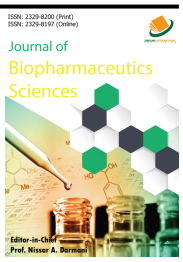
ZIBELINE INTERNATIONAL PUBLISHING

ISSN: 2329-8200 (Print)
ISSN: 2329-8197 (Online)

CODEN : JBSOC4

Journal of Biopharmaceutics Sciences (JBS)

DOI : http://doi.org/10.26480/jbs.01.2017.01.04



CrossMark

AN ATOMIC FORCE MICROSCOPE EVALUATION OF DENTAL EANAMEL SURFACE STRUCTURE FOLLOWING BEYOND COLD-LIGHT BLEACHING AND CPP-ACP TREATMENT

Yingli Zhang[1*], Wen Li[1], Lianmei Du[1], Shuo Yin[2], Jiming Liu[3]

[1]Department of Endodontics, Stomatology Hospital, Jilin University, Changchun 130021, China
[2]Department of Prosthodontics, Stomatology Hospital, Jilin University, Changchun 130021, China
[3]Department of Orthodontics, Stomatology Hospital, Jilin University, Changchun 130021, China
**Corresponding Author's E-mail: zhangyingli1989@163.com*

ARTICLE DETAILS

Article History:

Received 7 January 2017
Accepted 10 February 2017
Available online 15 February 2017

ABSTRACT

The study aimed to evaluate the dental enamel surface morphology following cold- light bleaching with/ without casein phosp-hopeptide-amorphous calcium phosphate (CPP-ACP) treatment. It provided important reference for that CPP-ACP's capability promoting a slight remineralization of enamel. Methods: 30 clinical patients were randomly divided into three groups, with ten people in each group. Control group: cold-light bleaching, CPP-ACP group: cold-light bleaching and CPP-ACP paster, NaF group: cold-light bleaching and NaF. Negative control group is pre-operation. The silicone rubber mold of the leading front teeth were made quickly before and after treatment. Then epoxy resin moulding was performed and the moulds were put in 37℃ incubator for 24 hours. The morphology of enamel surfaces were observed with AFM. Results: The enamel surface was smooth and a small pit-like lacunae porous can be observed with AFM before treatment. Control group: Enamel column and interstitial can not be observed with AFM after cold-light bleaching, the enamel surface structure became rough and porous surface was uneven. CPP-ACP group: The roughness of Enamel surface was reduced, Micro fiber and coarse fiber bundle merged and pores were reduced. NaF group: Granular structure of enamel surface became smooth, the roughness was slightly bigger than CPP-ACP group, but few pores were observed, micro fiber and coarse fiber bundles were found in parallel with each other. Among treatment specimens of control group and CPP-ACP group, control group and NaF group, preoperative group and postoperative control group, a statistically significant differences ($p<0.05$) were identified. CPP-ACP group and NaF group have no significant difference ($p>0.05$). Conclusion: Cold light bleaching will cause enamel microstructure to change and the surface roughness to be increased. Postoperative application of CPP-ACP and sodium fluoride will both promote enamel mineralization, yet the former one is superior to the latter one, improving cold light bleaching effect.

