COMPARATIVE EVALUATION OF CARIES PREVENTIVE EFFICACY OF RESIN INFILTRANT, CASEIN PHOSPHOPEPTIDE-AMORPHOUS CALCIUM PHOSPHATE AND NANO-HYDROXYAPATITE-AN IN VITRO STUDY

Dissertation submitted to

THE TAMILNADU Dr. M.G.R. MEDICAL UNIVERSITY

In partial fulfillment for the Degree of

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BRANCH IV – CONSERVATIVE DENTISTRY AND ENDODONTICS

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This Dissertation is submitted to **THE TAMILNADU Dr. M.G.R. MEDICAL UNIVERSITY**, *in partial fulfillment for the degree of* **MASTER OF DENTAL SURGERY in CONSERVATIVE DENTISTRY AND ENDODONTICS -BRANCH IV.** *It has not been submitted partially or fully for the award of any other degree or diploma.*

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LIST OF ABBREVIATIONS USED

(IN ALPHABETICAL ORDER)

ABBREVIATION	WORD EXPLANATION
ANOVA	Analysis Of Variance
CPP-ACP	Casein Phosphopeptide-Amorphous Calcium Phosphate
CLSM	Confocal Laser Scanning Microscope
CEJ	Cemento Enamel Junction
CDC	Centre for Disease Control
Demin	Demineralization
HA	Hydroxyapatite
HCl	Hydrochloric Acid
Nano-HA	Nanohydroxyapatite
n	Sample Size
OSHA	Occupational Safety and Health Administration
p value	Probability Value
Remin	Remineralization
SD	Standard Deviation
TEGDMA	Tri Ethylene Glycol Dimethacrylate
VHN	Vickers Hardness Number

Background

White spot lesions are the early signs of demineralization occuring under intact enamel which may or may not lead to the development of caries. Remineralization includes any repair to the crystal lattice in order to bring about a net mineral gain to the enamel subsurface lesion but it does not involve the precipitation of the solid phases onto the enamel surfaces. A new micro invasive treatment method suggested for the management of white spot lesions is the infiltration of a resin into the lesion. The aim of this invitro study is to compare and evaluate the caries preventive efficacy of a resin infiltrant (ICON), Casein phosphopeptide -Amorphous calcium phosphate (GC Tooth mousse) and Nano-hydroxapatite (Aclaim) on non cavitated enamel lesions.

Materials and methods

60 human maxillary incisors extracted for periodontal reasons were included in this study. The Sectioning was done at the middle third region of the crown for the 60 samples with approximate dimensions of (5x5x5mm). In order to create the artificial enamel lesions, the samples were demineralized by placing in a beaker containing the prepared demineralizing solution for 14 days. The Study samples were then divided into four groups which is Resin infiltrant (Group I), CPP-ACP (Group II), nano-HA (Group III) and control (Group IV) of 15 enamel samples in each group. The caries preventive efficacy of each group were evaluated using confocal laser scanning microscope and Vickers microhardness test for the depth of the penetration and surface microhardness after infiltrating the samples with resin infiltrant and remineralizing the

samples with CPP-ACP, nano-HA for a time period of 30days and also after acid challenge for a period of 14 days.

Statistical analysis

The Data were analyzed using Statistical Package for Social Sciences (SPSS) version 20.0. Measures of central tendency such as mean and measures of dispersion like Standard deviation were calculated for all the parameters tested. The Data collected were statistically analyzed using ANOVA and Post hoc bonferroni test was used for comparing intragroups and Tukey test was used to compare Intergroups.

Results

The mean values after demineralization of enamel samples in demineralizing solution is 245 µm for Group I(Resin infiltrant) , 246 µm for Group II (CPP-ACP), 250 µm for Group III (nano-HA) and 247 µm for Group IV (control). After remineralizing the enamel samples for a period of 30 days, the results are Group I (Resin infiltrant) 158 µm > Group II (CPP-ACP) 28.8µm ⩾ Group III (nano-HA) 26.3µm. After acid challenge for a period of 14 days the amount of material which was resistant to acid attack was Group I (Resin infiltrant) 114µm (72%) > Group III (CPP-ACP) 16.4µm (57%) ⩾ Group III (nano-HA)13.8µm (50%) . The untreated control group showed increased progression of lesion and least resistance to acid challenge. The p value were 0.993 after demineralization, <0.001 after remineralization and after acid challenge for 14 days when comparison was done between all the four groups. After

Post hoc Tukey inter group comparison Group I showed statistically significant difference which was greater when compared to group II (CPP-ACP) and Group III (nano-HA) where there were no difference.

The mean microhardness after demineralization of enamel samples in Group I (Resin infiltran) is 226VHN, Group II (CPP-ACP) 222VHN, Group III (nano-HA) (207 VHN, Group IV (Control) 215VHN. The pvalue were 0.143 which was not statistically significant. The mean microhardness value after Remineralization of enamel samples is Group I (Resin infiltrant) 316VHN >Group II (CPP-ACP) 282VHN ⩾ Group III (nano-HA) 267VHN > Group IV (Control) 218 VHN. The p value were <0.001 when comparing all the four groups. After Post hoc Tukey test there was statistically significant difference between Group I (Resin infiltrant) and other 3 groups. Group II (CPP-ACP) and Group III (nano-HA) showed no significant difference. The mean microhardness value after acid challenge of enamel samples for 14 days is Group I (Resin infiltrant) 292VHN > Group II (CPP-ACP) 254VHN ⩾ Group III (nano-HA) 237 VHN > Group IV (Control) 167 VHN. The p value was < 0.001 for groups. After Post hoc Tukey test there statistically significant difference between Group I (Resin infiltrant) and other 3 groups. Group II (CPP-ACP) and Group III (nano-HA) showed no significant difference.

Conclusion

Within the limitations of this invitro study it can be concluded that resin infiltrant showed higher caries inhibition potential than CPP-ACP and nano-HA. In addition, resin infiltrant showed superior acid resistance compared to CPP-ACP and nano-HA. It has a promising role in the management of early enamel carious lesion. It can be used as an alternative micro invasive approach.

Keywords

Resin infiltrant, casein phosphopeptide-amorphous calcium phosphate, nanohydroxyapatite, confocal laser scanning microscope, Vickers microhardness test

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Enamel carious lesions are characterized by the loss of mineral beneath an apparently intact surface layer. Hence, increased porosity within the carious lesion body causes the characteristic whitish appearance of these lesions. Therefore these lesions are often called as white spot lesions[1]. White spot lesions are the early signs of demineralization occuring under intact enamel which may or may not lead to the development of caries. It is initiated by the pathogenic bacteria that have breached the enamel layer and by the organic acids which are produced by them. These cause the removal of a certain amount of calcium and phosphate ions which fail to be replaced naturally during the remineralization process[2]. White spots can also be seen after the removal of orthodontic bands and brackets due to the inadequate oral hygiene resulting from the increased plaque accumulation, due to the high carbohydrate or acid content in the diet and also due to salivary hypofunction[3].

White spot lesions are commonly reversed by the process of remineralization mainly through the application of fluorides[4]. Remineralization is defined as the process whereby calcium and phosphate ions are supplied from a source external to the tooth in order to promote ion deposition into the crystal voids present in demineralized enamel and to produce a net mineral gain. The term void is used to define any accessible space in a crystal caused by the loss of ions due to the demineralization process. Remineralization, therefore includes any repair to the crystal lattice in order to bring about a net mineral gain to the enamel subsurface lesion but it does not involve the precipitation of the solid phases onto the enamel surfaces[5].

Deep enamel lesions show a tendency to remineralize only superficially. Consequently, the arrested lesions show a thick and highly mineralized surface layer[6] but the underlying lesion body is still porous and the whitish appearance often persists[7]. Moreover during remineralization stains can be incorporated into the lesion, leading to the formation of brown spots that might be even more unesthetic[8]. And, also with the deeper penetration of the lesion into the dentin, the remineralization process promoted by the application of fluoride has been seen to have considerable limitations[9].

The goal of caries management is therefore to stop or arrest the progression of the lesion. But, remineralization brought about by the topical application of fluoride requires multiple treatment sessions and a strict long term follow up which requires strong motivation and cooperation from the patient but is often seen to be difficult to achieve. In addition, the monitoring systems used for assessing the status and progression of the lesions over time are still being studied and are difficult to apply in every day clinical practice[10].

Remineralization of enamel subsurface lesions has been studied widely both in vitro and in situ as well as in numerous clinical studies[11.] Newer approaches for remineralization have been developed using both the stabilized and unstabilized calcium phosphate systems. One such system that has been developed uses casein phosphopeptide (CPP) in order to stabilize the calcium and phosphate ions at higher concentrations and to form an amorphous nanocomplexes namely casein phosphopeptide- amorphous calcium phosphate (CPP-ACP)[12]. The CPP allows for the higher concentrations of calcium, phosphate and fluoride ions to be stabilized in a metastable solution and in a form that is bioavailable for the promotion of remineralization[13].

In current practice nanohydroxy apatite has been widely used and studied as a biomimetic material both for the reconstruction of demineralized (mineral loss) enamel and also has been seen to be an effective anticaries agent mainly because of its unique potential to bring about remineralization[14]. The size of the calcium phosphate crystal also plays an important role in the formation of hard tissues and also has a significant impact on its intrinsic properties, solubility and biocompatibility[15]. A nano-apatite particle can simultaneously repair as well as prevent the initial erosive lesions in enamel compared to conventional hydroxyapatite (HA) crystals which are hundreds of nanometers in length[16].

A new micro invasive treatment method suggested for the management of white spot lesions is the infiltration of a resin into the lesion. The resin infiltrant prevents the further progression of the initial enamel caries lesion and by occluding the micro porosities within the lesion as it has a low viscosity. And as it is a light curing resin that can rapidly infiltrate into the porous enamel. The resin completely fills the pores within the tooth thereby replacing the lost tooth structure as well as arresting the caries progression [17.] This inhibition of caries progression is achieved by the sequential effects of 15% hydrochloric acid gel applied for two minutes and by the application of a low viscosity tri ethylene glycol dimethacrylate (TEG-DMA) resin which has a sufficiently high penetration coefficient. The unique feature of this technique is that, it is noninvasive, preserves tooth structure and it can be completed in a single visit.

Confocal microscopy is an useful tool used to study the infiltration of low-viscosity resins (infiltrants) into initial enamel carious lesion. The images obtained are high resolution optical images with depth selectivity. [18]. Currently there are no

studies available in the literature which compare the depth of penetration and the micro hardness of resin infiltrants to other remineralizing agents like casein phosphopeptide amorphous calcium phosphate (CPP-ACP) and nanohydroxyapatite (nano-HA).

In the current study the caries preventive efficacy of the resin infiltrant (ICON) with casein phosphopeptide amorphous calcium phosphate (GC Tooth mousse) and nano hydroxyapatite (Aclaim) were evaluated by using the confocal laser scanning microscope and Vickers microhardness analysis.

AIM

To compare and evaluate the caries preventive efficacy of a resin infiltrant (ICON), Casein phosphopeptide-Amorphous calcium phosphate (GC Tooth mousse) and Nano-hydroxapatite (Aclaim) on non cavitated enamel lesions.

OBJECTIVES

1) To compare the effects of the Resin infiltrant (ICON) and Casein phosphopeptide-amorphous calcium phosphate (GC Tooth mousse) and Nanohydroxyapatite (Aclaim) on the depth of lesion and lesion progression by using confocal laser scanning microscope.

2) To evaluate the influence of the Resin infiltrant (ICON), Casein phosphopeptide-Amorphous calcium phosphate (GC tooth mousse) and Nanohydroxyapatite (Aclaim) on microhardness using Vickers microhardness test.

Reynolds et al (1987)[19] determined the ability of casein and found that casein and tryptic peptides which prevent enamel demineralization are related to their incorporation into plaque, thereby increasing plaque calcium phosphate and acid-buffering capacity by the phosphoseryl, histidyl, glutamyl, and aspartyl residues and also indirectly through catabolism by plaque bacteria

Reynolds et al (1995)[20] stated that casein phosphopeptides (CPP) are produced from a tryptic digest of the milk protein casein by the aggregation with calcium phosphate and purification by ultrafiltration. The CPP have a remarkable ability to stabilise calcium phosphate in solution and substantially increase the level of calcium phosphate in dental plaque. Through their multiple phosphoseryl residues the CPP bind to clusters of amorphous calcium phosphate (ACP) in a metastable solution, preventing their growth to the critical size required for nucleation and precipitation.

Reynolds et al (1997)[13] conducted a study on the remineralization of enamel subsurface lesions by casein phosphopeptide-stabilized calcium phosphate solutions**.** Casein phosphopeptides (CPP) stabilize amorphous calcium phosphate (ACP), localize ACP in dental plaque, and are anticariogenic in animal and in situ human caries model. In this invitro study, CPP-stabilized calcium phosphate solutions were seen to remineralize subsurface lesions in human third-molar enamel samples. Solutions were used to examine the effect of CPP-amorphous calcium phosphate concentration on remineralization. Other solutions were used to examine the effect of

increasing pH, which decreased the concentrations of free calcium and phosphate ions and increased the level of CPP-bound ACP. Although most of the remineralizing solutions were supersaturated with respect to the amorphous and crystalline calcium phosphate phases, the solutions were stabilized by the CPP such that spontaneous precipitation of calcium phosphate did not occur. After a ten-day remineralization period, enamel lesions were sectioned, subjected to microradiography, and the mineral content determined by microdensitometry. All solutions deposited mineral into the bodies of the lesions, with the 1.0% CPP-calcium phosphate (pH 7.0) solution replacing 63.9 +/- 20.1% of mineral lost at an averaged rate of 3.9 +/- 0.8 x 10(-8) mol hydroxyapatite/m2/s. The remineralizing capacity was greater for the solutions with the higher levels of CPP-stabilized free calcium and phosphate ions. Remineralization was not significantly correlated with either the CPP-bound ACP of the degrees of saturation for hydroxyapatite, octacalcium phosphate, or ACP. However, remineralization was significantly correlated with the degree of saturation for dicalcium phosphate dihydrate (CaHPO4.2H2O), but his was attributed to the significant correlation of remineralization with the activity gradients from the solution into the lesion of some calcium phosphate ions and ion pairs, in particular the neutral ion pair CaHPO4. 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 this effect high rates of enamel remineralization

Robinson et al (2001)[21] conducted an in vitro study to determine the penetration of adhesive resins into artificial caries-like lesions. Artificial lesions of

enamel were created in extracted human teeth using acidified gels. A range of currently available adhesive materials were then used to infiltrate the porosities. The extent of occlusion of the lesion porosities was determined both qualitatively using light microscopy and quantitatively using a chloronaphthalene imbibition technique. The effect of such treatment upon subsequent exposure to acid gels was also investigated. Results showed that up to 60% of the lesion pore volume had been occluded following infiltration with some of the resin materials and that this treatment was capable of reducing further acid demineralization. The development of such treatment strategies could offer potential noninvasive means of treating early enamel lesions.

