COMPARATIVE EVALUATION OF DENTAL VARNISHES IN PREVENTION OF DEMINERALIZATION OF ENAMEL DURING FIXED ORTHODONTIC TREATMENT-A SPLIT MOUTH STUDY

DISSERTATION

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In the partial fulfillment of the requirements for the degree

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In

ORTHODONTICS AND DENTOFACIALORTHOPAEDICS

By

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-Dr. Swagat Verma

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SL. NO.	ABBREVIATEDFORM	FULL FORM
1.	WSL	White Spot Lesion
2.	FV	Fluoride Varnish
3.	CPP-ACP	Casein Phosphopetide Amorphous Calcium Phosphate
4.	PLM	Polarized Light Microscope
5.	KNH	Knoop hardness number
6.	NaF	Sodium Fluoride
7.	Ca++	Calcium ions
8.	$PO^{3}_{4}{}^{-}$	Phosphate ions
9.	F^{-}	Fluoride ions
10.	ANOVA	Analysis of variance

ABSTRACT

ABSTRACT

AIM: To evaluate and compare the effect of two different varnishes in prevention of demineralization of enamel when applied around Orthodontic brackets bonded to maxillary 1st premolar teeth scheduled for extraction, 3 months later, using polarized light microscope.

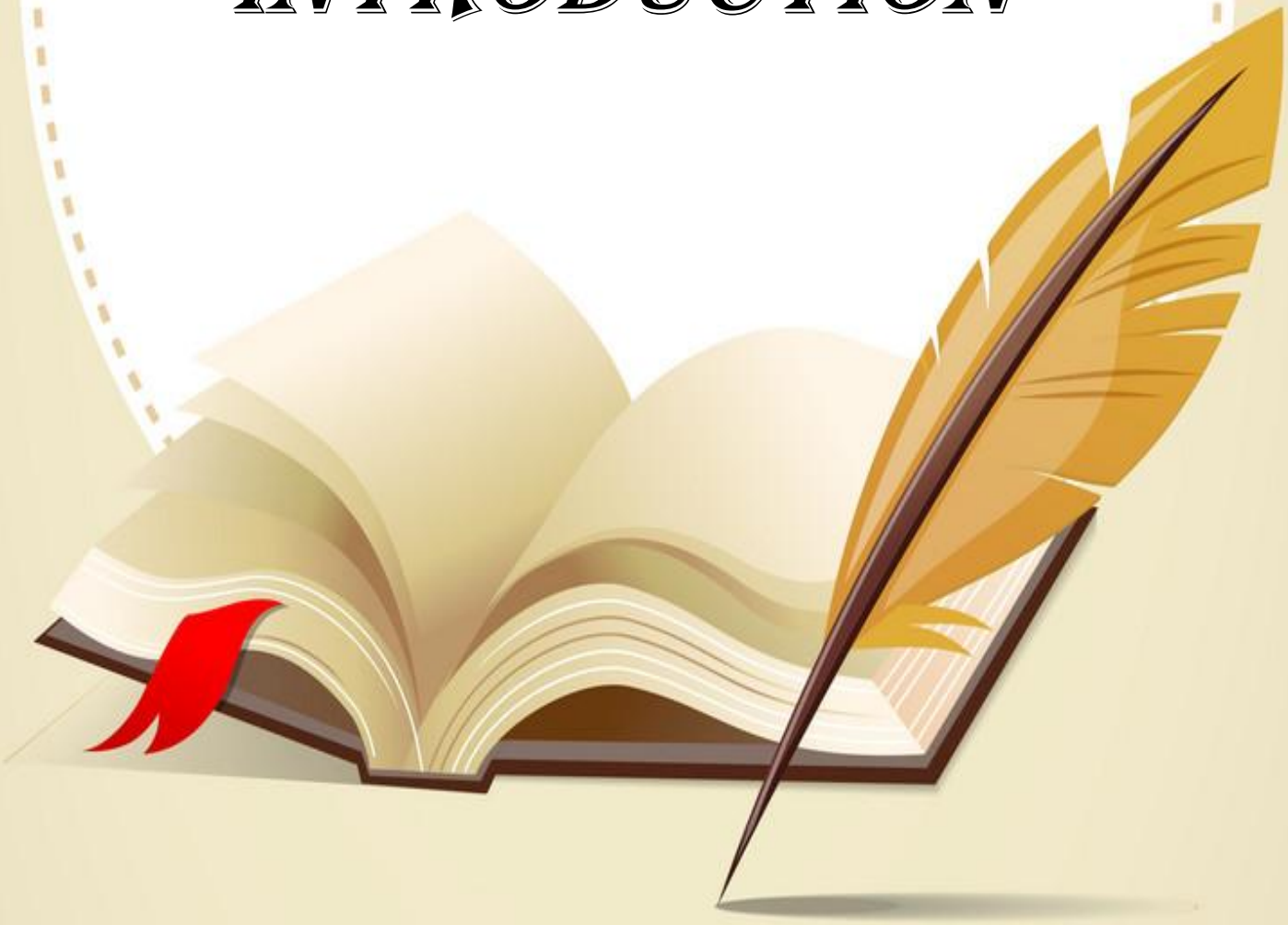
MATERIALS AND METHODS: This split mouth study consisted of 15 patients undergoing fixed Orthodontic treatment. The samples were divided into 3 groups, Group I was control (N=15) i.e., mandibular 1st premolar of right or left side where no varnish was applied, Group II (N=15) included maxillary 1st premolar of left side where VOCO Varnish was applied and in Group III (N=15) included maxillary 1st premolar of right side where MI Varnish was applied. Extractions were done after 3 months following application of two different varnishes on respected tooth. Buccolingual sections of teeth were made, mounted and visualized under polarized light microscope and 3 readings were taken for image of area showing deepest demineralization depth using image J software. Data was tabulated and adequate statistical comparisons were made.

RESULTS: The mean depth of demineralization was highest for Group I – Control group (1744.07±38.55 µm) > Group II – VOCO Varnish (1063.64±160.1 µm) > Group III – MI Varnish (940.44±96.06 µm) and difference was statistically significant. Amongst two different varnishes, Group III (MI Varnish) had less demineralization depth than Group II (VOCO Varnish).

CONCLUSION: Demineralization was evident with fixed Orthodontic treatment as seen in control and experimental groups. Both varnishes were efficacious in preventing white spot lesions.

KEY WORDS: Varnishes; Demineralization; White spot lesions; Split mouth study; Polarized light microscope.

INTRODUCTION



White spot lesions (WSLs), seen as an unfortunate sequel of Orthodontic treatment in areas around Orthodontic brackets is clinically defined as opaque, white areas caused by the loss of minerals from subsurface-enamel. Development of white spot lesions (WSLs) around Orthodontic brackets is a common problem jeopardizing the health and aesthetics of the teeth. The overall prevalence of white spot lesions among Orthodontic patients varies from 0 to 97% [1]. The incidence of white spot lesions in patients treated with fixed Orthodontic appliance is reported to be up to 50%, and these can be seen as early as 4 weeks after bracket placement [2]. The increase in prevalence and severity of enamel opacities is seen on the maxillary and mandibular 1st molars, the maxillary lateral incisors, and canines [3]. The greatest prevalence of white spot lesions was found in the mandibular 1st molar (30%), followed by the maxillary lateral incisors (29%), and the mandibular 2nd premolars (20%). The increase was greater on the cervical and middle third of the vestibular surface of these teeth [4].

Various methods had been used to prevent white spot lesions like mechanical plaque control, use of fluoride as mouth rinses, gels, tooth pastes, varnishes; pit and fissure sealants, incorporation of fluoride in luting cements, bonding agents, elastomeric modules; essential oil mouth rinses; argon lasers, chewing gums, use of CPP-ACP remineralizing agents, bioactive glass, etc [5].

One of the most commonly used method for preventing white spot lesions during fixed Orthodontic treatment is use of topical fluorides in form of fluoride incorporated in toothpaste and use of fluoride mouth rinses; fluoride pit and fissure sealants etc. Most topical fluoride products like toothpastes and mouth rinses rely on patient compliance; however, the application of fluoride varnish is a compliance-free method for the prevention of white spot lesions. The main action of fluoride products is preventing demineralization by promoting formation of fluorapatite crystals in enamel which is more resistant to acid attack. Fluoride also affects the activities of cariogenic bacteria and prevents caries. The varnishes had been proven to be efficacious in preventing caries as they adhere to the surface in a thin layer and release fluoride for long time [6,7]. There are many in vitro and in vivo reports in literature claiming that fluoride varnishes are effective in the prevention and reduction

in depth of white spot lesions [6,8–19]. However, a systematic review has reported that fluoride varnishes have only moderate efficacy in preventing enamel demineralization during comprehensive Orthodontic treatment [20]. Another systemic review article has reported that fluoride releasing materials near brackets seems to be an effective approach to reduce the risk of white spot lesions development in patients wearing fixed Orthodontic appliances and this reduction could be even more meaningful for patients who have difficulties following oral hygiene instructions and thus are under higher risk [21]. Fluoride varnish is more efficacious than fluoride gel in reducing enamel demineralization, but both the agents could not completely eliminate chances of enamel demineralization [22].

The second most important treatment modality for the prevention of white spot lesions is promotion of remineralization by various remineralizing agents (Bioactive glass, Tricalcium phosphate, Calcium sucrose phosphate, Xylitol, Casein phosphopeptide-amorphous calcium phosphate paste).

CPP-ACP is an amorphous form of calcium phosphate (ACP) stabilized by a phosphopeptide from the milk protein casein (CPP). CPP increases the level of calcium phosphate ions in plaque by adhering to ACP, thereby affecting demineralization and remineralization process of enamel. There are studies showing CPP inhibits the adherence and functioning of cariogenic bacteria (Streptococcus mutans) in mouth.

It has been reported that CPP-ACP interacts with fluoride ions to produce novel nanoclusters of calcium, fluoride phosphate ions and provided added anticariogenic effect. Various studies found CPP-ACP to be efficacious in preventing white spot lesions, when used as a paste [23,24], rinse [25,26], or varnish [27–37].

CPP-ACP products available as paste again needed patient compliance, hence availability of CPP-ACP complex along with fluoride as varnish might be better in preventing white spot lesions. Most of the previous studies that had evaluated efficacy of CPP-ACP varnish were in-vitro studies [27–34]. Most of the in-vitro studies had their inherent drawbacks like inability to simulate the complex biological process involved in remineralization and demineralization of enamel. Hence in-vivo study would be better to check the efficacy of different products used to prevent white spot lesions. Few in-vivo studies had been conducted evaluating efficacy of CPP-ACP varnish

using visualization method in early childhood caries [35] or during fixed Orthodontic treatment [37]; by measuring bacterial count [36]; or measuring demineralization using polarized light microscope or scanning electron microscope. None of the in-vivo studies had evaluated efficacy of commonly used varnishes (Sodium Fluoride varnish and CPP-ACP varnish) during fixed Orthodontic treatment. So, split mouth study design was used to evaluate and compare efficacy of two different varnishes in preventing white spot lesions when applied around the brackets bonded with non-fluoride adhesives to 1st maxillary premolars of different quadrants, scheduled for extraction for fixed Orthodontic treatment and comparing the results to control teeth i.e., mandibular 1st premolars bonded with same adhesive and scheduled for extractions. Thus, effect of two different varnishes in similar biological environment for the patient would help in comparative evaluation of efficacy of two different varnishes (MI Varnish and VOCO Varnish).

The efficacy of different materials in preventing white spot lesions is generally evaluated by measuring enamel demineralization depth with the help of light microscope, electron microscope, scanning electron microscope, transmission electron microscope, polarized light microscope, laser fluorescence, contact micro-radiography, etc.

Amongst these methods polarized light microscope is widely used to measure white spot lesions depth. Polarized light microscope is a contrast-enhancing technique that improves the quality of the image obtained with birefringent materials and is also capable of providing information on absorption colour and optical path boundaries between minerals of differing refractive indices, like enamel and dentin in tooth. Thus, it was selected for the present study.

Considering this the aim of present study was to make comparison between two varnishes by measuring depth of enamel demineralization around brackets bonded on 1st premolars, that were extracted after 3 months of varnish application, using polarized light microscope.

AIMS AND OBJECTIVES

AIM:

To evaluate and compare the effect of two different varnishes in prevention of demineralization of enamel by measuring depth of enamel demineralization, when applied around Orthodontic brackets bonded to 1st premolar teeth, that were extracted after 3 months, of varnish application using polarized light microscope.

OBJECTIVES:

1) To evaluate mean depth of demineralization using polarized light microscope around brackets of right maxillary 1st premolar on which GC MI Varnish was applied once, followed by extraction of the tooth after 3 months later.

2) To evaluate mean depth of demineralization using polarized light microscope around the brackets of left maxillary 1st premolar on which VOCO Profluoride Varnish was applied once, followed by extraction of the tooth after 3 months later.

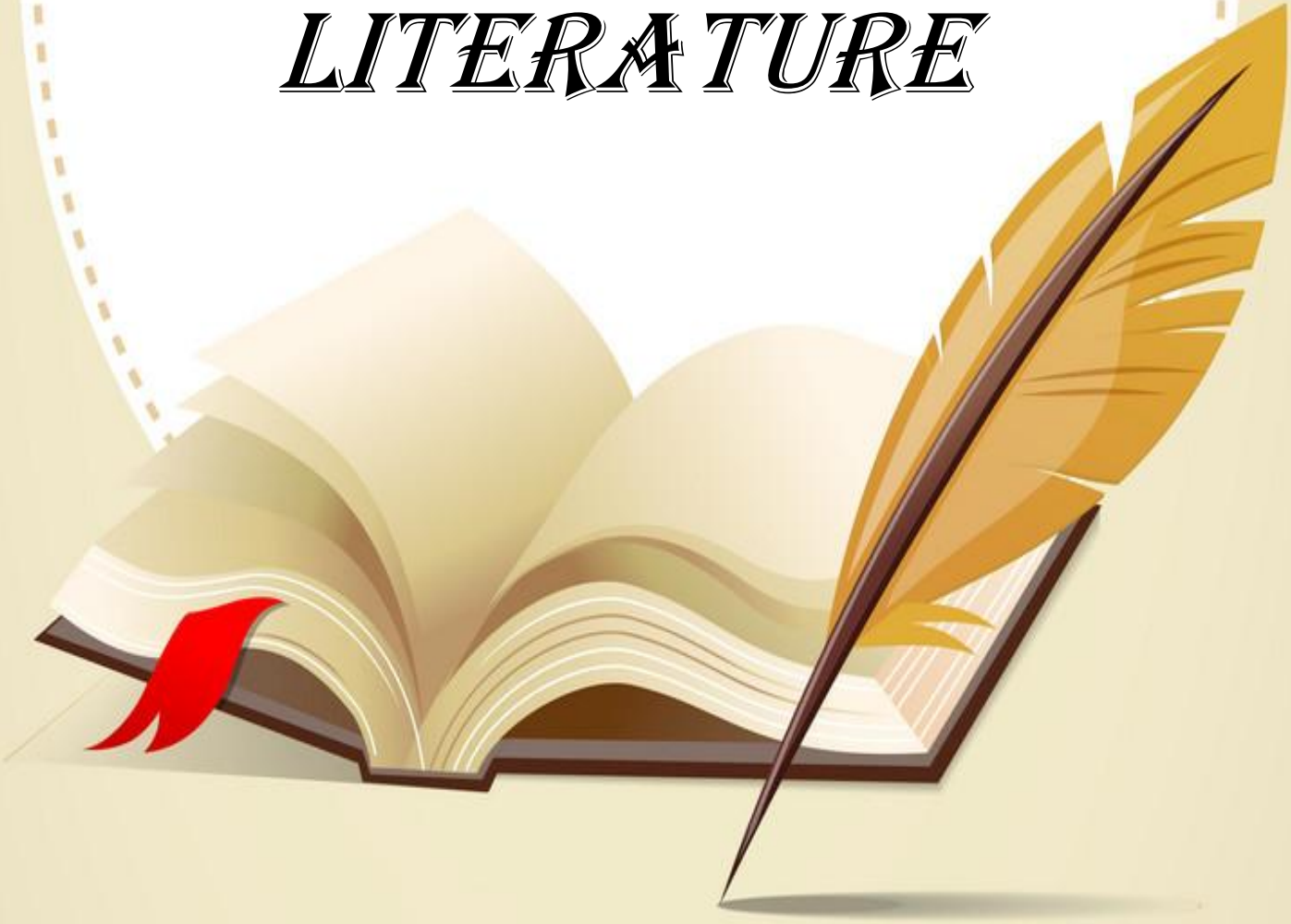
3) To evaluate mean depth of demineralization using polarized light microscope around the brackets of right or left mandibular first premolars where no varnish was applied and therefore it served as control, was extracted at the same time as maxillary premolars.

4) To compare the mean depth of demineralization among these groups.

Null Hypothesis:

There is no difference in the efficacy of MI and VOCO varnish in prevention of demineralization of enamel during fixed orthodontic treatment.

REVIEW OF LITERATURE



Stratemann MW, Shannon IL (1974)[38] : Designed a study with twofold purpose. One was the measurement of the customary incidence of decalcification in orthodontic patients. The second was an investigation of the effectiveness of the daily use of a 0.4 per cent SnF_2 gel in the reduction of mineral loss from enamel in these patients with banded teeth. A total of 209 patients were studied 99 on the test program and 110 on controls. Of 110 control patients, 58 percent presented areas of decalcification at the posttreatment evaluation. Of the 99 test patients, 27 percent had decalcified areas either under or around the bands

.

Fitzpatrick DA and Way DC (1977)[39]: Determine whether there is any difference in wear between etched and unetched enamel in vivo over a 12-week test period. Thirty-two teeth from eleven orthodontic patients requiring extractions were used in this study, in two groups. Group A consisted of five patients requiring the removable of the maxillary first premolars only. Etching caused an additional 3.0 micron loss of enamel over the normal wear of 1.6 microns in an 85 day period. Most of the additional were occurs the first 28 days and then levels off during the reminder of the test period.

TenCate JM and Arends J (1977)[40]: Investigated the mechanism of lesion remineralization, artificial (HEC) lesions in bovine enamel were remineralized in a pH and pF-stat controlled system at 37 °C. In all experiments 5 cm² lesion was immersed in 12.5 ml of a solution containing 2 mM Ca. 1.2 mM PO, and either O or 1 ppm F at pH 7.0. The mineral deposition was followed by monitoring the alkali and fluoride uptake and the solution calcium and phosphate concentration changes. From the ratio of the deposited ions (Ca/PO, Ca/F, Ca/OH, etc.) and from the analysis of the lesion material after remineralization (IR data, X-ray diffraction), the conclusion can be drawn that the material deposited is most likely (fluoridated) hydroxy- apatite (HAP). SEM experiments show that a crystalline material is deposited during re-mineralization, with rod-like crystals having a diameter of 200 The addition of 1 ppm F to the remineralizing solution causes an about twofold increase in rate of remineralization thus comparable with previous studies of the remineralization of

acid-etched enamel. Hard- ness experiments indicate that during the deposition studied a rehardening of the body of the lesion occurs.

Koch G, Petersson LG, Rydén H (1979)[41] : Compared the caries increment in schoolchildren exposed to a fluoride varnish (Duraphat) every six months and in children receiving the conventional weekly fluoride mouthrinsing programme with 0.2 per cent sodium fluoride over a two-year period. Two hundred 14-year-old children, divided into one test and one control group took part in the study. They were clinically and radiographically examined every year. Preexperimental data revealed no differences between the groups. During the experimental period the children in the fluoride varnish group developed a statistically significant lower number of new carious lesions compared with those in the mouthrinsing group. The difference in caries increment was about 30 per cent.

Gwinnett AJ and Ceen RF (1979)[42]: Determined the sites of plaque accumulation and extent to which plaque accumulated on mesh-back and perforated types of brackets and the resin associated with them. Ten subjects wore ten brackets to their maxillary incisors, canines, and premolars for a period of 90 days. Five metal mesh-back bracket were bonded with a lightly filled resin and five perforated metal brackets were bonded with a heavily filled resin. The clinical observations indicated that one of the most common sites for demineralization appear to lie at the junction between the bonding resin and the enamel, just peripheral and commonly gingival to the bracket base.

Mizrahi E (1982)[43]: Determined the prevalence and severity of enamel opacities in patients before and after orthodontic treatment. The sample consisted of 527 patients examined prior to and 269 patients examined after completion of multibanded orthodontic treatment. The results showed that there was a significant increase in both the prevalence (before, 72.3 per cent; after, 84.0 per cent) and severity (Opacity Index: before, 0.125; after, 0.200) following completion of orthodontic treatment. Male patients experienced a significantly higher increase in the severity of enamel opacities following orthodontic treatment. There was no significant sex differential in the prevalence of enamel opacities either before or after orthodontic treatment. This

study showed that orthodontic treatment with multibanded appliances contributed to the development of new areas of enamel demineralization and to an increase in the severity of enamel opacities as measured by the Opacity Index.

TenCate JM, Duijsters PPE (1983)[44]: Studied the demineralization of dental enamel was studied in undersaturated calcium phosphate solutions with different pH and fluoride concentrations. The combined data of the mineral dissolved show a dissolution pattern not significantly different from the stoichiometric hydroxyapatite (HAP) dissolution [Ca/P = 1.72 (0.01)]. However, during the very initial demineralization, calcium is removed preferentially. The rate of demineralization seems to be affected to a greater extent when the solution is supersaturated to CaF2 than to fluorapatite (FAP). This is attributed to a different morphology of the calcium fluoride deposition which effectively blocks the acid diffusion. During demineralization, fluoride is taken up by the enamel; the percentage of fluoride being removed from the solution is correlated with the amount of demineralization.

O'Reilly MM and Featherstone JDB (1987)[45]: Determined quantitatively the amount of demineralization and the ability of commercially available products to inhibit or reverse orthodontically related demineralization. The control group brushed only with the supplied dentifrice. In addition to brushing with the dentifrice, those in test group I rinsed once each night with a sodium fluoride (0.05%) mouthrinse; group II received a weekly topical APF treatment (1.2% fluoride); and Group Ill received a weekly topical APF treatment and rinsed once each night with the sodium fluoride mouthrinse. All premolars were extracted after 1 calendar month. Mineral profiles were determined on cross-sectioned teeth 50 to 75 micron occlusal and cervical to the brackets, directly underneath the brackets, and 500 F micron away from the brackets. The control teeth (dentifrice only) demonstrated up to 15% demineralization to a depth of 50 micron. All of the test teeth produced rehardening and/or inhibition of demineralization ($P < 0.01$). Those in test group III showed a particularly hard outer layer. The study demonstrated that measurable demineralization occurred around orthodontic appliances after only 1 month and this demineralization can be completely inhibited and/or reversed by the use of commercially available fluoride products.

DUCKWORTH RM, MORGAN SN, and MURRAY AM (1987)[46]: Determined the sensitivity of methodology for measuring the concentration of fluorine species in saliva and in plaque has been tested. Human subjects mouth-rinsed daily with aqueous solutions of NaF and Na2FPO3. Samples of unstimulated whole saliva and of plaque were collected twice weekly at least 18 hr after treatment application. Oral fluoride concentrations rose from placebo values for approximately two weeks before attaining equilibrium and returned to baseline when daily mouthrinsing was stopped. Mean elevated oralfluoride concentrations increased significantly with increasing applied NaF concentration in the range 0-1000 ppm F (0-0.053 mol/L). There appeared to be a linear relationship between saliva and plaque fluoride. The ability of fluoride treatments to sustain elevated oral fluoride levels between daily applications may be of major importance in caries control.

Ogaard B, Rolla G, and Arends J (1988)[47]: Conducted a clinical trial to investigate carious lesion development associated with fixed orthodontic therapy. Specially designed orthodontic bands for plaque accumulation were attached to premolars scheduled to be extracted as part of an orthodontic treatment. Visible white spot lesions were seen within 4 weeks in the absence of any fluoride supplementation. Both microradiographic and SEM examinations showed surface softening of the enamel surface-that is, a surface layer was not seen in the lesions. The clinical significance of the present study is that enamel demineralization associated with fixed orthodontic therapy is an extremely rapid process caused by a high and continuous cariogenic challenge in the plaque developed around brackets and underneath ill-fitting bands. Careful inspection of the appliance at every visit and preventive fluoride programs are therefore required.

Sonis AL and Snell W (1989)[48]: Compared a visible light-activated, fluoride-releasing bonding system with a visible light-activated conventional bonding system relative to bracket retention and prevalence of decalcification. Twenty-two patients were entered into the study, representing 206 experimental brackets and 206 control brackets. The average treatment period was 25 months. No significant differences in bracket retention' rates were found between the two systems. Significantly, 26 teeth in

the control group demonstrated decalcification (12.6%), whereas none of the teeth in the experimental group did. The results of this study suggested that a visible light-activated, fluoride-releasing bonding system is capable of adequately retaining brackets while aiding in 'the prevention of decalcification around bonded appliances.

Artun J, Odont., and Thylstrup A, Odont. (1989)[49]: Studied the surface features of incipient carious lesions around bonded orthodontic brackets were assessed during a 3-year period after appliance removal. At standardized intervals color slides and silicone impressions for replication were made of two maxillary incisors on each of six adolescent patients. The labial surfaces of the teeth had demineralized white areas around the bonded brackets. The color slides were projected and studied in a darkroom. The positive surface replicas were studied by scanning electron microscopy (SEM). At the time of debonding, large accumulations of dental plaque were observed in those areas with white, demineralized surfaces. During the posttreatment or experimental period, there was a reduction in the amount of plaque. The appearance of the lesions changed from chalky-white at time of debonding to a more diffuse opacity, particularly in the peripheral parts. Under SEM the surfaces of the lesions were less irregular 3 years after debonding. At higher magnification the labial surfaces showed signs of wear. The present study confirms that removal of cariogenic challenge results in arrest of further demineralization. The gradual regression of the lesion at the clinical level is believed to be primarily a result of surface abrasion.

