28 teeth were divided into four treatment groups (n = 7): (1) control negative (deionized water), (2) control positive (acidulated phosphate fluoride gel), (3) 6% TiF4 solution once daily, (4) 2% TiF4 solution three times daily. Chemical structure and surface microstructure analyses were made for sound, after demineralization then after treatments. **Results:** The fluoride content was greater in 6% TiF4, whereas the calcium content was greater in control positive followed by 6% TiF4 then 2% TiF4 groups. Surface microstructure revealed uniform, smooth, glaze-like surface with no irregularities in 6% TiF4 while localized areas of glaze-like depositions were noted in 2% TiF4. **Conclusions:** The TiF4 showed better minerals contents and uniform surface in its higher concentration and the frequency of applications was not important as concentration.

Keywords: Demineralization, enamel, TiF4, fluoride, calcium

Introduction

Dental caries is an infectious disease that infects various people in different areas throughout the world and if not stopped or treated in its early stages, the results will be pain and tooth loss.[1] This disease is considered as the most oral health problem that worsens the general health and quality of life. It is a dynamic process that consists of rapidly alternating periods of tooth demineralization and remineralization. This results in the initiation of specific caries lesions at certain anatomical predilection sites, particularly on the enamel of the teeth, if net demineralization occurs over sufficient time. Enamel is the most mineralized and hardest tissue in the body that covers the crowns of teeth. It is a cellular tissue so there is no physiological way of repair, with the exception of the possibility for protection and remineralization that is supplied by saliva. It is made up of hydroxyapatite crystals that are very well ordered and are crystalline in structure. These crystals were created mostly from calcium and phosphate.[2] It is important to balance the pathological and protective factors that influence the initiation and progression of dental caries. Protective factors promote remineralization and lesion arrest, whereas pathological factors shift the balance in the direction of dental caries and disease progression.[3] The loss of mineral leads to increased porosity, widening of the spaces between the enamel crystals and softening of the surface, which allows the acids to diffuse deeper into the tooth resulting in demineralization of the mineral below the surface (subsurface demineralization). The build-up of

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reaction products, mainly calcium and phosphate, from dissolution of the surface and subsurface raises the degree of saturation and can partially protect the surface layer from further demineralization. In addition, the presence of fluoride can inhibit the demineralization of the surface layer.[4] Fluoride is the most employed prophylactic agent to reduce and prevent enamel demineralization, remaining so far as the most effective agent for caries prevention. Sodium fluoride (NaF), amine fluoride, stannous fluoride, or acidulated phosphate fluoride (APF) are the most preventive compounds with fluoride which have been applied over years in caries prevention. Accordingly, the efficacy of other fluoride compounds with polyvalent metal ions, such as titanium tetrafluoride (TiF4) have shown an inhibitory effect, which is attributed not only to fluoride but also to the action of titanium. It is speculated that the titanium ions might play an important role, as they might substitute calcium in the apatite lattice and show a strong tendency to complex with phosphate groups. Moreover, it is suggested that titanium interacts with the enamel surface, because of the low pH of the agent, thus leading to an increased fluoride uptake by enamel.[5,6] Titanium ions may reduce demineralization by binding phosphate from apatite, forming a glaze-like layer on the surface.[7] TiF4 is also able to interact with the enamel surface, leading to an increase in Alkali_soluble fluoride analysis (KOH) fluoride deposition compared to NaF.[8] The TiF4 varnish was able to reduce surface and subsurface enamel demineralization under cariogenic challenge[9] and form a thicker protective layer that completely covered the eroded enamel surface.[10] Although TiF4 was first introduced in 1972 as an anti-erosive agent but a definite protocol to regulate its use has not been introduced, which makes it difficult to obtain in the local market.[11] The entire effect and the beneficial properties of this material may not completely understood. Studies are underway to try to further investigate its promising properties, effectiveness, duration of use, as well as its optimal concentration. In an attempt to provide greater insight into such issues, this study was carried out to evaluate the effectiveness of titanium tetrafluoride in different concentrations and frequent of applications against acid demineralization.

Materials and Methods

The sample

The sample was extracted from upper first premolars teeth for orthodontic treatment and collected in Iraqi private and governmental dental centers.

