International Journal of Dental Sciences
www.dentaljournal.in
E-ISSN: 2663-4708, P-ISSN: 2663-4694
Received Date: 06-03-2020 Accepted Date: 21-03-2020; Published: 04-04-2020
Volume 2; Issue 2; 2020; Page No. 24-29

Effect of light curable fluoride varnish and nanohydroxyapatite toothpaste in preventing whitespot lesions adjacent to orthodontic brackets: An In-vivo study

Jenifer Gnanasekar[1], Babukuttan pillai S[2], Koshi Philip[3]
[1] Senior Resident, Department of Orthodontics, Government Dental College, Thrissur. Kerala, India
[2] Associate Professor, Department of Orthodontics, Government Dental College, Thiruvananthapuram, kerala, India
[3] Professor, Department of Orthodontics, Government Dental College, Alappuzha, Kerala, India

Abstract
Objective: To evaluate the effect of light curable fluoride varnish and nanohydroxyapatite toothpaste in preventing enamel demineralization adjacent to orthodontic brackets.
Methods: Forty-five patients whose orthodontic treatment involved extraction of first premolars were recruited. The patients were assigned into three groups each of fifteen through consecutive sampling. In each patient one first upper premolar was considered the test teeth, brackets were bonded in all the teeth and normal orthodontic procedure was continued as per the treatment protocol. In Group 1 (control) the subjects followed regular oral hygiene regimen, in Group 2 subjects were advised to brush twice daily using nanohydroxyapatite toothpaste whereas in Group 3 they received single application of light curable fluoride varnish on the test teeth alone, The study was carried out for a period of 4 months after which the test teeth (premolar) was extracted and buccolingual sections were made to analyse the mean depth of demineralization under polarized light microscope. The statistical analysis was done using Kruskal Wallis one-way ANOVA followed by Mann Whitney test for intergroup comparison.
Results: Statistically significant difference in demineralization adjacent to orthodontic brackets were evident. The mean lesion depth were 175.18±58.4 µm in the control group, 88.75±22.5 µm in the nanohydroxyapatite toothpaste group and 37.86±16.3 µm in the light curable fluoride varnish group. The least demineralization was with light curable fluoride varnish followed by nanohydroxyapatite toothpaste group. Maximum demineralization occurred in control (untreated) group.
Conclusion: Light curable fluoride varnish showed 80% reduction in lesion depth followed by nanohydroxyapatite toothpaste with 50% reduction indicating that both are effective in preventing enamel demineralization adjacent to orthodontic brackets during orthodontic treatment. Light curable fluoride varnish could be an effective alternative in preventing enamel demineralization during orthodontic treatment especially for non-compliant and high-risk patients.

Keywords: demineralization; light curable fluoride varnish; nanohydroxyapatite toothpaste; polarized light microscope

1. Introduction

Enamel demineralization around orthodontic brackets remains the most concerning aspect of fixed appliance therapy. The complex structure of orthodontic brackets makes their periphery an amenable site for the retention of bacterial plaque and hence a potential risk for enamel demineralization. The decline in oral hygiene during orthodontic treatment leads to an increased risk for development of white spot lesions (WSL). Proliferation of facultative bacteria, including *Streptococcus mutans* and *Lactobacillus* increases during orthodontic treatment [1]. This leads to a decrease in pH that tips the demineralization-remineralization balance towards demineralization, leading to WSL [2]

White spot lesions (WSL) have been reported to occur in 25 to 96 % of the patients with poor oral hygiene [3, 4]. Initial enamel decalcification can be noticed as early as 4 weeks after the beginning of orthodontic treatment [5, 6]. Mechanical and crystallographic studies on white spot lesions revealed around 10% loss in mineral content, making the area softer and prone to enamel caries [7]. These enamel lesions caused by orthodontic treatment lasted for 5 years or more after appliance removal [8]. Various methods have been advocated to prevent the incidence of white spot lesions during orthodontic treatment.