Underwood ML, Rawls HR, and Zimmerman BF (1989)[50]: Examine the clinical durability and caries inhibition potential of a fluoride-exchanging resin (FER) when used as an orthodontic bracket-bonding adhesive. In the clinical durability investigation, orthodontic brackets were bonded to alternate teeth with the FER in 10 patients scheduled for routine orthodontic procedures. The remaining teeth were bonded with Concise orthodontic resin. Number of bonding failures and the site and mode (adhesive or cohesive) of failure were recorded. Also included in the study were 10 patients scheduled for orthodontic care with prescribed extraction of four first premolars. Bracketed teeth were extracted after 60 days and were sectioned and examined with polarized light microscopy using H,O and quinoline as imbibition

media. Failure rates for the FER and Concise were 10.8% and 7.3%, respectively. Occurrence of adhesive rather than cohesive, failure indicates that structural integrity was maintained for both adhesives. Microscopic examination of specimens with H,O showed lesion formation to be 2.78% for the FER and 1.73% for Concise. These lesions were large and not seen in positions near the brackets indicating presence before bonding. With quinoline, dark zone formation was 2.3% for the FER and 33.5% for Concise, indicating a 93% reduction in the first stages of enamel alteration. Results demonstrate that the fluoride-exchanging resin holds promise as a practical cartes-preventive adhesive.

Ten Cate JM, Featherstone JDB (1991)[51]: Critically reviewed the current information about tooth-fluoride interactions, both from laboratory and clinical studies. For many years after the discovery of its caries preventive effect, fluoride was thought to be primarily active by lowering the solubility of the apatitic mineral phase of the dental hard tissues. Recent findings have shed new light on the mechanisms by which fluoride inhibits or delays dental caries. Fluoride present in the oral fluids alters the rate of the naturally occurring dissolution and reprecipitation processes at the tooth oral fluid interface. Demineralization of enamel is inhibited by concentrations of fluoride in the sub-ppm range. Likewise, remineralization of incipient caries lesions (the earliest stage of enamel caries) is accelerated by trace amounts of fluoride. As these two processes comprise dental caries the physiological balance between hard tissue breakdown and repair is favourably shifted by fluoride. The driving force for both phenomena is thermodynamic, that is, fluorapatite or a fluoridated hydroxyapatite may form when fluoride is supplied at low concentrations.

Rosenbloom RG, and Tinanoff N (1991)[52]: The purpose of this study was to evaluate salivary *Streptococcus mutans* levels in patients before, during, and after orthodontic treatment. *S. mutans* levels were significantly elevated during active treatment. However, when sampled 6 to 15 weeks into the retention phase of treatment, the microbial levels decreased significantly to levels comparable to age-matched untreated controls. In addition, patients who were no longer wearing any retention appliances had *S. mutans* levels similar to those subjects sampled in the retention phase of treatment as well as to subjects in age-matched control groups. The

findings of the study suggest that orthodontic treatment does not result in any long-term elevations of *S. mutans* levels.

Boyd RL (1992)[53]: Compare the effectiveness of a 1100 ppm fluoride toothpaste used alone, or together with a 0.05% NaF rinse used once daily or a 0.4% SnF, gel applied twice daily, in controlling the decalcification that often accompanies orthodontic treatment. Ninety-five consecutively treated adolescent patients were matched for age and sex and assigned to one of these three regimens. Single blind assessments of decalcification were performed on all labial surfaces of all erupted teeth before appliances were placed and 3 months after appliances were removed. Because the first molars had the highest decalcification scores, data for the whole mouth and for first molars were analysed separately. When pre-treatment levels of decalcification were subtracted from post-treatment values, significantly lower decalcification scores ($p < 0.05$) were found for both whole mouth and first molars in the rinse and gel groups as compared with the control group (toothpaste alone). Although the gel group consistently had less decalcification than the rinse group, this difference only approached statistical significance. These results indicate that twice daily use of a 1100 ppm fluoride toothpaste and either a once-daily 0.05% NaF rinse or a twice-daily 0.4% SnF, gel provides additional protection against decalcification beyond that achieved with toothpaste alone.

Forsberg M, Oliveby A, and Lagerlof F (1992)[54]: Conducted a study for the purpose of establishing the possible influence of orthodontic therapy with fixed appliances on salivary clearance of sugar. Fifteen consecutive patients between the ages 12 and 17 years took part in the investigation. Unstimulated salivary flow rate, residual volume of saliva in the mouth after swallowing (RESID), and salivary clearance of sugar was determined on two occasions, before treatment commenced and after a minimum of 3 weeks of appliance wear. Analysis of the data showed that both RESID and salivary flow rate exhibited significantly increased levels during orthodontic therapy. The insertion of fixed appliances did not seem to have any effect on the rate of salivary clearance of sugar. It was assumed that this finding could be a consequence of the combined effects of the changes in salivary flow rate and RESID**.**

Geiger AM, Gorelick L, Gwinnett AJ and Benson BJ (1992)[55]: Conducted a clinical study to determine if rinsing frequency with a neutral 0.05% sodium fluoride rinse influenced white spot lesion formation associated with orthodontic brackets. Patients from two private orthodontic practices participated. Each received home-care instructions and were told to use 10 ml of sodium fluoride rinse daily before bedtime. The rinse was supplied free of charge to determine if this affected compliance with its prescribed use. Compliance was measured by recording the number of bottles used by each patient. As assessment of oral hygiene status was conducted, and at the time of debonding, white spot lesions were recorded. A significant dose response relationship was noted in which those who rinsed at least once every other day had fewer lesions (21%) than those who rinsed less frequently (49%). It was concluded that a significant reduction in enamel white spot lesions can be achieved during orthodontic therapy through the use of a 10 ml neutral sodium fluoride rinse. The more closely patients complied with the prescribed use, the more likely they could expect a decrease in the occurrence of lesions.

Reynold EC, Cain CJ, Webber FL, Black CL, Riley PF, Johnson IH, and Perich JW (1995)[56]: Determined the ability of CPP-CP to reduce caries activity by use of specific-pathogen-free rates inoculated with Streptococcus sobrinus. Solutions of the CPP-CP were applied to the molar teeth twice daily and other group animals received solution containing Fluoride. The anticariogenic effects of CPP-CP significantly reduce caries activity, being similar to that of fluoride. The anticariogenic effects of CPP-CP and Fluoride were additive, since animals receiving 0.5% CPP-CP plus 500ppm F had significantly lower caries activity than those animals receiving either CPP-CP or fluoride alone.

Schiipbachl P, Neeser JR, Golliard M, Rouvet M, and Guggenheiml B (1996) [57]: Evaluated the protective effects of milk and milk products against dental caries. This effect was mediated by micellar casein or caseinopeptide derivatives. A reduction in the Streptococcus sobrinus population in the oral microbiota of animals fed diets supplemented with these milk components was consistently observed. A possible

explanation for these findings was that milk components are incorporated into the salivary pellicle, thereby reducing the adherence of S. sobrinus.

Reynolds EC (1997)[58]: Determined CPP-stabilized calcium phosphate solutions remineralize subsurface lesions in human third-molar enamel. After a ten-day remineralization period, enamel lesions were sectioned, subjected to microradiography, and the mineral content determined by microdensitometry. The remineralizing capacity was greater for the solutions with the higher levels of CPP-stabilized free calcium and phosphate ions. The CPP, by stabilizing calcium phosphate in solution, maintain high-concentration gradients of calcium and phosphate ions and ion pairs into the subsurface lesion and thus effect high rates of enamel remineralization.

Todd MA, Staley RN, Kanellis MJ, Donly KJ, and Wefel JS (1999)[6]: Evaluated the ability of a fluoride varnish, Duraflor, to directly inhibit demineralization of enamel surrounding orthodontic brackets. Brackets were bonded to 36 extracted human canines and premolars with a traditional composite resin and randomly assigned to three equal groups of twelve. Group 1 served as the control with no topical application after bonding. Group 2 was treated with a single application of a nonfluoridated placebo varnish. Group 3 was treated with a single application of Duraflor. All groups were cycled in an artificial caries challenge for 1 hour two times daily for 37 days and were brushed with a medium bristled toothbrush to simulate mechanical wear of the varnish. Demineralization of enamel was evaluated in longitudinal buccolingual tooth sections using polarized light microscopy. Both average depth and area of demineralization were measured with a sonic digitizer. ANOVA ($P \leq .0001$) and Duncan's test ($P \leq .05$) indicated significant differences in depth and area of demineralized enamel. Those teeth treated with Duraflor exhibited 50% less demineralization than the control teeth and an even greater difference when compared to the placebo group. Fluoride varnishes should be considered for use as a preventive adjunct to reduce enamel demineralization adjacent to orthodontic brackets, particularly in patients who exhibit poor compliance with oral hygiene and home fluoride use.

Al-Khateeb S, Exterkate R, Angmar-MaÊnsson B, ten Cate B (2000)[59]: Investigated whether full remineralization would occur in white spot lesions when the surface porosity was increased by acid-etching. The effect of fluoride was also investigated. Enamel blocks with in vitro produced white spot lesions were used. Group A was exposed to a remineralizing solution only. In group B, the lesions were etched with 35% phosphoric acid for 30 s, then treated as in group A. Group C was treated as group A + daily treatment with a fluoride toothpaste slurry (1000 ppm) for 5 min. Group D was treated as group B + the daily fluoride treatment of group C. The remineralization was measured weekly with Quantitative Light-induced Fluorescence during the experimental period. After 10 weeks of remineralization, mineral profiles were assessed with transverse microradiography. The enamel fluorescence was partly regained. There were significant differences in the lesion depth, mineral content at the surface layer, and integrated mineral loss between the groups. Addition of fluoride accelerated the remineralization only in the beginning; in later stages the process leveled out and even reached a plateau in all the groups. It was concluded that full remineralization was not achieved by etching, by the addition of fluoride, nor by the combination of both treatments in this in vitro study.

Shen P , Cai F, Nowicki A, Vincent J, and Reynolds EC (2001)[60]: Determined the ability of CPP-ACP in sugarfree chewing gum to remineralize enamel subsurface lesions in a human in situ model. Thirty subjects in randomized, cross-over, double-blind studies wore removable palatal appliances with six human-enamel half-slabs inset containing subsurface demineralized lesions. The appliances were inserted immediately before gum-chewing for 20 min and then retained for another 20 min. This was performed four times per day for 14 days. At the completion of each treatment, the enamel half-slabs were paired with their respective demineralized control half-slabs, embedded, sectioned, and subjected to microradiography and densitometric image analysis, for measurement of the level of remineralization. The addition of CPPACP to either sorbitol- or xylitol-based gum resulted in a dose-related increase in enamel remineralization, with 0.19, 10.0, 18.8, and 56.4 mg of CPP-ACP producing an increase in enamel remineralization of 9, 63, 102, and 152%, respectively, relative to the control gum, independent of gum weight or type.

Reynolds EC, Cai F, Shen P, Walker GD (2003)[25]: Compare the ability of CPP-ACP, with that of other forms of calcium, to be retained in supragingival plaque and remineralize enamel subsurface lesions *in situ* when delivered in a mouthrinse or sugar-free gum in randomized, double-blind trials. In the mouthrinse study, only the CPP-ACP-containing mouthrinse significantly increased plaque calcium and inorganic phosphate levels, and the CPP were immunolocalized to the surfaces of bacterial cells as well as the intercellular matrix. In the chewing gum studies, the gum containing the CPP-ACP, although not containing the most calcium *per* piece of gum, produced the highest level of enamel remineralization independent of gum-chewing frequency and duration. The results showed that CPP-ACP were superior to other forms of calcium in remineralizing enamel subsurface lesions.

Cai F, Shen P, Morgan MV, Reynolds EC (2003)[61]: The study aimed to determined the effect of CPP-ACP incorporated into a sugar-free lozenge on enamel remineralization. This study was designed with 4 treatments: (i) a lozenge containing 56.4mg CPP-ACP; (ii) a lozenge containing 18.8 mg CPP-ACP; (iii) a lozenge not containing CPP-ACP; (iv) a no lozenge nil-treatment control. After microradiography & computer-assisted densitometric image analysis they found that incorporation of CPP-ACP into the lozenge significantly increased enamel surface lesion remineralization, relative to the control sugar-free lozenge.

Benson PE , Shah AA and Campbell IF (2003)[62]: The aim of the study was to investigated the effect of fluoridated elastomers on the quantity of disclosed dental plaque surrounding an orthodontic bracket in vivo. The subjects were 30 individuals about to start fixed orthodontic treatment. The study consisted of two experimental periods of 6 weeks with a washout period between. Fluoridated elastomers were randomly assigned at the first visit to be placed around brackets on 12, 11, 33 or 22, 21, 43. Non-fluoridated elastomers were placed on the contra-lateral teeth. After 6 weeks (visit 2) the elastomers were removed, the teeth disclosed and a photograph taken. Non-fluoridated elastomers were placed on all brackets for one visit to allow for a washout period. At visit 3, fluoridated elastomers were placed on the contra-lateral teeth to visit 1. At visit 4, the procedures at visit 2 were repeated. The

photographs were scanned, then the area and proportion of the buccal surface covered with disclosed plaque was measured using computerized image analysis. A mixed-effects ANOVA was carried out with the dependent variable being the area or percentage area of disclosed plaque. There was no evidence of a systematic error and substantial agreement for the repeat readings of the same images. The only significant independent variable for the area of disclosed plaque was the subject ($p<0.001$). The significant independent variables for the proportion of disclosed plaque were the subject ($p<0.001$) and the tooth type ($p=0.002$). The independent variable describing the use of fluoridated or non-fluoridated elastomers was not significant for either the area or the proportion of disclosed plaque.

Sengun A, Sari Z, Ramoglu SI, Malkoc S, Duran I (2004)[63]: Evaluated the influence of a xylitol lozenge on the dental plaque pH profile of fixed orthodontic patients. Twelve volunteers participated in this study. Before the measurement of plaque pH, subjects were asked to refrain from brushing their teeth for 48 hours and from eating and drinking for two hours. The subjects' baseline dental plaque pH was recorded using the touch technique. It was followed by a one-minute rinse with 15 ml of a 10% solution of sucrose, and subsequent plaque pH measurements were carried out during the next one hour. Xylitol lozenges were taken five times a day during a 14-day period. The variables of resting-plaque pH, minimum-plaque pH (MP pH), time required to reach MP pH (TMP), last-plaque (LP) pH at the end of one hour, pH area (CH), and pH at each test time were calculated for each pH test of the subjects. The paired sample *t*-test was used for statistical comparison. The mean MP pH values increased from 4.81 to 5.09 in the experimental measurement (*P* , .05). The mean TMP was not affected by the use of xylitol (*P* . .05). Although the LP pH showed an increase during the experimental period, the difference between control and experimental periods was not statistically significant (*P* . .05). The CH of the experimental period was significantly less than that of the control period (*P* , .05). As a result, the use of a xylitol lozenge after a sucrose challenge can be an advisable practice for fixed orthodontic patients to prevent future dental caries.

Soliman MM, Bishara SE, Wefel J, Heilman J, Warren JJ (2005)[64]:

Measured the rate and amount of fluoride ions released from the sealant over a period of 17 weeks and to determine whether the fluoride-releasing sealant has a recharging ability when fluoride ions are reintroduced into the environment. Disc-shaped specimens were prepared from two types of sealants: (1) 10 discs were made using a fluoride-releasing sealant and (2) 10 discs were made of a nonfluoride adhesive primer (control). An ion analyzer was used to measure the fluoride release using a fluoride ion–specific combination electrode. The results of the repeated measure analysis (F 5 7.76) indicated that the fluoride-containing sealant released fluoride ions into the solution in sustained but significantly (P 5 .014) decreasing rates from a high of 0.074 6 0.04 ppm/week/mm2 in the first week to a low of 0.015 6 0.017 ppm/week/mm2 at the end of the 17th week. Furthermore, the Pro Seal discs had the ability to be recharged with fluoride ions introduced from a foaming solution of acidulated phosphate fluoride. The mean fluoride release rate one week after recharging was 0.354 6 0.095 ppm/week/mm2 and decreased to 0.014 6 0.009 ppm/week/mm2 after eight weeks. The control sealant showed no significant fluoride release and was unable to absorb the fluoride ions available in the solution. The fluoride containing sealant Pro Seal released fluoride ions in sustained but significantly decreasing amounts. The Pro Seal discs had the ability to be recharged with fluoride ions.

Tu¨rkkahraman H, Sayın MO, Bozkurt FY, Yetkin Z, Kaya S, Onal S (2005)[65]: Determined the changes in microbial flora and periodontal status after orthodontic bonding and to determine whether two different archwire ligation techniques affect these changes. A total of 21 orthodontic patients scheduled for fixed orthodontic treatment were selected for this split-mouth study. Two commonly used auxiliaries (elastomeric rings and ligature wires) for tying archwires were tested. Microbial and periodontal records were obtained before bonding (T0), one week later (T1), and five weeks after bonding (T2). Paired *t*-test and Wilcoxon signed rank test were used to compare the groups statistically. Although, teeth ligated with elastomeric rings exhibited slightly greater numbers of microorganisms than teeth ligated with steel ligature wires, the differences were not statistically significant and could be ignored. The two archwire ligation techniques showed no significant differences in the gingival index, bonded bracket plaque index, or pocket depths of the bonded teeth.

However, teeth ligated with elastomeric rings were more prone to bleeding. Therefore, elastomeric ring use is not recommended in patients with poor oral hygiene.

Vivaldi-Rodrigues G, Demito CF, Bowman SJ, Ramos AL. (2006): Done prospective examination of 10 consecutively treated orthodontic patients was undertaken to examine the effectiveness of fluoride varnish in reducing enamel demineralization. Pairs of dental quadrants for each patient's mouth (ie, maxillary right and mandibular left; maxillary left and mandibular right) were randomly assigned to an experimental or control group. After placement of resin-bonded orthodontic brackets, fluoride varnish was applied to the 2 experimental dental quadrants for each patient. Subsequent applications were done every 3 months during 12 months of orthodontic treatment. A double-blinded examination of intraoral photographs of the 100 experimental and 100 control teeth was done. The presence of white spot lesions was registered using the enamel decalcification index and the 2 groups were compared using paired Student t tests with a significance level of 5% ($P < .05$). Most importantly, the change in mean enamel decalcification index was significantly smaller for the experimental group (0.34), compared to the control group (0.51).

Blicks CS, Renfors G, Oscarson ND, Bergstrand F, Twetman S (2007): Evaluated the efficacy of topical fluoride varnish applications on white spot lesion (WSL) formation in adolescents during treatment with fixed orthodontic appliances. The subjects were 273 consecutive 12- to 15-year-old children referred for maxillary treatment with fixed orthodontic appliances. The patients were randomly assigned to a test or a control group with topical applications of either a fluoride varnish (Fluor Protector) or a placebo varnish every 6th week during the treatment period. The outcome measures at debonding were incidence and progression of WSL on the upper incisors, cuspids and premolars as scored from digital photographs by 2 independent examiners. The results from the present study strongly suggest that regular topical fluoride varnish applications during treatment with fixed appliances may reduce the development of WSL adjacent to the bracket base.

Reynolds EC, Cai F, Cochrane NJ, Shen P, Walker GD, Morgan MV, and Reynolds C (2008)[67]: Determined the ability of CPP-ACP to increase the incorporation of fluoride into plaque and to promote enamel remineralization *in situ*. Randomized, doubleblind, cross-over studies involved mouthrinses and dentifrices containing CPP-ACP and fluoride. The mouthrinses were used for 60 sec, three times/day for 5 days, and supragingival plaque was collected and analyzed for F. The dentifrices were rinsed as a water slurry for 60 sec four times/day for 14 days in an *in situ* model. The addition of 2% CPPACP to the 450-ppm-F mouthrinse significantly increased the incorporation of fluoride into plaque. The dentifrice containing 2% CPP-ACP produced a level of remineralization similar to that achieved with a dentifrice containing 2800 ppm F. The dentifrice containing 2% CPP-ACP plus 1100 ppm F was superior to all other formulations.

Farhadian N, Miresmaeili A, Eslami B, and Mehrabi S (2008)[11]: Evaluated the effect of a fluoride varnish on enamel demineralization adjacent to bonded brackets. 15 patients who needed at least 2 premolars extracted for orthodontic reasons were selected. In each patient, 1 premolar was considered the test tooth, and the other was the control. Brackets were bonded, and T-loops were engaged on all premolars, but only the test teeth received fluoride varnish. The premolars were extracted after 85 to 95 days, and buccolingual sections 50 to 70 μm in thickness were evaluated with polarized light microscopy. The mean depth of demineralization in each lesion was measured 3 times on photographs by an operator blinded to the groups. There was significant reduction (approximately 40%) in depth of demineralization in the test group (P <.001).

Buren JL, Staley RN , Wefel J, and Qiand F (2008)[10]: Evaluated the effectiveness of a new enamel sealant, Pro Seal (Reliance Orthodontic Products, Itasca, Ill), on inhibiting enamel demineralization. Two materials that have demonstrated success in white spot prevention and do not require patient compliance were used for comparison. Thirty-two noncarious extracted molars were divided into 4 groups and received 1 of the following treatments: no treatment (control), fluoride varnish (Fluor Protector; Ivoclar Vivadent, Amherst, Mass), unfilled resin sealant (Delton; Dentsply Professional, York, Pa), and filled resinsealant (Pro Seal). The teeth were subjected to

15,000 simulated brush strokes followed by acidic challenge for 96 hours. They were examined macroscopically and sectioned for quantitative examination with polarized light microscopy. All surface treatments provided statistically significant (P <.05) reductions in lesion depth compared with controls. Pro Seal performed significantly better (P <.05) than the other products, decreasing lesion depth by 97% compared with the controls and completely inhibiting lesion formation in 3 specimens.

Tufekci E, Casagrande ZA, Lindauer SJ, Fowler CE, Williams KT (2008)[68]: Tested the null hypothesis that adding Listerine mouthrinse to the standard oral hygiene regimen has no added benefit for orthodontic patients in maintaining proper oral health. Patients within their first 6 months of orthodontic treatment were assigned either to the brushing _ flossing (N _ 25) or brushing _ flossing _ Listerine (N _ 25) group. Initially, all of the participants received a prophylaxis and instructions on how to brush and floss. Measurements were recorded for the bleeding, gingival, and plaque indices (BI, MGI, and PI, respectively) that provided baseline values (T1). Subsequent measurements were taken at 3 months (T2) and 6 months (T3). Mean BI, MGI, and PI at T1, T2, and T3 were compared statistically between the groups using repeated measures analysis of variance. The significance level was set at P _ .05. The response profiles for the BI, MGI, and PI over time were significantly different between the two groups. Patients who had Listerine in their daily oral hygiene regimen exhibited significantly lower scores for all three indices at T2 and T3 than the patients who only brushed and flossed.

Azarpazhooh A, Main PA (2008)[69] : Developed a scientifically current and evidence-based protocol for the use of fluoride varnish for the prevention of dental caries among high-risk children and adolescents. Previous systematic reviews on this topic were used as the basis for the current review. Ovid MEDLINE, CINAHL and several other relevant bibliographic databases were searched for English-language articles, with human subjects, published from 2000 to 2007. A total of 105 articles were identified by the literature search; relevance was determined by examining the title, abstract and body of the article. Seven original research studies met the inclusion criteria. These articles were read and scored independently by 2 reviewers, and evidence was extracted for systematic review.

Reynolds EC (2009)[70]: Determined the scientific evidence to support a role for new remineralization technology as an adjunct to fluoride treatment in the non-invasive management of early caries lesions. CPP-ACP has been shown to reduce caries development in the rat caries model. The technology has also been demonstrated to inhibit enamel and dentin demineralization and to promote remineralization in several independent *in vitro* and *in vivo* studies. Further, CPP-ACP has been shown to slow the progression of caries significantly and to promote the regression of early lesions in randomized, controlled clinical trials. Therefore, evidence exists to support the clinical use of the CPP-ACP technology as an adjunct to fluoride treatment in the non-invasive management of early caries lesions.

Bailey DL, Adams GG, Tsao CE, Hyslop A, Escobar K, Manton DJ, Reynolds EC, and Morgan MV (2009)[71]: Conducted a clinical trial to test whether, in a post-orthodontic population using fluoride toothpastes and receiving supervised fluoride mouthrinses, more lesions would regress in participants using a remineralizing cream containing casein phosphopeptide-amorphous calcium phosphate compared with a placebo. Forty-five participants (aged 12-18 yrs) with 408 white-spot lesions were recruited, with 23 participants randomized to the remineralizing cream and 22 to the placebo. Product was applied twice daily after fluoride toothpaste use for 12 weeks. Clinical assessments were performed according to ICDAS II criteria. Transitions between examinations were coded as progressing, regressing, or stable. Ninety-two percent of lesions were assessed as code 2 or 3. For these lesions, 31% more had regressed with the remineralizing cream than with the placebo (OR = 2.3, P = 0.04) at 12 weeks. Significantly more post-orthodontic whitespot lesions regressed with the remineralizing cream compared with a placebo over 12 weeks.