Teeth with hypoplasia, crack, incipient carious lesions/white spots, and filling were excluded after being checked by 10× magnifying lens.[12]

Teeth were cleaned, polished, and ultrasonicated then placed in a plastic screw cup filled with deionized water, to which 0.1% crystals of thymol were added, in order to avoid bacterial growth. All the plastic cups were kept in a fridge to avoid dehydration of teeth.[13] The center of the buccal surface of each tooth was covered by adhesive tape circle of 5mm in diameter while the remaining parts of surface remained uncovered and painted by acid-resistant nail varnish. The adhesive circle then removed to allowing paint free central window on the buccal surface of each tooth. Teeth were adjusted in an acrylic model (size of the model used was 25 mm × 15 mm). The grit papers (400, 600, and 2000, respectively) were used to grind and polish each window ten times in one circular direction for each paper size. This technique permitted a flat surface of each tooth for examination.[14]

Groups design

The total sample of 35teeth were divided randomly into four groups (A–D); each group consisted of seven teeth (five teeth for energy dispersive spectroscopy (EDS) analysis and the remaining two teeth for scanning electron microscopy (SEM) examination). The last seven teeth remained sound without treatment for SEM (two teeth) and EDS (five teeth) examination. SEM and EDS achieved before pH cycling, after pH cycling and after treatment with materials.

EDS and SEM analysis devices (Bruker, Germany) were used for examining the weight percentage (wt.%) of the following elements: oxygen (O), phosphate (P), calcium (Ca) and fluoride (F) as well as microscopical changes of enamel surface for all groups.

pH cycling

In order to induce demineralized enamel surface, the groups were subjected to 5 days of demineralizing and remineralizing solutions at 37°C.[15] Each cycle consisted of a three hour immersion of each sample in 20 mL of demineralizing solution at pH 4.3 (0.75-mM acetate buffer, 2.2-mM calcium chloride, and 2.2-mM sodium phosphate) followed by a twenty-one hour immersion in remineralizing solution at pH 7.0 (20 mM cacodylate buffer, 1.5 mM calcium chloride, 0.9 mM sodium phosphate, and 0.15 M-potassium chloride). All the solutions were subjected to renewal for every cycle.[16]

Sample treatment

After pH cycling and the sample demineralized, the four groups treated as followed:

Group (A) as control negative had no treatment just in deionized water.

Group (B) as control positive treated by acidulated phosphate fluoride gel 1.23% (ALPHA-PRO APF, USA) for 4 min by cotton applicator with dabbing movement on the windows of samples for 7 days.[13]

Group (C) treated by immersing the samples separately in 2% TiF4 solution (SIGMA-ALDRICH, USA) 4 min, three times daily for 7 days

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Group (D) treated by immersing the samples separately in 6% TiF_4 solution 4 min, one time daily for 7 days.

The TiF_4 solutions were prepared by dissolving solid TiF_4 powder (2 or 6 g) in 100 mL deionized water according to concentration needed 2% or 6%, respectively.[17] The pH values of solutions were 5.2 and 4.5, respectively.

Statistical analysis using Statistical Package for social Science (SPSS version 22, Chicago, IL) mean, minimum, maximum and standard deviation, inferential statistics is: one way ANOVA and Tukey's honestly significant difference. Level of significance is $P < 0.05$.

Ethical approval

The study was carried out in a manner that was consistent with the ethical principles that can be traced back to the Declaration of Helsinki. Before the patient's sample was taken, it was carried out with both the patient's verbal and written approval. In order to obtain this approval, the study protocol, as well as the subject information and consent form, was examined by a local ethics committee and given their stamp of approval on April 18, 2021, as indicated by the document number (332).

Results

The data obtained by EDS analysis for all groups are shown in Table 1.

The maximum mean of weight percentage (wt.%) of Ca was appeared in control positive followed by 6% TiF_4 groups was higher than in sound group $P < 0.05$. The maximum mean of wt.% of F was in 6% TiF_4 group. The sum mean wt % of Ca and F about 80% the total wt.% of all element in 6% TiF_4 group. The mean wt.% of P was higher in 2% TiF_4 treatment group but lower than in sound group with significance with all groups $P < 0.05$. The mean wt.% of O was higher in demineralized group while it lower in 6% TiF_4 group.

Multiple pairwise comparisons of wt.% among groups using Tukey's honestly significant difference shown in Table 2.

The 6% TiF_4 group showed significant differences with all groups for all elements except with Ca in control positive and 2% TiF_4 groups where it is not significantly different $P > 0.05$. The 2% TiF_4 group showed significant differences with control positive group for all elements, whereas it showed no significant differences with demineralized group except with element of O $P < 0.05$.