Fluoride is the most important agent preventing demineralization, as well as against the development and progression of these incipient enamel lesions [9, 10]. Fluoride ions can be incorporated into the hydroxyapatite structure of tooth enamel by the replacement of hydroxyl groups or by redeposition of dissolved hydroxyapatite as less soluble fluoridated forms, such as fluorapatite or fluorhydroxyapatite [11]. Fluorides have been used in the form of toothpastes, gels, mouth rinses etc., however the effectiveness of these products is directly related to patient compliance. Later fluoride releasing composites and glass ionomer cements have been introduced as a bonding agent to overcome this problem but the bond strength of these materials is less than conventional composite resin [12].

Another possible way is the application of resin sealant on the enamel surface around and beneath the orthodontic bracket to prevent demineralization. But research have shown that most of the chemically cured sealants do not effectively seal smooth enamel surfaces, because of oxygen inhibition of polymerization when the sealant is in contact with the air in a thin layer [13]. To overcome this inadequate polymerisation of resin sealants light cure sealants have been introduced but subsequent clinical studies did not support its effectiveness in preventing demineralization during orthodontic treatment [14].

Many studies have recommended the routine use of fluoride varnish during fixed orthodontic treatment to enhance enamel resistance against cariogenic challenges during orthodontic therapy [15, 16]. But the longevity and sustainability of the conventional fluoride varnish coatings are questionable and they require repeated applications at specific intervals; this is a cumbersome procedure [17]. Currently resin based fluoride varnish has been introduced for preventing WSL during orthodontic treatment. Recently introduced Clinpro XT varnish (3M, ESPE) is a highly filled, light cured resin-modified glass ionomer-based material, and the manufacturer claims that it provides a site-specific fluoride-releasing coating and remains intact for more than 6 months. The effectiveness of this varnish has been investigated for dentinal hypersensitivity and remineralization but its superiority over the other preventive methods to prevent enamel demineralization is not studied.

Nano-hydroxyapatite (n-HAp) is considered as one of the most biocompatible and bioactive materials and has gained wide acceptance in medicine and dentistry in recent years. Hydroxyapatite (HA) is the main constituent in enamel and dentine constituting 95wt% and 75wt%, respectively. Nanosized hydroxyapatite particles have similarity to the apatite crystals of tooth enamel in morphology and crystal structure and have better affinity towards hydroxyapatite crystals of enamel[18]. Research have shown that n-HAp have the potential to repair dental enamel[19]. Particles of n-HAp incorporated into dentifrices are the new products available in market. It is claimed to be effective in reducing hypersensitivity and promoting remineralization, but its effect in preventing white spot lesion around orthodontic brackets is not studied. Therefore, the prime objective of this study is to evaluate the effect of light curable fluoride varnish and commercially available nanohydroxyapatite incorporated toothpaste in preventing demineralization adjacent to orthodontic brackets and to compare their relative efficiency.

2. Material and Methods

2.1 Study design

Quasi experiment

2.2 Sample and Sampling Technique

Patients aged between 13- 20 years reported to the Department of Orthodontics, Government Dental College, Thiruvananthapuram seeking orthodontic treatment who fulfill the inclusion and exclusion criteria were selected for the study. The eligibility criteria includes: need of fixed orthodontic treatment with first premolar extractions, patients should consent for all first premolar extractions, all first premolars fully erupted and an intact buccal surface with no structural defects; absence of clinical evidence of demineralized lesions or fluorosis; and no history of previous orthodontic treatment. Forty-five patients were selected for the study with fifteen patients in each group. Patients were assigned to three different groups by consecutive sampling as and when the patient reports to the op. Informed written consent was taken from all the patients prior to the study. The research protocol was approved by the ethical committee of the institution.