Kersten et al (2001)[22] conducted a study on the fissure sealing optimization of sealant penetration and sealing properties. 48 extracted, non-carious human molars were sealed with the unfilled sealant (Heliobond) using the enamel adhesive technique (35% phosphoric acid gel, 120 s etching time, bond application, light-curing for 60 s). The following factors were tested in comparison to the control group (1): influence of a precuring time lapse of 20 s after sealant application (2); ultrasound application with a plastic tip during the etching procedure (3); a wetting agent in an acid vehicle (4); enamel drying with acetone after the etching procedure (5); and finally, the combination of ultrasound during etching; a drying procedure with acetone; and a 20 s precuring time lapse (all applied to the same sample). The sealed teeth were sectioned and evaluated by conventional light microscopy to determine the penetration depth into the fissure, and by confocal laser microscopy to investigate the quality of the adhesion zone. Strict adherence to a specified

penetration time, an intensified etching procedure with ultrasound, and the use of a drying procedure with acetone each showed a positive effect on the fissure penetration depth of the sealant and on the adhesion zone. The combination of these measures improved significantly the quality of the fissure sealing. Penetration depth increased to 92% of the fissure depth. From 95-100% of the total length of the analyzed adhesion zone shows excellent tags of sealant in the conditioned enamel surface

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Shen et al (2001)[23] conducted a study on the remineralization of enamel subsurface lesions by using sugar-free chewing gum containing casein phosphopeptide-amorphous calcium phosphate**.** Casein phosphopeptide-amorphous calcium phosphate nanocomplexes (CPP-ACP) exhibit anticariogenic potential in the laboratory, animal, and human in situ experiments. The aim of this study was to determine the ability of CPP-ACP in sugar-free 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 sub-surface 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 CPP-ACP 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.

Gray et al (2002)[24] conducted a study on the infiltration of resin into white spot caries-like lesions of enamel. The aim of this study was to determine the extent to which caries lesions can be infiltrated successfully with polymerizable resins. This could provide operators with an additional means of managing white spot lesions in high caries risk patients rather than relying on improved plaque control and fluoride application methods. Artificial caries lesions were produced in extracted premolar teeth using an acidified gel. The lesions were infiltrated using two of the resins currently available to the dental profession. The effects of acid etching the surface and of drying the lesion by two methods was also investigated. The degree of resin penetration was evaluated by scanning electron microscopy. The results showed that following a short etch (5s with 36% phosphoric acid), prior dehydration of the lesion with ethanol and the application of multiple layers of bonding resin, lesions could be infiltrated almost completely with organic resin. This approach could offer an alternative approach for the management of uncavitated lesions**.**

Reynolds et al (2003)[25] conducted a study on the retention in plaque and remineralization of enamel lesions by the various forms of calcium in a mouthrinse or in a sugar-free chewing gum. Casein phosphopeptide-amorphous calcium phosphate (CPP-ACP) nanocomplexes incorporated into sugar-free chewing gum have been shown to remineralize enamel subsurface lesions in situ. The aim of this study was to 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 the form of a mouthrinse or sugar-free gum in randomized, double-blind trials. In the mouthrinse study group, 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 CPP could be detected in plaque extracts 3 hrs after the subjects chewed the CPP-ACP-containing gum. The results showed that CPP-ACP was superior to other forms of calcium in remineralizing enamel subsurface lesions

Schmidlin et al (2004)[26] conducted a invitro study on the penetration depth of a bonding agent into de and remineralized enamel in vitro**.** Ten extracted human molars were mesiodistally sectioned. Buccal and lingual enamel surfaces were divided into four equal areas using sticky wax. The central two areas of each tooth (n = 20) were demineralized for 12 weeks using an acidic gel (pH 4.8). The lateral areas served as controls. After demineralization, ten specimens were remineralized in a saliva substitute for three weeks. An amine fluoride solution (Elmex Fluid) was applied on one half of each specimen before acid etching. After etching for 120 s, an enamel-bonding agent (Heliobond) containing 0.1% rhodamine was applied onto the test and control areas, and was light cured for 60 s. Subsequently, the specimens were sectioned and resin tag length was determined using a confocal laser scanning microscope (CLSM). Results were statistically compared with ANOVA followed by

Scheffe's and Bonferroni/Dunn post hoc tests. With a mean penetration depth of 68 +/- 22 microm, tags in demineralized enamel were significantly longer than in other groups ($p < or = 0.01$). Penetration decreased significantly in remineralized areas or when fluoride was used ($p < or = 0.01$), but was still significantly deeper than in control sites ($p < or = 0.01$).

Iijima et al (2004)[27] conducted a study on the acid resistance of enamel subsurface lesions remineralized by a sugar-free chewing gum containing casein phosphopeptide-amorphous calcium phosphate. The aim of this clinical study was to investigate the acid resistance of enamel lesions remineralized in situ by a sugar-free chewing gum containing casein phosphopeptide-amorphous calcium phosphate nanocomplexes (CPP-ACP: Recaldent). The study utilized a double-blind, randomized, crossover design with two treatments: (i) sugar-free gum containing 18.8 mg of CPP-ACP, and (ii) sugar-free gum not containing CPP-ACP as control. Subjects wore removable palatal appliances with insets of human enamel containing demineralized subsurface lesions and chewed the gum for a period of 20 min 4 times per day for a duration of 14 days. After each treatment the enamel slabs were removed and half of each lesion challenged with acid in vitro for 8 or 16 h. The level of remineralization was determined using microradiography. The gum containing CPP-ACP produced approximately twice the level of remineralization as the control sugar-free gum. The 8 and 16 hour acid challenge of the lesions remineralized with the control gum resulted in 65.4 % and 88.0% reductions, respectively, of deposited mineral, while for the CPP-ACP-remineralized lesions the corresponding reductions were 30.5% and 41.8%. The acid challenge after in situ remineralization for both the

control and CPP-ACP-treated lesions resulted in demineralization underneath the remineralized zone, indicating that the remineralized mineral was more resistant to subsequent acid challenge. The results show that sugar-free gum containing CPP-ACP is superior to an equivalent gum not containing CPP-ACP in remineralization of enamel subsurface lesions in situ with a mineral that is more resistant to subsequent acid challenge.

Paris et al (2006)[28] conducted a comparative study on the progression of sealed initial enamel lesions after exposure to a demineralizing solution in vitro. In each of 54 the bovine enamel specimens three subsurface lesions were created.Two of the lesions were etched with phosphoric acid and sealed with either fissure sealant or with various adhesives (heliobond, resulcinmonobond, excite, solobond M, adper prompt L –pop and fissure sealant helioseal) for 15sec or 30sec,where as one lesion remained as the untreated control. Half of the specimens were covered with nail varnish (baseline) and the other half was reexposed to a demineralizing solution for 14 days (experimental).The specimens were then cut perpendicularly to the surface and infiltrated with a low viscosity fluorescent resin and observed with a confocal laser scanning microscope. For lesions sealed with the fissure sealant and adhesives (1-3) the progression of lesion depth was significantly decreased compared with untreated control, the extended penetration time of 30seconds of adhesives resulted in a reduced lesion progression compared with a penetration time of 15 seconds

Meyer-Lueckal et al (2006)[29] conducted a study on the influence of the application time on the penetration of different dental adhesives and fissure sealant

into artificial subsurface lesions in bovine enamel. In each of the 54 specimens of bovine enamel, three windows were demineralized for a period of 14 days. Subsequently, two windows were etched with phosphoric acid for 5s in order to degrade the surface layer, whereas one window served as untreated control. The specimens were randomly divided into six groups and a fissure sealant as well as five different adhesives were applied onto the subsurface lesions and allowed to penetrate for either 15 or 30 s. Overlying material was wiped away and the resins were light cured. To visualize the penetrated resins and the remaining pore structures, the specimens were infiltrated with a low viscous fluorescent resin and studied using a Confocal Laser Scanning Microscope (CLSM). For Helioseal, Heliobond, Resulcin Monobond, and Excite an application time of 30s resulted in significantly higher ($p < 0.05$; t-test) penetration depths (47-105 microm) compared to 15s (29-49 microm.

Mueller et al (2006)[30] conducted a study to evaluate the Inhibition of lesion progression by the penetration of resins in vitro and the influence of the application procedure. This study compared the progression of sealed initial enamel lesions penetrated with a fissure sealant (Helioseal, Vivadent) or various adhesives (Heliobond, Excite, Vivadent; Resulcin, Merz; Solobond M, Voco; Prompt L-Pop, 3M-ESPE) after exposure to a demineralizing solution, in vitro. From 27 bovine teeth, 54 enamel specimens were prepared and covered with nail varnish (control), thus obtaining three windows for treatment. After demineralization (pH 5.0; 14 days), two of the windows (A, B) were etched with phosphoric acid (20%; 5 seconds); whereas, the third area served as the control (C). The specimens were divided randomly into six groups (n=9), and the material was applied (90 seconds)

either once (A) or twice (B). Light-curing followed each application. Half of the area of each specimen window was then covered with nail varnish, and the samples were again stored in the demineralizing solution (pH 5.0; 14 days). The specimens were cut perpendicular to the surface, and both enamel slabs were studied after infiltration using a fluorescent, low viscous resin (VIRIN) and confocal microscopy (CLSM). Lesion depths were calculated (ImageJ) from the surface to that point in the lesion where the grey values clearly changed to a darker grey. After demineralization, mean lesion depths (SD) (14 days) were measured at 105 (21) microm. The second demineralization led to a mean progression of the lesion depths of 52 (31)%. Adper Prompt L-Pop and Solobond M could not significantly prevent lesion progression after a single application ($p > 0.05$; t-test); however, the second application of Solobond M significantly decreased lesion progression ($p < 0.05$; t-test). Helioseal, Heliobond, Resulcin Monobond and Excite showed significantly better inhibition of the demineralization compared to the other materials ($p < 0.05$; Bonferroni). It can be concluded that the penetration of adhesives into initial lesions inhibited a further demineralization in vitro

Paris et al (2007)[31] conducted a study on Resin infiltration of natural caries lesions. The aim of this study was to evaluate the penetration of a conventional adhesive into natural enamel caries after pre-treatment with two different etching gels in vitro. Extracted human molars and premolars showing proximal white-spot lesions were cut across the lesions perpendicular to the surface. Corresponding lesion halves were etched for 120 sec with either 37% phosphoric acid gel (H_3PO_4) or 15% hydrochloric acid gel (HCl) and subsequently infiltrated with an adhesive. Specimens

were observed by confocal microscopy. Mean penetration depths (SD) in the HCl group [58 (37)µm] was significantly increased when compared with those of the H_3PO_4 group [18 (11)µm] ($p < 0.001$; Wilcoxon). It can be concluded that etching with 15% hydrochloric acid gel is a more suitable method than with 37% phosphoric acid gel as a pre-treatment for caries lesions intended to be infiltrated.

Paris et al (2007)[32] conducted a study on the Resin infiltration of artificial enamel caries lesions with a experimental light curing resins to prevent enamel lesions from further demineralization, a complete and homogeneous penetration of low-viscosity resins ('infiltrants') should be accomplished. With commercially available adhesives, this goal might not be achieved because of their penetration capabilities. On this note, the Penetration Coefficient (PC) describes the penetrativity of liquids and might be employed to develop optimal infiltrants. Thus, the aim of this study was to compare the penetration abilities of 12 experimental infiltrants (BisGMA/TEGDMA comonomers showing varying PCs) with a commercially available adhesive (Excite, Vivadent). In each of the 156 bovine enamel specimens, four subsurface lesions were created. Three of the four lesions were infiltrated with either the adhesive or one of 12 experimental resins for either 10, 22, or 40 seconds, and subsequently light-cured. Specimens were studied using confocal microscopy and penetration depths were determined. A good correlation between PC and penetration depth was thereby observed (Pearson's correlation coefficient, r=0.820).

Paris et al (2007)[33] conducted a study on the Penetration coefficients of commercially available and experimental composites intended to infiltrate enamel

carious lesions. The penetration coefficients (PCs) of five adhesives and a fissure sealant as well as 66 experimental composite resins was determined. To establish the resins' PCs the viscosities, surface tensions, and contact angles to the enamel was measured. For the commercially available products PCs from 4.0 to 278.9 cm/s were measured. Four of these materials showed a good correlation to the penetration depths obtained in a previous study. Experimental composites showed PCs from 0.2 to 474.9 cm/s. The addition of ethanol significantly increased the PCs due to a decrease of viscosity and contact angle. Highest PCs were found for mixtures containing TEGDMA, HEMA and 20% ethanol.

Cai et al (2007)[34] conducted a study on the effect of addition of citric acid and casein phosphopeptide-amorphous calcium phosphate to a sugar-free chewing gum on enamel remineralization in situ. Casein phosphopeptide-amorphous calcium phosphate (CPP-ACP) has been shown to remineralize enamel subsurface lesions in situ. The aim of this study was to investigate the effects of CPP-ACP in a fruit-flavoured sugar-free chewing gum containing citric acid on enamel remineralization, and acid resistance of the remineralized enamel, using an in situ remineralization model. The study utilized a double-blind, randomized, crossover design with three treatments: (i) sugar-free gum (2 pellets) containing 20 mg citric acid and 18.8 mg CPP-ACP, (ii) sugar-free gum containing 20 mg citric acid alone, (iii) sugar-free gum not containing CPP-ACP or citric acid. Ten subjects were instructed to wear removable palatal appliances, with 4 half-slab insets of human enamel containing demineralized subsurface lesions and to chew gum (2 pellets) for 20 min 4 times per day for 14 days. At the completion of each treatment the enamel half-slabs were

removed and half of the remineralized lesion treated with demineralization buffer for 16 h in vitro. The enamel slabs (remineralized, acid-challenged and control) were then embedded, sectioned and subjected to microradiography to determine the level of remineralization. Chewing with gum containing citric acid and CPP-ACP resulted in significantly higher remineralization (13.0 +/- 2.2%) than chewing with either gum containing no CPP-ACP or citric acid (9.4 +/- 1.2%) or gum containing citric acid alone (2.6 +/- 1.3%). The acid challenge of the remineralized lesions showed that the level of mineral after acid challenge was significantly greater for the lesions exposed to the gum containing CPP-ACP**.**

Ferrazzano et al (2007)[35] conducted a study on new strategies in dental caries prevention by experimental study on casein phosphopeptides. 59 samples of dental enamel were divided into 3 groups, which subsequently underwent 3 different chemical treatments: the samples from group I (control group) were preserved in distilled water; the samples from group II were treated with a demineralizing solution for producing artificial caries; the samples from group III underwent the same treatment as group II, but with the addition of CPPs. The effects of these procedures were evaluated by quantitative analysis (change in weight and calcium titration) and qualitative analysis (SEM). Statistical analysis of the results was performed using ANOVA. In presence of CPPs, acid dissolution of human enamel is reduced by over 50% in vitro. Our results demonstrate that CPPs could be a valid preventive system against demineralisation of early enamel lesions.