KEYWORDS

Solar Energy, Solar Cooling, Adsorption Cooling, Parabolic Through Collector

1. INTRODUCTION

Tooth discoloration is one of the most common diseases in oral medicine, Due to its adverse influence on patients' appearance and psychology patients, therefore, the treatment of discolored teeth has become a pressing issue much concerned by patients and explored by oral physicians. Based on a study, there are so many teeth whitening methods such as night bleaching technology, consulting room bleaching technology and bleaching products on the market [1,2]. Currently, beyond cold light whitening is a commonly used in clinical tooth whitening technique [3]. With high strength blue light irradiating the surface of enamel daubed with hydrogen peroxide teeth whitening agent, the high temperature and strong light made hydrogen peroxide penetrate through tooth enamel and dentine tubules in a short period of time and react with the pigment deposited in the surface or deep of teeth, thus turning the teeth to white [4,5]. Some scholars raised the concern that though a certain concentration of peroxide can bleach tooth, it may cause the change of microstructure of enamel surface, such as demineralization [6]. To address this concern, the current study was conducted in pursuit of an ideal drug to repair the demineralization. Traditionally, after tooth bleaching treatment, using the fluorine ion products or coating fluorine locally can promote restoration and mineralization of teeth [7,8]. Currently, fluoride toothpaste and mouth rinse is clinical commonly used, but the effect can only be limited to tiny lesions and too much fluoride will cause fluorosis [9,10]. In the 1990s, some scholars found a kind of GC Tooth Mousse in milk, cheese and other dairy products. It contains casein phosphopeptide-amorphous calci-um phosphate, namely CPP – ACP, which has an anti- caries effect. It can effectively combine with biofilm, plaque, bacteria, hydroxyapatite and provide adequate bioav- ailability of calcium and phosphorus so as to promote tooth remineralization [11]. Clinical researches have proved GC's role in facilitating the recovery of chalky damaged parts of orthodontic teeth [12]. Therefore, this research adopted CPP - ACP as a promoting factor of mineralization, applied atomic force microscope to observe the ultrastructure changes of enamel surface before and after the Beyond cold light whitening treatment and delivered a conclusion of GC's role in teeth remineralization.

2. MATERIALS AND METHODS

2.1 The Main Reagent and Instruments

Beyond cold light whitening instrument (Beyond Technology corp); Beyond cold light whitening agent (containing 35% hydrogen peroxide, manganese, magnesium oxide, calcium fluoride, silicon peroxide, ferric oxide and methyl fiber, etc.) (Beyond Tech Corp, USA); Vita 16 color

plate (Vita Inc, Germany); 3M silicone rubber (STD, Express TM 3M ESPE); Epoxy resin (ALDRICH Chemical Company, WI53201, USA); Polishing machine (26oE polishing, shandong, China); Atomic force microscopy (Digital Instruments company); Digital camera (S600, nikon); GC Tooth Mousse (GC corporation,Japan), 37 ℃ constant temperature box (MCO - 15 ac, SANYO CO2 INCUBATOR).

2.2 Study Subjects and Groups

A total of 30 patients who received beyond cold light whitening treatment in October 2012 to October 2014 in Stomatology Hospital of Jilin University were covered in this study. These subjects were randomly divided into blank control group, which received nothing after whitening treatment; CPP - ACP group, with GC application after whitening treatment; and NaF group, with NaF application after whitening treatment. Each of the 3 groups has 10 cases under study. Pretreatment was done for negative comparison.

2.3 Patient Enrollment Criteria

(1) Older than 18 years of age; (2) Patients with no pregnancy; (3) Teeth with no filler, no decay; (4) Endogenous coloring teeth; (5) All patients in an excellent state of mind; (6) All willing to sign the cold light whitening treatment informed consent.

2.4 Experimental Method

Following treatment of steps (1) - (10), nothing was done to blank control group, CPP - ACP group had GC evenly coated on the surface of teeth before 10 minutes' standing, and NaF group had NaF coated evenly on the surface of teeth before 10 minutes' standing. (2) Tell patients potential post-treatment adverse reactions and the cost of treatment; Guide patients to sign the informed consent of Beyond cold light whitening treatment; (2) Use Vita 16 colorimetric plate to record the color levels before bleaching and use the digital camera to collect images of teeth in a standard environment; (3) Use polishing sand in whitening agent kit to polish the teeth to be bleached and gargle; (4) Patients must wear goggles to prevent their eyes from being stimulated by the cold light source; (5) Apply lip oil on lips and wear mouth-gag, advise patients to swallow saliva and place cotton roll inside. Blow dry teeth surface and the gingival margin, apply protective agent on gingival margin, cover 3-4 mms to the gingival and use light curing lamp to irradiate it for curing; (6) Smear whitening gel evenly on the teeth to be bleached with a thickness of 2 mm; (7) Adjust the irradiation angle of cold light whitening instrument to make it 90 ° from the tooth surface, irradiate for ten minutes for the first time; (8) After irradiation, remove the teeth whitening gel on the surface and use a digital camera to take teeth images; (9) Repeat (6), (7), (8) steps for twice; (10) Remove gum protectant, cotton roll, mouth-gag and goggles, let patients gargle with warm water, and continue with shade matching and record color level. (11) Inform patients of issues to be heeded after the whitening post-treatment. Adopt silicone rubber second impression method to get the impression of maxillary anterior teeth immediately before and after the operation, then complete epoxy resin mould, put it in 37 ℃ constant temperature box for 24 hours, demould after the epoxy resin is cured.