2.3 Interventions

In every participant stainless steel pre-adjusted edgewise brackets were bonded on buccal surface of premolars using standard acid etch- adhesive system. Prior to bonding, all teeth were thoroughly cleansed using nonfluoridated pumice and water and air dried for 10 seconds. The enamel was etched with 37% phosphoric acid gel (EAZETCH) for 20 seconds and rinsed with water for 60 seconds and dried thoroughly around 10 seconds until a frosty appearance was observed. Particular care was taken in the etching procedure to ensure that only the area where the bracket would be placed would be etched and bonded. This is done to avoid the influence of etching procedure upon the enamel adjacent to the bracket.

In all the participants bonding was done according to the treatment protocol using Stainless steel pre-adjusted edgewise brackets having 0.022″ slot, MBT prescription with Transbond™ XT light-cured adhesive paste including the premolars which requires therapeutic extraction as a part of treatment plan. Excessive adhesive that appeared as flash around the bracket base were carefully removed using a dental scaler and were light cured for 20 seconds. This formed the control or baseline for the study, in addition to this certain intervention were done in other two groups. Group 1 (control) received no interventions - instructed to follow their regular oral hygiene practice and to brush twice daily using nonfluoridated toothpaste. Group 2 - participants were instructed to brush twice daily using nano-hydroxyapatite toothpaste (Aclaim- Group pharmaceuticals). Aclaim (Fig 1) is a product of Group Pharmaceuticals which contains nanocrystals of hydroxyapatite of approximately 100nm in size. To ensure the patient compliance, subjects noted the time of brushing on a checklist which was counter signed by the parent or the guardian. In Group 3 - enamel surface was etched around orthodontic bracket for 15 seconds. After washing and air drying, light-curable fluoride varnish (Clinpro™ XT; 3M ESPE, Pymble, New South Wales, Australia) was mixed as per manufacturer’s instructions, applied as a thin layer over the etched enamel surface around the maxillary first premolar brackets and light-cured for 20 seconds. Clinpro™ XT varnish (Fig 2) is a resin modified glass ionomer (RMGI) based on the patented methacrylate modified polyalkenoic acid. Patients were advised not to brush their teeth for at least 6 hours after varnish application and to use nonfluoridated toothpaste until the collection of samples.

Fig 1: Aclaim- Nanohydroxyapatite toothpaste

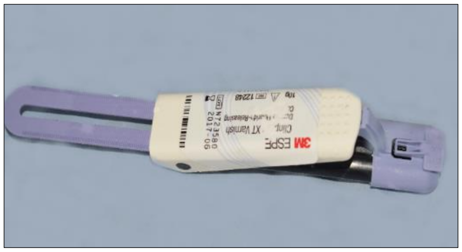

Fig 2: Clinpro XT light curable fluoride varnish

2.4 Collection of Samples

Regular review was done in all the patients under study and also assessed for any debonding of premolar brackets once in 3 weeks. In group 2 the checklist of patient compliance was also assessed. At the end of 4 months the brackets were carefully debonded and the premolars were extracted. Careful debonding procedure was used to ensure that there is no enamel microfractures around the bracket bases. Only one maxillary premolar was selected for the study in each patient. The roots of all the teeth were cleaned and stored in 0.1% Thymol solution.

2.5 Sectioning the specimen

Once all the samples were collected the crowns were cut 2mm apical to the cementoenamel junction and embedded individually in a mold with chemically cured resin to prevent fracturing during thickness reduction. Buccolingual longitudinal sections of the middle third of crown were made using Hard tissue Microtome (SP 1600, Leica, Nussloch, Germany) (Fig3). The thickness of each sections were further reduced to 50-70 µm by handgrinding.

Fig 3: A tooth section being cut in Hard Tissue microtome

2.6 Evaluation of Depth of Demineralization

The sections were evaluated under Polarized light microscope (CX21 Olympus, Tokyo, Japan) for analyzing the depth of demineralization using water as imbibing medium. In our study microphotographs of the gingival third of the buccal surface were taken in fixed ×4 magnification using Magcam DC5 digital camera attached to the polarized light microscope. The depth of demineralization was measured from the microphotographs in micrometers. The gingival third was further divided into gingival, middle and occlusal third and the demineralized areas were measured using the micrometer scale in the same magnification. The average these three measurements was recorded as the lesion depth for that teeth. This was recorded by an oral pathologist in a blinded situation. Figure 4-6 are microphotographs of samples from the control, Aclaim and the Clinpro™ XT group at the end of 4 month study period.