Meyer-lueckel et al (2008)[17] conducted a study on the progression of artificial enamel caries lesions after infiltration with experimental light curing resins**.**

The arrest of enamel caries lesions by infiltration with low-viscosity light curing resins might be a promising approach of microinvasive dentistry. However, no materials optimized for rapid lesion infiltration ('infiltrants') are commercially available today. The penetration coefficient (PC) of experimental resins has been shown to correlate with penetration speed and, therefore, might be an important feature of infiltrants. The aim of the present study was to evaluate the influence of PC and composition of experimental infiltrants on the progression of enamel lesions in a demineralizing environment. Artificial enamel lesions were prepared in a demineralization solution for 50 days and infiltrated with either one of twelve experimental infiltrants or a commercially available adhesive for 10, 22, and 40 s, respectively. Specimens were cut perpendicularly to the surface and one half of each specimen was exposed to a demineralizing solution for another 50 days, whereas the other half was used as baseline control. Lesion progression was analyzed using confocal microscopy (CLSM) and transversal microradiography (TMR). The square root of the product of PC and application time was negatively correlated with progression of lesion depth (CLSM: r = -0.741; TMR: r = -0.450). Therefore, infiltrants should preferably have high PCs to facilitate inhibition of lesion progression efficiently

Meyer-Lueckel et al (2008)[36] conducted a study on the Improved resin infiltration of natural caries lesions. In artificial lesions, improved penetration and the caries-inhibiting properties of infiltrating resins could be observed with increasing penetration coefficients (PCs). The aim of the present study was to compare the penetration abilities of an experimental 'infiltrant' into natural lesions with those of

an adhesive in vitro. Extracted human molars and premolars showing proximal white spots were cut across the lesions perpendicular to the surface. Corresponding lesion halves were etched for 120 sec with 15% hydrochloric acid gel and were subsequently treated with either an adhesive (PC: 31 cm/sec) or an infiltrant (PC: 273 cm/sec). Specimens were observed by confocal microscopy and transverse microradiography. Penetration depths of the adhesive were significantly lower compared with those of the infiltrant ($p < 0.001$; Wilcoxon). It can be concluded that resins with higher PCs (infiltrants) show superior ability to penetrate natural lesions compared with resins with lower PCs

Cohrane et al (2008)[37] conducted a study on the enamel subsurface lesion remineralization using casein phosphopeptide stabilized amorphous calcium phosphate (CPP-ACP) and amorphous calcium fluoride phosphate (CPP-ACFP).The mineral deposited on the subsurface lesions were analysed using transverse microradiography and electron microscope. Casein phosphopeptide – amorphous calcium fluoride phosphate (CPP-ACFP) solutions produced greater levels of remineralization than CPP-ACP solutions at a pH 5.5 and below.

Kumar et al (2008)[38] conducted a study on the effect of casein phosphopeptide-amorphous calcium phosphate on the remineralization effect of artificial caries-like lesions in an invitro study. Permanent extracted teeth were placed in demineralizing solution for 96 hours to produce artificial caries-like lesions of 120-200µm in depth. They were then sectioned into 100-150µm thick samples and

randomly assigned to five groups and for Group A, a fluoridated toothpaste (1100 ppm) was used as a positive control and in Group B, a non-fluoridated toothpaste was used as a negative control. Tooth Mousse containing CPP-ACP was tested by three different means: as a toothpaste (Group C); as a topical coating (Group D); and (Group E) as a topical coating after treating the sections with the same fluoridated toothpaste as in Group A**.** The lesion depth decreased significantly by 7 per cent in Group A, 10.1 per cent in Groups C and D, and 13.1 per cent in Group E (Paired t-test, $p < 0.05$), while in Group B the lesion depth increased significantly by 23 per cent. Based on the data obtained, CPP-ACP containing Tooth Mousse remineralized initial enamel lesions and it showed a higher remineralizing potential when applied as a topical coating after the use of a fluoridated toothpaste

Huang et al (2009)[39] conducted an invitro study on the effect of nano-hydroxyapatite concentration on remineralization of initial enamel lesion. The purpose of the study was to determine the effect of nano-hydroxyapatite concentrations on initial enamel lesions under dynamic pH-cycling conditions. Initial enamel lesions were prepared in bovine enamel with an acidic buffer. NaF (positive control), deionized water (negative control) and four different concentrations of nano-hydroxyapatite (1%, 5%, 10% and 15% wt%) were selected as the treatment agents. Surface microhardness (SMH) measurements were performed before and after demineralization and after 3, 6, 9 and 12 days of application and the percentage surface microhardness recovery (%SMHR) was calculated. The specimens were then examined by a scanning electron microscope. The %SMHR in nano-hydroxyapatite groups was significantly greater than that of the negative control. When the

concentration of nano-HA was under 10%, SMH and %SMHR increased with increasing nano-hydroxyapatite concentrations. There were no significant differences between the 10% and 15% groups at different time periods in the pH-cycling. The SEM analysis showed that nano-hydroxyapatite particles were regularly deposited on the cellular structure of the demineralized enamel surface, which appeared to form new surface layers. It was concluded that nano-hydroxyapatite had the potential to remineralize initial enamel lesions. A concentration of 10% nano-hydroxyapatite may be optimal for remineralization of early enamel caries.

Neuhaus et al (2009)[40] stated that dental products with casein phosphopeptide--amorphous calcium phosphate-nanocomplexes (CPP-ACP) are used in several tooth products (toothpastes, chewing gums, mouthrinses) and are also used in certain dental restorative material. CPP-ACP containing products are supposed to enhance remineralisation of dental hard tissues and thus might play a major role in the prevention and therapy of initial caries or erosively dissolved enamel.

Rao et al (2009)[41] conducted a Study to study the efficacy of a toothpaste containing casein phosphopeptide in the prevention of dental caries by a randomized controlled trial in 12- to 15-year-old high caries risk children in Bangalore, India**.** Casein phosphopeptide (CPP) has the potential to be added to mouth rinses, gels, toothpastes, chewing gums and confectioneries. Until now CPP has been studied in vitro, in situ and in animals, but clinical trials are still lacking. This study was conducted to evaluate the efficacy of CPP-containing toothpaste in preventing dental caries in schoolchildren. The study was conducted among 150 schoolchildren

randomly divided into three groups, each group using one of three types of toothpastes: (a) containing 2% w/w CPP; (b) containing 1,190 mg/kg fluoride as 0.76% sodium monofluorophosphate (SMFP); (c) placebo toothpaste without CPP or fluoride. Students brushed with the given toothpastes for 24 months. Oral hygiene and caries experience was assessed at baseline, 12 months and 24 months. The increments in caries lesions was calculated and analyzed to assess the caries-preventive effect. A significant reduction in the caries increment was observed among students using CPP toothpaste or SMFP toothpaste, compared with the group using the placebo toothpaste. The reduction in caries increment was not significantly different between the CPP and SMFP groups. Oral Hygiene Index score increased from the 12-month to the 24-month examination. It is concluded that CPP can be effectively incorporated into calcium carbonate-based toothpaste and that toothpaste containing CPP is effective in preventing caries. Toothpaste containing 2% CPP seemed to have an efficacy similar to that of a paste containing 1,190 mg/kg SMFP in the prevention of caries.

Meyer-Lueckel et al (2010)[42] conducted a study on the Infiltration of natural caries lesions with experimental resins differing in penetration coefficients and ethanol Resin infiltration of enamel caries lesions requires materials optimized for penetration into the capillary structures of the lesion body. With increasing penetration coefficients (PC) improved penetration and caries-inhibiting properties of low-viscosity resins (infiltrants) could be observed in artificial caries lesions. The aim of the present in vitro study was to compare the penetrativity of experimental

resins varying in PC and ethanol addition into natural caries lesions using this technique. Extracted human molars and premolars showing proximal white spot lesions (International Caries Detection and Assessment System: code 2) were etched for 2 min using 15% hydrochloric acid gel. After drying, the lesions were stained with tetramethylrhodamine isothiocyanate and 1 of 4 experimental resins (PC63; PC185; PC204; PC391) was applied for 5 min. The materials consisted of bisphenol-A-glycidyl-methacrylate (B), tri-ethylene-glycol-dimethacrylate (T) and ethanol (E) in ratios (B:T:E) of PC63: 25:75:0; PC185: 20:60:20; PC204: 0:100:0; PC391: 0:80:20. Excess material was removed before light curing. The teeth were sectioned perpendicularly to the lesion surfaces and unbound dye was bleached by immersion in hydrogen peroxide. The remaining lesion pores were stained with fluorescein solution. Lesion and penetration depths were analyzed using confocal microscopy (n = 60). At deep lesion sites the percentage penetration of PC204 was significantly higher compared to PC63 and PC391 ($p < 0.05$; Mann-Whitney test) but only slightly higher than PC185 ($p > 0.05$). It can be concluded that materials with high PC (infiltrants) are capable of penetrating almost completely into enamel parts of natural caries lesions in vitro. A solvent-free resin mainly consisting of triethylene glycol dimethacrylate seems to be preferable.

Huang et al (2010)[43] conducted a study on the Combined effects of nano-hydroxyapatite and Galla chinensis on remineralisation of initial enamel lesion in vitro bovine enamel blocks with in vitro produced initial lesion were used. The lesions were subjected to a pH-cycling regime for 12 days. Each daily cycle includes 4 x 3 min application with one of five treatments: NaF (positive control), deionised

water (negative control), crude aqueous extract of G. chinensis (GCE), nano-hydroxyapatite (nano-HA) and GCE with nano-HA. The samples were subsequently evaluated using a microhardness tester, polarised light microscopy (PLM), X-ray diffraction (XRD) and scanning electron microscopy (SEM

Vashisht et al (2010)[44] conducted a study on the remineralization of early enamel lesions using casein phosphopeptide amorphous calcium Phosphate: an ex-vivo study. This randomized study was conducted on 10 subjects undergoing orthodontic treatment with premolar extraction as part of their treatment. Artificial white lesions were created with the application of 37% phosphoric acid for 20 mins. Teeth were then divided into two groups: one experimental and the other control. Customised orthodontic band with a window was luted with intermediate restorative material in the experimental group whereas in the control group, band without a window was luted. The casein phosphopeptide amorphous calcium phosphate (GC tooth mousse) paste was then applied on the window region of the experimental group for 3 mins thrice daily after meals for 14 days, whereas no paste was applied in the control group. After 14 days, teeth were extracted and viewed under an SEM. The study groups showed remineralization of the lesions as compared with the control group in most of the samples. Casein phophopeptide could significantly remineralize the artificial enamel lesions in vivo.

Srinivasan et al (2010)[45] compared the remineralization potential of CPP–ACP and CPP–ACP with 900 ppm fluoride on eroded human enamel using Vickers surface microhardness measurements and scanning electron microscope

analysis(SEM). They found that both CPP–ACP and CPP–ACP with 900 ppm fluoride substantially remineralized the softened enamel, with the CPP–ACP and fluoride combination showing higher remineralization potential than CPP–ACP

Walker et al (2010)[46] conducted a study on Casein phosphopeptide-amorphous calcium phosphate incorporated into sugar confections inhibits the progression of enamel subsurface lesions in situ. Casein phosphopeptide-amorphous calcium phosphate (CPP-ACP) has been demonstrated to exhibit anticariogenic activity in randomized, controlled clinical trials of sugar-free gum and a tooth cream. Two randomized, double-blind, crossover studies were conducted to investigate the potential of CPP-ACP added to hard candy confections to slow the progression of enamel subsurface lesions in an in situ model. The confections studied were: (1) control sugar (65% sucrose + 33% glucose syrup); (2) control sugar-free; (3) sugar + 0.5% (w/w) CPP-ACP; (4) sugar + 1.0% (w/w) CPP-ACP; (5) sugar-free + 0.5% (w/w) CPP-ACP. Participants (10 and 14 in study 1 and 2) wore a removable palatal appliance containing enamel half-slabs with subsurface lesions, except for meals and oral hygiene procedures, and consumed 1 confection 6 times a day for 10 days. The enamel half-slabs were inset to allow the development of plaque on the enamel surface. Participants rested for 1 week before crossing over to another confection. The appliances were stored in a humid container at 37 degrees C when not in the mouth. After each treatment period, the enamel half-slabs were removed, paired with their demineralized control half-slabs, embedded, sectioned and then analysed using transverse microradiography. In both studies consumption of the control sugar confection resulted in significant demineralization (progression) of the enamel

subsurface lesions. However, consumption of the sugar confections containing CPP-ACP did not result in lesion progression, but in fact in significant remineralization (regression) of the lesions. Remineralization by consumption of the sugar + 1.0% CPP-ACP confection was significantly greater than that obtained with the sugar-free confection.

Huang et al (2011)[14] conducted a study on the remineralization potential of nano-hydroxyapatite on initial enamel lesions. Remineralization effect of nano-hydroxyapatite and micro-hydroxyapatite was investigated on demineralized bovine enamel under different PH cycling conditions through surface and cross-sectional microhardness test and polarized light microscopy.Results demonstrated that nano-hydroxy apatite provides better remineralization than micro-hydroxyapatite.Remineralization effect of nano-hydroxyapatite increased when PH was less than 7.0.

Tschoppe et al (2011)[47] conducted a study on Enamel and dentin remineralization by nano-hydroxyapatite toothpastes.This in vitro study evaluated the effects of nano-hydroxyapatite (n-HAp) toothpastes on remineralization of bovine enamel and dentine subsurface lesions. Specimens were demineralized, randomly divided into five groups, and exposed to an aqueous remineralizing solution for two and five weeks (37 °C). Brushing procedures were performed with the respective toothpaste/storage solution slurry twice daily (2 × 5 s; total contact time of the slurries 2 × 120s/d): storage in remineralizing solution only (0); additional brushing with B (20 wt% zinc carbonate nano-hydroxyapatite, ZnCO(3)/n-HAp); BS (24 wt%

ZnCO(3)/n-HAp); E (0.14 wt% amine fluoride); or A (7 wt% pure n-HAp). Differences in mineral loss (ΔΔZ) before and after storage/treatment were microradiographically evaluated. Dentine groups 0, B, BS, and A showed significantly higher ΔΔZ values compared to E ($p < 0.05$; ANOVA). Enamel ΔΔZ values of group A were significantly higher compared to group E ($p < 0.05$), whilst no significant differences of these groups could be observed compared to 0, B, and BS ($p > 0.05$) With the in vitro conditions chosen, toothpastes containing n-HAp revealed higher remineralizing effects compared to amine fluoride toothpastes with bovine dentine and comparable trends were obtained for enamel**.**

Hamba et al (2011)[48] evaluated the effects of casein phosphopeptide-amorphous calcium phosphate (CPP-ACP) and CPP-ACP with 900 ppm fluoride (CPP-ACPF) pastes on inhibition of enamel demineralization over time, using polychromatic micro-computed tomography (micro-CT). The application of CPP-ACP or CPPACPF pastes to sound enamel surfaces resulted in inhibition of enamel demineralization and a better effect was noted for the latter paste

Ferrazzano et al (2011)[49] conducted a study on the in vivo remineralising effect of GC tooth mousse on early dental enamel lesions. 40 volunteers (age range 10-16 years) were recruited and divided in two groups of 20 (Group A and B). In Group A subjects two demineralised enamel specimens were placed on the buccal surfaces of the first molars and subjects were instructed to apply a commercial product containing CPPs (GC Tooth Mousse) only on the right-sided specimen and a placebo mousse on the left, for 1 month. In Group B subjects two enamel specimens

were similarly placed into the mouth and used as controls. SEM analysis revealed a diffuse and homogeneous mineral coating, reducing the surface alterations only in the demineralised specimens treated with synthetic CPPs into the mouth. Results demonstrate that CPPs are able to promote remineralisation of early enamel lesions.