SEM of the enamel surface structure for normal as well for the study groups to better understand the changes of the surface after the treatments.

SEM of sound enamel surface before demineralization appeared normal, smooth, and intact devoid from any alteration as shown in Figure 1.

SEM of the enamel surfaces for the study groups is shown in Figure 2. The structure of enamel surface after demineralization was changed where the enamel lost its normal architecture and the prisms with huge irregularity.

Table 1: Descriptive and statistical test of weight percentages of elements among groups

Elements	Groups	Minimum	Maximum	Mean	SD	P
Oxygen	Sound	36.130	50.530	42.444	5.272	0.000*
	Demineralized	46.190	51.310	48.810	2.040	
	Control positive	12.090	22.830	17.444	4.034	
	6% TiF4	6.720	8.040	7.222	0.519	
	2% TiF4	35.980	37.290	36.804	0.503	
Phosphate	Sound	12.810	22.150	17.252	3.784	0.000*
	Demineralized	7.940	12.660	10.004	1.825	
	Control positive	4.460	8.720	6.096	1.790	
	6% TiF4	0.720	1.640	0.976	0.391	
	2% TiF4	7.460	17.020	12.488	4.162	
Calcium	Sound	23.250	42.760	33.594	8.050	0.000*
	Demineralized	17.120	28.700	24.012	4.521	
	Control positive	32.430	51.030	42.530	6.749	
	6% TiF4	30.900	39.470	35.450	3.117	
	2% TiF4	28.980	34.000	31.314	1.954	
Fluoride	Sound	0.000	0.090	0.050	0.039	0.000*
	Demineralized	0.000	0.060	0.020	0.028	
	Control positive	5.650	11.120	8.278	2.105	
	6% TiF4	39.980	50.130	45.352	3.635	
	2% TiF4	0.160	1.200	0.618	0.481	

SD = standard deviation, TiF_4= titanium tetrafluoride

*Significant $P < 0.05$

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Table 2: Multiple pairwise comparisons of weight percentages of elements among groups using Tukey's honestly significant difference

Groups		Oxygen		Phosphate		Calcium		Fluoride	
		MD	P value	MD	P value	MD	P value	MD	P value
Demineralized	+Ve	31.366	0.000*	3.908	0.046*	–18.518	0.000*	–8.258	0.000*
	6% TIF4	41.588	0.000*	9.028	0.001*	–11.438	0.005*	–45.332	0.000*
	2% TIF4	12.006	0.000*	–2.484–	0.405	–7.302	0.083	–0.598	0.969
Control positive	6% TIF4	10.222	0.000*	5.120	0.021*	7.080	0.096	–37.074	0.000*
	2% TIF4	–19.360–	0.000*	–6.392	0.004*	11.216	0.005*	7.660	0.000*
6% TIF4	2% TIF4	–29.582–	0.000*	–11.512	0.000*	4.136	0.479	44.734	0.000*

MD = mean difference, TiF4= titanium tetrafluoride

*Significant $P < 0.05$

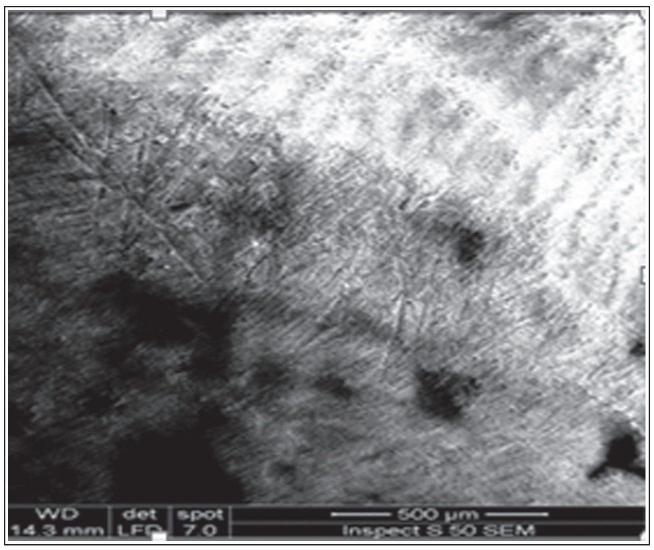

Figure 1: SEM of sound enamel surface

Numerous voids and micropores apparently were seen on the enamel surface. In group that treated with APF 1.23%, enamel surface appeared with accumulations of globules due to deposition of calcium fluoride on the surface. The enamel surface in group treated with 6% TiF4 solution appeared as uniform glaze-like surface with no apparent irregularities or pores. The enamel surface in group treated with 2% TiF4 solution appeared as localized areas of glaze-like depositions that accumulated in irregular crystalline manner.