2.5 Atomic Force Microscope Observation

Polish the palatal side of epoxy resin model with polishing machine, and put it under ultrasonic oscillation cleaning for 10s. Prepare samples of maxillary central incisor, upper incisor and eyetooth, and observe with atomic force microscope the 5 points respectively 1 mm, 2 mm, 3 mm, 4 mm and 5 mm above the middle incisal edge of labial surface of each sample. Scan in AFM tapping mode with the maximum scan range of 5μm×5μm. Set the scanning frequency to 0.5 Hz and typical mechanical parameters to 0.5 N/m. Use Digital Instruments NanoScope IIIa for image data acquisition and processing and analyze the surface roughness of samples.

2.6 Statistical Analysis

Roughness results of each sample using x ± s, analyse by SPSS 16.0, P<0.05 stands for statistically significance.

3. RESULTS

After cold light whitening, the color of teeth immediately improved 5 to 7 levels and patients were satisfied with the effect. (Figures 1A and 1B). Before the treatment, ultrastructure of enamel was shown clearer and more stereoscopic. Enamel surface was smooth and dense, microfiber and coarse fiber beam were visible, and there were rare pores (Figures 2A and 2B). After the treatment, structure of enamel surface becomes coarse, granular and distributed unevenly. Obvious pore structure and the quaternary structure of enamel are visible; micro fiber and fiber bundles are perpendicular to the surface, and the fibers are arranged in parallel with each other along the long axis (Figures 3A and 3B). With CPP - ACP daubed on the surface for 10 minutes after the treatment, the roughness of enamel surface was reduced, micro fiber and coarse fiber bundles merge together, pores became smaller (Figures 4A and 4B). With NaF daubed on the surface for 10 minutes after the treatment, there were fewer granular structures on enamel surface, but small pores were still observed, micro fiber and coarse fiber bundles were in parallel with each other (Fig.5A and B). By analysing the enamel surface roughness of the experimental group and control group, the results show: Enamel surface roughness was significantly different between pretreatment group and post-treatment control group ($p < 0.05$). It proved that the roughness of the enamel surface was increased after cold light whitening. Blank control group and CPP - ACP group, Blank control group and NaF group both had significant difference ($p < 0.05$), while there was no significant difference between CPP - ACP and NaF group ($p > 0.05$), indicating that using CPP - ACP and NaF will reduce the roughness of enamel surface after cold light whitening and they both can promote the mineralization of enamel surface (Table 1 and Table 2).

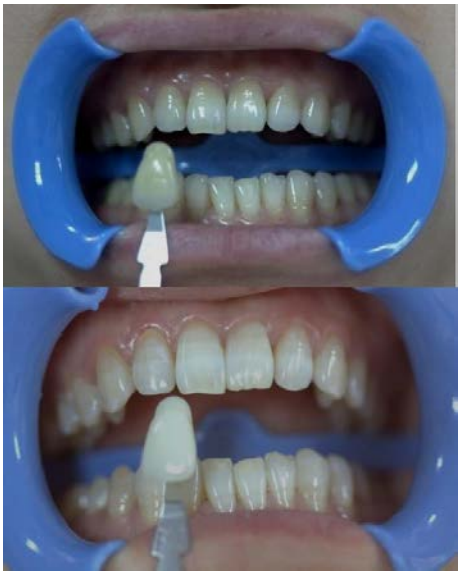


Figure 1: A is the image of pretreatment, B is the image of post-treatment.