Meyer-Lueckel et al (2011)[50] conducted a study to determine the influence of application time on penetration of an infiltrant into natural enamel caries. Caries infiltration is an innovative approach to treat medium stages of caries that bridges the gap between preventive and invasive measures, whereby hard tissues are preserved. Special low viscosity resins (infiltrants) showed almost complete penetration into natural lesions when applied for 5 min. Since shorter application times seem to be clinically more feasible, the aim of this in vitro study was to compare the penetration of an infiltrant (Icon pre-product; DMG, Hamburg, Germany) into natural caries lesions after various application times. Extracted permanent human posterior teeth showing non-cavitated proximal caries lesions were infiltrated for either 0.5, 1, 3, or 5 min (n=20) and light-cured. Specimens were prepared and lesion (LD) as well as penetration depths (PD) were analysed using dual fluorescence confocal microscopy. PD [median (Q25;Q75)] at maximum LD after 0.5 min [159 (27;340) μm] and 1 min [152 (69;375) μm] were significantly lower compared to those after 3 min [414 (338, 518) μm] and 5 min [407 (332;616) μm] ($p<0.05$). Deep lesion parts (PD>500 μm) could be penetrated almost completely after 3 min [98 (88;100)%] and 5 min [100 (81;100)%] application. Thus, 3 min application of an infiltrant seems to be sufficient to achieve an almost complete penetration of enamel caries.

Tahler et al (2012)[51] conducted a study on the influence of resin infiltration system on enamel microhardness and surface roughness. Twenty freshly extracted premolars were used. Sound enamel surfaces were treated with a resin infiltrant (Icon) or fissure sealant (Seal-Rite). The average roughness (R a, μm) of the specimens was measured with a profilometer (Surtronic 10 Tylor Hobson). Surface hardness was determined by utilizing Vicker's surface hardness (VHN) with a Micromet II Microhardness tester. Each specimen acted as its own control. Data were analyzed with 2-way analysis of variance (ANOVA), and mean values were compared with independent t-test. All analyses were performed with the SPSS program version 16 (USA). Differences with a P-value of $\leqslant 0.05$ were considered statistically significant. Comparison of enamel surfaces before and after application of resin infiltrant revealed no significant differences in surface hardness; however, enamel surfaces treated by infiltrant showed significantly higher VHN (244.0 ± 79.8) values than those treated with fissure sealant (37.5 ± 14.2). Surface roughness did not differ before and after application of either material to sound enamel. Enamel surfaces treated with fissure sealant (5.3 ± 1.4) were significantly smoother than those treated with infiltrant (6.9 ± 2.0. Within the limitations of the study, the results showed that enamels treated with the resin infiltrant showed approximately the same microhardness and surface roughness as sound enamel, indicating that this material might be suitable for the treatment of enamel subsurface lesions.

Torres et al (2012)[52] conducted a study on the effect of caries infiltration technique and fluoride therapy on microhardness of enamel carious lesions. Enamel

white spot subsurface lesions compromise esthetics and precede cavitation; therefore, they must be halted. The aim of this study was to evaluate the effect of a caries infiltration technique and fluoride therapy on the microhardness of enamel carious lesions. Subsurface carious lesions were produced in 60 bovine specimens with polished enamel surfaces. The specimens were divided into four groups (n=15), according to the treatment used: ICON, control-immersion in artificial saliva; DF, daily 0.05% fluoride solution; WF, weekly 2% fluoride gel; and IC, resin infiltration (Icon). The specimens were kept in artificial saliva and evaluated for microhardness at five points: baseline, after caries production, after four and eight weeks of treatment, and a final evaluation after being submitted to a new acid challenge. The repeated-measures analysis of variance showed significant differences according to the type of treatment (TREAT; p=0.001) and time of evaluation (EV; p=0.001). The results of the Tukey test were TREAT: CON = 45.18 (±29.17)a, DF = 107.75 (±67.38)b, WF = 83.25 (±51.17)c, and IC = 160.83 (±91.11)d. Analysis of correlation between the TREAT and EV factors showed no significant differences for DF (138.63 ± 38.94) and IC (160.99 ± 46.13) after the new acid challenge. The microhardness results in decreasing order after eight weeks were IC > DF > WF > CON. It was concluded that the microhardness of carious lesions increased with the infiltration of resin, while the final microhardness after a new acid challenge was similar for DF and IC.

Paris et al (2013)[53] conducted a study on the Micro-hardness and mineral loss of enamel lesions after infiltration with various resins: influence of infiltrant composition and application frequency in vitro. The aim of this in vitro study was to

evaluate the influence of infiltrant composition and application frequency on micro-hardness and lesion progression after resin infiltration of artificial enamel lesions. In each of 100 bovine enamel samples, three artificial caries lesions were created (pH=4.95, 50 days). After etching two of the lesions (37% phosphoric acid) specimens were randomly allocated to five infiltrants (four experimental infiltrants with different monomer and solvent compositions and penetration coefficients, and one commercial infiltrant [Icon, DMG]). Lesions were then infiltrated and light-cured, and infiltration repeated afterwards for one of the lesions. Infiltrated samples were cut into halves, with one half being demineralised for further 50 days. Micro-hardness (VHN) and integrated mineral loss (ΔZ) were evaluated at baseline and after second demineralisation. Repeated measures ANOVA and paired t-tests were used to analyse influence of material composition and application frequency on micro-hardness and lesion progression (integrated mineral loss difference ΔΔZ). Resin infiltration significantly increased micro-hardness and reduced lesion progression compared to untreated artificial lesions ($p<0.05$, t-test). Neither micro-hardness nor lesion progression were significantly influenced by material composition ($p>0.05$, ANOVA). In contrast, twice application resulted in significantly increased micro-hardness and demineralisation resistance of infiltrated lesions ($p<0.05$, ANOVA. Resin infiltration significantly improves micro-hardness and demineralisation resistance of enamel lesions; these effects are significantly enhanced if resins are applied twice. Experimental resins did not outperform the commercial infiltrant

Araujo et al (2013)[54] conducted a study on the evaluation of polymerization characteristics and penetration into enamel caries lesions of experimental infiltrants. Groups were set up as follows: G1 (TEGDMA 100%); G2 (TEGDMA 80%, Ethanol 20%); G3 (TEGDMA 80%, HEMA 20%); G4 (TEGDMA 75%, BisEMA 25%); G5 (TEGDMA 60%, BisEMA 20%, Ethanol 20%); G6 (TEGDMA 60%, BisEMA 20%, HEMA 20%); G7 (TEGDMA 75%, UDMA 25%); G8 (TEGDMA 60%, UDMA 20%, Ethanol 20%); G9 (TEGDMA 60%, UDMA 20%, HEMA 20%) and Icon(®). Ten specimens were comprised by each group for the following tests (n=10): degree of conversion (DC), elastic modulus (EM), Knoop hardness (KH), and softening ratio (SR). Infiltrant penetration was evaluated using confocal microscopy (CLSM). Data were subjected to two-way ANOVA and a Tukey's test (5%). Data comparing experimental materials and Icon(®) were analysed using ANOVA and Dunnett's test (5%).The highest DC values were found in G1, G7, G8, and G9. The lowest DC values were found in G2, G4, G5, and G6. EM and KHN were significantly lower in HEMA and with ethanol addition for all blends, except for G9. There was no significant difference among the groups regarding SR, and it was not possible to take KHN readings of G2, G5, and G8 after storage. There was no significant difference among groups for infiltrant penetration into enamel lesions.The addition of hydrophobic monomers and solvents into TEGDMA blends affected DC, EM, and KHN. UDMA added to TEGDMA resulted in an increase in DC, EM, and KHN. Overall, solvents added to monomer blends resulted in decreased properties. The addition of hydrophobic monomers and solvents into TEGDMA blends does not improve the penetration depth of the infiltrants.

Elkassas et al (2014)[55] conducted a study on the remineralizing efficacy of different calcium-phosphate and fluoride based delivery vehicles on artificial caries like enamel lesions. Artificial caries lesions were created on 115 extracted human molars. Specimens were assigned according to remineralizing agent into five groups: G1: Control (artificial saliva), G2: Clinpro™ white varnish, G3: Relief, G4: Tooth Mousse Plus, G5: Vanish™XT. Surface micro-hardness (SMH), surface roughness (Ra) and surface topography by scanning electron microscope (SEM) were evaluated at baseline, after demineralization, after 2 and 4 weeks remineralization and after acid challenge. Demineralized enamel showed the lowest SMH. By 2 weeks remineralization, SMH were ranked as follows: G2 (282.14±6.82)>G3 (269.37±7.25)>G5 (263.00±6.49)=G4 (251.83±8.26)>G1 (226.5±9.34). However, 4 weeks remineralization showed the following: G2 (304.09±6.65)>G3 (293.1±5.24)=G4 (285±7.29)>G5 (272.43±4.89)>G1 (233.33±9.12). By exposure to acid challenge, groups presented order of: G2 (279.71±5.99)=G3 (275.51±5.59)>G4 (262.29±6.65)>G5 (245.43±6.43)>G1 (190.27±8). Surface roughness showed the following mattress after 2 weeks remineralization: G1 (0.2488±0.0016)=G2 (0.2487±0.0007)=G3 (0.2476±0.0006)>G4 (0.2442±0.0004)>G5 (0.2396±0.0009). After 4 weeks remineralization: G1 (0.2469±0.0017)>G4 (0.244±0.0004)>G5 (0.2413±0.0008)=G3 (0.2405±0.0007)=G2 (0.2399±0.0006). After acid challenge; G1 (0.2582±0.0027)>G5 (0.2556±0.0007)>G4 (0.2484±0.0009)>G3 (0.2463±0.0007)>G2 (0.2443±0.0004). SEM revealed mineralized coating on the surfaces which resists dissolution by acid challenge at variable degrees according to remineralization regimen applied. Remineralizing agents containing different calcium-phosphate formulas and fluoride have increased remineralization potential

compared to artificial saliva. Clinpro™ varnish presented the highest remineralization tendency with greatest resistance for acid challenge.

The following armamentaria and materials were used in this study

Armamentarium

For mounting of enamel samples

1. 60 Human maxillary incisor teeth.
2. 10% formalin
3. Diamond disc
4. Micromotor straight handpiece (NSK,JAPAN)
5. Autopolymerizing resin (DPI)
6. Porcelain cup and spatula
7. LED light curing unit (Satelec mini LED, Aceton group Ltd.,Norwich,UK)

For storing of enamel samples

8. Artificial Saliva

Composition of artificial saliva-(Klimek et al.1982)[56]

a. Ascorbic acid - 0.002g
b. Glucose - 0.030g
c. Sodium chloride (NaCl) - 0.580g
d. Potassium Chloride (KCl) - 1.270g
e. Calcium Chloride ($CaCl_2$) - 0.170g
f. Ammonium Chloride (NH_4Cl) - 0.160g
g. Sodium thiocyanate (NaSCN) - 0.160g
h. Di hydrogen potassium phosphate (KH_2PO_4) - 0.03g
i. Urea - 0.02g

j. Sodium Hydrogen Phosphate(Na_2HPO_4) - 0.3g

k. Mucin - 2.7g

L. Distilled water to make up a volume of 1 litre.

For demineralizing enamel samples

9. Demineralizing solution **(Buskes et al.1985)**[57]

Composition of the demineralizing solution

a) Methylhydroxydiphosphonate - 6µM

b) Calcium Chloride Dihydrate ($CaCl_2\_2H_2O$) - 3mM

c) Potassium Dihydrogen Phosphate (KH_2PO_4) - 3mM

d) Acetic acid - 50mM

e) Traces of thymol - 0.2mM

The pH was checked daily and when necessary the pH of the demineralizing solution was adjusted by adding small amounts of either glacial acetic acid or potassium hydroxide solution (10 M)

For checking the pH of demineralizing solution

10. Digisun pH meter (Digisun electronic systems, Hyderabad, India)

For weighing chemicals for preparing artificial saliva and demineralizing solution:

11. Weighing balance **(Wensar weighing scales limited, Chennai, India)**

For brushing of the enamel samples

12. Powered tooth brush (ORAL-B)

For Testing of the enamel samples

13. Confocal Laser scanning microscope (Carl Zeiss, Germany)

14.Vickers microhardness tester (Bareiss, Digi test, V test, Germany)

Materials used in this study

Name	Manufacturer	Composition
Resin infiltrant (ICON)	ICON DMG (GERMANY)	**Icon-Etch:** Hydrochloric acid, pyrogenic silicic acid, surface-active substances **Icon-Dry**: 99% ethanol **Icon-Infiltrant**: Methacrylate-based resin matrix, initiators, additives
CPP-ACP (GC tooth mousse)	GC Corporation Tokyo, Japan	Glycerol -20.0% Propylene glycol- 2.0% CPP-ACP (casein phosphopeptide - amorphous calcium Phosphate) -10.0% D-glucitol- 5% Colloidal silica -2.0% Sodium carboxyl methylcellulose(CMC-Na)- 2% Titanium dioxide- 2% Xylitol- 2% Guar gum <0.1% Phosphoric acid <0.2% Sodium saccharin <0.2% Zinc oxide <0.1% Magnesium oxide <0.1% Ethyl 4-hydroxybenzoate

		<0.1%, Propyl4-hydroxybenzoate 0.1% Fluoride-900ppm
Nano hydroxyapatite(Aclaim)	Group pharmaceuticals limited, Mumbai	Nanohydroxyapatite Sorbitol Glycerin Silica Purified water Cocamido Propyl betaine Hydroxyl ethyl cellulose Titanium di oxide Flavor Sodium saccharin

STUDY METHODOLOGY

Collection of the teeth

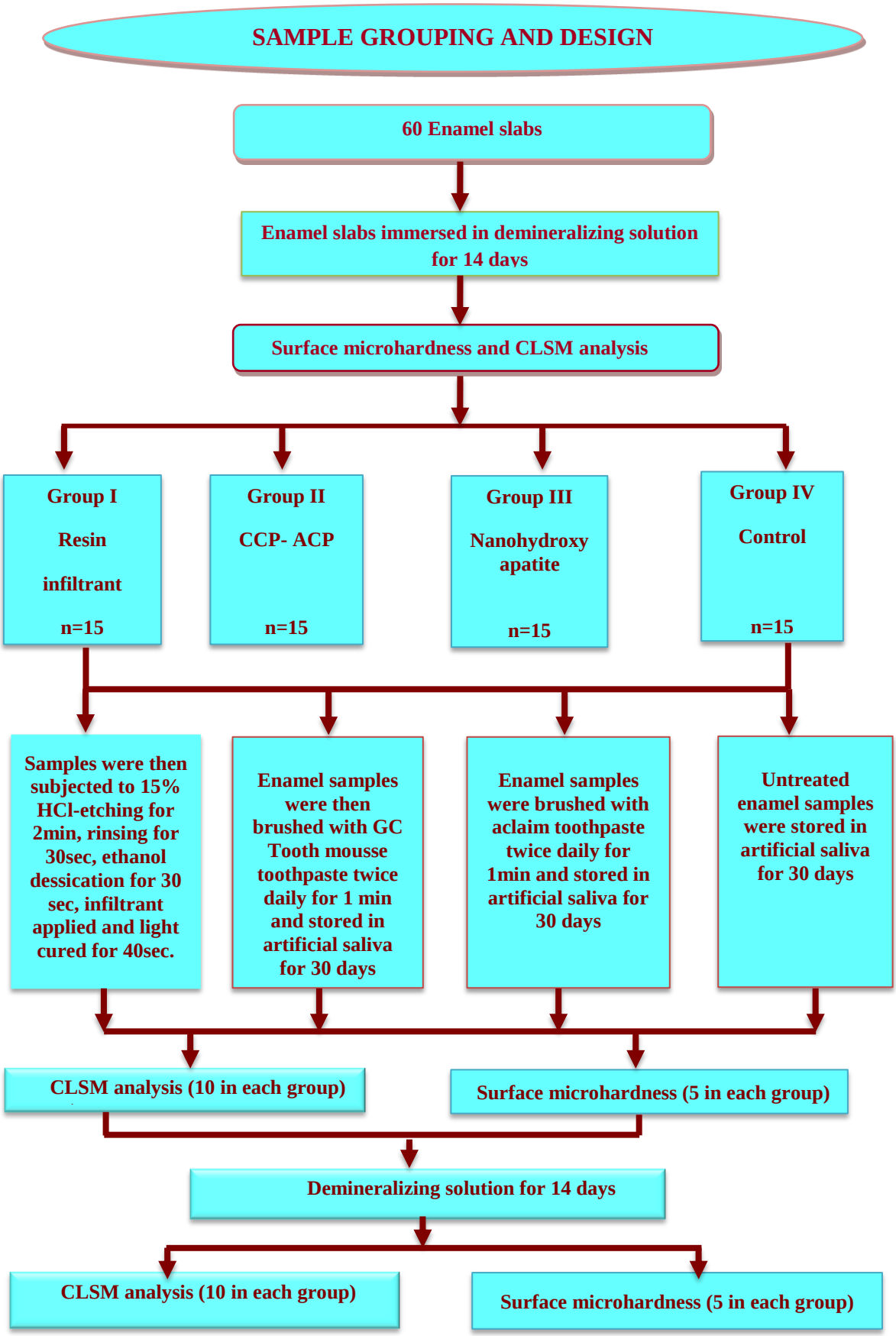
60 human maxillary incisors extracted for periodontal reasons were included in this study. Teeth with any visible caries, hypoplastic lesions and white spot lesions were excluded from this study. The teeth were stored in 10% formalin immediately after extraction.