Discussion

The results of this study agreed with the result of Al-Bazzaz[18] and showed reduction in the mean weight percentage of calcium and phosphorous after demineralization when compared to sound teeth. This result was confirmed by SEM for demineralized enamel surface that revealed numerous voids and micro spaces, because any reduction in pH of the surrounding environment below critical pH (5.5) will create an acidic medium that causes outward movement of the minerals of tooth mainly calcium and phosphorous leaving behind micro pores and voids. APF of 1.23% is a known effective compound against tooth decay. The acidic nature of this compound causes increased fluoride uptake and more production of fluorohydroxyapatite during its chemical reaction with tooth mineral. This result agreed with the result of Lee *et al.*,[19] which was confirmed by EDS analysis where the mean weight percentage of calcium and fluoride markedly increased when compared to sound teeth and demineralized. This result was confirmed by SEM, where the enamel surface of groups treated with APF 1.23% appeared with globules of calcium fluoride on the surface as illustrated in Figure 2A, so it can be considered as a suitable positive control for the evaluation of new and especially acidic fluoride compounds such as TiF4.

EDS analysis for the oxygen demonstrated that the weight percentages increased and decreased in adverse direction to that of calcium and phosphorous, which is difficult to be explained since the EDS analysis was used to express the elements in enamel surface in atomic percentage; therefore, the increase in percentage of one element would affect the percentages of other elements in the chemical composition of enamel so as to achieve balanced composition and the total percentage that does not exceed 100%.

The samples treated with 6% TiF4 demonstrated a smooth and uniform glaze-like layer on the enamel surface which is related to its titanium and fluoride ions as seen [Figure 2C]. TiF4 in the aqueous environment decomposed and changed into titanium dioxide (TiO2) which made a resistant glaze-like layer on the tooth surface as well as to the acidic compound of hydrated fluoride (HF). HF resulted in some surface porosities that increase the fluoride uptake on the tooth surface.[20] TiF4 protects the tooth by forming a protective barrier against noxious agents (mechanical protection), and increasing the fluoride uptake into the enamel surface (chemical protection).[21] This result confirmed by EDS analysis where the mean weight percentage of calcium

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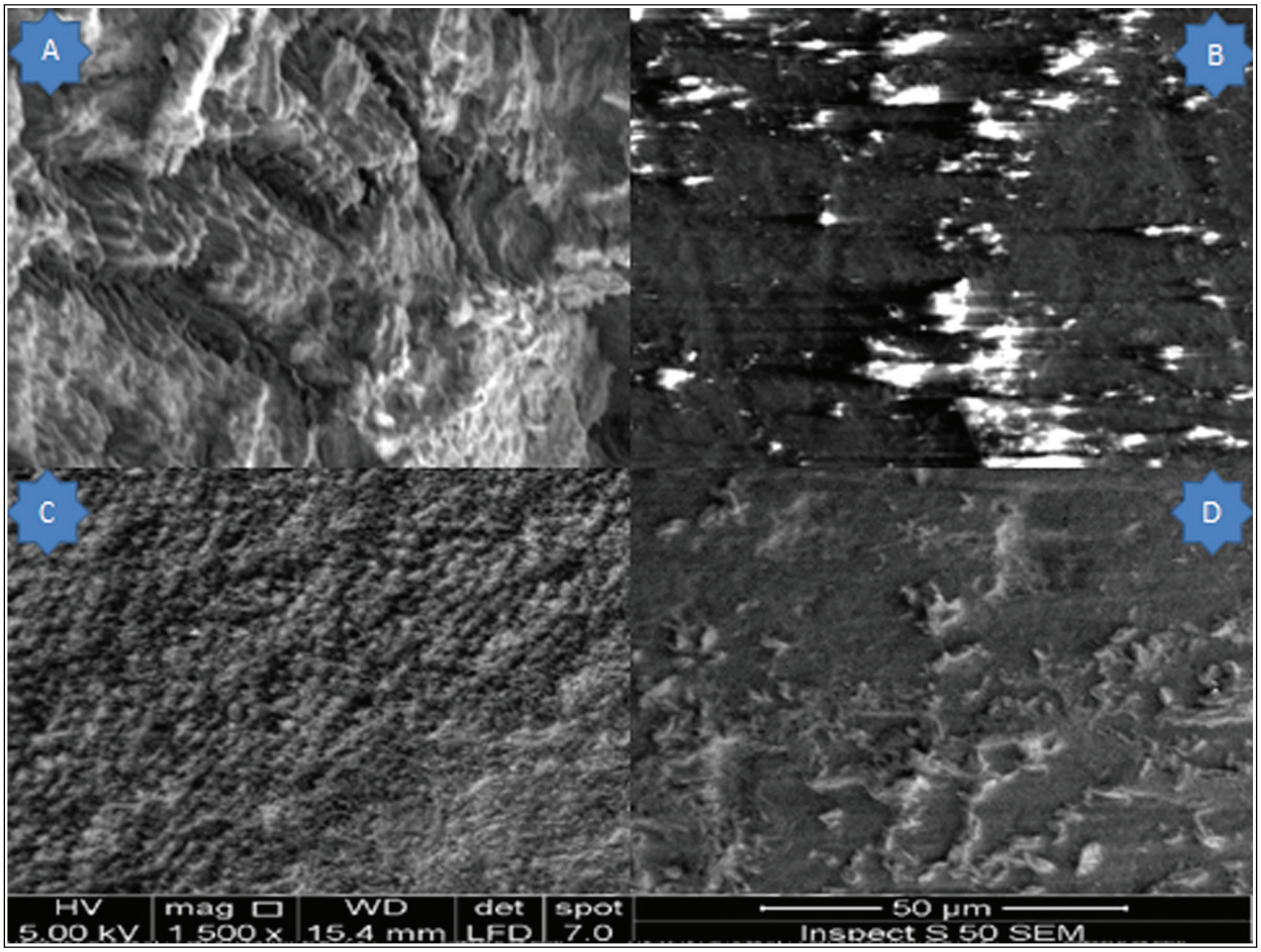