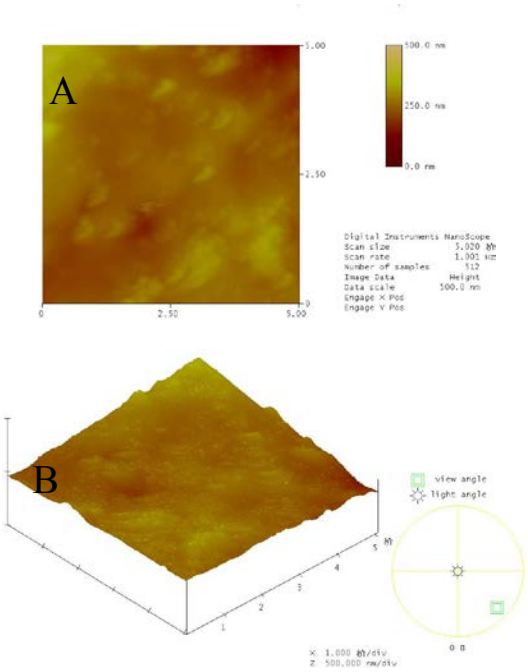


Figure 2: AFM images of enamel surface before operation. A is the topological graph for enamel. B is a three-dimensional image of A.

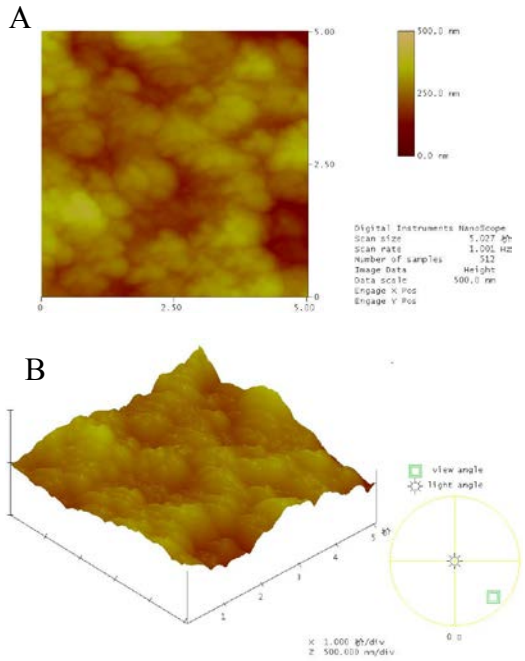


Figure 3: AFM results of enamel surface after treatment. A is the topological graph for enamel. B is a three-dimensional image of A.

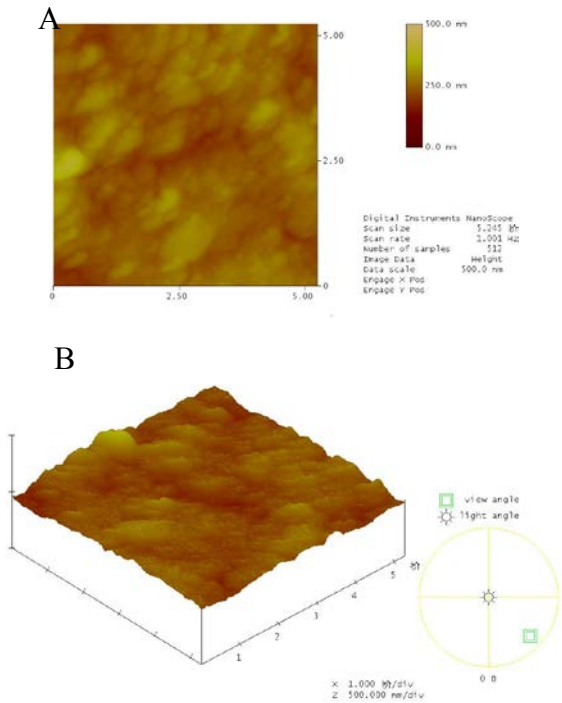
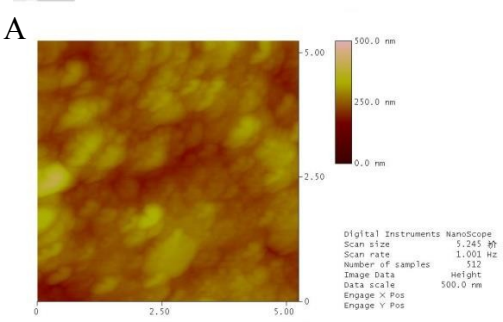


Figure 4: AFM results of enamel surface in CPP-ACP group after treatment. A is the topological graph for enamel. B is a three- dimensional image of A.