Enamel sample preparation

The teeth were thoroughly cleaned of all debris including calculus and tissue debris. Occupational Safety and Health Administration (OSHA) and Centre for Disease Control and Prevention (CDC) recommendations and guidelines were followed during the collection, storage, sterilization and handling of the extracted teeth. Then the radicular portions were removed by decoronating the teeth 2mm coronal to cement enamel junction(CEJ) using a diamond disk (Axis dental, Texas) attached to a slow speed micromotor straight handpiece rotating at 1500rpm. The Sectioning was done at the middle third region of the crown for the 60 samples with approximate dimensions of (5x5x5mm) and then stored in 10% formalin at room temperature of 37^{0}c and humidity.

Mounting of the enamel samples

60 Enamel slabs were then embedded on an acrylic resin block using a standardized mould having a dimension of (2x1.5x1cm) and the embedded blocks were then stored in artificial saliva at 37^{0}C.


SAMPLE GROUPING AND DESIGN
60 Enamel slabs
Enamel slabs immersed in demineralizing solution for 14 days
Surface microhardness and CLSM analysis
Group I
Resin
infiltrant
n=15
Group II
CCP- ACP
n=15
Group III
Nanohydroxy apatite
n=15
Group IV
Control
n=15
Samples were then subjected to 15% HCl-etching for 2min, rinsing for 30sec, ethanol dessication for 30 sec, infiltrant applied and light cured for 40sec.
Enamel samples were then brushed with GC Tooth mousse toothpaste twice daily for 1 min and stored in artificial saliva for 30 days
Enamel samples were brushed with aclaim toothpaste twice daily for 1min and stored in artificial saliva for 30 days
Untreated enamel samples were stored in artificial saliva for 30 days
CLSM analysis (10 in each group)
Surface microhardness (5 in each group)
Demineralizing solution for 14 days
CLSM analysis (10 in each group)
Surface microhardness (5 in each group)

Demineralization of enamel samples

In order to create the artificial enamel lesions, the samples were demineralized by placing in a beaker containing the prepared demineralizing solution (Buskes et al.1985)[57]. The study samples were then stored for a period of 14 days all the while maintaining a pH of 5.0 and at 37^{o}C temperature. The pH was checked daily using a pH meter and any variation in pH was corrected by adding either glacial acetic acid or potassium hydroxide solution. The study samples were then randomly divided into four groups containing 15 samples each.

Distribution of samples

The samples were then divided into four groups each containing 15 samples.

Group I- Resin infiltrant (ICON) (n=15)

Group II- CPP-ACP (GC Tooth mousse) (n=15)

Group III –Nano Hydroxyapatite (Aclaim) (n=15)

Group IV –Control (n=15)

Evaluation of microhardness

Vickers hardness number (VHN) was determined by making three indentations at different regions of each specimen using a square based diamond pyramid Vicker's indenter under a load of 100 g for 10 sec. The indentations were made 100 μm apart from each other to avoid residual stress. This procedure resulted in obtaining well defined indentations. The main criteria for accepting an indentation was clearness of outline and the absence of flaws in the tooth at the area of measurement. Microhardness testing was carried out for all the enamel samples and

results were obtained. In order to standardize the enamel samples, 60 samples with VHN in the range of 300.0 to 350.0 was selected for the study. 20 enamel samples (5 in each group) were then evaluated for microhardness after

1) Placing the samples in the demineralizing solution for 14 days

2) Exposing the samples to resin infiltrant, CPP-ACP, and nano-HA for 30days.

3) Re-exposing the treated enamel samples to the demineralizing solution for a period of 14 days

Evaluation of penetration

The infiltrated specimens were then observed using a confocal laser scanning microscope equipped with Argon/Krypton laser. The microscope was operated in the fluorescent mode. The excitation light had a maximum wavelength of 560nm and the detected light was passed through a 590 nm long pass filter in order to suppress the reflected and scattered light. The observed layer was then chosen 10μm beneath the specimen surfaces. The microscope setting was kept constant throughtout the investigation period.

The depth of the lesion was then measured with the confocal laser scanning microscope for all the study samples after being exposed to the demineralizing solution for 14 days. The samples were treated with the remineralizing agent for a time period of 30 days, the penetration depth of the treated enamel samples was then determined using the Confocal laser scanning microscope (Carl Zeiss, Germany) and also after re exposing the treated enamel samples to a demineralizing solution for 14 days and maintaining a pH of 5 at 37^{0} C.

Remineralization of enamel samples

GROUP I (Resin infiltrant)

15 enamel samples after demineralization were then infiltrated with the resin infiltrant (ICON, DMG) by first etching the enamel samples with 15% hydrochloric acid for 2minutes, after which rinsing with water was done for 30 seconds to remove the etchant. And after drying the sample, ethanol was applied for 30 seconds in order to dessicate the enamel sample and finally to infiltrate it with the resin infiltrant and allowed to remain for 3 minutes and the excess was removed. The curing was done with a LED curing unit for 40 seconds (Satelec mini LED, Aceton group Ltd.,Norwich,UK). The applicator tip was replaced and allowed to remain for 1 minute and light cured for 40 seconds.

GROUP II (CPP-ACP)

15 enamel samples after demineralization were brushed twice daily for 1 minute using a powered tooth brush(Oral-B) using GC tooth mousse for a time period of 30 days.

GROUP III (Nanohydroxyapatite)

Aclaim tooth paste was applied over the 15 enamel samples after demineralization twice daily for 1 minute using a powered tooth brush(Oral-B) for 30 days.

Group IV (Control)

The 15 enamel samples were placed in artificial saliva for 30 days and was taken as the control group.

The treated enamel samples were then subjected to microhardness testing (5 in each group) and confocal laser scanning microscope (10 in each group) and

analysis was done. The samples in all the groups were subjected to demineralizing solution for 14 days and the surface microhardness (5 in each group) was evaluated using Vickers microhardness test and the depth of the lesion (10 in each group) were analyzed using confocal laser scanning microscope.

Resin infiltrant (Group I)

Figure 1 a- ICON

CCP-ACP (Group II)

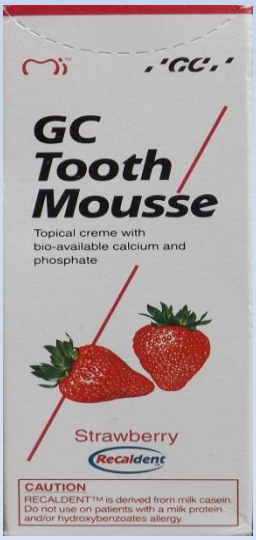


Figure 1b– GC tooth mousse

Nano-HA (Group III)

Figure 2a–Aclaim toothpaste

Demineralizing solution

Figure 2b chemicals for preparing demineralizing solution

Straight handpiece

Figure 3a – NSK Straight handpiece

Diamond disk

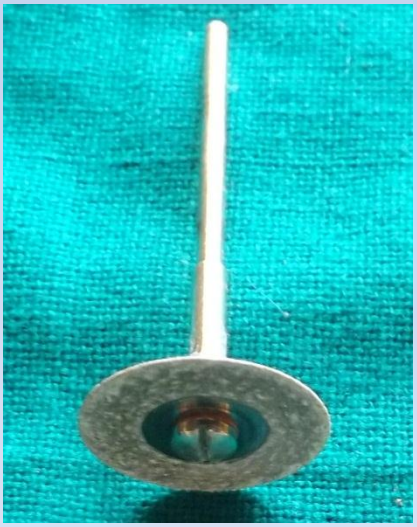


Figure 3b

Self cure acrylic resin

Figure 4a

Powered tooth brush

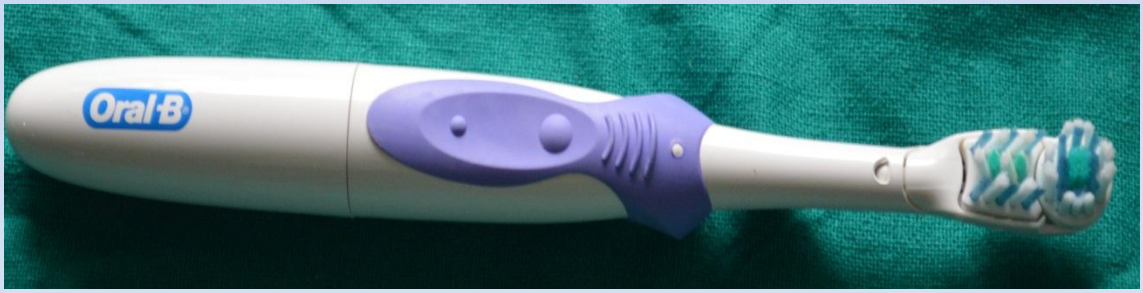


Figure 4b Oral-B

Artificial saliva

Figure 5a chemicals for preparing saliva

pH meter

Figure 5b

Weighing balance

Figure 6a Weighing balance for weighing chemicals for preparing artificial saliva and demineralizing solution