Benham AW, Campbell PM, and Peter H. Buschang PH (2009)[12]: Performed a pilot study to test the null hypothesis that highly filled (58%) resin sealants do not prevent white spot lesions in patients undergoing active orthodontic treatment. A split-mouth design was applied to 60 healthy patients, with the sealant randomly allocated to either the right or the left side of each jaw. The sealant was applied to the incisors and canines from the gingival surface of the bracket to the free gingival

margin. The contralateral teeth had the same type of bracket with no sealant. Sealants were placed on the experimental teeth 2 weeks to 3 months after initial bonding and were removed after 15 to 18 months. Intraoral photographs, visual assessments, and DIAGNOdent (KaVo Dental Corporation, Lake Zurich, Ill) measurements were used to assess white spot lesions after sealant removal. The teeth without sealants had 3.8 times the number of white spot lesions than were noted on the sealed teeth. These sealants showed no visible signs of discoloration. The DIAGNOdent measured statistically significant differences between sealed and unsealed teeth in the maxilla ($P < .001$) and in the mandible ($P = .010$). DIAGNOdent measurements also showed a difference between sealed and unsealed teeth after the 28 teeth with visible lesions were excluded.

Kronenberga O, Lussib A; Ruf S (2009)[14] : Test the null hypotheses: (1) there is no difference in the caries protective effect of ozone and Cervitec/Fluor Protector during multibracket (MB) appliance therapy, and (2) DIAGNOdent and quantitative light-induced fluorescence (QLF) are not superior to a visual evaluation of initial caries lesions. Twenty right-handed patients with a very poor oral hygiene who required full MB appliance therapy were analyzed during 26 months. In a split-mouth-design, the four quadrants of each patient were either treated with ozone, a combination of Cervitec and Fluor Protector, or served as untreated controls. The visible plaque index (VPI) and white spot formation were analyzed clinically. DIAGNOdent and QLF were used for a quantitative assessment of white spot formation. The average VPI in all four dental arch quadrants amounted to 55.6% and was independent of the preventive measure undertaken. In the quadrants treated with Cervitec/Fluor Protector,only 0.7% of the areas developed new, clinically visible white spots. This was significantly (P_ .05) less than in the quadrants treated with ozone (3.2%). The lesions detected with QLF only partially corresponded to the clinically detected white spots, while DIAGNOdent proved to be unable to detect any changes at all.

Chapman JA, Roberts WE, Eckert GJ, Kula KS, and Cabezas CG (2010)[72]: Conducted a retrospectively to determine the incidence and severity of WSLs in orthodontics by examining pretreatment and posttreatment digital photographs.. A total of 332 consecutive finished patients from a university graduate orthodontic clinic

were evaluated. Initial and final digital images were compared to assess WSLs. The facial surfaces of the anterior 8 maxillary teeth were analyzed. The incidence of at least 1 WSL on the labial surface of the anterior 8 maxillary teeth was 36%. The order of incidence was lateral incisor (34%), canine (31%), premolar (28%), and central incisor (17%).

Robertson MA, Kau CH, English JD, Lee RP, Powers J and Nguyen T (2011)[23]: Determined the effectiveness of MI Paste Plus, in the prevention or reduction of white spot lesions in orthodontic patients. Total 50 patients (26 using MI Paste Plus & 24 patient using placebo paste) completed the study. The enamel decalcification index scores in the MI paste Plus group reduced by 53.5%, whereas the placebo group increases by 91.1% during the study period.

Tufekci E, Dixon JS, Gunsolley JC, Lindauer SJ (2011)[73]: Determined the prevalence of white spot lesions (WSLs) in orthodontic patients at 6 and 12 months into treatment using the visual examination method. Patients 6 and 12 months into treatment were examined for the presence of WSLs. The control group consisted of patients who were examined for WSLs immediately after bonding. Upon clinical evaluation, teeth were given a visual score based on the extent of demineralization. The percentages of individuals having at least one WSL were 38%, 46%, and 11% for the 6-month, 12-month, and control groups, respectively. This clinical study showed a sharp increase in the number of WSLs during the first 6 months of treatment that continued to rise at a slower rate to 12 months.

Brown ML; Davis HB; Tufekci E; Crowe JJ; Covell DA; Mitchell JC (2011)[74]: Measured ion release from four sol-gel bioactive glass–containing orthodontic resin bonding agents (BAG-Bonds) following immersion into simulated body fluid (SBF) at pH values of 4 and 7. Four BAG-Bonds, two containing fluoride, were developed. Commercially available Transbond-XT was used as the control. Three disks (10 mm 3 2 mm) of each material were individually suspended in 3.5 mL of SBF at pH 4 and pH 7. SBF was analyzed to measure pH and ions released at 1 hour, 10 hours, and 100 hours. Calcium was measured by atomic absorption analysis, phosphate by ultraviolet

visible spectrometry, and fluoride by an ion-specific electrode. Significant differences in calcium and phosphate ion release were found between the four BAG-Bonds and the control at multiple time points. Significant changes in pH were also found. There was no measurable release of fluoride from any of the materials.

Bergstrand F and Twetman S (2011)[75]: Conducted a study to update the evidence for primary and secondary prevention (treatment) of white spot lesions (WSL) adjacent to fixed orthodontic appliances. A search for relevant human clinical trials published in English between 2004 and March 2011 retrieved 25 publications that fulfilled the inclusion criteria. The findings consolidated the use of topical fluorides in addition to fluoride toothpaste as the best evidence-based way to avoid WSL. For the treatment of post-orthodontic WSL, home-care applications of a remineralizing cream, based on casein phosphopeptide-stabilized amorphous calcium phosphate, as adjunct to fluoride toothpaste could be beneficial but the findings were equivocal.

Knösel M, Forslund L, Jung K, Ziebolz D (2012)[14]: Assessed the efficacy in enamel demineralization prevention of two fluoride-containing enamel varnishes compared to a non-fluoride varnish, weekly fluoride gel application, and a non-treated control group. Enamel specimens obtained from 75 human upper permanent incisors were randomly allocated to five trial groups (each n = 15): A), ProSeal (Reliance), B), Maximum Cure® (Reliance), C), CervitecPlus (Ivoclar Vivadent, Schaan, Liech-tenstein), D) elmex® gelée (GABA, Lörrach, Germany), and E), a non-treated control group. Groups A–C received a baseline varnish application, whereas group D specimens received a once weekly gel application for 2 min. Six demineralization cycles per day were carried out for 5 min each using 0.05 M citric acid, with the specimens stored in remineralization solution between cycles. Lesion depth expressed in percentage fluorescence loss (Δ-F in %) compared to baseline (T0) was assessed quantitatively with light-induced fluorescence (QLF) after 3 (T1), 7 (T2), 14 (T3), and 30 (T4) days globally and for each time point, and analyzed for compounds. Significant fluorescence loss revealing greater lesion depth was detected in the untreated controls (E) at T3, and in groups A (ProSeal) and C (CervitecPlus) at T4. No significant Δ-F changes were seen in the specimens from groups B (Maximum Cure®) and D (elmex® gelée).

Akina M and Basciftci FA (2012)[76]: Compared the effects of sodium fluoride mouth rinse, casein phosphopeptideamorphous calcium phosphate (CPP-ACP), and the microabrasion technique in treating white spot lesions. The study population consisted of 80 patients (46 females, 34 males; 966 affected teeth) who had developed multiple decalcified enamel lesions after fixed orthodontic therapy. The study population was divided into four groups of 20 patients each. The control group (group I) participants were to just brush their teeth, the fluoride group (group II) participants were instructed to use 20 ml of neutral 0.025% sodium fluoride rinse, the participants in the CPP-ACP group (group III) were instructed to use tooth mousse twice a day in addition to fluoride toothpaste for 6 months, and the participants in the microabrasion group (group IV) were to undergo treatment by the microabrasion technique, which is a commonly used mixture of 18% hydrochloric acid. The area of the white spot lesions decreased significantly in all groups. Inter group differences in the treatment success rates were significant. The highest success rate was observed for group IV (97%). The success rate of group III (58%) was significantly higher than that of groups II (48%) and I (45%).

Gurunathan D, Somasundaram S, Kumar SA (2012)[26]: Revealed CPP-ACP as a safe and novel carrier for calcium, phosphate and fluoride ions to promote enamel remineralization. The calcium phosphate- based remineralization technologies show promise as adjunctive treatment to fluoride therapy in the management of early caries lesions. Biomimetic approaches to stabilization of bioavailable calcium, phosphate and fluoride ions, and the localization of these ions to non-cavitated caries lesions for controlled remineralization show promise for the non-invasive management of dental caries. Hence, CPP-ACP has application in oral care products, dental professional products and foodstuffs.

Srivastava K, Tikku T, Khanna R, and Sachan K (2013)[5]: Reviewed the risk factors, preventive methods and fate of the orthodontics scars. They concluded that importance of excellent oral hygiene practice during fixed orthodontic treatment be explained. Preventive programs must be emphasized to all orthodontic patients.

Suggestions are offered in the literature for ways to prevent this condition from manifesting itself.

Hang GJ, Chiang BR, Mills BE, Salma S, Spiekerman C, Korpak AM, Starrett JL, Greenlee GM, Drangsholt RJ, and Matunas JC (2013): Assessed the effectiveness of 2 agents commonly used to ameliorate white spot lesions compared with a normal homecare regimen. The subjects were randomized to 1of 3 arms: (i) an 8 week regimen of MI Paste Plus, (ii) a single application of PreviDent fluoride varnish, and (iii) usual homecare (control). Photographs were taken at enrolment & 8 weeks later. Total 115 subjects completed the study and the mean improvement assessed by the professional panel was 21%, 29%, and 27% respectively.

Juliena KC, Buschangb PH, Campbellc PM (2013)[1]: Quantified the prevalence of white spot lesions (WSLs) on the anterior teeth and, secondarily, to evaluate risk factors and predictors. Digital photographs and records of 885 randomly chosen patients were evaluated before and after treatment. Chart information included gender, age, as well as banding and debanding dates. Fluorosis and oral hygiene before and after treatment were also evaluated. Pre-existing and posttreatment WSLs were recorded and compared for all 12 anterior teeth. Risk ratios (RR) and absolute risk (AR) were calculated to determine the likelihood and risk of WSL formation. Overall, 23.4% of the patients developed at least one WSL during their course of treatment. Maxillary anterior teeth were affected more than mandibular teeth. The maxillary laterals and canines and the mandibular canines were the most susceptible. There was no significant difference in WSLs between genders.

Manfred L; Covell DA; Crowe JJ; Tufekci E; Mitchell JC (2013)[77]: Compared changes in enamel microhardness adjacent to orthodontic brackets after using bonding agents containing various compositions of bioactive glass compared to a traditional resin adhesive following a simulated caries challenge. Extracted human third molars (n 5 10 per group) had orthodontic brackets bonded using one of four novel bioactive glass (BAG)-containing orthodontic bonding agents (BAG-Bonds) or commercially

available Transbond-XT. The four new adhesives contained BAG in varying percentages incorporated into a traditional resin monomer mixture. Teeth were cycled through low-pH demineralizing and physiologic-pH remineralizing solutions once each day over 14 days. Microhardness was measured on longitudinal sections of the teeth 100, 200, and 300 mm from the bracket edge and beneath the brackets, at depths of 25 to 200 mm from the enamel surface. Normalized hardness values were compared using three-way analysis of variance. Significantly less reduction in enamel microhardness was found with the experimental adhesives at depths of 25 and 50 mm at all distances from the bracket edge. In all groups, there were no significant changes in enamel microhardness past 125-mm depth. Results varied with the different BAG-Bonds, with 81BAG-Bond showing the smallest decrease in enamel microhardness.

Cochrane NJ, Shen P, Yuan Y, Reynolds EC (2014)[79]: Analysed the fluoride, calcium & organic phosphate ions release from: (i) MI Varnish containing (CPP-ACP); (ii) Clinpro White containing (fTCP); (iii) Enamel Pro containing amorphous calcium phosphate; (iv) Bifluoride 5 containing calcium fluoride; and Duraphat. All varnishes released measurable fluoride & calcium, however only MI Varnish and Enamel Pro released significant levels of inorganic phosphate.

Farooq I, Moheet IA, Imran Z, Farooq U (2013)[78]: Reviewed literature and found that CPP–ACP products have provided a new direction to preventive dentistry. The role of CPP–ACP in the prevention of dental caries is quite evident and therefore its incorporation in various dental materials should be encouraged.

Pithon MM, Santos MJd, Andrade CSS, Filho JCBL, Braz AKS, Araujo REd, et al (2014)[18]: Evaluated the efficiency of varnish containing CPP and ACP in prevention of caries lesions around orthodontic brackets. For this purpose 8 groups were formed: (i) brushing, (ii) brushing + mouthwash, (iii) Duraphat varnish, (iv) Duraphat + brushing + mouthwash, (vi) MI Varnish, (vii) MI Varnish + brushing, (viii) MI Varnish + brushing + mouthwash. After evaluation by OCT, MI Varnish showed more effectiveness in diminishing caries lesion depth, compared with Duraphat.

Brown MD, Campbell PM, Schneiderman ED, Buschang PH (2015)[81]: Evaluated white spot lesions among treated orthodontic patients using alumni- centered, practice-based research network. 20 randomly selected alumni participated, providing 158 treated cases. Approximately 28% of the patients developed WSLs. The average patient developed 2.4 white spots, affecting 12.7% of the teeth examined.

Dehailan LA, Martinez-Mier EA & Lippert F (2015)[88]: Investigated the effect of five commercially available fluoride varnishes (FV) on caries lesions. Ninety bovine enamel specimens were assigned to five varnish groups (n=18). Early caries lesions were created in the specimens and characterized using Vickers surface microhardness number (VHN). FV was applied to each group of specimens. Immediately afterwards, 7.5 ml of artificial saliva (AS) were pipetted over each group of specimens, collected and renewed every 15 min for 6 h. AS samples were analyzed for fluoride using an ion-specific electrode. Enamel fluoride uptake (EFU) was determined using the acid etch technique. Each group was then subjected to a pH cycling regimen for 5 days after which VHN was determined again. One-way analysis of variance (ANOVA) was used for data analysis. FVs differed in their rehardening capability (highest mean value was for Enamel Pro=32.3±5.8 and lowest mean value was for Vanish=18.9±11.3). No significant difference in EFU was found among groups. Total fluoride release over 6 h was in the order of MI Varnish (303 μg/ml)>Enamel Pro (217 μg/ml)>Flor-Opal (153 μg/ml)>PreviDent(84 μg/ml)> Vanish(28 μg/ml).

Rajan R, Krishnan R, Bhaskaran B, Kumar SV (2015)[24]: Compared the remineralizing potential of four commercially available products namely SHY-NM, GC Tooth Mousse Plus, ReminPro and Colgate strong teeth on demineralized human teeth. The study included 50 extracted premolars having 3 × 3 mm window prepared on the middle third of the tooth, which was then subjected to demineralization for 48 hours at 37ºC. Teeth were randomly selected and grouped into five study groups of 10 teeth in each. Each group was treated with respective remineralizing agent and sectioned using hard-tissue microtome. Each section obtained was visualized under polarized light microscope and analyzed using Image J software. The statistically

evaluated results revealed that SHY-NM has the most remineralizing potential followed by ReminPro, GC Tooth Mousse Plus and fluoridated toothpaste.

Perrini F, Lombardo L, Arreghini A, Medori S, and Siciliani G (2016)[82]: Evaluated the efficacy of a fluoridated varnish in preventing white spot lesions in patients with fixed appliances. A laser-induced fluorescence device was used to determine the degree of demineralization on varnish application. A split mouth study design was used for 24 orthodontic patients. Analysis showed periodic application of fluoride varnish can offer some protection against white spots.

Bilgin G, Yanikoglu F, Tagtekin D, Stookey GK, Schemeron BR, Hayran O (2016)[83]: Evaluated the effectiveness of casein and hydroxyapatite on remineralization of white spot enamel lesion and assessment by a new caries detection device, FluoreCam System. Demineralized human enamel specimens were measured for baseline surface. Ten specimens in each of four groups were used in this in vitro recycling study with the following treatments applied three times daily for 1 min: 1) Sodium fluoride (NaF) dentifrice, Ipana, 2) Casein phosphor peptide-amorphous calcium phosphate (CPP-ACP) agent, Tooth Mousse, 3) hydroxyapatite and fluoride agent, Remin Pro, 4) Fluoride varnish, Pro fluorid. The recycling demininerali zation-remineralization treatment regimens were continued for 21 days. Significant differences between treatments were observed by micro hardness; compared to the positive control group (NaF dentifrice) enhanced and significantly greater remineralization was observed with the Remin Pro treatment . However no significant differences between groups were observed using the fluorescence assessments.

Tuloglu N, Bayrak S, Tunc ES and Ozer F (2016)[29]: Conducted a study to investigate the effects of a fluoride varnish with added Casein Phosphopeptide-Amorphous Calcium Phosphate (CPP-ACP) treatments on acid resistance of primary teeth enamel. Enamel specimens obtained from 40 primary incisors (for surface microhardness testing) and 40 primary molars (for demineralization depth measurement) were randomly divided into four groups (n = 10 incisors and 10 molars) each according to surface treatment: no treatment (control), MI varnish (1–8

% sodium fluoride and 1–5 % CPP-ACP), Clinpro White (1–5 % sodium fluoride and <5 % modified tricalcium phosphate), Duraphat (<5 % sodium fluoride). Specimens were stored for 24 h in a moist environment. After varnish residues were removed, specimens were subjected to pH cycling. The effects of fluoride varnishes were evaluated according to surface microhardness, lesion depth and structural changes. The lowest changes in surface microhardness and lesion depth occurred in MI varnish group, followed by the Clinpro White, Duraphat and no treatment (control) group. Statistically significant differences in both surface microhardness and lesion depth were observed among all groups ($P < 0.05$). Fluoride varnish containing CPP-ACP was more effective in increasing the acid resistance of primary enamel than other fluoride varnishes.

Shah M, Paramshivam G, Mehta A, Singh S, Chugh A, Prashar A, and Chugh VK (2017)[84]: Evaluated the effects of single application of a Conventional versus Light curable fluoride varnish (LCFV) on prevention of enamel demineralization during fixed orthodontic treatment over 4 month period. Total 22 patients with 88 teeth were enrolled in the trial. The result of this study indicated that single application of LCFV can prevent enamel demineralization for longer duration of time as compared to conventional fluoride varnish.

Rahimi F, Sadeghi M, Mozaffari HR (2017)[85]: Evaluated the efficacy of fluoride varnish compared with other agents for preventing WSLs during orthodontic treatment. Studies were searched from four databases- PubMed, Scopus, Web of Science and Cochrane Library- from January 1980 to May 2017; only studies with English abstracts were included. Out of 432 studies searched from the databases, 33 studies were evaluated for eligibility. Of the 33 studies, 19 were excluded with reasons and 14 studies were included in the systematic review. Parameters of WSLs (decalcification score, prevalence, incidence, progression score, ΔQ and ΔZ and *DiagnoDent* (DD) pen score) were compared for the various treatments. Although there were some limitations for this systematic review study, the review showed that fluoride varnish combined with *chlorhexidine* (CHX) may be a good treatment for WSLs after orthodontic treatment, especially for a 6-month period, and that resin infiltration might also be effective for preventing WSLs. More studies are needed to further investigate these observations.

Shahmoradi M, Hunter N, and Swain M (2017)[33]: Investigated the efficacy of various fluoride varnishes in the protection of the structural and nanomechanical properties of dental enamel. Demineralized enamel specimens were imaged using a high-resolution micro- CT system and lesion parameters including mineral density and lesion depth were extracted from mineral density profiles. Nanoindentation elastic modulus and hardness were calculated as a function of penetration depth from the load-displacement curves. The average depth of the lesion in specimens with no prior fluoride varnish treatment was 86±7.19 μmwhereas the varnish treated specimens had an average depth of 67 ± 7.03 μm ($P < 0.05$). The mineral density of enamel lesions with no fluoride varnish treatment had an average of 1.85 gr/cm3 which was 25% lower than the corresponding value in varnish treated enamel and 37% lower than sound enamel. While, in the varnish treated group, elastic modulus and hardness values had decreased by 18% and 23%, respectively, the corresponding values in the non-varnish treated specimens had a reduction of 43% and 54% compared to the sound enamel. The findings from this study highlight the preventive role of fluoride varnishes. Addition of calcium and phosphate does not seem to enhance or inhibit the prevention or remineralization performance of fluoride varnishes.

Sharma H, Gupta C, Thakur S, Srivastava S (2017)[86]: Evaluated the efficacy of MI varnish and Clinpro XT varnish in reducing dentinal hypersensitivity. Patients with cervical dentinal hypersensitivity were selected for the study. The teeth to be tested were isolated. Then, a blast of air and ice cold water was applied on the tooth surface, and the score was measured by visual analog scale. The patients were randomly assigned to one of the treatment groups (Group 1: MI varnish; Group 2; Clinpro XT varnish). The manufacturer's instructions were followed. The sensitivity scores were recorded immediately and after 1 week of therapy. Mann–Whitney U‐test and Wilcoxon‐matched pairs test were used for the analysis. Although both varnishes were shown to reduce the dentinal hypersensitivity in patients, according to statistics, MI Varnish was a better agent to reduce dentinal hypersensitivity than Clinpro XT varnish.

Bakrya AS, Abbassy MA (2018)[87]: Compared the remineralization efficacy of using the MI paste plus according to manufacturer's instructions to MI varnish and to using a modified method of MI-paste plus application. 100 enamel specimens were obtained from the buccal and lingual surfaces of 50 extracted human non-caries third molars. All specimens were challenged by a buffered demineralization solution for 4 days, and were divided in 4 groups with 25 specimens in each group. 25 demineralized specimens had MI paste plus applied for 4 min and then wiped out (MI), 25 specimens had MI paste applied followed by application of SE-bonding agent (MI+Bond), 25 specimens had MI Varnish applied according to manufacturer instructions (MI Varnish) the rest of specimens served as controls (C). All specimens were stored for 7 days in artificial saliva. All specimens had their surface hardness (SH) measured by micro-hardness tester before/after the acidic challenge and after the treatment procedures. After the SH test all specimens were crosssectioned to obtain 100–150 micron thickness specimens to observe the lesion depth before/after treatment by the TMR (Transverse Micro Radiography) technique. TMR experiment showed that (MI+Bond) and (MI varnish) groups recorded significant decrease in lesion depth and mineral loss of the tested subsurface lesion $p < 0.05$. (MI+Bond) group scored the highest significant regain of surface micro hardness results $p < 0.05$. Conclusion: (MI varnish) and the modified application of MI paste are methods that can increase the efficacy of CPP-ACP in remineralizing the enamel surface lesions.

Abufarwa M, Noureldin A, Campbell PM, Buschang PH (2019)[27]: Conducted a study was to test how long Casein phosphopeptide amorphous calcium phosphate varnish prevents enamel demineralization in vitro. Human molars & premolars were sectioned buccolingually & evaluated under polarized light microscope. Samples revealed no significant demineralization during the first 4 weeks & significant increases thereafter.

Dehailan LA, Martinez-Mier EA, Eckert GJ, Lippert F (2019)[88]: Investigated the anticaries efficacy, measured as fluoride release into artificial saliva (AS); change in surface microhardness of early enamel caries lesions; and enamel fluoride uptake (EFU) of 14 commercially available FVs and two control groups. Bovine enamel

specimens (535 mm) were prepared and assigned to 18 groups (n=12). Early caries lesions were created in the specimens and characterized using Vickers microhardness (VHNlesion). FV was applied to each group of specimens. Immediately afterward, specimens were incubated in 4 mL of AS for 18 hours, which were collected and renewed every hour for the first six hours. AS samples were analyzed for fluoride using an ion-specific electrode. Specimens were then brushed for 20 seconds with toothpaste slurry and subjected to pH cycling consisting of a four-hour/day acid challenge and one-minute treatments with 1100 ppm F dentifrice for five days. Microhardness was measured following pH cycling (VHNpost). EFU was determined using microbiopsy. Acid resistance (eight-hour demin challenge) was performed after pH cycling, and microhardness was measured (VHNart) and compared with baseline values to test the FV impact after pH cycling. One-way analysis of variance was used for data analysis (a=0.05). FVs differed in their release characteristics (mean 6 SD ranged from 14.97 6 2.38 lg/mL to 0.50 6 0.15 lg/mL), rehardening capability (mean 6 SD ranged from 24.3 6 15.1 to 11.7 6 12.7), and ability to deliver fluoride to demineralized lesions (mean 6 SD ranged from 3303 6 789 lg/cm3 to 707 6 238 lg/cm3). Statistically significant but weak linear associations were found between DVHN(post – lesion), EFU,and fluoride release (correlations 0.21-0.36). The results of this study demonstrated that differences in FV composition can affect their efficacy in in vitro conditions.

Yadav S, Sachdev V, Malik M, Chopra R (2019)[36]: Assessed and compared the reduction in Streptococcus mutans count in biofilm samples after topical application of three different varnishes and to evaluate the effect of oral prophylaxis prior to fluoride varnish application. Sixty healthy children with no active caries, in the age group of 2 – 8 years, were randomly divided into Group A = fluoride varnish containing CPP-ACP; Group B = fluoride varnish containing xylitol; and Group C = fluoride varnish with 0.9%. difluorosilane; further, the groups were divided into subgroups, namely A1, B1, and C1 with prior oral prophylaxis and A2, B2, and C2 without oral prophylaxis. Plaque samples were collected at baseline, 1st month, and 3rd month; cultured; and incubated, and CFU/ml was calculated. Data were compiled, and CFU/ml was analysed by independent t-test, paired t-test, and one-way ANOVA. There was no statistical difference between the fluoride groups. Furthermore, no

statistically significant difference was seen between the subgroups. Fluoride varnish containing CPP-ACP showed higher reduction in S. mutans count followed by xylitol-containing fluoride varnish and fluor protector.