Figure 2: SEM of study groups (A) demineralized enamel surface (after pH cycling), (B) control positive (treated with APF), (C) group treated with 6% TiF4 solution and (D) Group treated with 2% TiF4 solution

and fluoride highly increased when compared with sound or demineralized groups. The mean weight percentage of fluoride exceeded more than that in control positive. This study demonstrated very little increase in weight percentage of fluoride and more increase in phosphate weight percentage in 2% TiF4 group that may be related to the action of titanium that seems to interact with the phosphate from tooth apatite forming a "glaze"-like crystals on the tooth surface other than CaF 2 -like layer formed in 6% TiF4 group which has been shown to be more acid resistant.[22] This result disagreed with the study of Wang *et al*.,[23] which concluded 2% TiF4 solution showed better remineralizing potency than higher concentration. The results of frequent application of low concentration of TiF4 disagreed with result of Vieira *et al*.,[24] which concluded multiple applications of a 0.5% TiF4 solution significantly reduced enamel demineralization *in vitro* and caused no enamel loss at application. The mean weight percentage of oxygen was highly increased in 2% TiF4 compared to 6% TiF4 and this may due to TiF4 in low concentration and frequent use decomposed and changed into TiO2 more than calcium fluoride which makes a resistant glaze-like layer on the tooth surface where appeared as localized areas of glaze-like depositions as in Figure 2D.

Conclusion

This study showed that titanium tetrafluoride solutions with higher concentration provide better results against acid demineralization and the frequency of applications were not important as concentrations.

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Not applicable.

Conflicts of interest

There are no conflicts of interest.

References

1. Mohammed HA, Sahi NM, Ahmed RT, Al-Rubaye AF. Antimicrobial activity of some nanoparticles synthesized by laser ablation technique against some bacteria isolated from oral cavity. Med J Babylon 2022;19:601-608.
2. Ali RA, Radeef SM, Mohammed NB, Diab BS. Oral health related quality of life among dental implant patients in relation to temporomandibular joint function. Med J Babylon 2022;19:609-614.