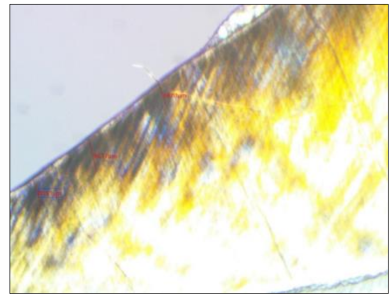

Fig 4: Microphotograph of demineralized enamel lesion in control group

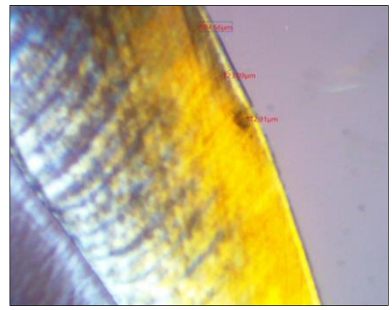

Fig 5: Microphotograph of demineralized enamel lesion in Aclaim group

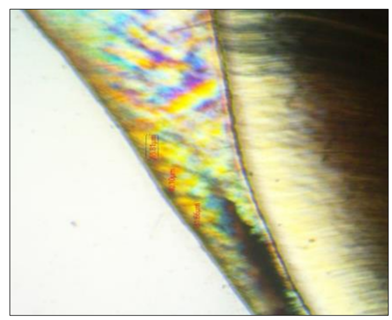

Fig 6: Microphotograph of demineralized enamel lesion in Clinpro XT group

3. Results

Forty-five patients who fulfilled the inclusion criteria became the part of the study. There were no drop outs in the study and no tooth was lost during sectioning process. Hence all the 45 samples with 15 per group were used to study the depth of demineralization during orthodontic procedure. None of the participants across the study either reported or exhibited any adverse or allergic reactions to any of the dental products to which they were exposed

3.1 Evaluation of Depth of demineralization

The mean and standard deviation of depth of demineralization of each groups were given in Group 1 (control), Group 2 (Aclaim) and Group 3 (Clinpro XT) were 175.18±58.49 µm, 88.75±22.54 µm and 37.86±16.31 µm respectively (Table 1). It is evident from the results that every tooth in the control and experimental groups showed some amount of demineralization during orthodontic treatment. The probability distribution curve does not show a normal distribution hence nonparametric test – Kruskal Wallis

one-way ANOVA is used for comparison of lesion depth between the study groups followed by two group comparisons by Mann-Whitney U test.
Kruskal Wallis one-way ANOVA for cluster level analysis showed significant differences ($p < 0.001$) in the depth of demineralization among the study groups (Table 2). Intergroup comparison using Mann Whitney U test also showed significant differences ($p < 0.0001$) between the interventional groups (Table 3). This revealed a greater amount of demineralization in untreated or control group compared to the experimental groups with Clinpro XT (Varnish) group showing very less amount demineralization compared to Aclaim (Toothpaste) group.