Varma V, Hegde KS, Bhat SS, Sargod SS, Rao HTA (2019)[30]: Evaluated the remineralization potential of Clinpro XT varnish containing tricalcium phosphate (TCP) and MI varnish containing casein phosphopeptide (casein phosphopeptide–amorphous calcium phosphate, CPP–ACP). Thirty premolar teeth were taken and divided into three groups. Samples were sliced mesiodistally into buccal and lingual halves using a diamond disk bur. The buccal halves of the teeth were used for the study. Artificial caries like lesions were produced and evaluated with Diagnodent. The samples in each group were treated with the respective remineralizing agent (except for the control group) at every 24 hours for 7 days and the surfaces were assessed using Diagnodent to record the values after the remineralization procedure. The Diagnodent values obtained were tabulated and statistically analyzed using one-way ANOVA and Tukey's multiple comparison tests. There was a significant difference between the values calculated before and after remineralization in all the three groups. The study findings showed that MI varnish containing CPP–ACP had the highest release of fluoride as compared to the Clinpro fluoride releasing varnish.

Sonesson M, Brechter A, Abdulraheem S, Lindman R and Twetman S (2019)[89]: Evaluated the effectiveness of a new fluoride varnish formula containing 1.5% ammonium fluoride in preventing white spot lesions (WSLs) in adolescents undergoing multibracket orthodontic treatment. The study employed a randomized controlled triple-blinded design with two parallel arms. One hundred eighty-two healthy adolescents (12–18 years) referred to three orthodontic specialist clinics were eligible and consecutively enrolled. Informed consent was obtained from 166 patients and they were randomly allocated to a test or a placebo group (with aid of a computer program, generating sequence numbers in blocks of 15). In the test group, fluoride varnish was applied in a thin layer around the bracket base every sixth week during the orthodontic treatment, while patients in the placebo group received a varnish without fluoride. The intervention started at onset of the fixed appliances and continued until debonding. The endpoint was prevalence and severity of WSLs on the

labial surfaces of the maxillary incisors, canines, and premolars as scored from high-resolution pre- and post-treatment digital photos with aid of a fourlevel score. One hundred forty-eight patients completed the trial, 75 in the test group and 73 in the placebo group (dropout rate 10.8%). The total prevalence of WSL's on subject level after debonding was 41.8% in the test group and 43.8% in the placebo group. The number of patients exhibiting more severe lesions (score 3 + 4) was higher in the placebo group ($P < 0.05$); the absolute risk reduction was 14% and the number needed to treat was 7.1. Regular applications of an ammonium fluoride varnish reduced the prevalence of advanced WSL during treatment with fixed orthodontic appliances.

Babu KLG, Subramaniam P, Teleti S (2020)[90]: Evaluated and compared the effect of varnish containing casein phosphopeptides-amorphous calcium phosphate (CPP-ACP) and fluoride (MI Varnish) with that of varnish containing only fluoride (Fluor Protector) on surface microhardness (SMH) of enamel. Enamel blocks were cut from the 90 premolar teeth samples. The samples were divided into three Groups (A, B, and C) consisting of 30 blocks each. Varnish containing CPP-ACP with fluoride was applied on samples of Group A and varnish containing only fluoride was applied on the samples of Group B. Group C was used as control group; without any varnish application. After varnish application, these samples were subjected to pH cycling. Following, SMH was assessed using SMH tester machine. The mean values of SMH for Group A were 488 ± 6 vickers hardness number (VHN), Group B were 485 ± 12 VHN, and Group C were 448 ± 12 VHN. There was no significant difference in the SMH of enamel between the varnish containing CPP-ACP and fluoride, with that of varnish containing only fluoride.

Bapat S A, Shashikiran ND, Gugawad S, Gaonkar N, Taur S, Hadakar S, Chaudhari (2020)[34]: Evaluated and compared the anti-microbial efficacy against *S. mutans* and enamel surface microhardness after application of two fluoride varnishes containing Casein Phosphopeptide-Amorphous Calcium Phosphate (CPP-ACP) and xylitol coated calcium phosphate ions as active ingredients. This was an in-vitro study involving one parameter on agar discs and one parameter on human enamel samples from November 2019 to January 2020. The anti-microbial efficacy of MI Varnish

(GC Dental) and Embrace Varnish (Pulpdent) was comparatively assessed. Ten wells of each varnish were assessed using disc diffusion method on Trypticase-Yeast-Cysteine-Sucrose-Bacitracin (TYCSB) agar plate. Zone of inhibition around each sample was measured after 48 hours of agar plate anaerobic incubation and the value was recorded using digital Vernier calliper. The change in enamel surface microhardness with and without application of fluoride varnish was assessed using Vickers Microhardness method on 30 samples. The results were compiled and compared using ANOVA. MI Varnish showed the higher antibacterial effects, compared to Pulpdent Embrace Varnish against *S. mutans* ($p<0.05$). The MI Varnish group showed significantly high value of enamel surface microhardness as compared to Pulpdent Embrace group and Control group ($p<0.05$). MI Varnish showed higher anti-bacterial efficacy as compared to Pulpdent Embrace Varnish against *S. mutans* by disc diffusion method on TYCSB agar. The surface microhardness of enamel group treated with MI Varnish was significantly greater than Pulpdent Embrace and control group.

Radha S, Kayalvizhi G, Adimoulame S, Prathima GS, Muthusamy K, Ezhumalai G, Jagadesaan N (2020)[35]: Assessed the remineralizing efficacy of fluoride and its combination varnishes on white spot lesion (WSL) in children with early childhood caries (ECC). Sixty children with active WSL on primary maxillary anterior teeth were randomly selected. At baseline, the WSL activity was evaluated using ICDAS II [lesion activity assessment (LAA)] and its dimensions through photographic method. They were allocated to group I (GI) (5% NaF), group II (GII) [5% NaF with amorphous calcium phosphate (ACP)], and group III (GIII) [5% NaF with casein phosphopeptides – amorphous calcium phosphate (CPP –ACP)]. First, oral hygiene instructions and diet counseling were given followed by application of fluoride varnishes in their respective groups. The same parameters were recorded at follow-up of 2, 4, 12, and 24 weeks intervals. Data were collected and subjected to statistical analysis using Friedman Chi-square and Mann–Whitney tests. Overall, the active WSL changed to inactive over a period of 24 weeks in GI was 90%, GII was 95%, and 100% in GIII. There was a significant reduction in dimension of WSL in GI from 4.119 to 2.525 ($p = 0.0001$). Likewise, there was a significant reduction in dimension of WSL in GII and GIII from 4.586 to 3.258 and 4.696 to 1.2155, respectively ($p =$

0.0001, p = 0.0001). Comparatively, group III (MI varnish) showed statistically significant reduction in the dimension of WSL from baseline to 24 weeks (p = 0.002). But the results were statistically insignificant with change of active lesions to its inactivity (p = 0.349). Fluoride varnish with CPP–ACP was found to be an effective preventive strategy in reversing WSL in children with ECC.

Rani K, Ramanna PK, Mailankote S, Joy AK, Thomas AA, Baby M (2021)[31]: Assessed the anticariogenic effectiveness of different fluoride varnishes on artificially induced enamel lesions employing scanning electron microscope. Eighty healthy, normal premolars without dental caries that were extracted in course of orthodontic therapy with all the surfaces intact were included in this study. A window, 4 × 4 mm, was made discernible on the buccal surface of each sample tooth. A demineralizing solution at 37°C was used to immerse the teeth for 48 hours to induce artificial lesions on the surface of the enamel. Following preparation of the artificial enamel lesions, the 80 premolar teeth were allocated into the four groups (20 each) depending on the fluoride varnish system used as Group I: control, Group II: Duraphat varnish, Group III: MI Varnish, and Group IV: Clinpro White Varnish. The anticariogenic effectiveness of different fluoride varnishes was evaluated employing a scanning electron microscope (SEM). The MI Varnish (fluoride varnish) group exhibited slightly greater (127.20 ± 0.14) mean demineralized lesions, pursued by Clinpro White Varnish use (126.88 ± 0.09), the control group (126.36 ± 0.10) and the Duraphat varnish (124.14 ± 0.08) in that order. Greater mean areas of remineralization were found with use of MI Varnish (92.40 ± 0.09), pursued by the Duraphat varnish use (106.68 ± 0.12), use of Clinpro White Varnish (112.36 ± 0.08), and then the control group (123.08 ± 0.18) in that order. Statistically significant differences were noted between the experimental groups employing the various fluoride varnishes (p <0.001). The current research concluded that the MI Varnish group presented a superior protective potential in comparison with Duraphat varnish and Clinpro White Varnish groups.

Edunoori R, Dasari AK, Chagam MR, Velpula DR, Kakuloor JS and Renuka G (2022)[91]: Compared the efficacy of Icon resin infiltration and Clinpro XT varnish on remineralization of white spot lesions using a polarized light microscope (PLM).

Artificial white spot lesions were created on a sample of 40 extracted human premolar teeth by immersing in a demineralizing solution. All samples were randomly allocated to two groups of 20 each; Group A: Icon resin infiltration and Group B: Clinpro XT varnish. Teeth were sectioned along the buccolingual plane using a diamond disc. Specimens were observed under the PLM (4× magnification) at three deepest measurements and their averages were calculated to obtain the mean penetration depth. Icon resin infiltration showed a significantly higher penetration depth and is more effective on remineralization of white spot lesions when compared to Clinpro XT varnish.

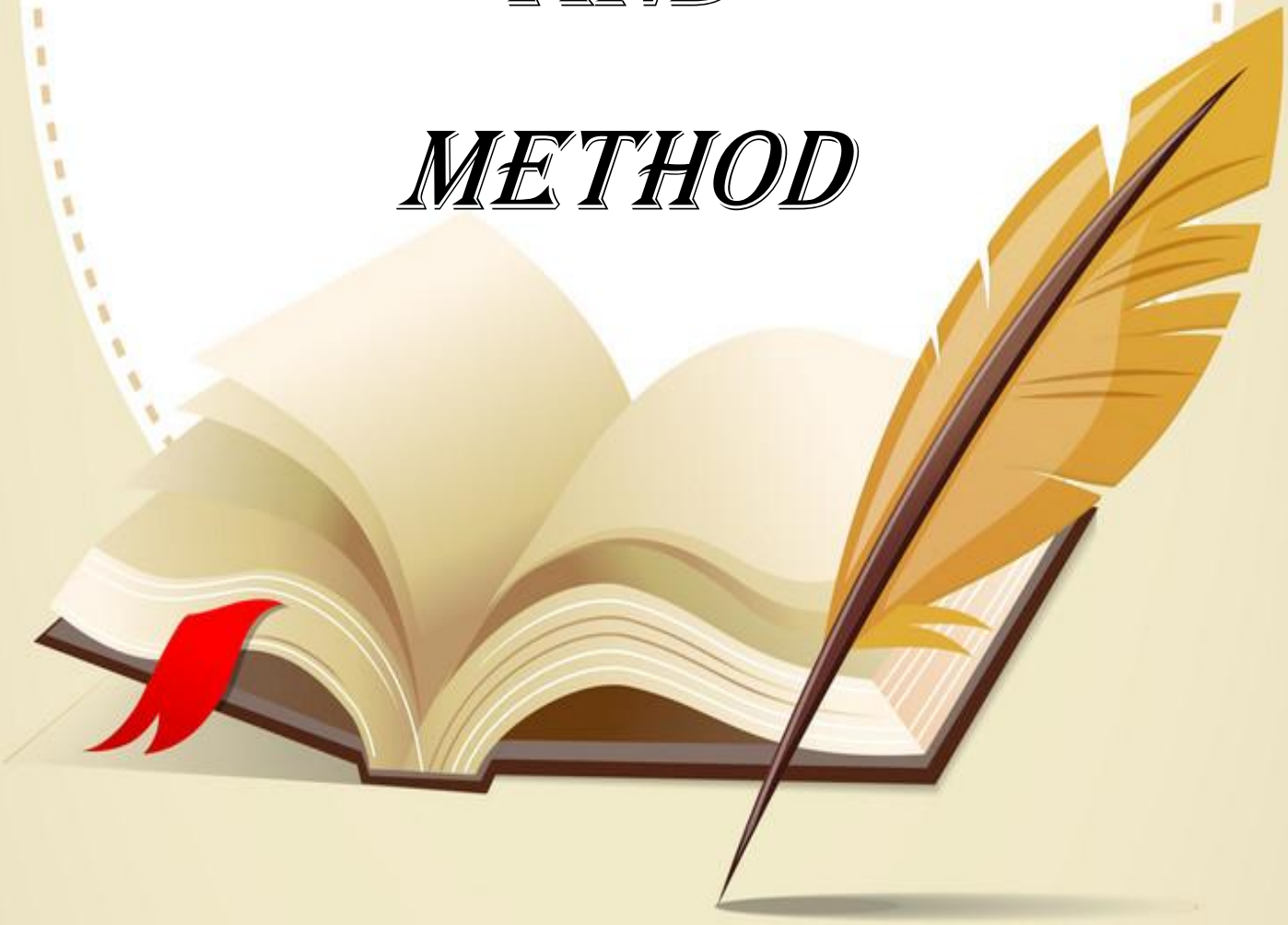
Nadar BG, Yavagal PC, Velangi CS, Yavagal4 CM, Basavaraj SP (2022): Conducted a systematic review to evaluate the efficacy of casein phosphopeptide-amorphous calcium phosphate (CPP-ACPF) varnish for remineralization of white spot lesions (WSLs) "*in vitro*" in human teeth. Literature search included three databases, namely Medline (via PubMed), The Cochrane Controlled Clinical Trials Register, and Google Scholar from 2010 to January 2021. The studies assessing WSL depth, calcium, phosphate ion release, and microhardness due to artificial demineralization or remineralization were considered for review. Reference articles were retrieved, and a customized risk assessment tool was used. The Cochrane risk of bias assessment tool was used to generate the risk of bias summary graph. Meta-analysis was performed using RevMan 5.4. Heterogeneity was evaluated by Cochrane's test, and random effects model was used to pool estimate of effect and its 95% confidence intervals (CIs) for surface microhardness. Eighteen studies were selected for review based on the eligibility criteria. Four studies showed superior remineralizing effect of CPP-ACPF compared to fluoride varnishes. Four studies involving 120 human permanent teeth samples were included in the meta-analysis. Efficacy of CPP-ACPF varnish was equivalent to other fluoride varnishes in improving surface microhardness after remineralization during 7-day period (mean surface microhardness: 3.94, 95% CI [−9.08–1.21], I2: 75%, $P = 0.13$). Major risks of bias associated with the studies included in the review were inadequate sample size, improper sample preparation, and unexplained blinding. CPP-ACPF varnish appears to be equally effective as other fluoride varnishes in remineralizing artificially induced WSLs, but quality of evidence is low.

Habeeb I, Shetty KN, Harshitha V, Sudhakar SS, Sarah H (2022)[37]: Evaluated the efficacy of MI varnish in preventing white spot lesions in patients with fixed orthodontic therapy. Method The study was performed where 100 patients undergoing orthodontics treatment were allocated randomly to 2 subgroups with differing frequencies of MI varnish application. Degree of mineralisation was measured on the vestibular surfaces of 12 teeth (6 varnish & 6 unvarnished controls) Measurement were taken at 4 sites using camera EOS 750D and then subjected to statistical analysis. The statistical analysis showed difference in the degree of demineralization between treated and untreated teeth, and was statistically significant in terms of time point, frequency of application or specific tooth site. Periodic application of MI varnish can offer some protection against white spot lesions and it is not statistically significant degree if patients have excellent oral hygiene. Additional protection is conferred by applying the product every 3 months compared with every 6 months.

Mashhour A, Allam G and Wassel M (2023)[32]: Compared the effect of Clinpro™ White varnish containing 5% sodium fluoride (NaF) and functionalized tricalcium phosphate, MI varnish containing 5% NaF and casein phosphopeptide-amorphous calcium phosphate (CPP-ACP), and 38% Silver diamine fluoride (SDF) in preventing demineralization of treated white spot lesions (WSLs) in enamel of primary teeth. Forty-eight primary molars with artificial WSLs were allocated into four groups as follows: Group 1: Clinpro white varnish, Group 2: MI varnish, Group 3: SDF, and Group 4: control (no treatment). The three surface treatments were applied for 24 h and then enamel specimens were subjected to pH cycling. Thereafter, the mineral content of specimens was evaluated by Energy Dispersive X-ray Spectrometer and the lesion depth was assessed via Polarized Light Microscope. One-way ANOVA followed by Tukey's post hoc test were used at $p \leq 0.05$ to identify significant. Insignificant difference in mineral content was observed among treatment groups. Treatment groups exhibited significantly higher mineral content compared to control except for Fluoride (F). MI varnish showed the highest mean calcium (Ca) ion content (66.57±0.63), and Ca/P (2.19 ± 0.11), followed by Clinpro white varnish, and SDF. MI varnish also displayed the highest phosphate (P) ion content (31.46 ± 0.56), followed by SDF (30.93 ± 1.02), and Clinpro white varnish (30.53 ± 2.19). Fluoride content was highest in SDF (0.93 ± 1.18), followed by MI (0.89 ± 0.34) and Clinpro

(0.66 ± 0.68) varnishes. Significant difference in lesion depth was observed among all groups ($p < 0.001$). The lowest mean lesion depth (μm) was found in MI varnish (226.23 ± 44.25) which was significantly lower than Clinpro white varnish (285.43 ± 44.70), SDF (293.32 ± 46.82), and control (576.69 ± 42.66). Insignificant difference in lesion depth was found between SDF and Clinpro varnish. In primary teeth, WSLs treated with MI varnish displayed better resistance to demineralization compared to WSLs treated with Clinpro white varnish and SDF.

MATERIAL AND METHOD



This split mouth study was conducted in the Department of Orthodontics and Dentofacial Orthopedics, Babu Banarasi Das College of Dental Sciences, Lucknow in collaboration with Department of Oral Pathology, with an aim to evaluate and compare the effect of two different varnishes (MI Varnish and VOCO Varnish) in prevention of demineralization of enamel when applied around Orthodontic brackets bonded to maxillary 1st premolar teeth scheduled for extraction after 3 months using polarized light microscope.

The approval was taken from the Ethical Committee of Babu Banarasi Das College of Dental Sciences, before conducting the study and informed consent was taken from all the subjects decided to participate in this study, who were undergoing fixed Orthodontic treatment voluntarily.

MATERIALS

1. Sample

The sample consisted of 15 patients with varying malocclusion coming to the Department of Orthodontics, BBDCODS, for fixed Orthodontic treatment in whom all four 1st premolar teeth had been scheduled for extraction as a part of the fixed Orthodontic treatment. A total of 60 teeth (30 maxillary and 30 mandibular 1st premolars), were obtained on extraction after 3 months following application of two different varnishes on right and left premolars of maxillary arch. Out of 30 extracted mandibular premolars 15 were randomly selected and used in the study.

2. Eligibility Criteria

Inclusion Criteria –

1. All patients who were advised fixed Orthodontic treatment following extraction of all 1st premolars using either MBT or Roth technique.
2. Patients having fully erupted premolars.
3. All the patient whose 1st premolars were intact, without presence of hypoplastic areas, caries, fracture or cracks visible to the naked eye.
4. Patients who were internally motivated to participate in the study.

Exclusion Criteria –

1. Patients having history of previous Orthodontic treatment that involved bonding on the 1st premolar tooth.
2. Patients having history of restorations on labial surface, crowns or root canal treatments of the sample teeth.
3. Patients having history of trauma or any structural alteration on the tooth surfaces of the sample teeth.
4. Patient who had history of undergoing bleaching of teeth.

3. Sample size estimation

Sample size estimation was done by using **GPower software (version 3.0).** Sample size was estimated for ANOVA: Fixed effects, main effects and interactions

A minimum total sample size of 44 was found to be sufficient for an alpha of 0.05, power of 80 %, 0.64 as effect size (assessed from a similar study).

tests - ANOVA: Fixed effects, main effects and interactions

Analysis:	A priori: Compute required sample size
Input:	Effect size f=0.64
α err prob	= 0.05
Power (1-β err prob)	= 0.80
Numerator df	= 10
Number of groups	= 3
Number of covariates	= 1
Output:	Noncentrality parameter λ=20.0704000
Critical F	= 2.0487395
Denominator df	= 40
Total sample size	= 44
Actual power	= 0.8093722

Hence sample was rounded off to 45.

4. **Distribution of sample**

Following table shows distribution of sample based on type of varnish application

Table 1: Distribution of sample in different groups

Groups	Sample size (N)	Tooth	Type of Varnish
Group I (Control)	**15**	**Mandibular right/left premolar**	**None**
Group II (Experiment)	**15**	**Maxillary left premolar**	**VOCO Varnish**
Group III (Experiment)	**15**	**Maxillary right premolar**	**MI Varnish**

5. **Materials used for Bonding (Fig.1)**

A) Cheek Retractor: Supplied by Captain Orthodontics.

B) Pumice: Keystone Industries (it is composed of silicon dioxide, aluminium oxide, potassium oxide, sodium oxide, calcium oxide, ferric oxide, magnesium oxide, titanium oxide, manganese oxide, sulphur trioxide, carbon dioxide, phosphorous pentoxide).

C) Contra-angle Handpiece with polishing cup: NSK-Nakanishi Japan.

D) Cotton roll: Doctor's Choice absorbent cotton wool.

E) Etchant: Anabond Eazetch gel (37% phosphoric acid) .

F) Applicator tip: 3M ESPE Disposable Applicator tip**.**

G) Primer: Transbond XT primer (3M Unitek Corporation, USA).

H) Adhesive: Light curable Tansbond XT adhesive (3M Unitek Corporation, USA) supplied as single paste contained in a syringe was used.

I) Light cure Unit: Woodpecker Curing Light, LED D (Gullin Woodpecker Medical instrument Co. Ltd).

J) Brackets: MBT or ROTH prescription (3M Unitek, USA) with slot configuration of 0.22" X 0.028".

K) Bracket holding tweezer: Supplied by Welcare Orthodontics.

L) Bracket height gauge: Supplied by Edge Orthodontics.

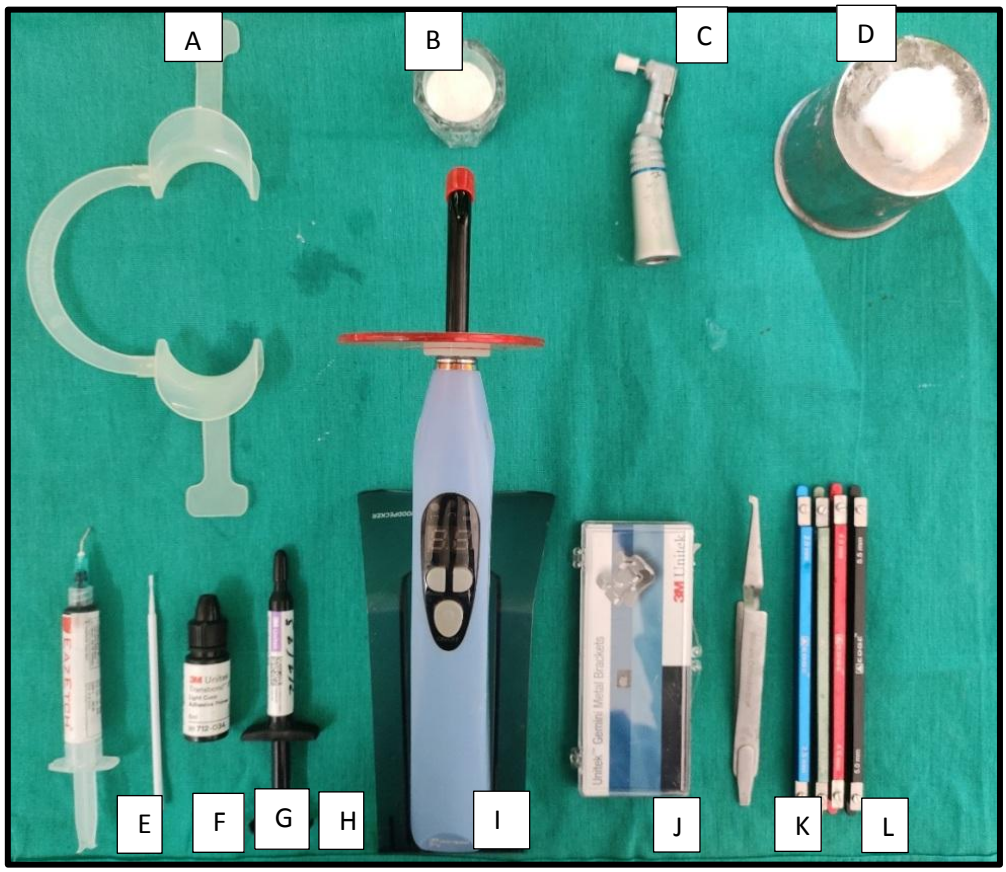


Fig. 1: Materials used for bonding

A) Cheek retractor
B) Pumice
C) Contra-angle handpiece with polishing cup
D) Cotton roll with cotton
E) Etchant
F) Applicator tip
G) Primer
H) Adhesive
I) Light cure unit
J) Brackets
K) Bracket holding tweezer
L) Bracket height gauge

6. Materials used to prevent White spot lesions (Fig. 2)

A) MI Varnish (GC America):

It is composed of following ingredients -

i. Medical ingredients – Sodium fluoride 5% wt/vol, CPP-ACP (Casein Phosphopetide Amorphous Calcium Phosphate) 2% wt/vol.

ii. Non-medical ingredients – Polyvinyl acetate, Ethanol, Ethylene Glycol Monoethyl Ether, Hydrogenated Rosin, Silicon Dioxide, Flavor, Sucralose.

B) Profluoride Varnish (VOCO Dental Germany):

It is composed of following ingredients -

i) Medical ingredients – Sodium fluoride 5% wt/vol (22,600 ppm fluoride).

ii) Non-medical ingredients- Ethanol, Artificial flavours, Xylitol.