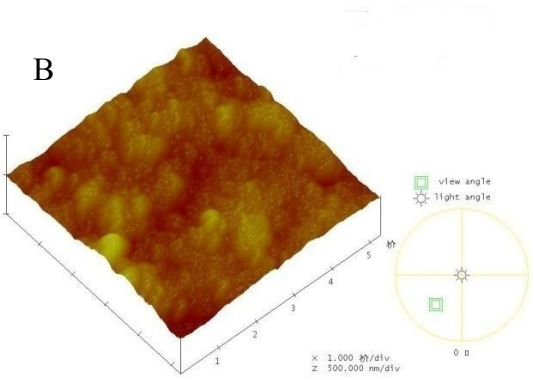


Figure 5: AFM results of enamel surface in NaF group after treatment. A is the topological graph for enamel. B is a three- dimensional image of A.

Table 1: Enamel roughness value.

Negative Control Group	Blank Control Group	CPP-ACP Group	NaF Group
49	69	62	58
41	64	34	37
64	52	33	36
88	78	36	45
51	58	80	48
55	73	32	90
31	69	35	73
32	47	44	35
52	75	75	55
47	59	55	47
57	74	72	45
29	60	54	63

Table 2: The results of statistics.

	Average Value	P
Negative Control Group	49.67±0.18	0.004*
Blank Control Group	64.83±0.18	0.032**
CPP-ACP Group	51.00±0.18	0.014***
NaF Group	52.67±0.18	0.826****

P* is the comparison value of negative control group and blank control group ($P < 0.05$). There was statistical significance. P * * is the comparison value of negative control group and the CPP - ACP group ($P < 0.05$) and there was statistical significance. P * * * is the comparison value of negative control group and the NaF group ($P < 0.05$), and there was statistical significance. P * * * * is the comparison value of CPP - ACP group and NaF group ($P > 0.05$), and there was no statistical significance.

4. DISCUSSION

According to research, in the process of teeth development and eruption, any adverse factor will cause tooth discoloration, which can be mainly categorized into intrinsic discoloration and extrinsic discoloration [13,14]. Intrinsic discoloration is mainly caused by local or systemic reasons such as being infected with some systemic disease or taking drugs, both of which can give rise to changes of the internal mineralized tissues. Extrinsic discoloration is largely due to smoking, food, medicine and pigment in the tooth surface [15,16]. Discolored teeth treatment has become quite a pressing task for medical resolution, while peroxide bleaching technique has no obvious damage to hard tissues and rarely causes irreversible changes of dental pulp, making it a popular way in tooth bleaching [17,18]. Whitening agent of Beyond cold light whitening is hydrogen peroxide, and contains silicon, manganese, calcium fluoride, magnesium peroxide, methyl fiber, ferric oxide and other compounds with diameter less than 20nm. Its whitening instrument excludes all harmful light and prevents the pulp from being stimulated by the high temperature of other lights. At the same time, the cold blue light with a wavelength of 480 ~ 520 nm was irradiated to the