Table 1: Descriptive Statistics of depth of demineralization in µm among the study groups

Groups	N	Minimum	Median	Maximum	Mean	SD	Interquartile range	95% confidence Interval(µm)
Control	15	91.84	157.49	287.36	175.18	58.49	77.28	142.79-207.57
Aclaim	15	51.50	83.25	120.26	88.75	22.54	33.00	76.26-101.23
Clinpro XT	15	0	37.17	70.20	37.86	16.31	21.61	28.82-46.90

Table 2: Comparison of demineralization depths between the control and the two experimental groups using Kruskal-Wallis One-way ANOVA

Groups	Depth of Demineralization(µm)			Kruskal Wallis one-way ANOVA	
	Mean	Median	SD		
Control	175.18	157.49	58.49	df 2	p-value 0.0001*
Aclaim	88.75	83.25	22.54		
Clinpro XT	37.86	37.17	16.31		

* p– value < 0.05 is significant

Table 3: Intergroup Comparison of depth of demineralization between study groups

Groups	Mann-Whitney U test	p-value
Control & Clinpro XT	0.00	0.0001*
Aclaim & Clinpro XT	5.00	0.0001*
Control & Aclaim	11.00	0.0001*

* p– value < 0.05 is significant

4. Discussion
Demineralization of enamel is an inevitable side-effect associated with fixed orthodontic treatment. Such enamel lesions may occur as early as 4 weeks which is typically the time period between one orthodontic appointment and the next [6]. Considering the short duration of time within which white spot lesions (WSL) can develop and become irreversible, early diagnosis and prevention is essential, as modern dentistry is focusing on preventive approaches.
Fluoride is the most important agent in preventing demineralization and the lesions from developing and progressing. Though there is a widespread use of fluoride products as toothpastes, mouth rinses, varnish etc. during orthodontic treatment, there is little evidence as to which method or combination of methods to deliver the fluoride is the most effective. The use of fluoride varnish is being investigated as an effective preventive measure for white spots lesions, especially for ease of professional application in long-term clinical situations. The role of fluoride varnish in inhibiting enamel demineralization around fixed appliances is increasingly being accepted [20, 21].
Though these fluoride varnish reduces the incidence of WSL the effectiveness mainly depends on multiple applications. Evrenol *et al*. suggested that frequency is more important than concentration [22]. Demito *et al.* recommended application of fluoride varnish (Durafluor) every 3 months [20]. Hence conventional varnish application requires additional chair side time for clinician and unsightly appearance of enamel due to repeated exposure of fluoride.
Clinpro XT (3M ESPE, Pymble, New South Wales, Australia) is a recently introduced highly filled, resin-modified glass ionomer-based light-curable fluoride varnish (LCFV). The manufacturers claim that the varnish layer remains intact for more than 5000 brushing strokes and can resist toothbrush abrasion and normal wear for 6 months or longer. The effectiveness of Clinpro XT varnish has been investigated for dentinal hypersensitivity and remineralization [23]. In this study the effect of LCFV in preventing enamel demineralization adjacent to orthodontic brackets is evaluated for a period of 4 months
The other major breakthrough in enamel repair came after introduction of n-HAp incorporated tooth paste in commercial form. Being the same content as that of dental enamel, it is claimed that these n-HAp incorporated tooth pastes give the tooth its bright white appearance and eliminates the diffused reflection of light by filling up the fine pores or soft lesions on the tooth surface and when it penetrates the pores in tooth tissues, it acts as a template in the remineralization process by continuously attracting large amounts of calcium and phosphate ions. The remineralization is more pronounced if the particle size of hydroxyapatite is less than micron size. Shetty *et al* in his study has shown the importance of particle size of n-HAp in Aclaim. He mentioned that the nanoparticles with particle size <100 nm can be kinetically protected on account of their sizes and can remain relatively stable under undersaturated condition; therefore, the prevention of enamel erosion is enhanced by the new nanolayer and is insensitive to dissolution, thus the enamel surface is protected under the acidic condition [24]. The present study evaluated the effect of nanohydroxyapatite incorporated (particle <100nm) toothpaste in preventing enamel demineralization adjacent to orthodontic brackets.
The study was undertaken for a period of four months for evaluation of demineralization during orthodontic treatment without interfering treatment progress. The compliance of the participants was high and none of the participants withdrew due to any adverse effects (e.g., allergies, gingival inflammation, enamel-staining, and accelerated plaque accumulation) and no detrimental effects were observed with any of the treatment protocols during the course of the study.