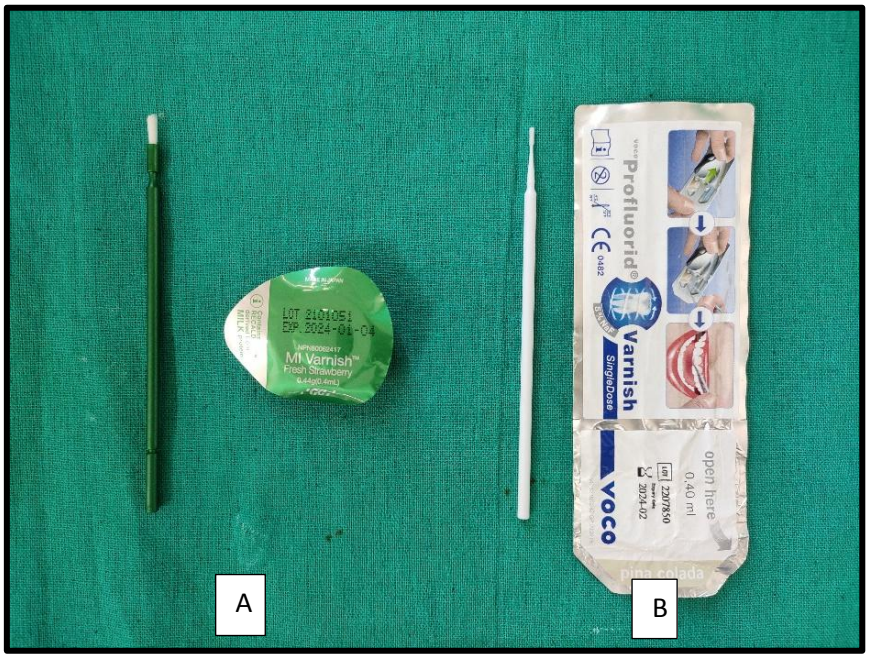


Fig. 2: Materials used to prevent white spot lesions

A) MI Varnish with applicator brush

B) Profluoride Varnish with applicator brush

7. Materials used for extraction of teeth (Fig. 3)

A) PPE: facemask, headcap, gloves

B) Debonding plier: Welcare Orthodontics

C) Local anasthesia: LIGNOX 2% A (manufactured by Indoco Remidies ltd.). It contains Lignocaine 2% with Adrenaline 1:80000

D) Syringe: Dispovan Syringe 5 ml

E) Periosteal elevator: GDC Dental Instruments

F) Upper premolar forceps: GDC Dental Instruments

G) Lower premolar forceps: GDC Dental Instruments

H) Cotton roll: Doctor's Choice absorbent cotton wool

I) Betadine: Povicare 5%

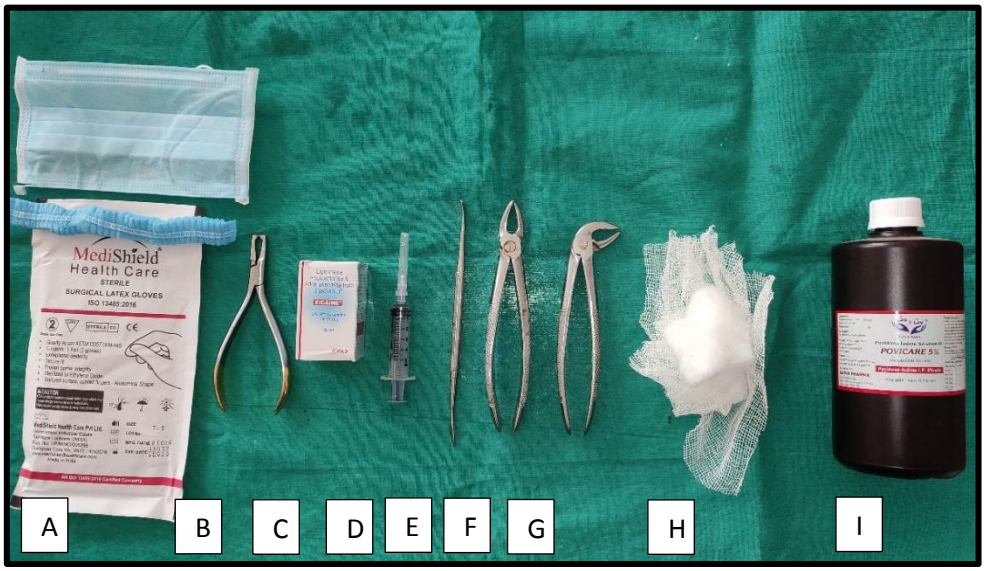


Fig. 3: Materials used for extraction of teeth

A) PPE (facemask, head cap, gloves)
B) Debonding plier
C) Local anasthesia
D) Syringe
E) Periosteal elevator
F) Upper premolar extraction forceps
G) Lower premolar extraction forceps
H) Cotton and gauze piece
I) Betadine

8. Materials used for storage of teeth (Fig. 4)

A) Bottles: Colour coded bottles (red, yellow, green) for 3 different groups.

B) Storage medium: 10% formalin

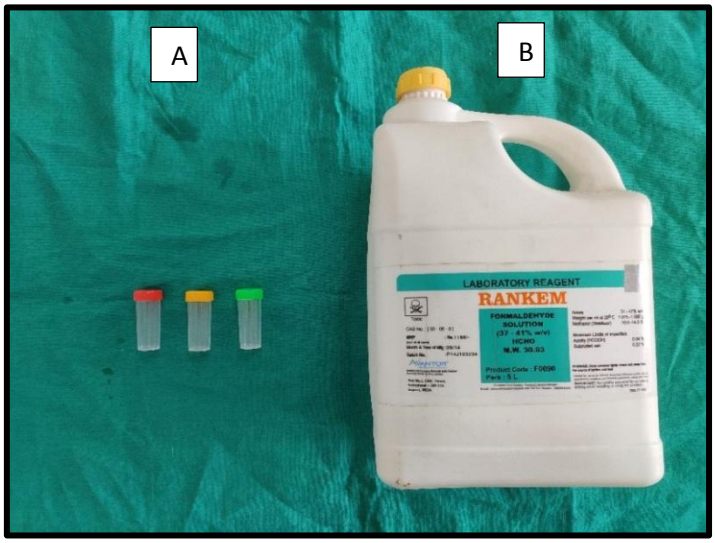


Fig. 4: Materials used for storage of teeth

A) Bottles
B) Storage medium (10% formalin)

9. Materials used for preparing tooth section (Fig. 5)

A) Micromotor with straight handpiece and cutting disk

B) Pumice powder

C) Arkansas stone

D) Vernier Calliper

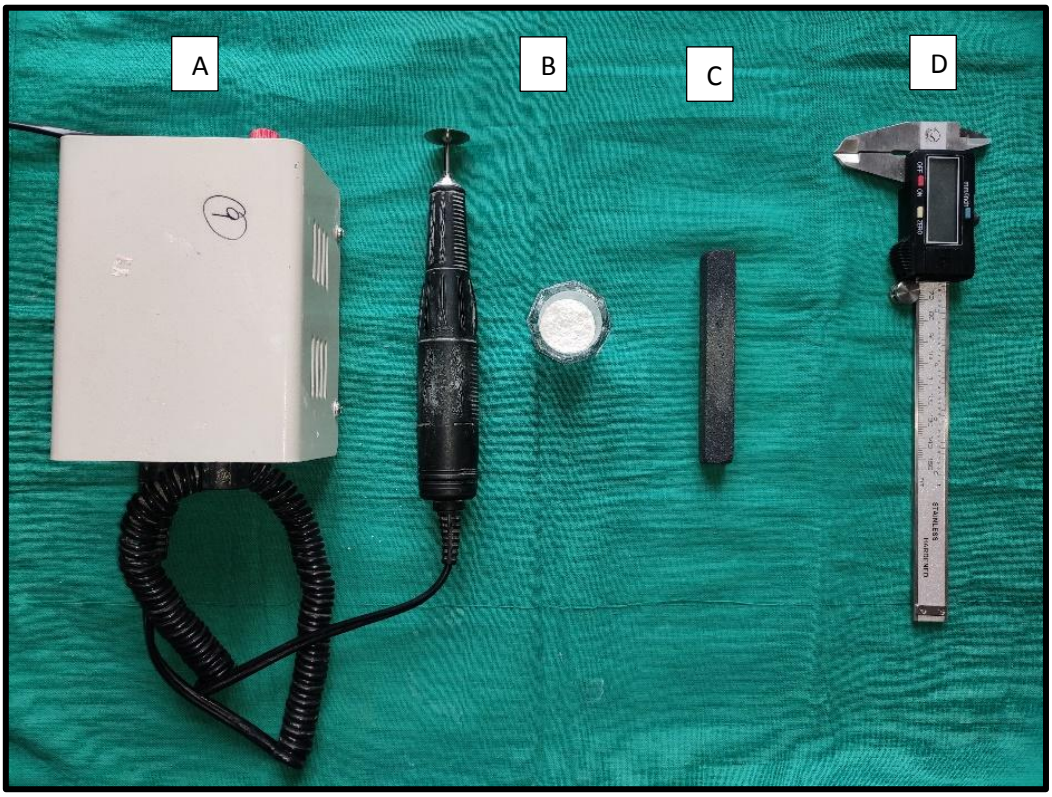


Fig. 5: Materials used for preparing tooth section

A) Micromotor with straight handpiece and cutting disc
B) Pumice powder
C) Arkansas stone
D) Vernier Calliper

10. Materials used to prepare slides (Fig. 6)

A) Cover slips: Rectangular cover glasses (Corning glass, USA).

B) Glass slides: Blue Star micro slide (Polar industrial corporation, Mumbai).

C) Xylene: (Laboratory Reagent Rankem)

D) Alcohol: Iso-propyl alcohol (Laboratory Reagent Rankem)

E) Mounting media: D.P.X Mountant (Laboratory Reagent Rankem).

Fig. 6: Materials used to prepare slides

A) Cover slips
B) Glass slides
C) Xylene
D) Alcohol
E) Mounting media

11. Visualization of demineralization depth around bracket base (Fig. 7)

A) Polarized light microscope: PLM BX51-P with optical system-UIS2 with attachment or detachment of Bertrand lens capable of focussing 360c rotatable, minimum graduation with stroke of 35mm with fine stroke per rotation 0.1mm in the department of Oral Pathology, BBDCODS, Lucknow.

B) Image analysis software: ImageJ 1.53t (Wayne Rasband and contributors, National Institute of Health, USA).

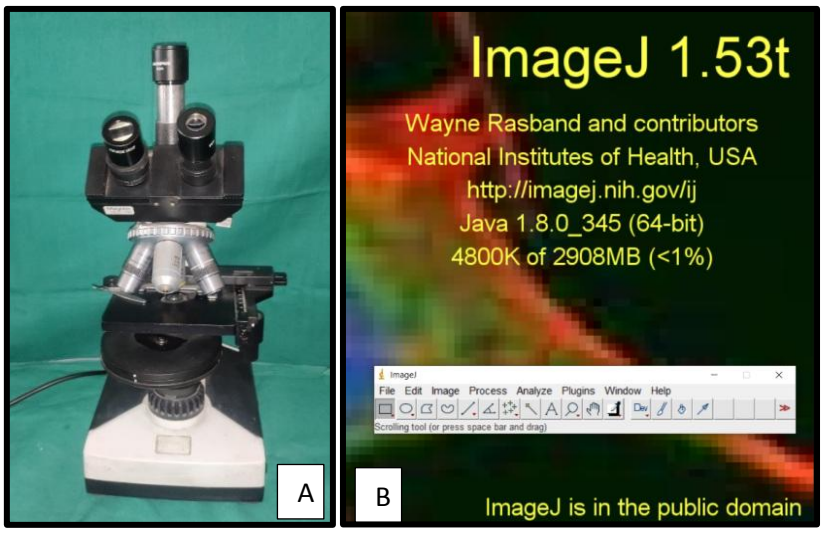


Fig. 7: Materials used for visualization of demineralization depth around bracket base

A) Polarized light microscope B) Image analysis software

12. Methodology (Fig. 8)

i) Bonding procedure:

A) Cleaning: The cleaning of enamel surface of all teeth was performed with rubber cup using low speed micro-motor (>20000 RPM) and Contra-angle handpiece (NSK Dental) .

B) Isolation of teeth before bonding: Cheek retractors, cotton rolls and vacuum suction was used to isolate the teeth.

C) Enamel conditioning: 37% phosphoric acid (Anabond Eazetch gel) was applied to the enamel area at the desired site only where bracket had to be placed for 15 to 30 seconds.

D) Washing of etchant: All the teeth were washed with water for 15 seconds and dried with oil free air spray, till frosty white appearance of enamel was visible.

E) Application of primer: A thin layer of primer (Transbond XT primer, 3M Unitek Corporation, USA) was applied with micro-brush on the etched enamel surface and cured for10 seconds with LED curing light.

F) Adhesive application and bracket positioning: Sufficient amount of bonding adhesive (Tansbond XT adhesive, 3M Unitek Corporation, USA) was applied uniformly over bracket mesh after application of primer bracket was placed on the specific tooth, and checked for horizontal and vertical placement accuracy. The excess flash was removed.

G) Curing of adhesive: The area was then cured using LED light for 20 seconds each on mesial and distal aspect per tooth.

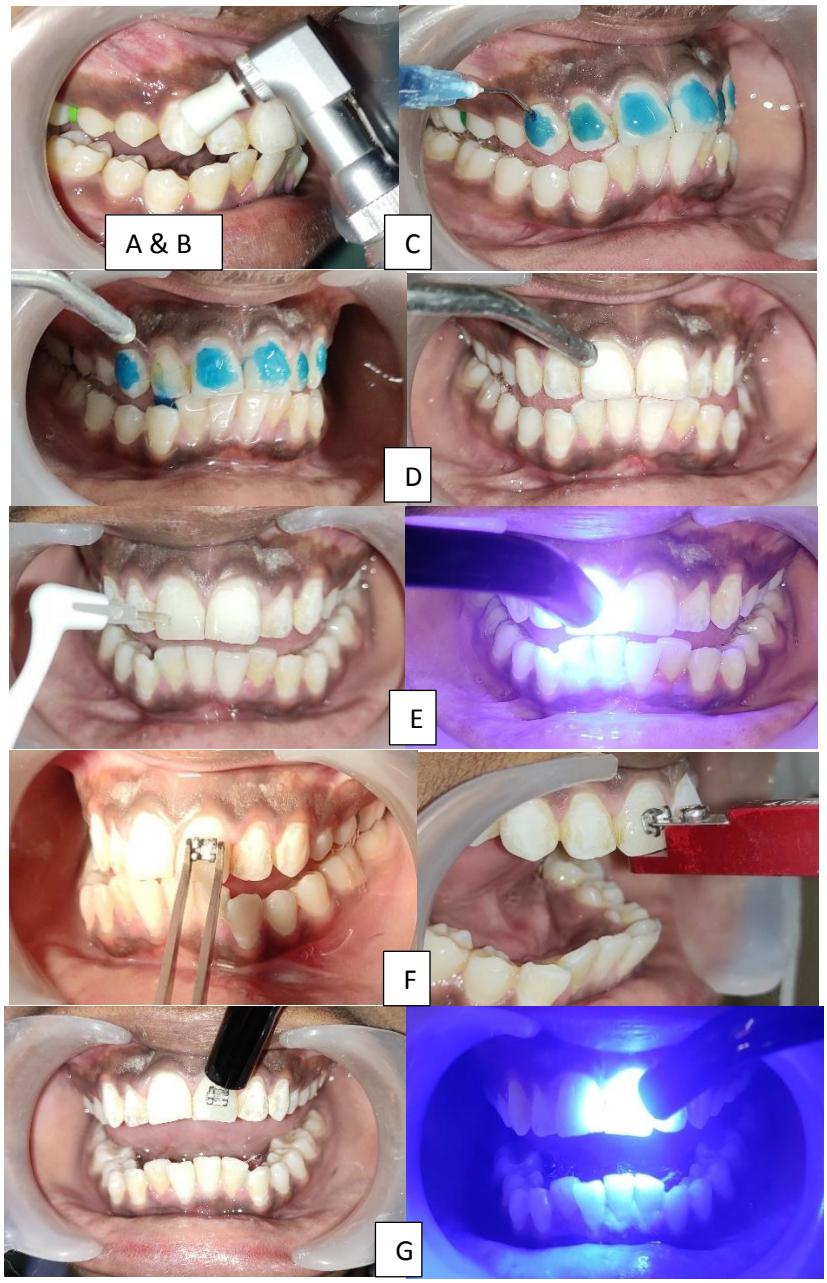


Fig. 8: Bonding procedure

A) Cleaning
B) Isolation of teeth
C) Enamel conditioning
Curing of adhesive
D) Washing of etchant
E) Application of primer
F) Adhesive application and bracket positioning

ii) Application of varnishes (Fig. 9)

- MI Varnish [5% Sodium fluoride varnish with Recaldent (CPP-ACP), GC America, USA] was applied in a thin layer around the base of the bracket of the maxillary right 1st premolar with the help of fresh applicator tip.
- VOCO Profluoride Varnish [5% Sodium fluoride (22,600 ppm fluoride)] was applied in a thin layer around the base of the bracket of maxillary left 1st premolar with the help of fresh applicator tip.
- No varnish was applied on mandibular 1st premolars as it served as control.
- After application of varnishes, patients were instructed not to brush or floss and avoid hard, hot or sticky food for at least 4 hours.
- Regular oral hygiene regimen was followed twice daily with non-fluoridated toothpaste as provided by operator.

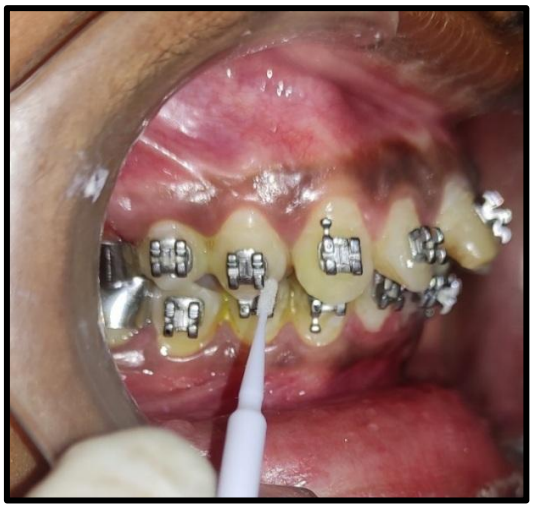


Fig. 9: Application of Varnish

iii) Extraction procedure:

- Before extraction brackets were debonded using debonding pliers at the base of the brackets and lifting the brackets off with a peel force (Fig. 10).
- The extraction of one side was done for both maxillary and mandibular 1st premolars in the department of Oral & Maxillofacial Surgery, BBDCODS, Lucknow.
- After 3 days the extractions of opposite quadrants were carried out in the same way.
- Upper and lower premolar forceps was used for extractions.

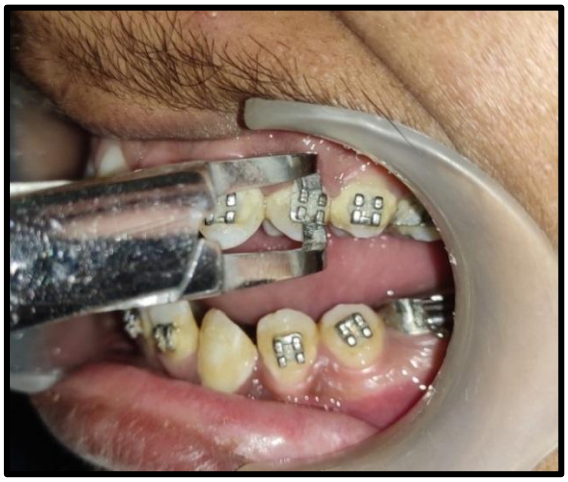


Fig. 10: Debonding of premolar bracket

iv) Storage of the extracted teeth (Fig. 11)

- The extracted tooth was cleaned off any blood stains, debris or soft tissue and were stored in 10% formalin to maintain a hydrated state till the sectioning was done.
- A total 45 containers (15 Red; 15 Green; 15 Yellow) were used to store all the extracted teeth for Group I, II and III.
- Teeth were stored in their respective bottles, along with the patient's name. Red coloured bottle contains teeth where no varnish was applied and denoted as Group I. Green coloured bottles contains teeth which were treated with VOCO Varnish and denoted as Group II. Yellow coloured bottles contains teeth which were treated with MI Varnish and denoted as Group III.

Group I (Red)

Group II (Green)

Group III (Yellow)

Fig. 11: Storage of extracted teeth in colour coded bottles

v) Sectioning of the teeth (Fig. 12)

- The teeth were sectioned buccolingually using micromotor and disc.
- Further reduction of the sections were done using pumice powder, glycerine and Arkansas stone to get the desired thickness of 50 microns without damaging the enamel surface.
- The sections were then cross checked before mounting.

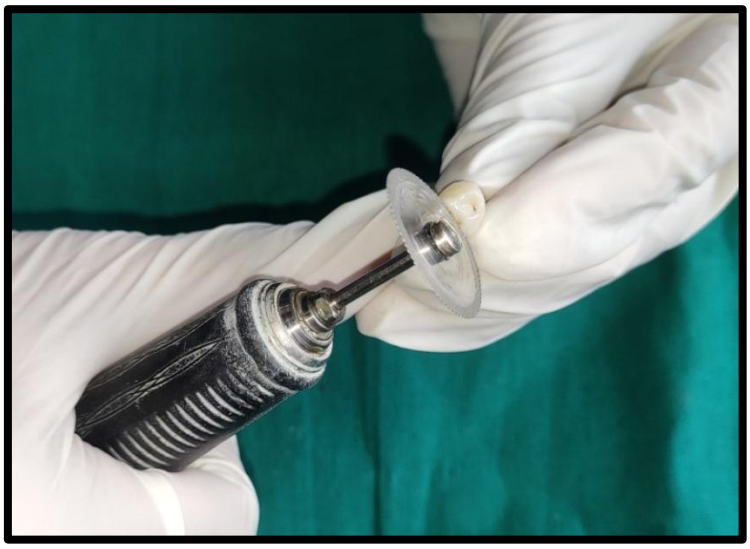


Fig. 12: Sectioning of tooth

vi) Preparation of the slides :

- The obtained sections were cleaned in the alcohol for 10 seconds and xylene for 30 seconds to remove debris and mount on glass slides using DPX (Fig. 13 A-C).
- The slides prepared from the premolars of the patient were labelled as Group I, II and III along with name of patient (Fig. 14).
- The mounted tooth sections were viewed under polarized light microscope, to confirm the zones of demineralization in all the samples.