Confocal laser scanning microscope

Figure 6b

Vickers microhardness tester

Figure 7a

60 Maxillary incisors

Figure 8a

Sectioning of teeth

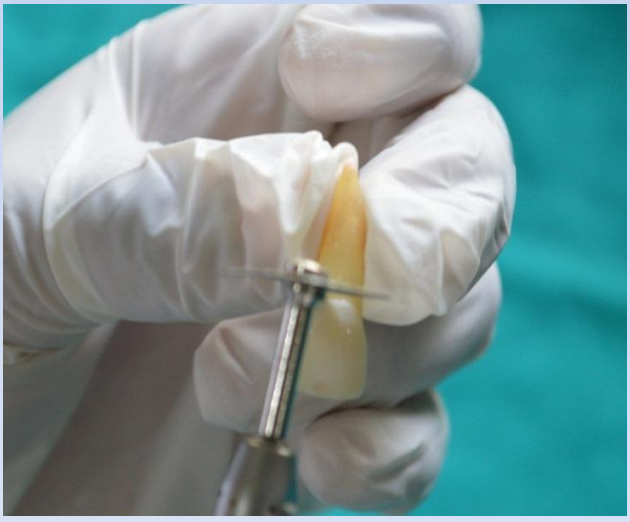


figure 8b

Resin infiltrant (Group I) enamel samples

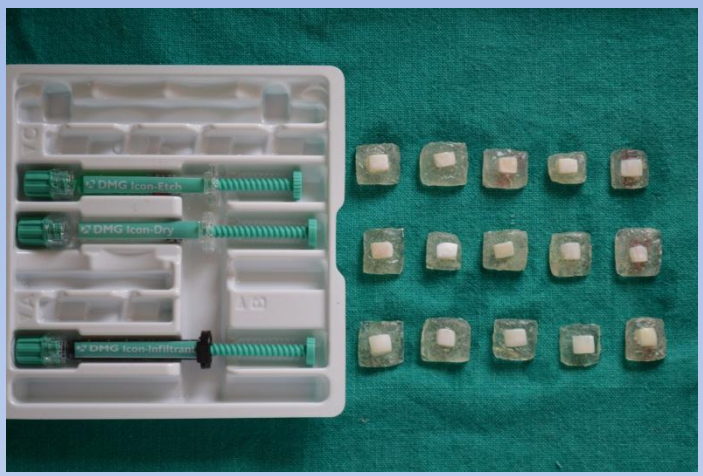


Figure 9a - Embedded in resin blocks

CCP-ACP (Group II) enamel samples

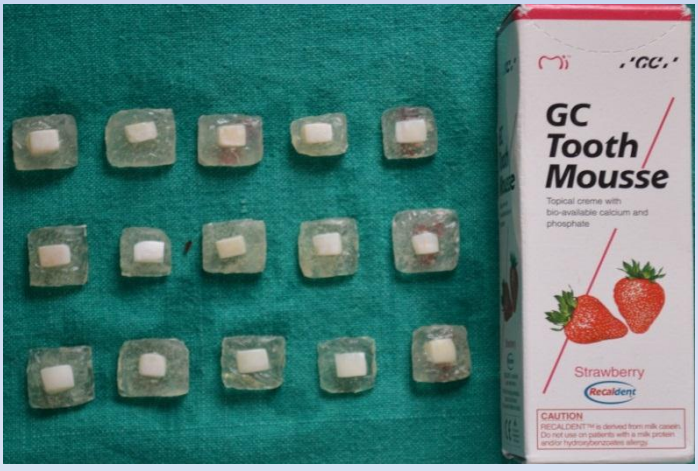


figure 9b CCP-ACP

Nano-HA (Group III) enamel samples

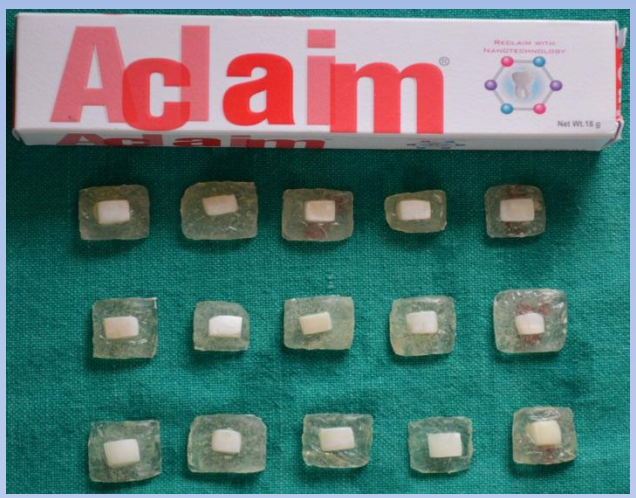


Figure 10a

Control (Group IV) enamel samples

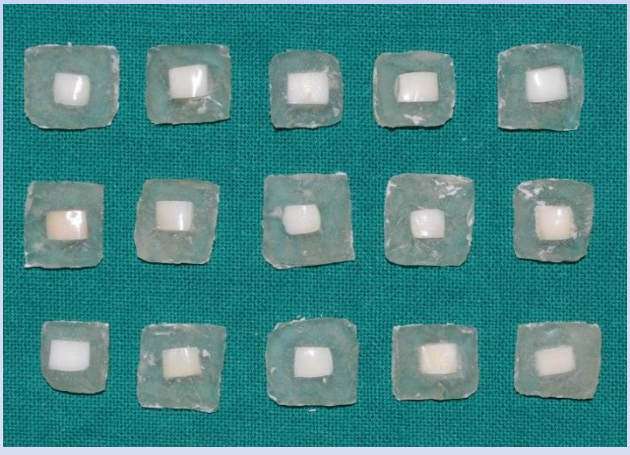


figure 10b

Prepared demineralizing solution

Figure 11 a

Prepared artifical saliva

figure 11 b

CLSM analysis of samples

Figure 12 a

Sample analysis through vickers microhardness tester

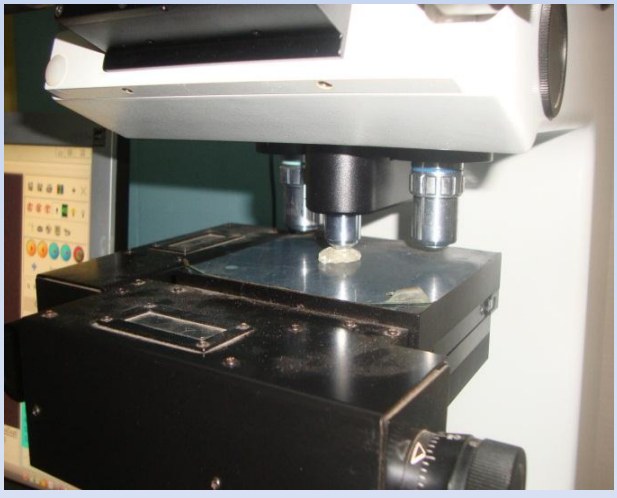


figure 12 b

CLSM images of infiltrated resin for Group I (Resin infiltrant)

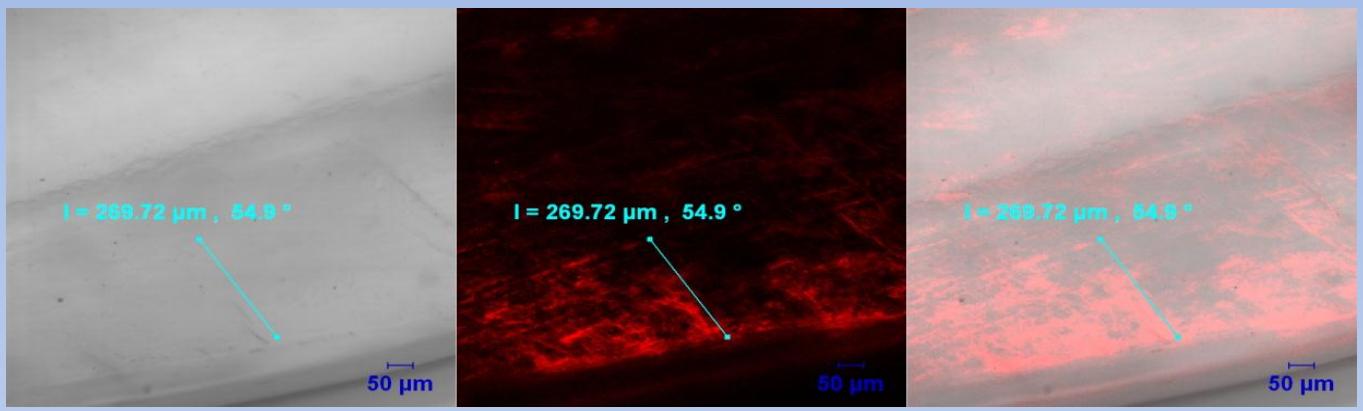


Figure 13 a

CLSM images after acid challenge for 14 days for Group I (Resin infiltrant)

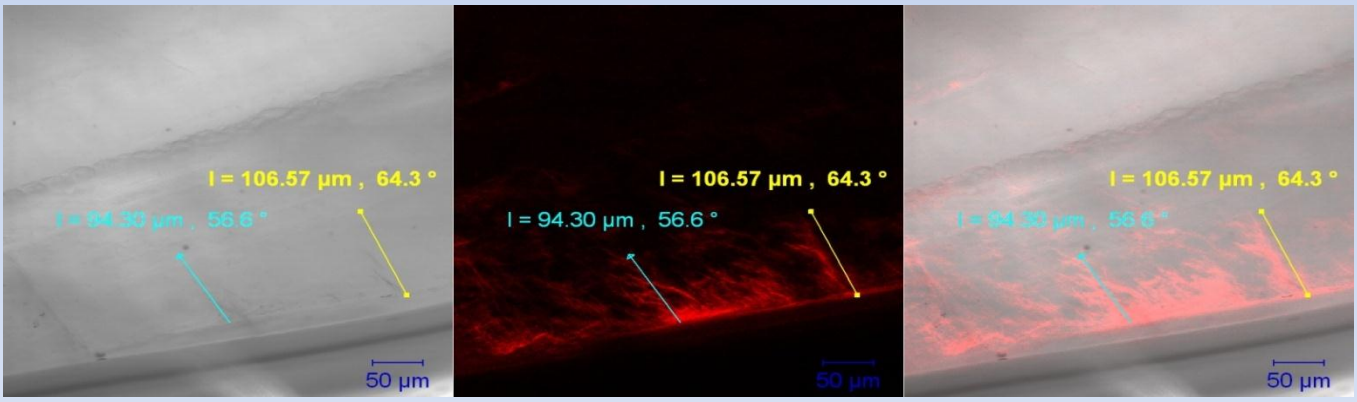


Figure 13 b

CLSM images of Group II (CPP-ACP) after Remineralization

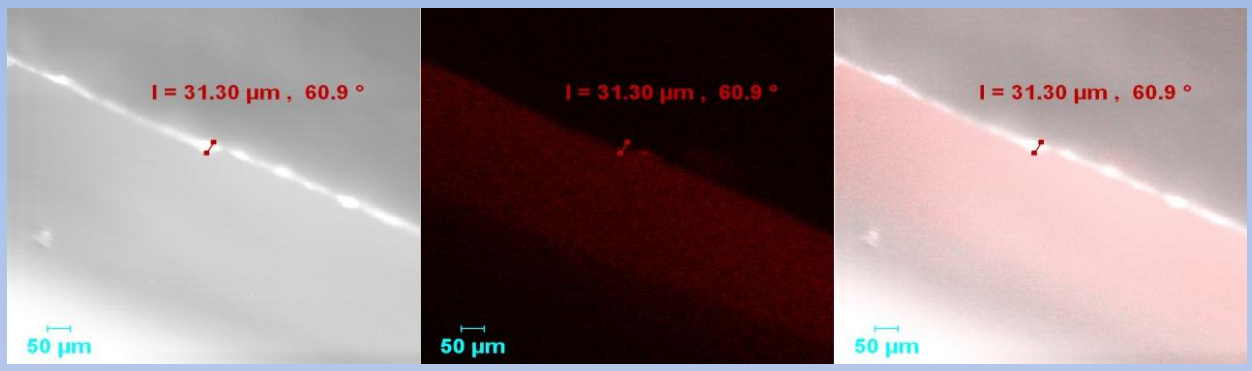


Figure 14 a

CLSM images after acid challenge for 14 days for Group II (CCP-ACP)

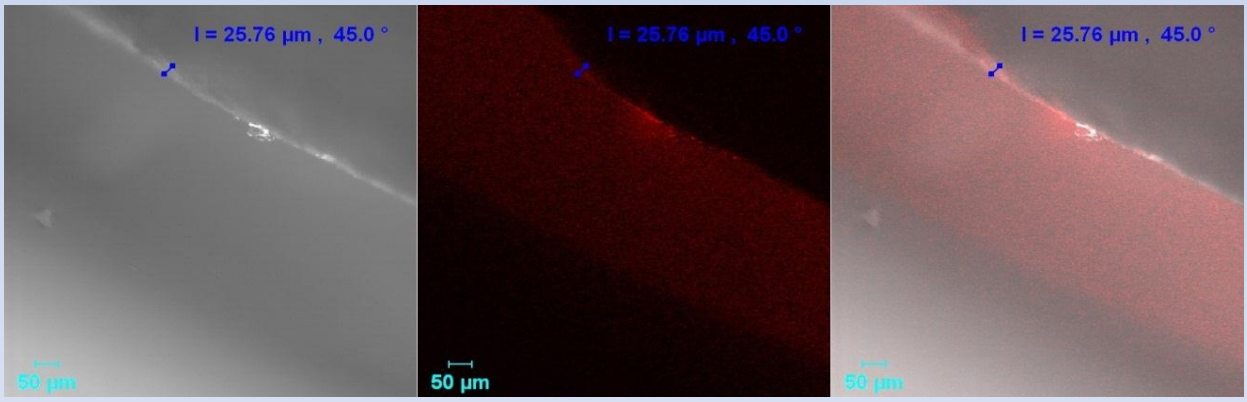


Figure 14 b

CLSM images of Group III (Nano-HA) after remineralization

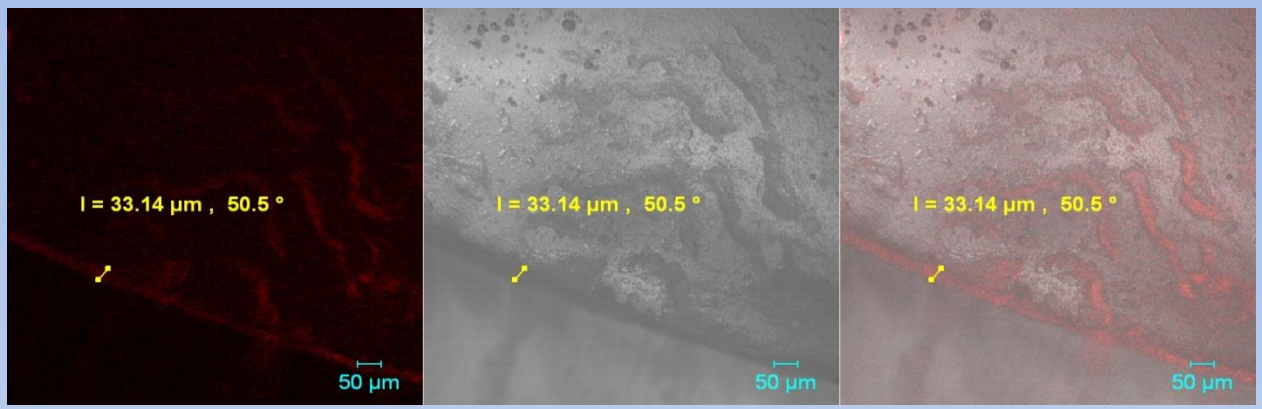


Figure 15 a

CLSM images after acid challenge for 14 days for Group III (nano-HA)

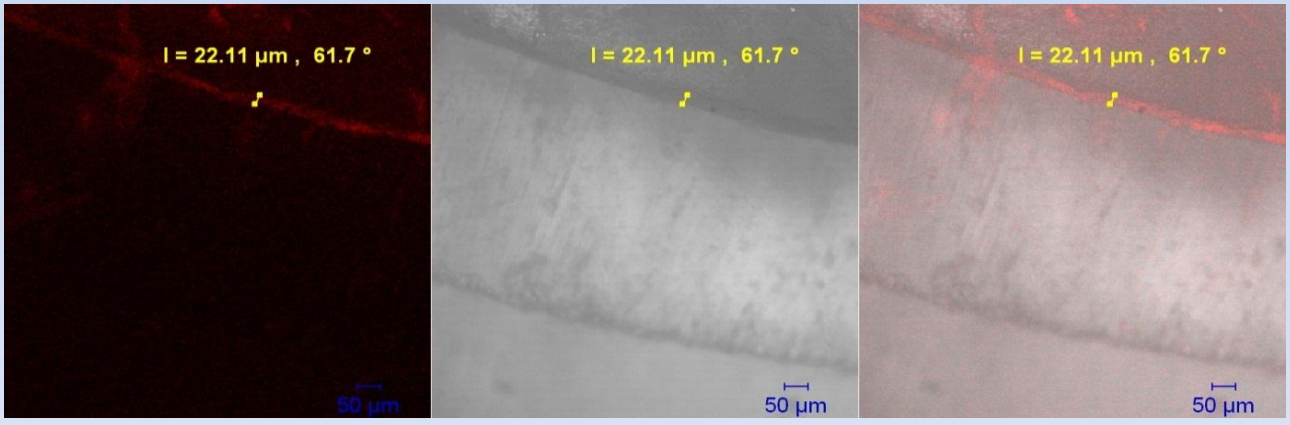


Figure 15 b

CLSM images of Group IV (control group) after demineralization

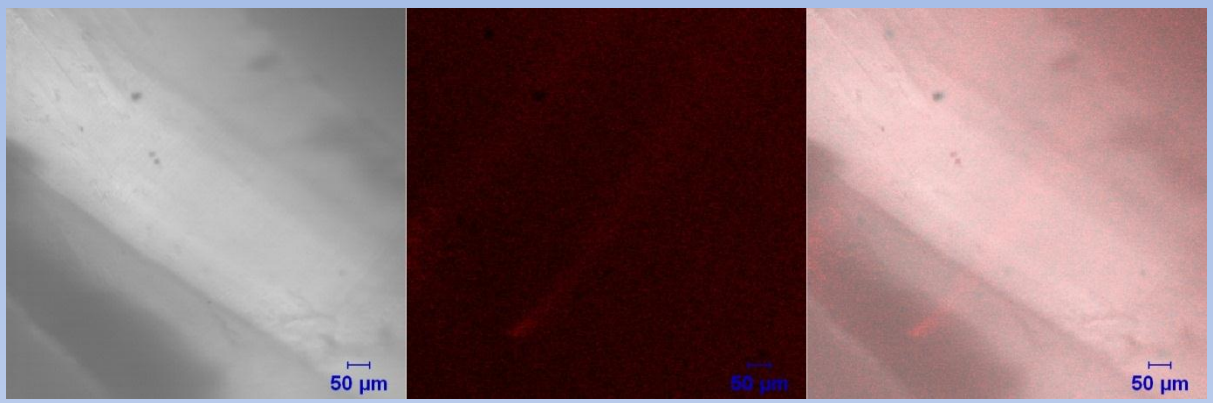


Figure 16 a

CLSM images after acid challenge for 14 days for Group IV (control)