tooth surface, accelerating the decomposition of hydrogen peroxide and the speed of oxygen free radicals entering the dentin tubule. Study showed the whitening agent reacted with the pigment deposited on the surface or down deep inside the teeth, turning the teeth white [19]. It was found in this study that after a course of treatment, the teeth were bleached fast and their color levels were increased 3 to 7 units, which has further proved the effect of beyond cold light whitening. However, Lopes' study shows that in family whitening, 3% hydrogen peroxide will cause slight demineralization of enamel surface and decrease the hardness of enamel surface [20]. In this study, we used epoxy resin specimen accurately reproduced enamel surface configuration before and after the treatment. By using AFM, we observed the enamel surface was uniform and smooth before the treatment and pore structures were rarely seen (Figure 2); structure of enamel surface became coarse and granula were distributed unevenly, pore structures were obviously seen and the quaternary structure of enamel was visible (Figure 3). Soares DG, etc.'s idea that peroxide bleaching may cause microscopic change of the enamel surface has been verified in the study [21]. GC Tooth Mousse is a new type of remineralization agent. It's safe compounds of casein peptide phosphate (casein phos - phopeptides, CPPs) and amorphous calcium phosphate (amorphouscalcium phosphate, ACP) with no side effects. CPP - ACP not only restrains demineralization and promotes mineralization, but also sterilizes and seals dentin tubule [22-24]. After treating the enamel with CPP - ACP for 10 min after whitening, significant changes took place on the enamel surface: the granular structure of enamel surface became even and smooth, the micro fiber and coarse fiber bundles were in parallel with each other, and change in roughness was clearly observed (Figure 4). Although NaF also changed the enamel surface roughness, the effect was not as good as GC Tooth Mousse (CPP - ACP). From the theoretical perspective of remineralization, fluorine functions in a way of simple absorption. It can accelerate the deposition of calcium and phosphate on the enamel surface, thus preventing ions from going further into the lesions. Yet fluorine's functioning can only be limited to tiny lesions. CPP - ACP decomposes phosphate and calcium ion, which further combine to form biphosphate. Biphophate buffers off some acid, inhibits demineralization of enamel surface and promotes the diffusion of ions to enamel surface [25,26]. Calcium and phosphate ions spread gradually into the internal tissue, absorb the moisture to generate mineral composition, and ultimately improved the resistance of demineralization, playing a role in the repair of teeth surface demineralization. This repair process is not a simple adsorption. Overcoming the side effects of fluoride products on teeth and human body, GC Tooth Mousse can be an ideal substitute of fluoride products.

ACKNOWLEDGMENT

We thank Lianmei Du and Shuo Yin for their useful discussions and technical assistance. We also thank Professor Zhang Yingli for her critical advice and support by Stomatology Hospital of Jilin University

REFERENCES

[1] Efeoglu, N., Wood, D.J., Efeoglu, C,T. 2007. Thirty-five percent carbamide peroxide pplication causes in vitro demineralization of enamel, Dental Materials, 23, (7), 900-904.

[2] Tanaka, R., Shibata, Y., Manabe, A., Miyazaki, T. 2010. Micro-structural integrity ofdental enamel subjected to two tooth whitening regimes, Archives of Oral Biology, 55, (4), 300-308.

[3] Wang, W., Zhu, Y., Li, J., Liao, S., Ai, H. 2013. Efficacy of cold light bleaching using different bleaching times and their effects on human enamel, Dental Materials Journal, 32, (5), 761-766.

[4] Shi, X.C., Ma, H., Zhou, J.L., Li, W. 2012. The effect of cold- light-activated bleachingtreatment on enamel surfaces in vitro, International Journal of Oral Science, 4, (4), 208-213.

[5] Huang, J.W., Shi, X.C., Zhou, J.L., Li, W. 2010. Effect of cold-light bleaching technique on human permanent teeth enamel surface, Hua Xi Kou Qiang Yi Xue Za Zhi, 28, (4), 361-363.

[6] Bollineni, S., Janga, R.K., Venugopal, L., Reddy, I.R., Babu, P.R., Kumar, S.S. 2014. Role of fluoridated carbamide peroxide whitening gel in the remineralization of demineralized enamel, An in vitro study, Journal of International Society of Preventive and Community Dentistry, 4, (2), 117-121.

[7] Tschoppe, P., Neumann, K., Mueller, J., Kielbassa, A.M. 2009. Effect of fluoridatedbleaching gels on the remineralization of predemineralized bovine enamel in vitro, Journal of Dentistry, 37, (2), 156-162

[8] Armênio, R.V., Fitarelli, F., Armênio, M.F., Demarco, F.F., Reis, A., Loguercio, A.D. 2008. The effect of fluoride gel use on bleaching sensitivity, a double-blind randomized controlled clinical trial, Journal of the American Dental Association, 139, (5), 592-597.

[9] Zaze, A.C., Dias, A.P., Amaral, J.G., Miyasaki, M.L., Sassaki, K.T., Delbem, A.C. 2014. In situevaluation of low- fluoride toothpastes associated to calcium glycerophosphate onenamel remineralization, Journal of Dentistry, 42, (12), 1621-1625.