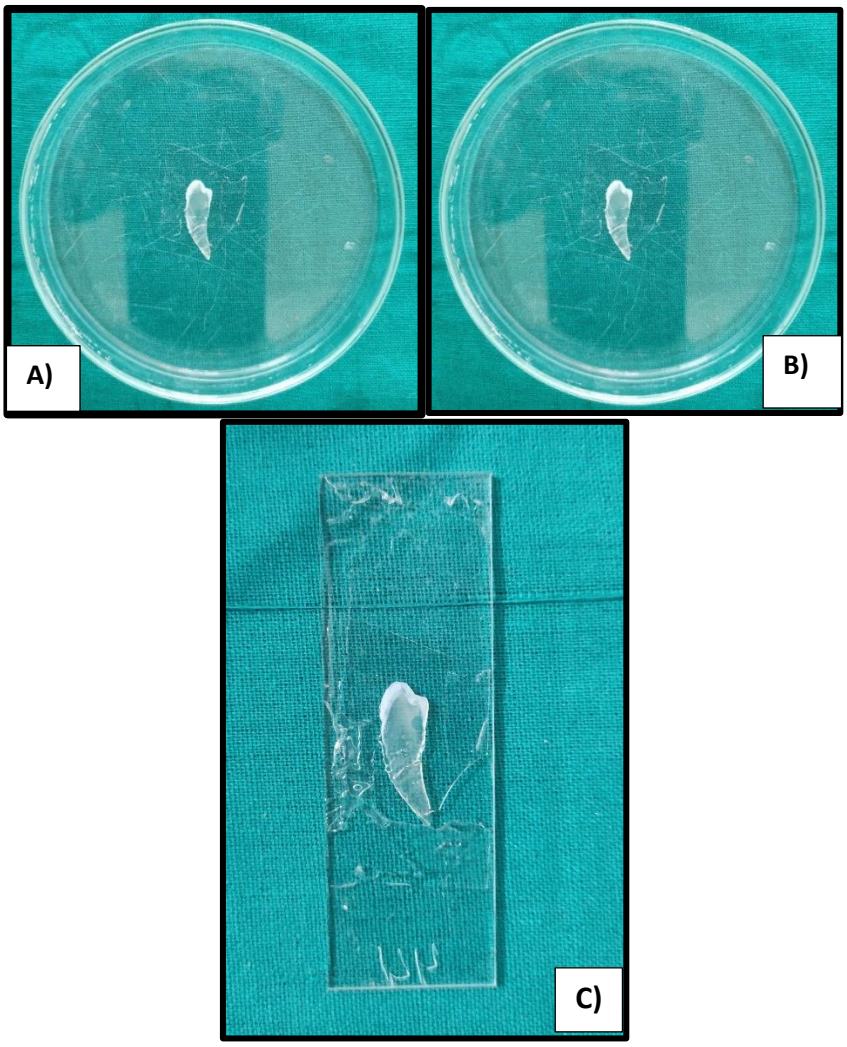


Fig. 13: Preparation of slide

A) Sectioned tooth dipped in alcohol
B) Sectioned tooth dipped in xylene
C) Mounting of sectioned tooth using DPX

Group I

Group II

Group III

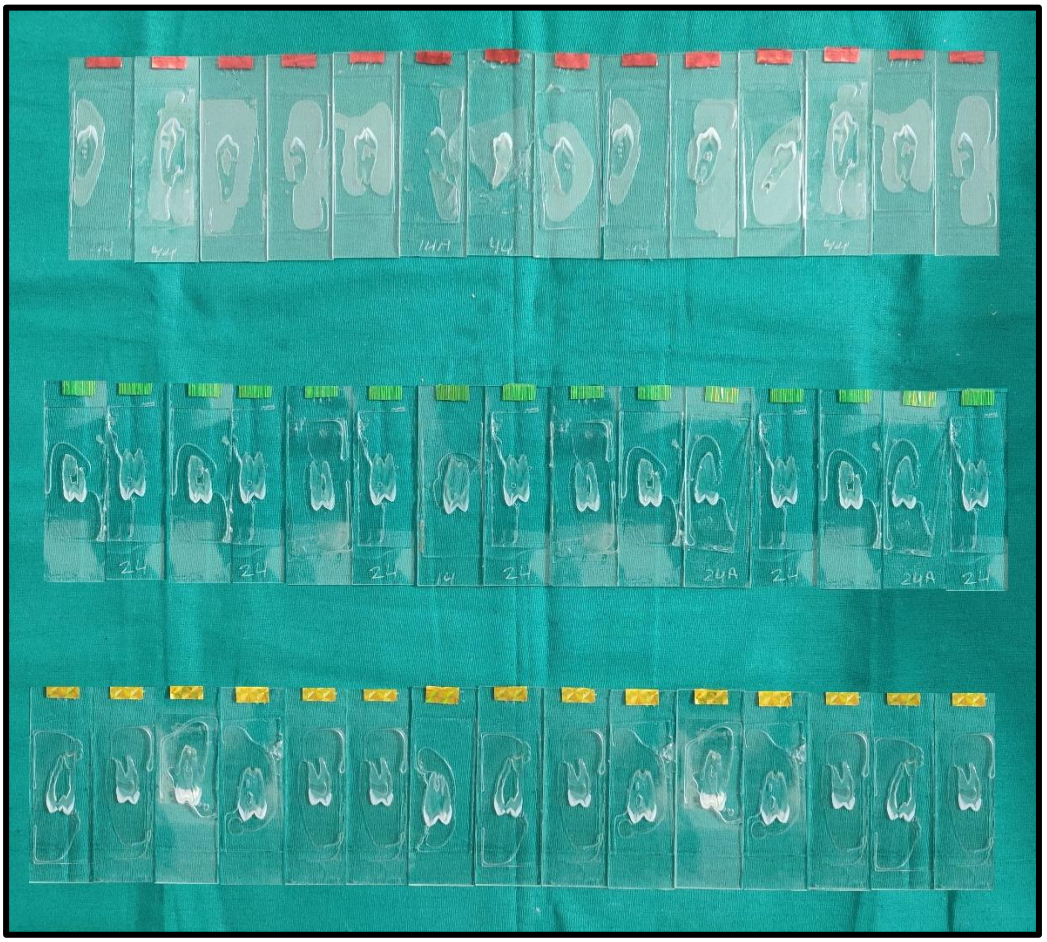


Fig.14:Slide with mounted section of specimen of all the groups

A) Group I (Red): Control group with no varnish

B) Group II (Green): Experiment group with application of Profluoride Varnish

C) Group III (Yellow): Experimental group with application of MI Varnish

vii) Measurement of the lesion depth

- The demineralized enamel lesions were examined under the polarized light microscope (PLM BX51-P) with water as imbibed medium.
- The polarized light enabled differentiation of the enamel lesions in colours.
- The maximum depth of demineralization, whether seen gingivally or occlusally to the area of bonded brackets was selected as appropriate site for microphotographs with fixed magnification of 25 times (Fig. 15 A).
- The lesion depth was measured from the surface of the tooth to the maximum depth using the Image J software (Java based image processing program) (Fig. 15 B).

- The mean of 3 measurements was recorded as the mean depth demineralization for that section.
    - The obtained data was tabulated and various statistical analysis was do

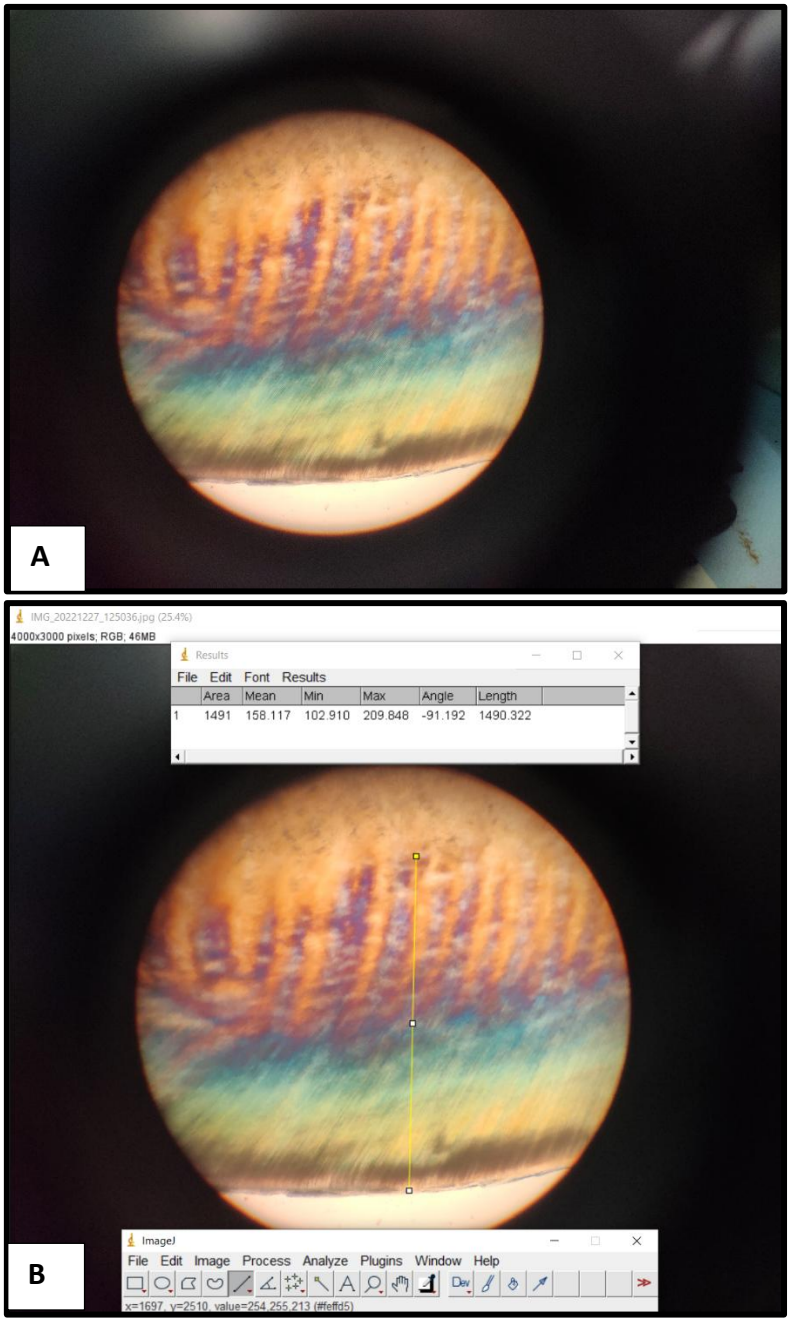


Fig. 15: Visualization and measurement of demineralization depth

A) Microphotography of area as visualized in polarized light microscope

B) Measurement of demineralization depth using ImageJ software

TOOLS FOR STATISTICAL ANALYSIS

Formula used for the analysis

A. The Arithmetic Mean

The most widely used measure of central tendency is arithmetic mean, usually referred to simply as the mean, calculated as

$$X = \frac{\sum_{i=1}^{n} X_i}{n}$$

B. The Standard Deviation

The standard deviation (SD) is the positive square root of the variance, and calculated as

$$SD = \sqrt{\frac{\sum X_i^2 - \frac{(\sum X_i)^2}{n}}{n-1}}$$

where, n= no. of observations

and also denoted by subtracting minimum value from maximum value as below

C. Tests of significance

Test of significance are used to estimate the probability that the relationship observed in the data occurred purely by chance was there a relationship between the variables. They are used to test the hypothesis proposed at the start of the study.

In this study Parametric tests were used

a) **The data was normally distributed**
b) **The data was obtained from the sample which is randomly selected**
c) **The data was quantitative data**

I. ANALYSIS OF VARIANCE

Analysis of variance (ANOVA) is used when we compare more than two groups simultaneously. The purpose of one-way ANOVA is to find out whether data from several groups have a common mean. That is, to determine whether the groups are actually different in the measured characteristic. One way ANOVA is a simple special case of the linear model. For more than two independent groups, simple parametric ANOVA is used when variables under consideration follows Continuous exercise group distribution and groups variances are homogeneous otherwise non parametric alternative Kruskal-Wallis (H) ANOVA by ranks is used. The one way ANOVA form of the model is

$Y_{ij} = \alpha_{.j} + \varepsilon_{ij}$

where:

- Y_{ij} is a matrix of observations in which each column represents a different group.
- $\alpha_{.j}$ is a matrix whose columns are the group means (the "dot j" notation means that α applies to all rows of the j^{th} column i.e. the value α_{ij} is the same for all i).
- ε_{ij} is a matrix of random disturbances.

The model posits that the columns of Y are a constant plus a random disturbance. We want to know if the constants are all the same.

Assumptions are:

a) Response variable must be normally distributed (or approximately normally distributed).
b) Samples are independent.
c) Variances of populations are equal.
d) The sample is a simple random sample (SRS).

Two-way anova is used when we have one measurement variable and two nominal variables, and each value of one nominal variable is found in combination with each value of the other nominal variable. It tests three null hypotheses: that the means of the measurement variable are equal for different values of the first nominal variable; that the means are equal for different values of the second nominal variable; and that there is no interaction (the effects of one nominal variable don't depend on the value of the other nominal variable). When we have a quantitative continuous outcome and two categorical explanatory variables, we may consider two kinds of relationship between two categorical variables, In this relationship we can distinguish effect of one factor from that of the other factor. This type of model is called a **main effect model** or **no interaction** model.

Tukey Multiple Comparison Test

After performing ANOVA, Tukey HSD (honestly significant difference) post hoc test is generally used to calculate differences between group means as

where,

$$q = \frac{\bar{X}_1 - \bar{X}_2}{SE}$$

$$SE = \sqrt{\frac{S^2}{2}\left(\frac{1}{n_1} + \frac{1}{n_2}\right)}$$

S^2 is the error mean square from the analysis of variance and n_1 and n_2 are number of data in group 1 and 2 respectively.

Statistical significance

Level of significance "p" is level of significance signifies as below:

$p > 0.05$ Non significant (ns)

$p < 0.05$ significant (*)

Measurement of reliability

To check the reliability of measurement, depth of demineralization was measured again for the 5 randomly selected sample.

Table 2: table for measurement of reliability

SL No.	1st measurement	2nd measurement
1.	1037.57	1037.08
2.	922.70	923.57
3.	912.42	911.97
4.	861.06	860.53
5.	828.08	828.22
P value	P = 0.128	

P > 0.05 (Non significant)

No significant difference was seen in the depth of demineralization between first and second set of measure thereby suggesting that measurements were accurate and reliable.

OBSERVATION AND RESULTS

This split mouth study was conducted in the Department of Orthodontics and Dentofacial Orthopedics, Babu Banarasi Das College of Dental Sciences, Lucknow in collaboration with Department of Oral Pathology, with an aim to evaluate and compare the effect of two different varnishes (MI Varnish and VOCO Varnish) in prevention of demineralization of enamel when applied around orthodontic brackets bonded to maxillary 1st premolar teeth scheduled for extraction after 3 months using polarized light microscope.

Mounted sections were visualized under the polarized light microscope and area that showed deepest demineralization depth were selected and photographed. Three readings were measured for picture of each specimen on Image J software and mean was taken as mean demineralization depth for that specimen.

Table 3: Descriptive statistics for Group I, II & III and inter group comparison using ANOVA

Group	N	Mean ± SD (µm)	Std. Error (µm)	95% Confidence Interval for Mean		Minimum (µm)	Maximum (µm)
				Lower Bound	Upper Bound		
Group I (Control)	15	1744.0703 ± 149.31	38.55186	1661.3847	1826.7558	1490.87	1935.31
Group II (VOCO Varnish)	15	1063.6498 ± 160.1	41.34004	974.9842	1152.3154	817.86	1354.05
Group III (MI Varnish)	15	940.4497 ± 96.06	24.80322	887.2521	993.6473	787.45	1139.68
Total	45	1249.3899 ± 381.97	56.94218	1134.6305	1364.1493	787.45	1935.31
P value							0.001*

P>0.05 Non significant, P<0.05 Just significant, P<0.01 Significant, P<0.001 Highly significant

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Table 2 showed that demineralization was evident in control group as well as in both experimental groups. There was increased demineralization in control group that was effectively reduced by both the varnishes. The mean depth of demineralization was highest for Group I and was 1744.07±38.55 µm with 1490.87 µm as minimum and 1935.31 µm as maximum value in this group. This was followed by Group II that had mean depth of demineralization as 1063.64±160.1 µm with 817.86 µm as minimum and 1354 µm as maximum value. Group III had the least mean depth of demineralization i.e., 940.44±96.06 µm with minimum value as 787.45 µm and maximum value as 1139.68 µm.

Overall intergroup comparison for efficacy of varnish used in the prevention of demineralization of enamel during fixed Orthodontic treatment showed statistically highly significant difference in the demineralization depth of the lesion between the groups when compared using one way ANOVA test (P value = 0.001).

Table 4: Post hoc pairwise comparison between Groups for efficacy of varnishes used in the prevention of demineralization of enamel during fixed Orthodontic treatment

Group	Mean Difference (µm)	Std. Error (µm)	P value	95% Confidence Interval	
				Lower Bound	Upper Bound
Group I vs Group II	680.42047	50.40135	.001*	557.9707	802.8702
Group I vs Group III	803.62060	50.40135	.001*	681.1708	926.0704
Group II vs Group III	123.20013	50.40135	.048*	.7504	245.6499

P>0.05 Non significant, P<0.05 Just significant, P<0.01 Significant, P<0.001 Highly significant

Table 3 showed that on individual intergroup comparison using post hoc pairwise statistical test, minimum lesion depth was seen in MI Varnish group followed by VOCO Varnish and maximum lesion depth was seen in control group. Group I had higher mean depth of demineralization than Group II (Group I > Group II) with the mean difference of 680.42 µm, and mean difference was statistically highly significant (P value = 0.001). Group I had higher depth of demineralization than

Group III (Group I > Group III) with the mean difference was 803.62 µm, and mean difference was statistically highly significant (P value = 0.001). Group II had higher depth of demineralization than Group III (Group II > Group III) with a mean difference of 123.2 µm, and mean difference was statistically significant (P value = 0.048).

DISCUSSION

White spot lesions (WSLs), seen as an unfortunate sequel of Orthodontic treatment in areas around Orthodontic brackets, is clinically defined as opaque, white areas caused by the loss of minerals from sub surface-enamel. This occurs as a result of accumulation of acidic by products of bacteria and plaque which create acidic environment in the oral cavity resulting in demineralization of enamel surface. As pH returns to normal levels, an ion from saliva deposit on the enamel surface and it is remineralized. This process of demineralization and remineralization is a continuous and dynamic process that maintains the integrity of enamel in the oral cavity. This balance can be disturbed readily in individual undergoing fixed Orthodontic treatment as attachment of brackets and bands on the teeth act as retentive sites for plaque deposition thereby promoting demineralization.

Fluoride had been mainstay of treatment of white spot lesions. Most of the previous studies had demonstrated the efficacy of fluoride mouthwashes, gels and varnishes in preventing demineralization and dissolution of enamel to variable extent. Fluoride mouth rinses are not site specific and are low in concentration to provide a desired effect. Fluoride gels have higher concentration than mouth rinses but cannot adhere to the tooth structure hence, fluoride varnishes that have higher concentration than gels and can adhere to tooth surface in a thin layer had been selected in the present study. The main action of fluoride products is preventing demineralization by promoting formation of fluorapatite crystals in enamel which is more resistant to acid attack. Fluoride also affects the activities of cariogenic bacteria and prevents caries. However, role of F^- as remineralizing agents is limited as it can't provide Ca++ and PO_4 ions required for remineralization.

Thus, the second most important treatment modality for prevention of white spot lesions is decreasing demineralization and promotion of remineralization by various remineralizing agents (Bioactive glass, Tricalcium phosphate, Calcium sucrose phosphate, Xylitol, Casein phosphopeptide amorphous calcium phosphate). CPP-ACP in a paste form had been used commonly as remineralizing agent and had been found to be efficacious. CPP-ACP is an amorphous form of calcium phosphate (ACP) stabilized by a phosphopeptide from the milk protein casein (CPP). CPP increases the level of calcium phosphate ions in plaque by adhering to ACP, thereby affecting demineralization and remineralization process of enamel. It has been

reported that CPP-ACP interacts with fluoride ions to produce novel nanoclusters of calcium, phosphate, fluoride ions and providing added anticariogenic effect. Various studies found CPP-ACP to be efficacious in preventing white spot lesions. CPP-ACP products available as a paste, again needed patient compliance, hence availability of CPP-ACP complex along with fluoride as varnish might be better in preventing white spot lesions, so it was decided to evaluate its efficacy in this study.

The efficacy of different materials in preventing white spot lesions is generally evaluated by visualizing or measuring extent of enamel demineralization with the help of light microscope, electron microscope, scanning electron microscope, transmission electron microscope, polarized light microscope, laser fluorescence, contact micro-radiography, etc. Amongst these methods polarized light microscope, that is widely used to measure depth of demineralization of white spot lesions was selected in present study. Polarized light microscope is a contrast-enhancing technique that improves the quality of the image obtained with birefringent materials and is also capable of providing information on absorption colour and optical path boundaries between minerals of differing refractive indices, like enamel and dentin in tooth. Birefringence from enamel comes from the minerals (intrinsic birefringence, with negative sign) and the non-mineral volumes (from birefringence, with positive signs), so that the image of enamel in Polarized light microscope (the observed birefringence) is a result of the sum of these two types of birefringence. Thus, it was selected for the present study.

In-vitro studies have the advantage of standardization of research study with less number of confounding factors present in in-vivo studies like bias because of sex, oral hygiene maintenance, cooperation, tooth structure variability, fluoride uptake from other sources, composition of saliva etc. However, in-vitro studies had various limitations as, clinically the tooth is never exposed to such acidic pH like that of artificial demineralizing solutions, and the role of modifying factors like diet, pH and viscosity of the saliva etc cannot be considered in these studies. To overcome these limitations, in-vivo studies or split mouth study design had been used to assess the efficacy of various agents to prevent white spot lesions. Visual inspections involved assessment of coronal portion of teeth that is graded using International Caries Detection and Assesment System (ICDAS). DIAGNOdent works by illuminating the tooth using LASERS to stimulate fluorescence in the near infrared region. The clean

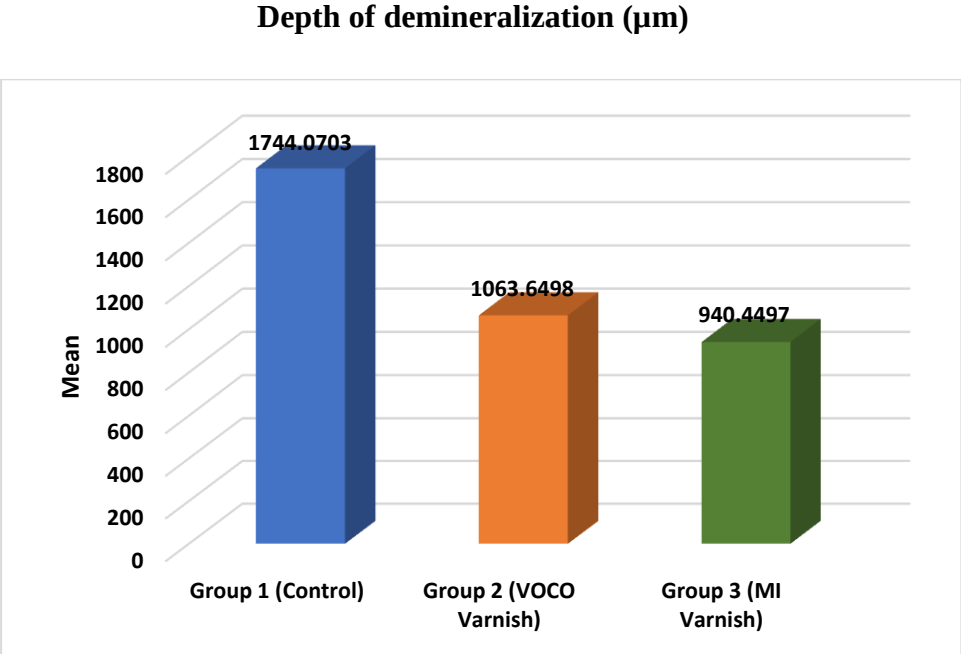
healthy tooth structure exhibits little or no fluorescence, whereas, carious tooth structure exhibits fluorescence, proportionate to the degree of caries. Quantitative Light-induced Fluorescence (QLF) is a phenomenon by which an object is excited by a particular wavelength and emits light at higher wavelength. When the excitation light is in the visible spectrum, the auto-fluorescence will be of a different color. Demineralization of enamel results in a reduction of this auto- fluorescence. The results were conflicting in studies where single method was used. Hence, **Pretty, Lussi et al**[92,93], suggested that the combined use of technology-based methods that is DIAGNOdent or QLF along with visual assessment would be the best approach for evaluating WSLs. Despite of this, the studies where two methods were used the results were still conflicting. Some of these studies did not show statistically significant reduction in WSLs in comparison to that of control, whereas in-vitro studies always demonstrated statistically significant improvement in the severity of WSL treated by various remineralizing agents. The reason for this could be that these clinical trials or in-vivo studies used visual method of evaluation and histological changes were not assessed. Whereas, in in-vitro studies teeth were exposed to harsher acidogenic challenges and histological changes that begins in subsurface enamel is much than what is seen clinically and were evaluated using different microscopic techniques. Hence, the in-vivo studies where patients apply remineralizing agents clinically and changes are seen histologically would best assess the efficacy of remineralizing agents. The split mouth study design is a type of in-vivo study where different quadrants of mouth are used for applying different preventive agents, and this was selected in present study to evaluate and compare efficacy of two different varnishes in preventing white spot lesions in comparison to control.

The present study was conducted in the Department of Orthodontics and Dentofacial Orthopedics, Babu Banarasi Das College of Dental Sciences, Lucknow in collaboration with Department of Oral pathology with an aim to compare the effect of two different varnishes (MI Varnish and VOCO Varnish) in prevention of demineralization of enamel when applied around Orthodontic brackets bonded to maxillary 1st premolar teeth scheduled for extraction after 3 months using polarized light microscope. The sample consisted of 15 patients with varying malocclusion coming to the Department of Orthodontics, for fixed Orthodontic treatment in whom all four 1st premolar teeth had been scheduled for extraction as a part of fixed

Orthodontic treatment. The sample was divided into 3 groups, Group I was control i.e., mandibular 1st premolar of right & left side where no varnish was applied. Group II was experiment group which included maxillary 1st premolar of left side where VOCO Varnish was applied and in Group III, also an experimental group included maxillary 1st premolar of right side where MI Varnish was applied. The procedure included bonding of 1st premolar following standard protocol with other teeth in beginning of fixed Orthodontic treatment, selected varnish was applied on their respective maxillary first premolar in a thin layer around the base of the brackets. A total of 60 teeth (30 maxillary and 30 mandibular 1st premolars), were obtained on extraction after 3 months following application of two different varnishes on right or left premolars of maxillary arch. Out of 30 extracted mandibular premolars 15 were used in the study. The extracted teeth were cleaned of debris, stored properly in 10% formalin, and finally were sectioned (buccolingually) using micro-motor. The obtained ground sections were cleared in alcohol and xylene and were mounted on the glass slide.

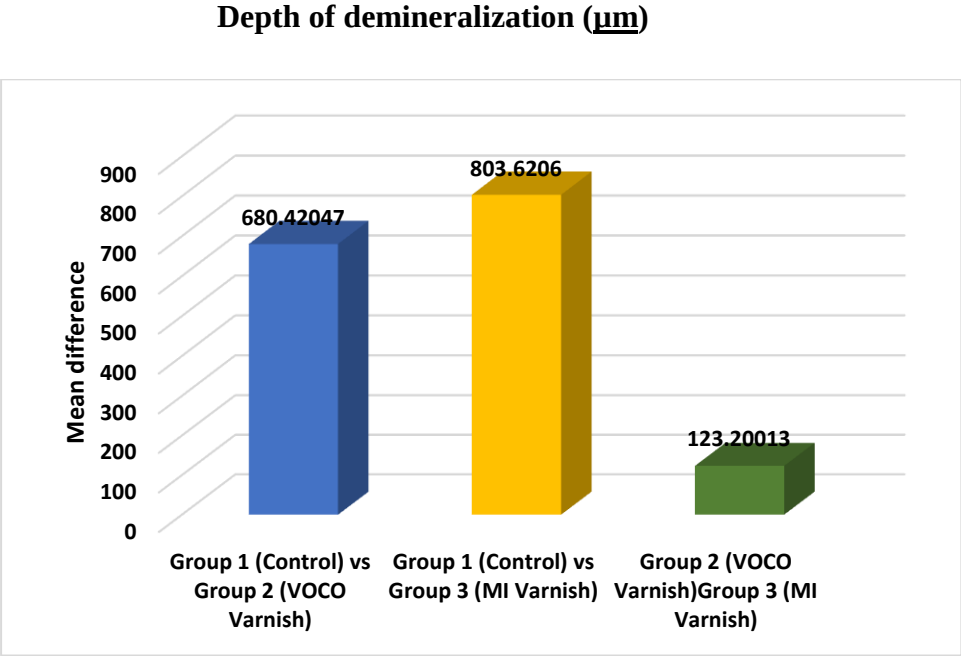
Mounted sections were visualized under the polarized light microscope and area that showed deepest demineralization depth were selected and photographed. Three readings were measured for picture of each specimen on ImageJ software and mean was taken as demineralization depth for that specimen. Data was tabulated and adequate statistical comparisons was made between groups.