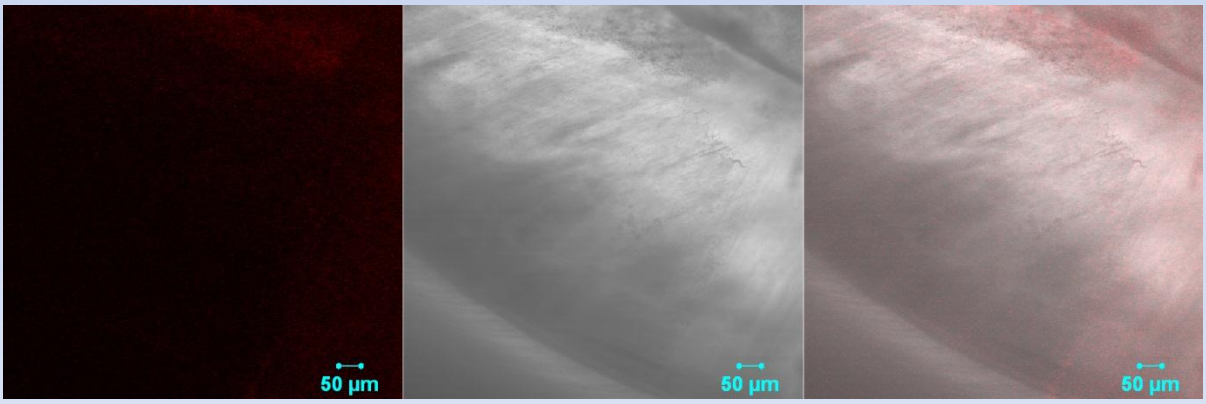


Figure 16 b

Microhardness images for Group I (Resin infiltrant)

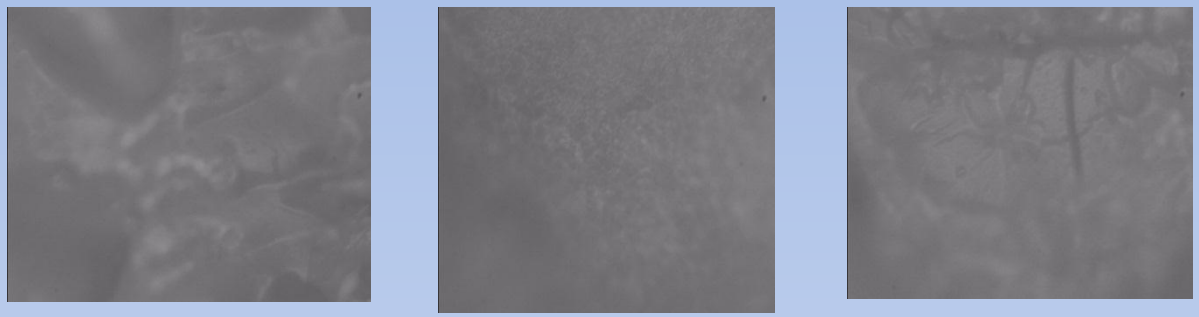


Figure 17 a

Microhardness images for Group II (CCP-ACP)

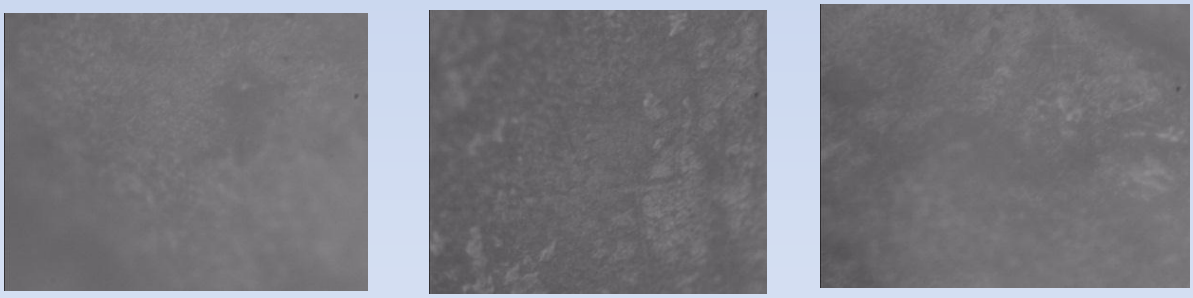


Figure 17 b

Microhardness images for Group III (nano-HA)

Figure 18 a

Microhardness images for Group IV (control)

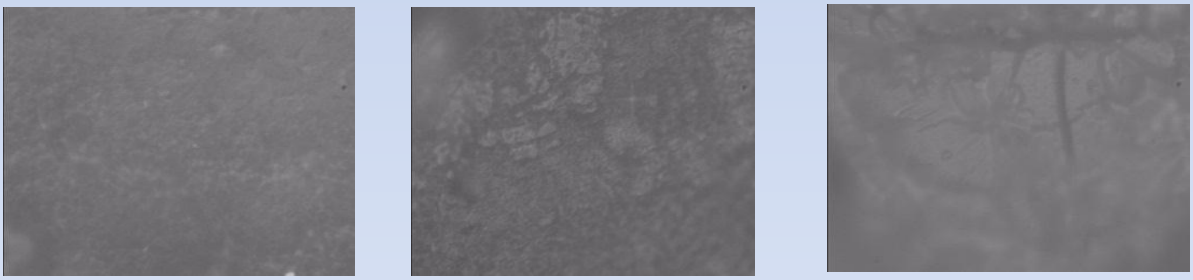


Figure 18 b

Statistical Analysis

Data were analyzed using Statistical Package for Social Sciences (SPSS) version 20.0. Measures of central tendency such as mean and measures of dispersion like Standard deviation were calculated for all the parameters. Datas were statistically analyzed using ANOVA. And post hoc bonferroni test were used for comparing intragroups and Tukey test were used to compare Intergroups.

Interpretation of results

Table 1 shows the intra group comparison for Group I (Resin infiltrant) after demineralization, infiltration, and acid challenge. The mean values after demineralization of enamel samples in the demineralizing solution is 245µm, after infiltrating with resin infiltrant the mean depth of penetration was 158µm. After acid challenge for a period of 14 days the amount of remaining resin infiltrant is 114µm (72%). The p value were <0.001for all the parameters tested. After Bonferroni Post hoc intra group comparison there was statistically significant difference between demineralization, remineralization and after acid challenge.

Table 2 shows the intra group comparison for Group II (CPP-ACP) after demineralization, remineralization and acid challenge. The mean values after demineralization of enamel samples in the demineralizing solution is 246µm. After remineralizing the enamel samples for a period of 30 days, there was a surface remineralization of 28µm. After acid challenge for a period of 14 days the amount of remaining CPP-ACP was 28.8µm (57%). The p value were <0.001for all the parameters tested. After Bonferroni Post hoc intra group comparison there was a statistically significant difference between demineralization, remineralization and after acid challenge.

Table 3 shows the intragroup comparison for Group III (nano-HA) after demineralization, remineralization and acid challenge. The mean values after demineralization of enamel samples in demineralizing solution is 250µm. After remineralizing the enamel samples for a period of 30 days, there was a surface

remineralization of 26.3µm. After acid challenge for a period of 14 days the amount of remaining nano-HA was 13.8µm (50%). The p value were <0.001 for all the parameters tested. After Bonferroni Post hoc intra group comparison there was statistically significant between demineralization, remineralization and after acid challenge.

Table 4 shows the inter group comparison for all the groups after demineralization, remineralization and acid challenge for confocal group. The mean values after demineralization of enamel samples in demineralizing solution is 245 µm for Group I (Resin infiltrant), 246 µm for Group II (CPP-ACP), 250 µm for Group III (nano-HA) and 247 µm for Group IV (control). After remineralizing the enamel samples for a period of 30 days, the results are Group I (Resin infiltrant) 158 µm > Group II (CPP-ACP) 28.8µm ⩾ Group III (nano-HA) 26.3µm. After acid challenge for a period of 14 days there was Group I (Resin infiltrant) 114µm (72%) > GROUP II (CPP-ACP) 16.4µm (57%) ⩾ Group IV (nano-HA) 13.8µm (50%) of remaining material. The untreated control group had least resistance with mean of 8 µm. The p value were 0.993 after demineralization, <0.001 after remineralization and after acid challenge for 14 days when comparison was done between all the four groups. After Post hoc Tukey inter group comparison Group I showed statistically significant difference which was greater when compared to group II (CPP-ACP) and Group III (nano-HA) where there were no difference.

Table 5 shows the intra group comparison of surface microhardness for Group I (Resin infiltrant). The mean microhardness value after demineralization of

enamel samples is 226VHN. The mean hardness after infiltrating with resin infiltrant increased to 316 VHN. The mean value after acid challenge for 14 days the hardness decreased to 292VHN. The p value were <0.001 for all the parameters. After Post hoc Bonferroni test there was statistically significant difference between demineralization and after acid challenge. There was also significant difference between remineralization and after acid challenge. There was no statistically significant difference between remineralization and demineralization.

Table 6 shows the intra group comparison of surface microhardness for Group II (CPP-ACP). The mean microhardness value after demineralization of enamel samples is 215VHN. The mean value after remineralizing the enamel samples for a period of 30days the hardness increased to 282 VHN. The mean value after acid challenge for 14 days the hardness value decreased to 254VHN. The p value were <0.001 for all the parameters. After Post hoc Bonferroni test there was statistically significant difference between demineralization, remineralization and after acid challenge.

Table 7 shows the intra group comparison of surface microhardness for Group III (nano-HA). The mean microhardness value after demineralization of enamel samples is 222VHN. The mean value after remineralizing the enamel samples for a period of 30days the hardness increased to 267 VHN. The mean value after acid challenge the hardness decreased to 237VHN. The p value were <0.001 for all the parameters. After Post hoc Bonferroni test there was statistically significant difference between demineralization, remineralization and after acid challenge.

Table 8 shows the intra group comparison of surface microhardness for Group IV (Control). The mean microhardness value after demineralization of enamel samples is 207VHN. The mean after remineralizing the enamel samples for a period of 30days in artificial saliva, the hardness increased to 218 VHN. The mean after acid challenge the hardness decreased to 167VHN. The p value were <0.001 for all the parameters. After Post hoc Bonferroni test there was statistically significant difference between demineralization, remineralization and after acid challenge. There was no statistically significant difference between demineralization and acid challenge.

Table 9 shows the inter group comparison of surface microhardness for all the four groups after demineralization. The mean microhardness value after demineralization of enamel samples is Group I (Resin infiltrant) 226VHN, Group II (CPP-ACP) 222VHN, Group III (nano-HA) 207 VHN, Group IV (control) 215VHN. The p value was 0.143 which was not statistically significant.

Table 10 shows the inter group comparison of surface microhardness for all the four groups after Remineralization. The mean microhardness value after Remineralization of enamel samples is Group I (Resin infiltrant) 316VHN > Group II (CPP-ACP) 282VHN ⩾ Group III (nano-HA) 267VHN > Group IV (control) 218 VHN. The p value were <0.001 when comparing all the four groups. After Post hoc Tukey test there was statistically significant difference between Group I (Resin

infiltrant) and other 3 groups. Group II (CPP-ACP) and Group III (nano-HA) showed no significant difference.

Table 11 shows the inter group comparison of surface microhardness for all the four groups after acid challenge. The mean microhardness after acid challenge of enamel samples for 14 days is Group I (Resin infiltrant) 292VHN > Group II (CPP-ACP) 254VHN ⩾ Group III (nano-HA) 237VHN > Group IV (control) 167 VHN. The p value was < 0.001 for groups. After Post hoc Tukey test there statistically significant difference between resin Group I (Resin infiltrant) and other 3 groups. Group II (CPP-ACP) and Group III (nano-HA) showed no significant difference.

Graph 1 shows intra group comparison for Group I (Resin infiltrant) after demineralization, remineralization and acid challenge. The different parameters tested demineralixation, remineralization and acid challenge were plotted along x-axis and mean value (µm) were plotted along y-axis.

Graph 2 shows intra group comparison for Group II (CPP-ACP) after demineralization, remineralization and acid challenge. The different parameters tested demineralixation, remineralization and acid challenge were plotted along x-axis and mean value (µm) were plotted along y-axis.

Graph 3 shows intra group comparison for Group II (nano-HA) after demineralization, remineralization and acid challenge. The different parameters

tested demineralization, remineralization and acid challenge were plotted along x-axis and mean value (µm) were plotted along y-axis.

Graph 4 shows inter group comparison for all the four Groups namely Group I (Resin infiltrant), Group II (CPP-ACP), Group III (nano-HA) and Group IV (control) after demineralization, remineralization and acid challenge. The different groups were plotted along x-axis and mean value (µm) were plotted along y-axis.

Graph 5 shows intra group comparison for the Group I (Resin infiltrant) for surface microhardness after demineralization, remineralization and acid challenge. The different parameters tested demineralization, remineralization and acid challenge were plotted along x-axis and mean hardness (VHN) were plotted along y-axis.

Graph 6 shows intra group comparison for Group II (CPP-ACP) for surface microhardness after demineralization, remineralization and acid challenge. The different parameters tested demineralization, remineralization and acid challenge were plotted along x-axis and mean hardness (VHN) were plotted along y-axis.

Graph 7 shows the intra group comparison for Group III (nano-HA) for surface microhardness after demineralization, remineralization and acid challenge. The different parameters tested demineralization, remineralization and acid challenge were plotted along x-axis and mean hardness (VHN) were plotted along y-axis.

Graph 8 shows the inter group comparison for all the four groups Group I (Resin infiltrant), Group II (CPP-ACP), Group III (nano-HA) and Group IV (control) for surface microhardness after demineralization, remineralization and acid challenge. The different groups were plotted in x-axis and mean hardness (VHN) were plotted along y-axis.