The advantage of this study is that the oral environment is simulated which is routinely encountered in clinical settings. For conducting this study, no alteration was made to the treatment plan or its execution. Previously, many studies have been conducted to assess the preventive effect of fluoride releasing materials in demineralization using a split-mouth design [25, 26]. But this design has a disadvantage of carry across effect of fluoride or calcium release to other sites too. In the present study, the subjects in the experimental groups were randomly divided into three equal groups and each received only one intervention during the assessment period. As suggested by Pascotto *et al.* [27] the current experimental design was chosen instead of the split mouth technique to avoid the carry-across effect due to fluoride or calcium and phosphate release by the agents on enamel around the brackets.

The closest in-vivo study to the present study was one by Mehta *et al.* [26] who evaluated the effect of LCFV (Clinpro XT) on enamel demineralization adjacent to orthodontic brackets for a period of 3 months and found around 90% protection against WSL. But he did not compare the relative efficiency of LCFV with other agents that prevent enamel demineralization. The present study shows similar results to his study in reducing the lesion depth by 80% in LCFV group compared to the untreated controls and also compares the relative efficiency of LCFV with commercially available nanohydroxyapatite incorporated tooth paste (Aclaim) in preventing WSL using polarized light microscope. At the end of 4 months almost every teeth showed some amount of demineralization. The mean lesion depths were 37.86 µm in the LCFV group, 88.75 in the nanohydroxyapatite toothpaste group and 175.18 in the control group after 4 months of orthodontic treatment Analysing the mean lesion depth of all three groups it can be inferred that the mean depth of demineralization in the LCFV (Clinpro XT) and nanohydroxyapatite toothpaste (Aclaim) group was around 80% and 50 % less than control group respectively. The results of the present study showed that the depth of demineralization is more in untreated control group compared to the interventional groups (LCFV and Aclaim toothpaste). This is in accordance with other previous studies [25, 26, 28].

Invariably 99.9% of the samples under investigation shows some amount of demineralization except one tooth under LCFV group which could be probably due to good oral hygiene or alteration in oral microbial flora or histological variations etc. Also confounding factors such as sex, oral hygiene, cooperation, tooth structure, fluoride uptake from other sources, composition of saliva might have some effects in the achieved results. Whereas in the study by Mehta *et al.* [26] using Clinpro XT varnish he found demineralization only in three teeth on a sample of nine after 120 days. This could be probably attributed to difference in oral hygiene between the patients or may be because of less samples (n=9) assessed at the end of 120 days in his study.

In a recent randomized controlled trial the efficacy of a fluoride varnish and ACP-CPP paste were compared to assess the improvement in WSL and found no significant difference between the varnish and paste group [29]. But the present study shows significant difference between the varnish and the nanohydroxyapatite toothpaste group showing better results with fluoride varnish group. Intergroup comparison of experimental groups (LCFV and Aclaim toothpaste) shows that the depth of demineralization is approximately 50% less in LCFV group compared to nanohydroxyapatite toothpaste group. This could be probably due to the effect of independency of patient compliance in varnish group, difference in assessment methods between the studies, he assessed the reduction of white spot lesions using photographs and it is mainly subjective whereas the present study used microscopic evaluation for detecting demineralization. Also, his study evaluated the remineralizing aspect of varnish and paste when brackets were not in place (post debonding) but the present study evaluated the prevention of demineralization by these agents during orthodontic treatment.

On the basis of the results, the present study recommends daily use of nanohydroxyapatite toothpaste twice daily while undergoing orthodontic treatment for patients who maintains good oral hygiene, but strongly suggests application of LCFV for high risk and noncompliant patients. The increased chair side time may be a concern for the clinicians to use LCFV but the results are more convincing regarding prevention of WSL during orthodontic treatment. The fluoride varnish should be applied at the time of initial bracket placement in order to prevent enamel demineralization. Future research could determine whether increased benefit would result from more frequent applications of fluoridated varnish, including what intervals between applications would be most beneficial.