The results of the present study suggested that with fixed Orthodontic treatment demineralization was evident, both in control group as well as experiment groups and both varnishes were found effective in reducing demineralization in experimental groups. The mean depth of demineralization was highest for Group I – Control group (1744.07±38.55 µm), followed by Group II – VOCO Varnish (1063.64±160.1 µm) and least in Group III – MI Varnish (940.44±96.06 µm). (Group I > Group II > Group III) and difference between the groups was statistically significant (P value = 0.001), (Table 2; Graph 1).



Graph 1: Bar diagram depicting mean depth of demineralization of three groups

On individual intergroup comparison using (Post Hoc pairwise statistical test), it was seen that Group I had higher mean depth of demineralization than Group II (Group I > Group II) with the mean difference of 680.42 µm, Group I had higher depth of demineralization than Group III also (Group I > Group III) with the mean difference was 803.62 µm, and this was statistically significant (P value < 0.001). Group II had higher depth of demineralization than Group III (Group II > Group III) with a mean difference of 123.2 µm, which was statistically non-significant (P value = 0.48), (Table 3; Graph 2).



Graph 2: Bar diagram depicting comparison of efficacy of varnishes between groups

The results of present the present study was compared with various in-vitro and in-vivo studies conducted using different varnishes in preventing white spot lesions and efficacy had been evaluated assessing depth of demineralization using Polarized light microscope or Scanning electron microscope or microhardness or visual examination or bacterial count. Also, result was compared to other studies that checked efficacy of different modalities in preventing white spot lesions.

An in-vivo study using similar split mouth design conducted by **Farhadian et al**[11] where 15 patients who needed atleast 2 premolars extraction for Orthodontic reasons were selected and only test group (1st premolar) received fluoride varnish (Bifluoride12, 6% calcium fluoride and 6% sodium fluoride; Voco; Cuxhaven, Germany) whereas other side served as control. The premolars were extracted after 85 to 95 days, and buccolingual sections were evaluated with polarized light microscopy. The mean depth was 57.0±5.5 µm in the test group and 94.3±6.7 µm in the control group. Similar to present study, there was significant reduction in depth of demineralization in the test group. The mean depth of demineralization was lesser than our study, for fluoride group that could be due to variability in adjunctive regimen in form of fluoridated toothpaste (250 ppm) in their study whereas non-fluoridated toothpaste was provided in present study.

Habeeb et al[37] conducted a split-mouth study and used 100 orthodontic patients, allocated randomly into 2 subgroups with various frequencies of MI Varnish application (Group I – once every 3 months and Group II – once every 6 months). Varnish was applied to 1st and 3rd quadrants, with quadrant 2nd and 4th as untreated control. Measurements were taken for group I and group II with interval of 3 and 6 months respectively for one year using digital vernier calliper and then subject to statistical analysis. Statistically significant difference was present among group I and group II when MI Varnish was applied 2 times or 4 times in a year. After 6 months at 9 month and 12 month there was no significant difference in the demineralization between group I and group II.

Another in-vitro study conducted by **Abufarwa et al[27]** where efficacy of MI varnish (CPP-ACP) as used in our study was assessed in comparison to control group on extracted molars and premolars. Standardized pre-treatment images of enamel surface were obtained using FluoreCam and repeated at 2, 4, 6, 8, and 12 weeks, and specimens were placed in toothbrushing simulator, thermocycled, at regular intervals. Also, few samples were sectioned and examined under polarized light microscope (PLM) evaluation. On examination using FluoreCam, it was seen that groups did not differ for area, intensity, or impact at pre-treatment, however in control group demineralization increased at every point significantly, whereas in experimental group, area decreased significant till 4 weeks and effect of varnish was nullified after 4 weeks with no difference as compared to pre-treatment and later demineralization increased as time passed i.e., 8 and 12 weeks. PLM of the control and experiment group revealed lesion depth 90±34 µm and 37±9 µm, respectively (control group > experimental group) but did not apply any statistical test as measurements were made only for 5 samples.

An in-vitro study was conducted by **Rani et al[31]** on 80 healthy premolars where a window of 4x4 mm was made on buccal surface of each tooth and was immersed for 48 hours in demineralizing solution and then allocated into the four groups : Group I – (Control), Group II – (Duraphat Varnish), Group III – (MI Varnish), and Group 4 – (Clinpro White Varnish). The anticariogenic effectiveness of different fluoride varnishes was evaluated using scanning electron microscope. Demineralization as assessed after creating artificial caries like lesion was statistically non-significant between the groups, thus similar amount of demineralization was seen

in all groups pre to application of varnish. Depth of demineralization reduced after application of various agents, least depth of demineralization was seen with the use of MI Varnish (92.40 ± 0.09 µm), followed by the Duraphat varnish (106.68 ± 0.12 µm), Clinpro White Varnish (112.36 ± 0.08 µm),and then the control group (123.08 ± 0.18 µm), respectively. Statistically significant differences were noted between the experimental groups employing the various fluoride varnishes (p <0.001). The current research concluded that MI Varnish group presented a superior protective potential in comparison with Duraphat Varnish and Clinpro White Varnish group as also seen in present study where MI Varnish had less demineralization depth than fluoride varnish and control group.

Shahmoradi et al[33] investigated the efficacy of various fluoride varnishes by randomly allocating 60 extracted premolars into 5 different groups - Group 1: Control, Group 2: Clinpro White Varnish, Group 3: Duraphat fluoride varnish, Group 4: MI Varnish, and Group 5: Duraphat single dose fluoride varnish. Micro-CT and colour-coded images of the enamel lesions after varnish application and initial acid attack were taken. The average depth of the lesion with no varnish treatment was 86±7.19 µm that reduced maximally by 19.74±4.51 µm in Clinpro White Varnish group, followed by MI Varnish 18.50±2.39 µm, followed by Duraphat single dose 17.11±5.33 µm, followed by control 2.52±1.79 µm. Comparison of the depth and mineral density of the lesions treated with different varnish types indicated no significant difference in depth reduction and mineral density preservation among different groups (P > 0.05) treated with any type of varnish, thus all were effective in preventing demineralization. The results of this was contrary to the results of the present study as we found statistically significant in efficacy of MI Varnish and VOCO Varnish.

An in-vitro study conducted by **Mashhour et al[32]** on 48 extracted primary molars divided into four groups as - Group1: Clinpro white varnish, Group 2: MI Varnish, Group 3: Silver Diamine Fluoride (SDF), and Group 4: Control. After pH cycling, the mineral content of specimen was evaluated by Energy Dispersive X-ray Spectrometer and the lesion depth was assessed by Polarized Light Microscope. MI varnish showed the highest mean calcium (Ca) ion content (66.57 ± 0.63µm), and Ca/P (2.19 ± 0.11 µm), followed by Clinpro white varnish, and SDF and then control. Similarly, MI varnish also displayed the highest phosphate (P) ion content (31.46 ±

0.56 µm), followed by SDF (30.93 ± 1.02 µm), and Clinpro white varnish (30.53 ± 2.19 µm). The lowest mean lesion depth was found in MI varnish (226.23 ± 44.25 µm) which was significantly lower than Clinpro white varnish (285.43 ± 44.70 µm), SDF (293.32 ± 46.82 µm), and control (576.69 ± 42.66 µm) and statistically significant difference in lesion depth was found between different groups. This is similar to our study where MI Varnish had lesser demineralization depth than fluoride varnish and others.

An in-vitro study was conducted by **Varma et al[30]** on 30 freshly extracted maxillary/mandibular premolar teeth. The teeth were immersed in 30 separate plastic containers containing 4 ml of demineralizing solution to produce artificial caries like lesion. Teeth were then taken out and divided into three groups. Group I – coated with MI Varnish, Group II – coated with Clinpro XT Varnish, and Group III – Control group. After seven cycles of remineralization, the surface was assessed using Diagnodent. The maximum remineralization was seen in group I (4), followed by group II (5.8) and then group III (7.5). There was a significant difference among the three study groups ($p<0.0001$). The finding showed that MI Varnish containing CPP-ACP had the highest release of fluoride as compared to the Clinpro fluoride releasing varnish.

Contrary results were seen in a split mouth study conducted by **Perrini et al[82]** where 24 Orthodontic patients, allocated randomly into 2 subgroups with differing frequencies of Duraphat Varnish application (every 3 months and every 6 months). Measurements was taken for visualized demineralization at vestibular surface (gingival, occlusal, mesial, distal) of six selected teeth i.e. central incisor, canine, and 2nd premolar using a DIAGNOdent Pen 2190 laser. The statistically significant reduction in demineralization was seen on maxillary and mandibular central incisor but not in other teeth thus overall comparison between treated and untreated teeth did not show statistically significant reduction in demineralization. Also, they hardly found any difference between 3 months, and 6 months fluoride application. The contrary results could be attributed to difference in way of detecting enamel demineralization i.e. using DIAGNOdent pen vs. polarized light microscope in present study. Also at each follow up visits, vestibular surfaces of all the teeth were cleaned using a brush mounted on low-velocity contra-angle handpiece, and residual varnish was removed with an air polishing system to prevent its being as false

positive. However, this might also have reduced efficacy of fluoride varnish later on whereas in present study, no clean-up was performed during test period (3 months).

Sonesson et al[89] conducted a randomized controlled triple-blinded trial on 182 healthy adolescents (12-18 years), randomly allocated to a test or a placebo group. All patients were seen every 6th week for wire adjustments. At the end of each visit test (Fluor protector S-7700 ppm fluoride) or placebo varnish (no added fluoride) was applied in a thin layer around the bracket base on the maxillary teeth. The number of actual varnish applications was recorded in the digital records of each patient. The primary outcome was assessed from digital photographs. The labial surfaces of incisors, cuspids, and premolars were experimental sites and scored as: 1 = no white spot formation; 2 = slight white spot formation; 3 = excessive white spot formation; 4 = white spot formation with cavitation. The prevalence of WSL on surface level was 21.3% with no difference between the groups. The lateral incisors and the 1st premolars were most commonly affected. The mean number of new lesions per patient was 2.0 (SD 2.6) and 1.9 (SD 2.5) in test and placebo group, respectively. The difference between the group for test score 3 was statistically significant suggestive of the fact that fluoride varnish reduced active white spot lesion comparison to placebo varnish. This could be compared to significant reduction in demineralization depth of both the varnishes in comparison to control that had highest depth of demineralization in present study.

A randomized control trial was conducted by **Yadav et al[36]** on 60 healthy children of 2-8 years, randomly allocated to three groups equally. Group A = MI Varnish, Group B = Profluoride, and Group C = Fluor Protector. Each group had, 2 subgroups, subgroup 1 – with prior oral prophylaxis and subgroup 2 – without oral prophylaxis. Plaque sample was collected from buccal surface of the maxillary right primary molar tooth, in sterile tubes containing reduced transport fluid initially (sample 1) followed by application of fluoride varnish and collection of samples at 1 month and 3 months (sample 2 and sample 3, respectively). MI Varnish group showed maximum reduction (41.20%) in the S. mutans (CFU/ml) from baseline to the 3rd month, followed by Profluoride (37.90%) and Fluor Protector (33.29%). There was no effect of prior oral prophylaxis on the efficacy of fluoride varnish.

An in-vitro study conducted by **Bapat et al[34]** assessed the antibacterial effects of two sodium fluoride varnishes, namely MI Varnish containing CPP-ACP and

Embrace Varnish containing Xylitol coated calcium and phosphate ions on agar disc and microhardness of extracted premolars. Disc diffusion method using on Trypticase-Yeast-Cysteine-Sucrose-Bacitracin (TYCSB) agar plate was used. On comparison of mean zone of inhibition around the discs coated with fluoride varnishes (mm), MI Varnish had a greater diameter of no growth around the sample wells thus MI Varnish showed significantly higher antibacterial effect, compared to Pulpdent Embrace Varnish ($p \leq 0.05$). For enamel surface microhardness Group A (MI Varnish) showed the highest microhardness as compared to Group B (Pulpdent Embrace Varnish) and Control.

Sharma et al[86] conducted a randomized controlled clinical trial to evaluate the efficacy of MI Varnish and Clinpro XT Varnish in reducing dentinal hypersensitivity. The teeth to be tested were isolated, then a blast of air and ice cold water was applied to measure visual analog score. The sensitivity scores were recorded immediately and after 1 week of therapy. The group for MI Varnish has a statistically significant difference in reducing dentinal hypersensitivity, suggestive of its better penetration than Clinpro XT Varnish.

An in-vitro study by **Dehailan et al[88]** to investigated the anticaries efficacy, measured as fluoride release into artificial saliva (AS); found MI Varnish's cumulative fluoride release as 14.97 µm/mL and peak fluoride concentration as 9.71 µm/mL which was higher than other fluoride varnishes. This suggests a synergistic effect of adding casein complexes to fluoride varnishes.

Another randomized clinical trial was conducted by **Radha et al[35]** to assess the remineralizing efficacy of fluoride and its combination varnishes on WSL in children with early childhood caries. It was conducted among children between 3-6 years. Children were randomly allocated to – Group I: 5% Sodium fluoride (Profluoride); Group II: 5% Sodium fluoride with ACP (Enamel Pro); and Group III: 5% Sodium fluoride with CPP-ACP (MI Varnish). In each group, a sample of 20 active WSL was included at the baseline and over a period of 24 weeks; the active status was measured at 2^{nd} week, 4^{th} week, 12^{th} week, and 24^{th} week. For MI Varnish group, there was steady reduction in number of active WSLs from 20 at baseline to 19 at 2 weeks, 10 at 4 weeks, 3 at 12 weeks and 0 at 24 weeks. This reduction was statistically significant at 4 and 12 weeks between groups, but it was

not statistically significant at 24 weeks. The reduction in dimension of WSLs was high in Group III compared to Group I and Group II.

Few studies evaluated remineralization potential of agents by measuring penetration depth of concerned material. An in-vitro study was conducted by **Edunoori et al[91]** on a sample of 40 freshly extracted premolar teeth on which artificial WSLs were created on buccal surface of each tooth by immersing them in a demineralization solution. Then the samples were randomly allocated to two groups of 20 each. In Group A (Icon Resin Infiltration) was applied followed by light curing and in Group B (Clinpro XT Varnish) was applied followed by light curing. Polarized light microscope at 4x magnification used for determining the depth of penetration of the materials. The penetration depth was determined by measuring the width of remineralization at three different areas in a lesion and the average value was considered as the mean penetration depth of the materials into the lesion. In Group A sample, the mean penetration depth in first, second, and third areas were 19.70 µm, and 14.19 µm, and 17.32 respectively, whereas in Group B sample, the mean penetration depth in the first, second, and third area were 7.92 µm, 7.63 µm, and 7.49 µm, respectively. When compared to Clinpro XT group (7.68±1.81 µm), the Icon group showed a greater mean penetration depth (17.07±4.35 µm), which indicate that icon group had greater infiltrative and remineralizing capacity when applied to demineralized enamel.

Tuloglu et al[29] conducted an in-vitro study with an aim to investigate the effects of three fluoride varnish MI Varnish (1–8 % sodium fluoride and 1–5 % CPP-ACP), Clinpro White (1–5 % sodium fluoride and <5 % modified tricalcium phosphate), Duraphat (<5 % sodium fluoride). Enamel specimens was 40 primary incisors (for surface microhardness testing) and 40 primary molars (for demineralization depth measurement). The lowest changes in surface microhardness occurred in MI varnish group (-20.80 VHN), followed by the Clinpro White (-34.60 VHN), Duraphat (-57.80 VHN) and then no treatment (control) group (-73.40 VHN). Statistically significant difference was in microhardness was seen from pre to post pH cycling for all the varnishes. Similar to present study demineralization depth was least in MI varnish group (23.60 µm), followed by Clinpro white (29.85 µm), Duraphat (40.37 µm) and control group (54.56 µm) and difference in mean depth of demineralization was statistically significant between groups. Scanning electron

microscopy examination revealed smooth surface of enamel in varnish group and cracks were seen in control group.

An in-vitro study was conducted by **Todd et al[6]** where brackets were bonded to 36 extracted human canines and premolars with traditional composite resin and randomly assigned to three equal groups of twelve. Group 1 served as control with no topical application after bonding. Group 2 was treated with single application of nonfluoridated placebo varnish. Group 3 was treated with a single application of fluoride varnish (Duraflor). All groups were cycled in a artificial caries challenge for 1 hour two times daily for 37 days and were brushed with a medium bristled toothbrush to simulate mechanical wear of the varnish. Demineralization of enamel were evaluated in longitudinal buccolingual tooth sections using polarized light microscope with mean depth of demineralization for control group (166.8µm), placebo varnish group (197.8µm), and fluoridated varnish group as (84.4µm). Demineralization depth was least in fluoride varnish group and was highest in placebo varnish group. The placebo varnish group in this study had a significantly greater amount of demineralization than that of non-fluoridated control group. The increased demineralization with the placebo varnish group over control group may be explained by the fact that the colonophony base of varnish has acidic properties. The low pH and the length of contact time with enamel explains why the placebo varnish group exhibited more demineralization than the control group that received no varnish and no fluoride.

An in-vitro study was conducted by **Babu et al[90]**, where they compared surface microhardness (SMH) on 90 extracted premolar teeth samples for three different varnishes. Varnish containing CPP-ACP with fluoride was applied on samples of group 1 and varnish containing only fluoride was applied on samples of group B and Group C was served as control group; without any varnish application. The mean values of VHN (Vickers Hardness Number) for Group A were 488±6, Group B were 485±12, and Group C were 448±12. On intercomparison between three groups, the mean difference of SMH of enamel Group A and B was not statistically significant (P = 0.35), however the mean difference between Group A and C and between Group B and C were statistically significant.

Similar to results of present study where VOCO varnish, a type of fluoride varnish was used and had better efficacy in reducing demineralization depth than control,

previous studies had also found fluoride varnish to be efficacious in terms of reduction of decalcification score, prevalence of visible white spot lesions, progression score of WSLs during Orthodontic treatment, demineralization depth. Its proven that fluoride or CPP-ACP in form of toothpaste, lozenges, mouthrinses, chewing gums, sealants, etc had effectively reduced WSLs and can be used to prevent or treat developing WSLs (**Cai et al[61], Reynolds et al[25], Rajan et al[24], Rebertson et al[23], Gurunath et al [26]).**

Overall conclusions drawn from comparing with previous studies is that efficacy of fluoride varnish or CPP-ACP varnish with fluoride was conflicting. The studies where visual examination was done or were examined using fluorescence as in DIGNOdent etc., difference was not statistically significant with control in all studies. However, in studies where demineralization depth was measured, irrespective of whether in-vitro in-vivo study design, there was statistically significant reduction in WSLs with these varnishes. The reason for better efficacy of MI Varnish over fluoride varnish could be understood better after knowing their mechanism of action.

The greater localized concentrations in the fluoride varnish produce deposits of calcium fluoride, depositing fluoride in porosities and micro-channels at different cariogenic sites in the enamel. These fluoride reservoirs gradually release fluoride into dental plaque, saliva or apatite structure of the tooth when the pH drops. Thus, the main action of fluoride products is preventing demineralization by promoting formation of fluorapatite crystals in enamel which is more resistant to acid attack. Fluoride also affects the activities of cariogenic bacteria and prevents caries. However, role of F^- as remineralizing agents is limited as it can't provide Ca++ and PO_4 ions required for remineralization.

The addition of calcium and phosphate ions for remineralization in fluoride varnish has not been successful in the past, due to the low solubility of calcium phosphates, particularly in the presence of fluoride ions. Insoluble calcium phosphates are not easily applied, do not localize effectively at the tooth surface, and require acid for solubility to produce ions capable of diffusing into enamel subsurface lesions. In contrast, soluble calcium and phosphate ions can be used only at very low concentrations, due to the intrinsic insolubility of the calcium phosphates, particularly the calcium fluorophosphates. Soluble calcium and phosphate ions do not

substantially incorporate into dental plaque or localize at the tooth surface to produce effective concentration gradients to drive diffusion into the subsurface enamel. A development of delivery system for bioavailable calcium and phosphate ions therefore may have a role as an adjunct to fluoride treatment in the management of white spot lesions. This included stabilization of Ca^2PO_4 ions needed for remineralization by ACP in presence of casein phophopeptide, a derivative of milk protein as paste, mouthwash, or varnish in market. CPP containing the active sequence –Ser(P)-Ser(P)-Ser(P)-Glu- Glu- has a remarkable ability to stabilize calcium and phosphate as nanoclusters of ions in metastable solution (Cochrane *et al.*, 2008)* and provide high concentration of Ca^2PO_4 ions at the toothpaste surface by binding to pellicle and plaque. Through the active sequence, the CPP binds to forming nanoclusters of calcium and phosphate ions to form nanocomplexes of around 1.5 nm radius, preventing the growth of the nanoclusters to the critical size required for nucleation and phase transformation. Although the calcium and phosphate ions are stabilized by the CPP, the ions are freely bioavailable to diffuse down concentration gradients into enamel subsurface lesions, thereby effectively promoting remineralization *in vivo*. CPP helps the ACP to bind with the dental enamel and also it decreases the count of Streptococcus mutans by integrating in the pellicle and inhibiting the adherence and functioning of cariogenic bacteria (Streptococcus mutans). Also, casein is able to buffer plaque acid either directly or indirectly through bacterial catabolism. This agent can release amino acids and thus accept protons acting as buffers and prevents demineralization. On addition of fluoride ions to CPP-ACP formation, besides availability of Ca^2PO_4 ions for remineralization, bioavailability of fluoride ions provides synergistic effect by reducing demineralization.

Within the limitations of present study, it can be suggested as MI Varnish reduced demineralization depth more efficiently than fluoride varnish in present as well as previous studies. MI Varnish provided synergistic effects of reducing demineralization and promoting remineralization whereas fluoride varnish, reduced demineralization with little or no remineralizing potential. According to Luccheses and Gherlone (2012)* it takes around 6 months for caries to progress in a patient not submitted to Orthodontic therapy, it takes around 1 month for those who are undergoing fixed Orthodontic treatment. Hence, the first 6 months are particular importance in development of WSLs because the majority of adolescent patients need

to adapt their hygienic practice to the requirements of Orthodontic therapy. Considering this, it is always advisable to use fluoride or CPP-ACP varnishes in subjects undergoing fixed Orthodontic treatment.

The patients having high caries index, poor oral hygiene, differently disabled or subjects who had xerostomia and are more prone to demineralization leading to development of white spot lesions during fixed Orthodontic treatment, could be benefited significantly by using MI Varnish as it was found to be more efficacious than fluoride varnishes. As application of varnishes every 3 months for 6 months had better efficacy in reducing demineralization depth than one time application in 3 months, it can be suggested to use the same for fluoride or CPP-ACP varnish clinically. The main contraindication for using CPP-ACP products is its use in subjects with lactose intolerance as CPP is a milk product, hence in such subjects conventional fluoride varnish could be used.

The major limitation of present study is that, Ca_2PO_4 or F^- release from either varnish on saliva could affect the other quadrants as well, however these limitations along with other variable like ion concentration in saliva, viscosity of saliva, extent of oral hygiene maintenance in different subjects could not be controlled in such split mouth study designs.

Future studies could aim at conducting Randomized controlled trials (RCT) on larger sample or checking efficacy of one time application in three months versus two time application every three months for first six months of fixed orthodontic treatment of fluoride varnish as well as CPP-ACP varnish with added fluoride. Also efficacy of CPP-ACP by its incorporation in other materials used during fixed orthodontic treatment like sealants, bonding agents, e-chains etc.

CONCLUSION

Following conclusion were drawn from the present study conducted with an aim to evaluate and compare the effect of two different varnishes in prevention of demineralization of enamel when applied around Orthodontic brackets bonded to maxillary 1st premolar teeth scheduled for extraction after 3 months using polarized light microscope –

1. Demineralization was evident with fixed Orthodontic treatment as highest depth of demineralization was seen in Control group.
2. Both, MI Varnish and VOCO Profluoride Varnish were effective in reducing depth of demineralization in comparison to control.
3. Depth of demineralization was highest in Group I (Control), followed by Group II (VOCO Profluoride Varnish), and least in Group III (MI Varnish) [Group I > Group II > Group III] and the difference between all the groups were statistically significant.
4. Amongst two varnishes, MI Varnish was significantly more effective than VOCO Profluoride Varnish in reducing depth of demineralization.

Within the limitation of this study, it can be suggested that MI Varnish could be considered to prevent WSLs during fixed Orthodontic treatment especially in patients having high caries index or poor oral hygiene or differently disabled or subjects who had xerostomia making more prone to demineralization.

SUMMARY

White spot lesions (WSLs), seen as an unfortunate sequel of Orthodontic treatment in areas around Orthodontic brackets, is clinically defined as opaque, white areas caused by the loss of minerals from sub surface-enamel. The process of demineralization and remineralization is a continuous and dynamic process that maintains the integrity of enamel in the oral cavity. This balance can be disturbed readily in individual undergoing fixed Orthodontic treatment as attachment of brackets and bands on the teeth act as retentive sites for plaque deposition thereby promoting demineralization.