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3. Pitts NB, Zero DT, Phil DM, Kim E, Jane AW. Dental caries. Nat Rev Dis Primers 2017;3:17030.
4. Pitts NB, Zero DT. White paper on dental caries prevention and management. FDI World Dental Federation; 2016. Available from: http://www.fdiworlddental.org/sites/default/files/media/documents-fdi_cpp-white_paper.
5. Magalhães AC, Santos MG, Livia PC. Effect of a single application of TiF4 varnish versus daily use of a low-concentrated TiF4/NaF solution on tooth erosion prevention in vitro. Caries Res 2016;50:462-70.
6. Vieira TI, Adílis KA, Lilian HA, Aline de AN, Ricardo TL, Lúcio MC, *et al*. Cyclodextrin and TiF4 nanocomplex on enamel demineralization. Braz Dent J 2020;31:423-30.
7. Magalhães AC, Comar LP, Al-Ahj LP, Silva TL, Buzalaf MA. Synthesis and characterization of hydroxyapatite treated with TiF4 in vitro. Caries Res 2013;7:515.
8. Comar LP, Souza BM, Grizzo LT, Buzalaf MA, Magalhães AC. Sound and demineralized enamel treated with TiF4 in vitro: KOH-soluble fluoride deposition and SEM-EDXS analysis. Caries Res 2013;47:516.
9. Alexandria AK, Nasur C, Nóbrega CBC, Valença AMG, Rosalen PL, Maia LC. In situ effect of titanium tetrafluoride varnish on enamel demineralization. Braz Oral Res 2017; 31:e86.
10. Medeiros MID, Carlo HL, Lacerda-Santos R, Lima BAG, Souza FB, Rodrigues JA, *et al*. Thickness and nanomechanical properties of protective layer formed by TiF4 varnish on enamel after erosion. Braz Oral Res 2016;30:e75.
11. Wahengbam P, Tikku AP, Lee WB. Role of titanium tetrafluoride (TiF4) in conservative dentistry: A systematic review. J Conserv Dent 2011;14:98-102.
12. Al-Mamoori RM, Al Haidar AH. Effect of resin infiltration and microabrasion on the microhardness of the artificial white spot lesions (an in vitro study). J Bagh Coll Dent 2022;34:44-50.
13. Al-Hasnawi KIM. The Impact of Selected Topical Fluoride Materials and Nd-YAG LASER on Dentine and Some Salivary Bacteria (In Vitro study), PhD Thesis. College of Dentistry, University of Baghdad; 2019.
14. Al-Bazzaz FA. The Effect of CO_2 LASER, Hydroxyapatite and Iron Oxide Nano Particles on Human Dental Enamel and Streptococcus Mutans (In Vitro study), PhD Thesis. College of Dentistry, University of Baghdad; 2017.
15. Argenta RMO, Tabchoury CPM, Cury JA. A modified pH-cycling model to evaluate fluoride effect on enamel demineralization. Pesqui Odontol Bras 2003;17:241-6.
16. Rodrigues LKA, Cury JA, Santos MN. The effect of gamma radiation on enamel micro hardness and its resistance to demineralization in vitro. J Oral Sci 2004;46:215-20.
17. Yassin HN, Al-Dahan ZA, Hamod MA. Evaluation of antibacterial effect of irrigant solutions (titanium tetra fluoride, green tea, sodium hypochlorite, normal saline) using real-time quantitative—polymerase chain reaction. J Bagh Coll Dent 2015;27:140-145.
18. Li X, Wang J, Junior A, Chang J. The remineralization of enamel: a review of literature. J Dent 2014;30:12-20.
19. Lee YE, Baek HJ, Choi YH, Jeong SH, Park YD, Song KB. Comparison of remineralization effect of three topical fluoride regimens on enamel initial carious lesions. J Dent 2010;38:166-71.
20. Wiegand A, Waldheim E, Sener B, Magalhaes AC, Attin T. Comparison of the effects of TiF4 and NaF solutions at pH 1.2 and 3.5 on enamel erosion *in vitro*. Caries Res 2009;43:269-77.
21. Wiegand A, Magalhaes AC, Attin T. Is titanium tetrafluoride (TiF4) effective to prevent carious and erosive lesions? A review of the literature. Oral Health Prev Dent 2010;8:159-64.
22. Comar L, Souza B, Al-Ahj L. Mechanism of action of TiF4 on dental enamel surface: SEM/EDX, KOH-soluble F, and X-ray diffraction analysis. Caries Res 2017;51:554-67.
23. Wang P, Gao J, Wang D, Snead Malcolm L, Li J, Ruan J. Optimizing concentration of titanium tetrafluoride solution for human dentine remineralization. Arch Oral Biol 2017;83:7-12.
24. Vieira AM, Ruben JL, Bronkhorst EM, Huysmans MC. In vitro reduction of dental erosion by low-concentration TiF4 solutions. Caries Res 2011;45:142-7.