[10] Atia, G.S., May, J. 2013. Dental fluorosis in the paediatric patient, Dental Update, 40, (10), 836-839.

[11] Nathoo, S.A. 1997. The chemistry and mechanisms of extrinsic and intrinsic, Journal of the American Dental Association, 6S-10S

[12] Yanagisawa, T., Miake, Y. 2003. High-resolution electron microscopy of enamel-crystal demineralization and remineralization in carious lesions, Journal of Electron Microscopy, 52, (6), 605-613.

[13] Shmuly, T., Zini, A., Yitschaky, M., Yitschaky, O. 2014. Can black extrinsic toothdiscoloration predict a lower caries score rate in young adults? Quintessence International, 45, (5), 439-444.

[14] Price, R.B., Loney, R.W., Doyle, M.G., Moulding, M.B. 2003. An evaluation of a technique toremove stains from teeth using microabrasion, Journal of the American Dental Association, 134, (8), 1066-1071.

[15] Krastl, G., Allgayer, N., Lenherr, P., Filippi, A., Taneja, P., Weiger, R. 2003. Tooth discoloration induced by endodontic Materials, a literature review, Dental Traumatology, 29, (1), 2-7.

[16] Hattab, F.N., Qudeimat, M.A., Al-Rimawi, H.S. 1999. Dental discoloration, an overview, Journal of Esthetic Dentistry, 11, (6), 291-310.

[17] Yu, H., Li, Q., Attin, T., Wang, Y. 2010. Protective effect of resin coating on themicroleakage of Class V restorations following treatment with carbamide peroxidein vitro, Operative Dentistry, 35, (6), 634-640.

[18] Barcellos, D.C., Benetti, P., Jr Fernandes, V.V., Valera, M.C. 2010. Effect of carbamideperoxide bleaching gel concentration on the bond strength of dental substrates and resin composite, Operative Dentistry, 35, (4), 463-469.

[19] Swoger, J., Pampaloni, F., Stelzer, E.H. 2014. Light-sheet-based fluorescence microscopy for three-dimensional imaging of biological samples, Cold Spring Harbor Protocols, 1, (1), 1-8

[20] Silikas, N., Watts, D.C., England, K.E., Jandt, K.D. 1999. Surface fine structure of treated dentine investigated with tapping mode atomic force microscopy (TMAFM), Journal of Dentistry, 27, (2), 137-144.

[21] Soares, D.G., Ribeiro, A.P., Sacono, N.T., Loguércio, A.D., Hebling, J., Costa, C.A. 2013. Mineralloss and morphological changes in dental enamel induced by a 16% carbamideperoxide bleaching gel, Brazilian Dental Journal, 24, (5), 517-521.

[22] Sim, C.P., Wee, J., Xu, Y., Cheung, Y.B., Song, Y.L., Manton, D.J. 2015. Anti-caries effect of CPP-ACP in irradiated nasopharyngeal carcinoma patients, Clinical Oral Investigations, 19, (5), 1005-1011.

[23] Gupta, R., Prakash, V. 2011. CPP-ACP complex as a new adjunctive agent for remineralisation, a review, Oral Health and Preventive Dentistry, 9, (2), 151-165.

[24] Ranjitkar, S., Kaidonis, J.A., Richards, L.C., Townsend, G.C. 2009. The effect of CPP-ACP onenamel wear under severe erosive conditions, Archives of Oral Biology, 54, (6), 527-532.

[25] Oliveira, G.M., Ritter, A.V., Heymann, H.O., Jr Swift, E., Donovan, T., Brock, G., Wright, T. 2014. Remineralization effect of CPP-ACP and fluoride for white spot lesions in vitro, Journal of Dentistry, 42, (12), 1592-1602.

[26] Ivanoff, C.S., Hottel, T.L., Garcia-Godoy, F. 2013. Microhardnessrecovery of demineralizedenamel after treatment with fluoride gel or CPP-ACP paste applied topically or with dielectrophoresis, American Journal of Dentistry, 25, (2), 109-113.