Fluoride had been mainstay of treatment of white spot lesions. Most of the previous studies had demonstrated the efficacy of fluoride mouthwashes, gels and varnishes in preventing demineralization and dissolution of enamel to variable extent. Fluoride mouth rinses are not site specific and are low in concentration to provide a desired effect. Fluoride gels have higher concentration than mouth rinses but cannot adhere to the tooth structure hence, fluoride varnishes that have higher concentration than gels and can adhere to tooth surface in a thin layer had been selected in the present study. Fluorides have a limited remineralization potential.

Thus, the second most important treatment modality for prevention of white spot lesions is decreasing demineralization and promotion of remineralization by various remineralizing agents (Bioactive glass, Tricalcium phosphate, Calcium sucrose phosphate, Xylitol, Casein phosphopeptide amorphous calcium phosphate). CPP-ACP in a paste form had been used commonly as remineralizing agent and had been found to be efficacious. CPP-ACP is an amorphous form of calcium phosphate (ACP) stabilized by a phosphopeptide from the milk protein

casein (CPP). CPP increases the level of calcium phosphate ions in plaque by adhering to ACP, thereby affecting demineralization and remineralization process of enamel. It has been reported that CPP-ACP interacts with fluoride ions has a synergistic effect on prevention and treatment of WSL'S. CPP-ACP products available as a paste, again needed patient compliance, hence availability of CPP-ACP complex along with fluoride as varnish might be better in preventing white spot lesions, so it was decided to evaluate its efficacy in this study.

Amongst the various methods to evaluate demineralization depth, polarized light microscope, that is widely used, was selected in present study. In-vitro studies have the advantage of standardization of research study with less number of confounding factors present in in-vivo studies like bias because of sex, oral hygiene maintenance, cooperation, tooth structure variability, fluoride uptake from other sources, composition of saliva etc. However, in-vitro studies had various limitations as, clinically the tooth is never exposed to such acidic pH like that of artificial demineralizing solutions, and the role of modifying factors like diet, pH and viscosity of the saliva etc cannot be considered in these studies. To overcome these limitations, in-vivo studies or split mouth study design that had been used to assess the efficacy of various agents to prevent white spot lesions was selected in present study.

Considering this aim to evaluate and compare the effect of two different varnishes in prevention of demineralization of enamel when applied around Orthodontic brackets bonded to maxillary 1st premolar teeth scheduled for extraction, 3 months later using polarized light microscope.

The present study was conducted in the Department of Orthodontics and Dentofacial Orthopedics, Babu Banarasi Das College of

Dental Sciences, Lucknow in collaboration with Department of Oral pathology The sample consisted of 15 patients with varying malocclusion coming to the Department of Orthodontics, for fixed Orthodontic treatment in whom all four 1st premolar teeth had been scheduled for extraction as a part of fixed Orthodontic treatment. The sample was divided into 3 groups, Group I (N=15)was control i.e., mandibular 1st premolar of right or left side where no varnish was applied. Group II was experimental group which included maxillary 1st premolar of left side where VOCO Varnish was applied and in Group III, also an experimental group included maxillary 1st premolar of right side where MI Varnish was applied. The procedure included bonding of 1st premolar following standard protocol followed by application of MI Varnish and VOCO Varnish on respective premolars in a thin layer around the base of the brackets. Patients was asked to follow normal oral hygiene regime with non-fluoride tooth paste.

All 1st premolars were extracted after 3 months . teeth were cleaned of debris and were sectioned (buccolingually) using micro-motor, mounted on the glass slide and visualized under the polarized light microscope. Area that showed deepest demineralization depth were selected and photographed. Three readings were measured for image of each specimen on ImageJ software and mean was taken as demineralization depth for that specimen. Data was tabulated and adequate statistical comparisons was made between groups.

Following conclusion were drawn from the present study –

1. Demineralization was evident with fixed Orthodontic treatment as highest depth of demineralization was seen in Control group.
2. Both, MI Varnish and VOCO Profluoride Varnish were effective in reducing depth of demineralization in comparison to control.
3. Depth of demineralization was highest in Group I (Control), followed by Group II (VOCO Profluoride Varnish), and least in Group III (MI Varnish) [Group I > Group II > Group III] and the difference between all the groups were statistically significant.
4. Amongst two varnishes, MI Varnish was significantly more effective than VOCO Profluoride Varnish in reducing depth of demineralization.

Future studies could aim at conducting Randomized controlled trials (RCT) on larger sample or checking efficacy of one-time application in three months versus two-time application every three months for first six months of fixed orthodontic treatment of fluoride varnish as well as CPP-ACP varnish with added fluoride. Also, efficacy of CPP-ACP by its incorporation in other materials used during fixed orthodontic treatment like sealants, bonding agents, e-chains etc.

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ANNEXURES

ANNEXURE -I

BABU BANARASI DAS COLLEGE OF DENTAL SCIENCES (FACULTY OF BBD UNIVERSITY), LUCKNOW

INSTITUTIONAL RESEARCH COMMITTEE APPROVAL

The project titled **"Comparative Evaluation of Dental Varnishes in Prevention of Demineralization of Enamel during Fixed Orthodontic Treatment – A Split Mouth Study"** submitted by **Dr Swagat Verma** Post graduate student from the **Department of Orthodontics and Dentofacial Orthopaedics** as part of MDS Curriculum for the academic year 2020-2023 with the accompanying proforma was reviewed by the Institutional Research Committee present on **12th October 2021** at BBDCODS.

The Committee has granted approval on the scientific content of the project. The proposal may now be reviewed by the Institutional Ethics Committee for granting ethical approval.

Prof. Vandana A Pant
Co-Chairperson

Prof. B. Rajkumar
Chairperson

ANNEXURE-II

Babu Banarasi Das University
Babu Banarasi Das College of Dental Sciences,
BBD City, Faizabad Road, Lucknow – 226028 (INDIA)

Dr. Lakshmi Bala
Professor and Head Biochemistry and
Member-Secretary, Institutional Ethics Committee

Communication of the Decision of the IXth Institutional Ethics Sub-Committee

IEC Code: 05 **BBDCODS/04/2022**

Title of the Project: Comparative Evaluation of Dental Varnishes in Prevention of Demineralization of Enamel during Fixed Orthodontic Treatment – A Split Mouth Study.

Principal Investigator: Dr Swagat Verma **Department:** Orthodontics & Dentofacial Orthopaedics

Name and Address of the Institution: BBD College of Dental Sciences Lucknow.

Type of Submission: New, MDS Project Protocol

Dear Dr Swagat Verma,

The Institutional Ethics Sub-Committee meeting comprising following four members was held on 07th April. 2022.

1.	Dr. Lakshmi Bala Member Secretary	Prof. and Head, Department of Biochemistry, BBDCODS, Lucknow
2.	Dr. Amrit Tandan Member	Prof. & Head, Department of Prosthodontics and Crown & Bridge, BBDCODS, Lucknow
3.	Dr. Rana Pratap Maurya Member	Reader, Department of Orthodontics, BBDCODS, Lucknow
4.	Dr. Akanksha Bhatt Member	Reader, Department of Conservative Dentistry & Endodontics, BBDCODS, Lucknow

The committee reviewed and discussed your submitted documents of the current MDS Project Protocol in the meeting.
The comments were communicated to PI thereafter it was revised.

Decisions: The committee approved the above protocol from ethics point of view.

Forwarded by:

Lakshmi Bala

(Dr. Lakshmi Bala)
Member-Secretary
IEC

Member-Secretary
Institutional Ethic Committee
BBD College of Dental Sciences
BBD University
Faizabad Road, Lucknow-226028

(Dr. Puneet Ahuja)
Principal
BBDCODS

PRINCIPAL
Babu Banarasi Das College of Dental Sciences
(Babu Banarasi Das University)
BBD City, Faizabad Road, Lucknow-226028

ANNEXURE - III

Babu Banarasi Das College of Dental Sciences

(Babu Banarasi Das University)
BBD City, Faizabad Road, Lucknow – 227105 (INDIA)

Guidelines for Devising a Participant / Legally Acceptable Representative Information Document (PID) in English

Study Title-Comparative evaluation of dental varnishes in prevention of demineralization of enamel during fixed Orthodontic treatment - A split mouth study.Invitation Paragraph You are being invited to take part in a research study. Before you decide it is important for you to understand why the research/study is being done and what it will involve. Please take time to read the following information carefully and discuss it with friends, relatives and your treating physician/family doctor if you wish. Ask us if there is anything that is not clear or if you would like more information. Take time to decide whether or not you wish to take part.

1. **What is the purpose of the study?**
   The purpose of the study is to evaluate and compare the effect of two different varnishes in prevention of demineralization of enamel when applied around orthodontic brackets bonded to maxillary 1st premolar teeth scheduled for extraction after 3 months using polarized light microscope.

2. **Why have I been chosen?**
   You have been chosen because you fulfill all the parameters for this study.

3. **Do I have to take part?**
   Yes

4. **What will happen to me if I take part?**
   Nothing will happen to you and it will be your decision after knowing the details.

5. **What do I have to do?**
   You have to do nothing.

6. **What is the procedure that is being tested?**
   Two different varnishes will be applied around the brackets of maxillary 1st premolars of different quadrant scheduled for extraction after 3 months.

7. What are the interventions for the study?

After 3 months your all 1st premolars will be extracted as scheduled for fixed

Orthodontic treatment.

8. What are the side effects of taking part?

Varnish will be applied.

9. What are the possible disadvantages and risks of taking part?

No disadvantages and risks

10. What are the possible benefits of taking part?

Prevention of white spot lesions

11. What if new information becomes available?

Sometimes during the course of a research project, new information becomes available about the research being studied. If this happens, your researcher will tell you about it and discuss with you whether you want to continue in the study. If you decide to withdraw, your researcher/investigator will make arrangements for your withdrawal. If you decide to continue in the study, you may be asked to sign an updated consent form.

12. What happens when the research study stops?

If the study stops/finishes before the stipulated time, this will be explained to the patient/volunteer.

13. What if something goes wrong?

If any severe adverse event occurs, or something goes wrong during the study, the

complaints will be handled by reporting to the institution (s), and Institutional ethical

community.

14. Will my taking part in this study be kept confidential?

Yes

15. What will happen to the results of the research study?

The results of the study will be used to be compare two different varnishes in prevention of demineralization of enamel.

16. Who is organizing the research?

This research study is organized by the academic institution (BBDCODS).

17. Will the results of the study be made available after study is over?

Yes

18. Who has reviewed the study?

The study has been reviewed and approved by the Head of the Dept, and the IEC/IRC of

the institution.

19. Contact for further information

Dr. Swagat Verma

Department of Orthodontics and Dentofacial Orthopedics

Babu Banarasi College of Dental Sciences.

Lucknow-227105

Mob-7617805013

Dr. Kamna Srivastava (Reader)

Department of Orthodontics and Dentofacial Orthopedics

Babu Banarasi College of Dental Sciences.

Lucknow-227105

Mob-9653006704

Dr. Rohit Khanna (HOD)

Department of Orthodontics and Dentofacial Orthopedics

Babu Banarasi College of Dental Sciences.

Lucknow-227105

Mob-9415037011

Signature of PI……

Name……..

Date……..

ANNEXURE - IV

Babu Banarasi Das College of Dental Sciences

(Babu Banarasi Das University, Lucknow)

BBD City, Faizabad Road, Lucknow – 227105 (INDIA)

प्रतिभागी के लिए सूचना पत्र

1. अध्ययन शीर्षक

दो विभिन्न वारनिश का दंन्त क्षरण रोकने की क्षमता का आकलन।

2. निमंत्रण अनुच्छेद

आपको एक शोध अध्ययन में भाग लेने के लिए आमंत्रित किया जा रहा है। निर्णय लेने से पहले आपके लिए यह समझना महत्वपूर्ण है कि शोध/अध्ययन क्यों किया जा रहा है और इसमें क्या शामिल होगा। कृपया निम्नलिखित जानकारी को ध्यान से पढ़ने के लिए समय निकालें और यदि आप चाहें तो दोस्तों, रिश्तेदारों और अपने इलाज करने वाले चिकित्सक/पारिवारिक चिकित्सक के साथ इस पर चर्चा करें। हमसे पूछें कि क्या कुछ ऐसा है जो स्पष्ट नहीं है या यदि आप अधिक जानकारी चाहते हैं। यह तय करने के लिए समय निकालें कि आप भाग लेना चाहते हैं या नहीं।

3. अध्ययन का उद्देश्य क्या है ?

दो तरह की वारनिश का उपयोग होगा जो कि ऊपरी जवडे के अलग–अलग साईड के प्रीमोलर पर लगाया जायेगा और दंत क्षरण रोकने की क्षमता का आकलन किया जायेगा।

4. मुझे इस अध्ययन के लिए क्यों चुना गया है?

आपको इसलिये चुना गया क्योंकि आप अध्ययन के सभी मानकों को पुरा करते हैं।

5. **क्या इसमें मुझे भाग लेना चाहिए ?**

हाँ।

6. **मुझे क्या होगा यदि मैं इस अध्ययन में भाग लेता हूं।**

आपको कुछ नहीं होगा पर सभी जानकारी पढने के बाद यह अपका निर्णय होगा।

7. **मुझे क्या करना है?**

आपको कुछ नहीं करना है।

8. **किस प्रक्रिया का अध्ययन किया जा रहा है?**

दो तरह की वारनिश का उपयोग होगा जो कि ऊपरी जवडे के अलग–अलग साईड के प्रीमोलर पर लगाया जायेगा और दंत क्षरण रोकने की क्षमता का आकलन किया जायेगा।

9. **इस शोध में कौन से हस्तक्षेप दिए जाएंगे?**

तीन महिने बाद आपके चारो प्रिमोलर निकाले जायेंगे जैसे कि दाँतों को पिछे ले जाने की प्रक्रिया के लिये निश्चित किया गया था।

10. **इस अध्ययन में भाग लेने के क्या दुष्प्रभाव हैं ?**

वारनिश लगया जायेगा।

11. **इस अध्ययन में भाग लेने के संभावित जोखिम और नुकसान क्या है?**

कुछ नहीं।

12. **अध्ययन में भाग लेने के संभावित लाभ क्या है?**

दाँतों की क्षरण प्रक्रिया का रोकथाम संभव है।

13. क्या होगा यदि कोई नई जानकारी उपलब्ध हो जाती है ?

कभी-कभी एक शोध परियोजना के दौरान, अध्ययन किए जा रहे शोध के बारे में नई जानकारी उपलब्ध हो जाती है। यदि ऐसा होता है, तो आपका शोधकर्ता आपको इसके बारे में बताएगा और आपसे चर्चा करेगा कि क्या आप अध्ययन जारी रखना चाहते हैं। यदि आप वापस लेने का निर्णय लेते हैं, तो आपका शोधकर्ता/अन्वेषक आपकी निकासी की व्यवस्था करेगा। यदि आप अध्ययन जारी रखने का निर्णय लेते हैं, तो आपको एक अद्यतन सहमति फॉर्म पर हस्ताक्षर करने के लिए कहा जा सकता है।

14. क्या होता है जब अध्ययन / शोध परीक्षण बंद हो जाता है।

यदि अध्ययन निर्धारित समय से पहले रुक जाता है / समाप्त हो जाता है, तो इसे समझाया जाएगा।

15. क्या होगा अगर कुछ गलत हो जाता है?

यदि कोई गंभीर प्रतिकूल घटना होती है, या अध्ययन के दौरान कुछ गलत होता है, तो संस्थान (एस), और संस्थागत नैतिक समुदाय को रिपोर्ट करके शिकायतों को नियंत्रित किया जाएगा।

16. क्या इस अध्ययन में मेरा हिस्सा गोपनीय रखा जाएगा?

हाँ।

17. अध्ययन / शोध परीक्षण के परिमाण का क्या होगा?

कौन सा वारनिश दाँत का क्षरण रोकने में बेहतर है, उसका पता चलेगा।

18. इस अध्ययन को कौन आयोजित कर रहा है और इस परीक्षण के लिए धन कहां से आएगा।

यह शोध अध्ययन शैक्षणिक संस्थान (बीबीडीसीओडीएस) द्वारा आयोजित किया जाता है।

19.क्या सेवाएं शोध खत्म हो जाने के बाद उपलब्ध रहेगी या नहीं

हाँ।

20.अध्ययन की समीक्षा किसने की है?

अध्ययन की समीक्षा की गई है और विभाग के प्रमुख, और आईईसी/आईआरसी के द्वारा अनुमोदित किया गया है। निम्न लोगों से संपर्क करें

21.अधिक जानकारी के लिए संपर्क करें।

डॉ० स्वागत वर्मा
ऑर्थोडोंटिक्स और डेंटोफेशियल ऑर्थोपेडिक्स विभाग

बाबू बनारसी दास कॉलेज ऑफ डेंटल साइंसेज।

लखनऊ-227105

मोब- 7617805013

डॉ० कामना श्रीवास्तव (रीडर)

ऑर्थोडोंटिक्स और डेंटोफेशियल ऑर्थोपेडिक्स विभाग
बाबू बनारसी दास कॉलेज ऑफ डेंटल साइंसेज।
लखनऊ-227105
मोब-9653006704

डा0 रोहित खन्ना (एचओडी)

ऑर्थोडोंटिक्स और डेंटोफेशियल ऑर्थोपेडिक्स विभाग

बाबू बनारसी दास कॉलेज ऑफ डेंटल साइंसेज।

लखनऊ-227105

मोब-9415037011

bbdcods.iec@gmail.com

पीआईकाहस्ताक्षर .....

नाम .....

दिनांक.....

ANNEXURE-V

Consent Form (English)

Title of the Study: Comparative evaluation of dental varnishes in prevention of demineralization of enamel during fixed orthodontic treatment.

Study Number……..

Subject's Full Name…….

Date of Birth/Age ……

Address of the Subject…….

Phone no. and e-mail address……

Qualification ……

Occupation: Student / Self Employed / Service / Housewife/

Other (Please tick as appropriate)

Annual income of the Subject……

Name and of the nominees(s) and his relation to the subject…… (For the purpose of

compensation in case of trial related death).

I confirm that I have read and understood the Participant Information Document dated ……..for the above study and have had the opportunity to ask questions. **OR** I have been explained the nature of the study by the Investigator and had the opportunity to ask questions.

I understand that my participation in the study is voluntary and given with free will without any duress and that I am free to withdraw at any time, without giving any reason and without my medical care or legal rights being affected.

I understand that the sponsor of the project, others working on the Sponsor's behalf, the Ethics Committee and the regulatory authorities will not need my permission to look at my health records both in respect of the current study and any further research that may be conducted in relation to it, even if I withdraw from the trial. However, I understand that my Identity will not be revealed in any information released to third parties or published.

I agree not to restrict the use of any data or results that arise from this study provided such a use is only for scientific purpose(s).

I permit the use of stored samples (tooth/tissue/blood) for future research. **Yes [ ] No [ ] Not Applicable [ ]**

I agree to participate in the above study. I have been explained about the complications and side effects, if any, and have fully understood them. I have also read and understood the participant/volunteer's information document given to me.

Signature (or Thumb impression) of the Subject/Legally Acceptable Representative:……..

Signatory's Name……. Date …….

Signature of the Investigator…… Date……..

Study Investigator's Name..... Date……..

Signature of the witness…… Date……..

Name of the witness……

Received a signed copy of the PID and duly filled consent form

Signature/thumb impression of the subject or legally Date……..

Acceptable representative

ANNEXURE-VI

सहमति फॉर्म

अध्ययन का शीर्षकफिक्स्ड ऑर्थोडॉन्टिक उपचार के दौरान इनेमल के डिमिनरलाइजेशन की रोकथाम में डेंटल : वार्निश का तुलनात्मक मूल्यांकन।

अध्ययन संख्या……..

विषय का पूरा नाम…….

जन्म तिथि आयु /……

विषय का पता ……।

फोन नंबर। और ईमेल पता ……

योग्यता ……

व्यवसाय/गृहिणी/सेवा/नियोजित-स्व/छात्र :

अन्य (कृपया जो उपयुक्त हो उस पर सही का निशान लगाएं)

विषय की वार्षिक आय ……

नाम और नामांकित व्यक्तियों और विषय से उनका संबंध (…… (के प्रयोजन के लिए

मुकदमे से संबंधित मौत के मामले में मुआवजा।(

.1मैं पुष्टि करता हूं कि मैंने उपरोक्त अध्ययन के लिए प्रतिभागी सूचना दस्तावेज़ दिनांक …….. को पढ़ और समझ लिया है और मुझे प्रश्न पूछने का अवसर मिला है। या मुझे अन्वेषक द्वारा अध्ययन की प्रकृति के बारे में समझाया गया है और प्रश्न पूछने का अवसर मिला है।

.2मैं समझता हूं कि अध्ययन में मेरी भागीदारी स्वैच्छिक है और बिना किसी दबाव के स्वतंत्र इच्छा के साथ दी गई है और मैं किसी भी समय, बिना कोई कारण बताए और मेरी चिकित्सा देखभाल या कानूनी अधिकारों को प्रभावित किए बिना वापस लेने के लिए स्वतंत्र हूं।

.3मैं समझता हूं कि परियोजना के प्रायोजक, प्रायोजक की ओर से काम करने वाले अन्य लोगों, आचार समिति और नियामक अधिकारियों को वर्तमान अध्ययन और किसी भी अन्य शोध के संबंध में मेरे स्वास्थ्य रिकॉर्ड को देखने के लिए मेरी अनुमति की आवश्यकता नहीं होगी। इसके संबंध में आयोजित किया जा सकता है, भले ही मैं परीक्षण से हट जाऊं। हालांकि, मैं समझता हूं कि तीसरे पक्ष को जारी या प्रकाशित किसी भी जानकारी में मेरी पहचान प्रकट नहीं की जाएगी।

.4मैं इस अध्ययन से उत्पन्न होने वाले किसी भी डेटा या परिणाम के उपयोग को प्रतिबंधित नहीं करने के लिए सहमत हूं, बशर्ते ऐसा उपयोग केवल वैज्ञानिक उद्देश्यों के लिए हो।

.5मैं भविष्य के शोध के लिए संग्रहीत नमूनों के उपयोग की अनुमत (रक्त/ऊतक/दांत)ि देता हूं। हाँ नहीं [ ] [ ] लागू नहीं [ ]

.6मैं उपरोक्त अध्ययन में भाग लेने के लिए सहमत हूं। मुझे जटिलताओं और दुष्प्रभावों के बारे में समझाया गया है, यदि कोई हो, और उन्हें पूरी तरह से समझ लिया है। मैंने प्रतिभागीस्वयंसेवक द्वारा मुझे दिए गए / सूचना दस्तावेज़ को भी पढ़ और समझ लिया है।

विषय:(या अंगूठे का निशान) कानूनी रूप से स्वीकार्य प्रतिनिधि के हस्ताक्षर/……..

हस्ताक्षरकर्ता का नाम …….. तारीख ……।

अन्वेषक के हस्ताक्षर …… दिनांक …… ..

अध्ययन अन्वेषक का नाम ..... दिनांक .....

गवाह के हस्ताक्षर …… दिनांक …… ..

गवाह का नाम ……

पीआईडी की एक हस्ताक्षरित प्रति और विधिवत भरा हुआ सहमति फॉर्म प्राप्त किया

विषय के हस्ताक्षर अंगूठे का निशान या कानूनी रूप से दिनांक/……..

स्वीकार्य प्रतिनिधि

ANNEXURE-VII

Ouriginal by Turnitin

Document Information

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Sources included in the report

W	URL: https://scialert.net/fulltext/?doi=jms.2013.146.150 Fetched: 12/30/2021 11:15:05 AM	6
W	URL: https://www.researchgate.net/publication/248866050_White_Spot_Lesions_After_Orthodontic_Treatment Fetched: 9/26/2019 3:18:41 PM	3
W	URL: https://www.researchgate.net/publication/49845987_Randomized_controlled_trial_on_fluoride_varn... Fetched: 11/28/2019 2:55:53 PM	5
W	URL: https://bmcoralhealth.biomedcentral.com/articles/10.1186/s12903-023-02799-1 Fetched: 3/7/2023 3:18:50 AM	6
SA	**final - Copy.docx** Document final - Copy.docx (D154581418)	3
W	URL: https://www.hindawi.com/journals/bmri/2017/7834905/ Fetched: 12/23/2020 6:48:41 AM	3
SA	**thesis intro-merged.pdf** Document thesis intro-merged.pdf (D61068147)	1
W	URL: https://www.thieme-connect.com/products/ejournals/pdf/10.4103/ejd.ejd_127_17.pdf Fetched: 1/14/2022 5:35:34 AM	3
W	URL: https://www.ncbi.nlm.nih.gov/pmc/articles/PMC7586479/ Fetched: 12/10/2020 10:05:56 PM	4
J	**BMC Oral Health** URL: f9b9509e-25dc-43a2-8a02-c55ce1b2659a Fetched: 10/19/2019 1:32:55 AM	3
W	URL: https://pubmed.ncbi.nlm.nih.gov/10434089/ Fetched: 12/30/2021 11:14:46 AM	1

ANNEXURE-VIII

Master chart for demineralization depth for all the groups:

SL. NO.	Control group (µm)	VOCO Varnish group (µm)	MI Varnish group (µm)
1.	1688.01	1060.77	1037.08
2.	1532.02	1134.35	923.57
3.	1490.87	1230.01	911.97
4.	1757.40	1193.975	860.53
5.	1866.51	962.588	828.22
6.	1864.56	1028.395	969.78
7.	1705.31	1148.401	849.30
8.	1935.31	1296.77	990.76
9.	1852.42	1354.04	927.40
10.	1609.91	1011.28	908.82
11.	1515.79	988.49	1082.72
12.	1775.84	935.43	787.45
13.	1769.29	968.87	1139.68
14.	1880.04	817.85	986.69
15.	1917.72	823.48	902.70