5. Limitation of the study

1. The study was conducted for a period of 4 months, longer duration would be helpful to identify the long-term benefits of these agents.
2. Complete randomization was not done which could have increased the chance of selection bias.
3. The study was conducted primarily in a South Indian setting at a single centre and that generalizability to other populations is unknown, though the study was sufficiently powered. So a multi-centric study with large sample size could further validate the result of our study.

6. Conclusion

1. Both Light curable fluoride varnish and nanohydroxyapatite incorporated toothpaste showed efficacy in reducing lesion depth compared to untreated controls.
2. Single application of Light curable fluoride varnish reduces demineralization around orthodontic brackets by 80% when compared to the untreated controls for the period of 4 months.
3. Nanohydroxyapatite incorporated toothpaste reduced depth of demineralization by 50% compared to untreated controls.
4. Light curable fluoride varnish shows better efficiency compared to nanohydroxyapatite toothpaste in preventing demineralization around orthodontic brackets and hence could be an efficient method in preventing WSL especially in non-compliant high-risk patients.

7. Conflict of Interest

None to declare.

8. Acknowledgements

We would like to thank Dr. Sherline, Department of Oral pathology, Saveetha Dental College and Research Institute, Chennai, TamilNadu, India for assisting us in preparing and evaluating histologic sections.

9. References

1. Lundström F, Krasse B. Streptococcus mutans and lactobacilli frequency in orthodontic patients; the effect of chlorhexidine treatments. Eur J Orthod. 1987; 9(1):109-116.
2. Featherstone JD. The caries balance: contributing factors and early detection. CDA. 2003; 31(2):129-134.
3. Mitchell L. Decalcification during orthodontic treatment with fixed appliances—an overview. Br J Orthod. 1992; 19(3):199-205.
4. Artun J, Brobakken BO. Prevalence of carious white spots after orthodontic treatment with multibonded appliances. Eur J Orthod. 1986; 8(4):229-234.
5. Øgaard B, Rølla G, Arends J. Orthodontic appliances and enamel demineralization: Part 1. Lesion development. Am J Orthod Dentofacial Orthop. 1988; 94(1):68-73.
6. O'reilly MM, Featherstone JDB. Demineralization and remineralization around orthodontic appliances: an in vivo study. Am J Orthod Dentofacial Orthop. 1987; 92(1):33-40.
7. Quantitative measurements of remineralization of incipient caries. - PubMed - NCBI [Internet]. [cited 2018 Jul 28]. Available from: https://www.ncbi.nlm.nih.gov/pubmed/8972804
8. Øgaard B. Prevalence of white spot lesions in 19-near-olds: A study on untreated and orthodontically treated persons 5 years after treatment. Am J Orthod Dentofacial Orthop. 1989; 96(5):423-427.
9. Schmit JL, Staley RN, Wefel JS, Kanellis M, Jakobsen JR, Keenan PJ, *et al*. Effect of fluoride varnish on demineralization adjacent to brackets bonded with RMGI cement. Am J Orthod Dentofacial Orthop. 2002; 122(2):125-134.
10. Trairatvorakul C, Kladkaew S, Songsiripradabboon S. Active management of incipient caries and choice of materials. J Dent Res. 2008; 87(3):228-232.
11. De Leeuw NH. Resisting the onset of hydroxyapatite dissolution through the incorporation of fluoride. J Phys Chem B. 2004; 108(6):1809-1811.
12. Fox NA, McCabe JF, Gordon PH. Bond strengths of orthodontic bonding materials: an in-vitro study. Br J Orthod. 1991; 18(2):125-130.
13. Zachrisson BU, Heimg\a ard E, Ruyter IE, Mjör IA. Problems with sealants for bracket bonding. Am J Orthod. 1979; 75(6):641-649.
14. Banks PA, Richmond S. Enamel sealants: a clinical evaluation of their value during fixed appliance therapy. Eur J Orthod. 1994; 16(1):19-25.
15. He T, Li X, Dong Y, Zhang N, Zhong Y, Yin W, *et al*. Comparative assessment of fluoride varnish and fluoride film for remineralization of postorthodontic white spot lesions in adolescents and adults over a 6-month period: A single-center, randomized controlled clinical trial. Am J Orthod Dentofacial Orthop. 2016; 149(6):810-819.
16. Benson PE, Parkin N, Dyer F, Millett DT, Furness S, Germain P. Fluorides for the prevention of early tooth decay (demineralised white lesions) during fixed brace treatment. Cochrane Database Syst Rev, 2013, 2013(12).
17. Chambers C, Stewart S, Su B, Sandy J, Ireland A. Prevention and treatment of demineralisation during fixed appliance therapy: a review of current methods and future applications. Br Dent J. 2013; 215(10):505.
18. Roveri N, Battistella E, Bianchi CL, Foltran I, Foresti E, Iafisco M, *et al*. Surface enamel remineralization: biomimetic apatite nanocrystals and fluoride ions different effects. J Nanomater, 2009, 2009:8.
19. Huang SB, Gao SS, Yu HY. Effect of nano-hydroxyapatite concentration on remineralization of initial enamel lesion in vitro. Biomed Mater. 2009; 4(3):034104.
20. Demito CF, Vivaldi-Rodrigues G, Ramos AL, Bowman SJ. The efficacy of a fluoride varnish in reducing enamel demineralization adjacent to orthodontic brackets: an in vitro study. Orthod Craniofac Res. 2004; 7(4):205-210.
21. Bichu YM, Kamat N, Chandra PK, Kapoor A, Aravind NKS. Prevention of enamel demineralization during orthodontic treatment: An in vitro comparative study. Orthod Art Pract Dentofac Enhanc, 2013, 14(1).
22. Evrenol BI, Kucukkeles N, Arun T, Yarat A. Fluoride release capacities of four different orthodontic adhesives. J Clin Pediatr Dent. 1999; 23(4):315-319.
23. Bhandary S, Hegde MN. A clinical comparison of in-office management of dentin hypersensitivity in a short-term treatment period. IJBAR. 2012; 3(03):69-174.
24. Shetty A, Shetty D, Shetty S. A Comparison of Clinical Efficacy of Dentifrices Containing Calcium Sodium Phosphosilicate, Nanoparticle Hydroxyapatite and a Dentifrice Containing Casein Phosphopeptide Amorphous Calcium Phosphate on Dentinal Hypersensitivity-A Comparative Triple Blind Randomized Study. Adv Hum Biol. 2014; 4(2):57.
25. Farhadian N, Miresmaeili A, Eslami B, Mehrabi S. Effect of fluoride varnish on enamel demineralization around brackets: an in-vivo study. Am J Orthod Dentofacial Orthop. 2008; 133(4):S95-S98.
26. Mehta A, Paramshivam G, Chugh VK, Singh S, Halkai S, Kumar S. Effect of light-curable fluoride varnish on enamel demineralization adjacent to orthodontic brackets: an in-vivo study. Am J Orthod Dentofacial Orthop. 2015; 148(5):814-820.
27. Pascotto RC, de Lima Navarro MF, Capelozza Filho L, Cury JA. In vivo effect of a resin-modified glass ionomer cement on enamel demineralization around orthodontic brackets. Am J Orthod Dentofacial Orthop. 2004; 125(1):36-41.
28. Todd MA, Staley RN, Kanellis MJ, Donly KJ, Wefel JS. Effect of a fluoride varnish on demineralization adjacent to orthodontic brackets. Am J Orthod Dentofacial Orthop. 1999; 116(2):159-167.
29. Gontijo L, Cruz R de A, Brandão PRG. Dental enamel around fixed orthodontic appliances after fluoride varnish application. Braz Dent J. 2007; 18(1):49-53.