I. Results for Confocal Group

Table 1: Results for Group I (Resin infiltrant)

Resin infiltrant	Mean	Std. Error	95% Confidence Interval		p value	Post Hoc
			Lower Bound	Upper Bound		
Demin	245.000	22.571	193.941	296.059	<0.001	Demin*>Remin*> Acid challenge
Remin	158.100	16.903	119.862	196.338		
Acid challenge	114.000	13.013	84.563	143.437		

* -Statistically significant p<0.05

Table 2. Results for Group II (CCP-ACP)

CPP-ACP	Mean	Std. Error	95% Confidence Interval		p value	Post Hoc
			Lower Bound	Upper Bound		
Demin	246.000	23.200	193.519	298.481	<0.001	Demin*>Remin*> Acid challenge
Remin	28.800	.742	27.121	30.479		
Acid challenge	16.400	.542	15.175	17.625		

* -Statistically significant p<0.05

Table 3:Results for Group III (nano-HA)

Nanohydroxy apatite	Mean	Std. Error	95% Confidence Interval		p value	Post Hoc
			Lower Bound	Upper Bound		
Demin	250.500	21.889	200.983	300.017	<0.001	Demin*>Remin*>Acid challenge
Remin	26.300	.367	25.471	27.129		
Acid challenge	13.800	.663	12.299	15.301		

* -Statistically significant p<0.05

Table 4: Inter Group Comparisons for all the study groups

Group		N	Mean	Std. Deviation	95% Confidence Interval		Minimum	Maximum	p value	Post Hoc (Tukey test)
					Lower Bound	Upper Bound				
Demin	Resin infiltrant	10	245.00	71.37538	193.9411	296.0589	150.00	370.00	0.853	
	CPP-ACP	10	246.00	73.36363	193.5188	298.4812	150.00	380.00		-
	Nano-HA	10	250.50	69.21986	200.9831	300.0169	180.00	370.00		
	Control	10	230.50	54.93412	186.5674	204.0713	160	350		
	Total	40	247.17	68.87875	221.4469	272.8864	150.00	380.00		
Remin	Resin infiltrant	10	158.10	53.45289	119.8621	196.3379	94.00	269.00		
	CPP-ACP	10	28.80	2.34758	27.1206	30.4794	25.00	31.00	<0.001	ICON*> (CC=ACC)*> Control
	nano-HA	10	26.30	1.15950	25.4705	27.1295	25.00	28.00		
	Control	10	23.00	0.90711	34.4562	21.7861	18.00	25.00		
	Total	40	71.06	69.33921	45.1750	96.9584	25.00	269.00		
Acid challenge	Resin infiltrant	10	114.00	41.15013	84.5630	143.4370	60.00	180.00	<0.001	ICON*> (CC=ACC)*> Control
	CPP-ACP	10	16.40	1.71270	15.1748	17.6252	14.00	18.00		
	Nano-HA	10	13.80	2.09762	12.2995	15.3005	12.00	18.00		
	Control	10	8.502	0.696	9.005	11.891	5.00	11.00		
	Total	40	48.06	52.70211	28.3874	67.7460	12.00	180.00		

* -Statistically significant $p<0.05$

II. Results for the Microhardness Group

Table 5 : Results for Group I (Resin infiltrant)

Resin infiltrant	Mean	Std. Error	95% Confidence Interval		p value	Post Hoc Tests Bonferroni method
			Lower Bound	Upper Bound		
Demin	226.000	9.274	200.252	251.748	<0.001*	Demin<acid challenge*
Remin	316.000	9.274	290.252	341.748		Remin>acid challenge*
Acid Challenge	292.000	7.348	271.597	312.403		Remin/demin (NS)

* -Statistically significant p<0.05
NS-not significant

Table 6: Results for Group II (CPP- ACP)

CPP-ACP	Mean	Std. Error	95% Confidence Interval		p value	Post Hoc Tests Bonferroni method
			Lower Bound	Upper Bound		
Demin	215.200	3.040	206.760	223.640	<0.001*	Demin<Remin*, Demin<Acid challenge*
Remin	282.600	3.842	271.933	293.267		Remin>demin* Remin>Acid challenge*
Acid Challenge	254.000	5.099	239.843	268.157		Acid challenge>Demin* Acid challenge<Remin*

* -Statistically significant p<0.05

Table 7: Results for Group III (nano-HA)

Nanohydroxyapatite	Mean	Std. Error	95% Confidence Interval		p value	Post Hoc Tests Bonferroni method
			Lower Bound	Upper Bound		
Demin	222.000	6.819	203.067	240.933	<0.001	Demin<Remin*, Demin<Acid challenge*
Remin	267.000	7.000	247.565	286.435		Remin>demin* Remin>Acid challenge*
Acid challenge	237.000	6.442	219.114	254.886		Acid challenge>Demin* Acid challenge<Remin*

* -Statistically significant p<0.05

Table 8 : Results for Group IV (control)

Control	Mean	Std. Error	95% Confidence Interval		p value	Post Hoc Tests Bonferroni method
			Lower Bound	Upper Bound		
Demin	207.000	5.385	192.048	221.952	<0.001	Demin<Remin*
Remin	218.000	6.042	201.226	234.774		Remin> Acid challenge*
Acid challenge	167.000	5.385	152.048	181.952		Demin/Acid challenge (NS)

* -Statistically significant p<0.05

Table 9: Comparison of the microhardness for all the study groups after demineralization

Demin	N	Mean	Std. Deviation	95% Confidence Interval for Mean		Minimum	Maximum	p value
				Lower Bound	Upper Bound			
Resin infiltrant	5	226.00	20.73644	200.2523	251.7477	200.00	250.00	0.143 (NS)
CPP-ACP	5	222.00	15.24795	203.0672	240.9328	200.00	240.00	
nano-HA	5	207.00	12.04159	192.0484	221.9516	190.00	220.00	
Control	5	215.50	16.93644	207.2523	223.7477	190.00	250.00	

* -Statistically significant p<0.05

Table 10: Comparison of microhardness for all the groups after Remineralization

Remin	N	Mean	Std. Deviation	95% Confidence Interval for Mean		Minimum	Maximum	p value	Post Hoc
				Lower Bound	Upper Bound				
Resin infiltrant	5	316.00	20.73644	290.2523	341.7477	290.00	340.00	<0.001	Resin infiltrant*> (CPP-ACP=nano-HA)*>control
CPP-ACP	5	282.60	8.59069	271.9332	293.2668	270.00	290.00		
nano-HA	5	267.00	15.65248	247.5649	286.4351	250.00	290.00		
Control	5	218.00	13.50926	201.2260	234.7740	200.00	235.00		

* -Statistically significant p<0.05

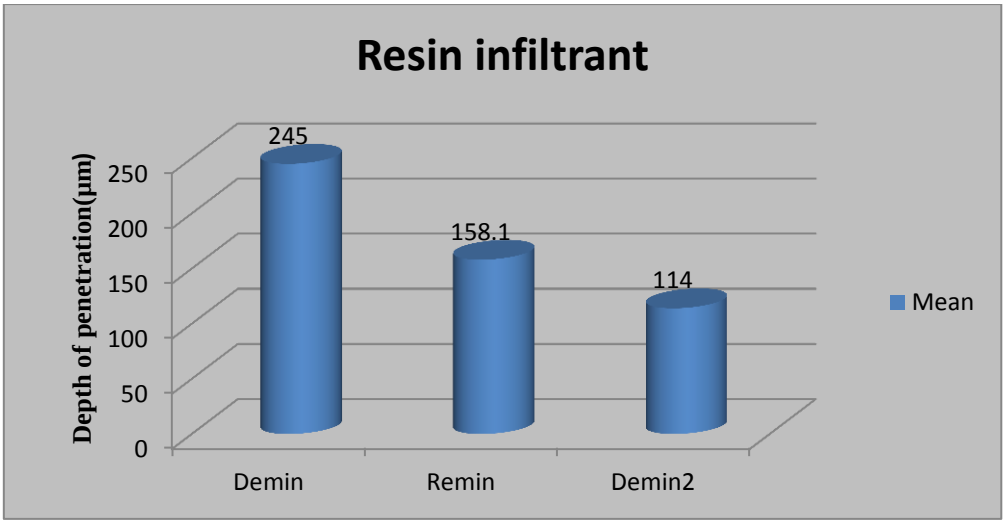
Table 11:Comparison of Microhardness for all the groups after Acid Challenge

Acid challenge	N	Mean	Std. Deviation	Std. Error	95% Confidence Interval		Minimum	Maximum	p value	Post Hoc
					Lower Bound	Upper Bound				
Resin infiltrant	5	292.00	16.43168	7.34847	271.5974	312.4026	270.00	310.00	<0.001	Resin infiltrant*> (CPP-ACP=nano-HA)*>control
CPP-ACP	5	254.00	11.40175	5.09902	239.8429	268.1571	240.00	270.00		
nano-HA	5	237.00	14.40486	6.44205	219.1140	254.8860	220.00	255.00		
Control	5	167.00	12.04159	5.38516	152.0484	181.9516	150.00	180.00		

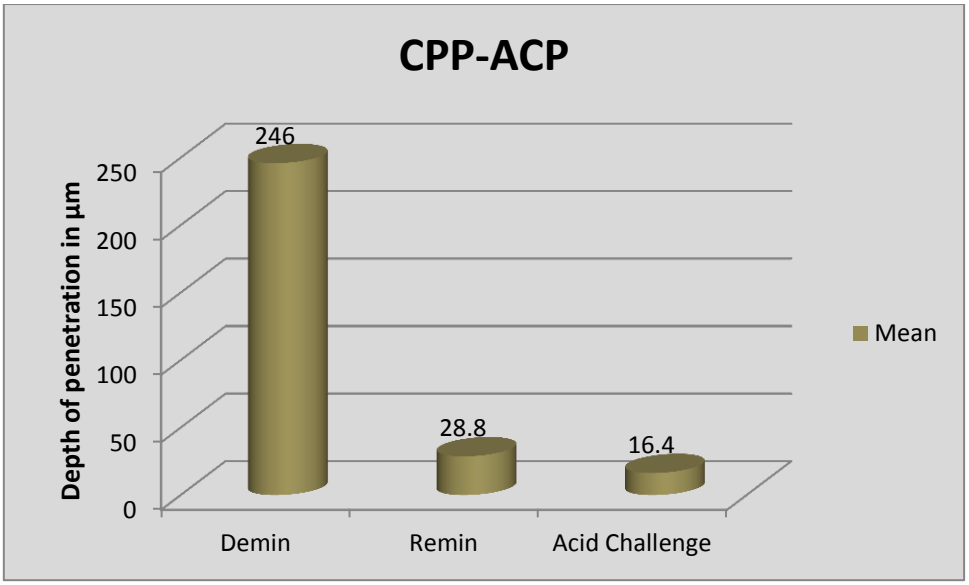
* -Statistically significant $p<0.05$

I.Graphs for Confocal group

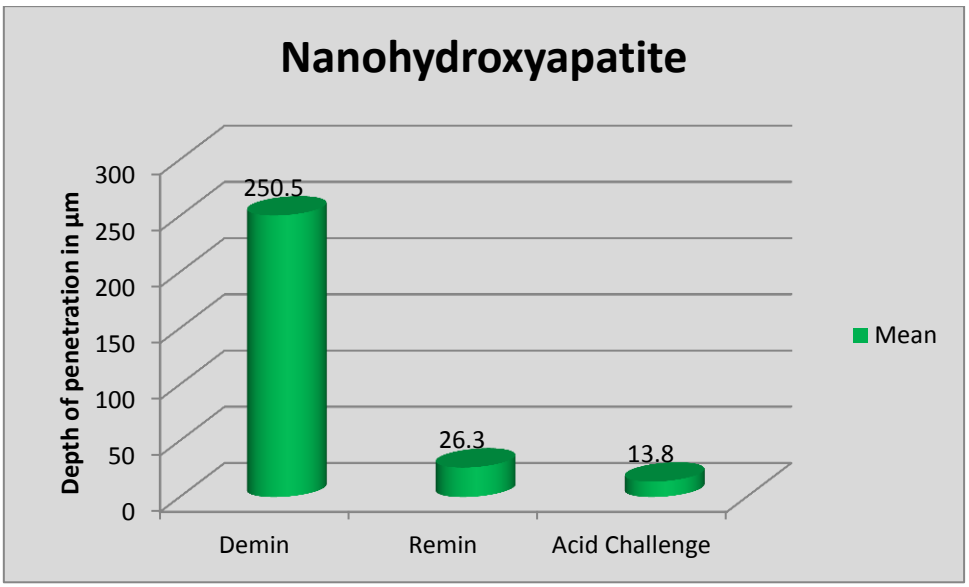
Graph 1 : Intra group comparison for Group I (Resin infiltrant)



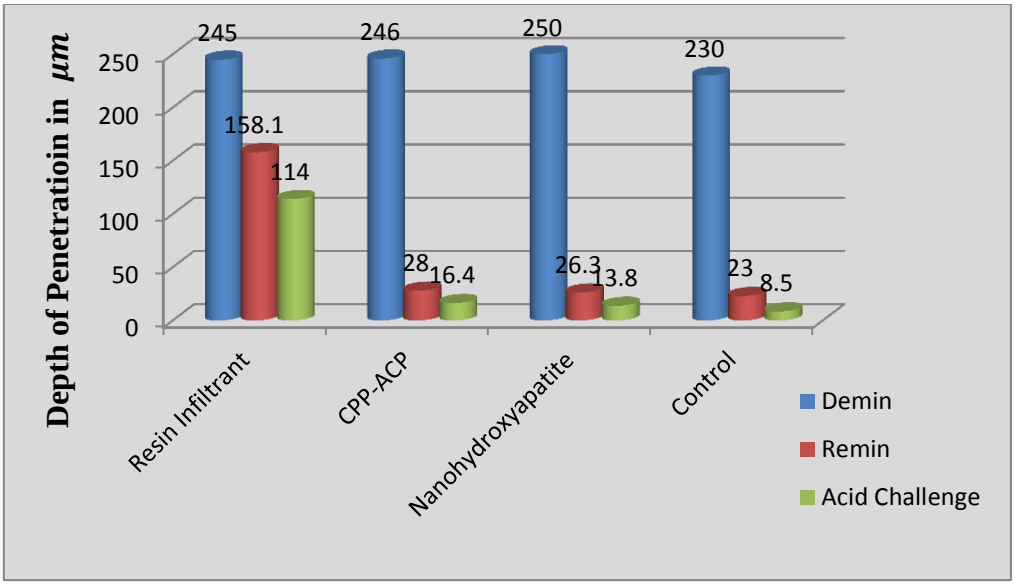
Graph 2 : Intra group comparison for Group II (CPP-ACP)



Graph 3 : Intra group comparison for Group III (nano-HA)

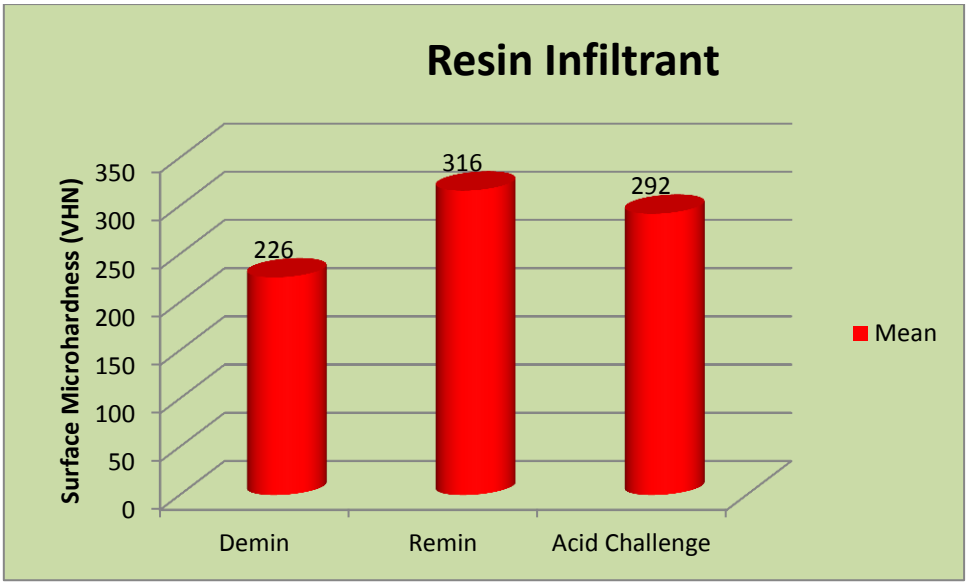


Graph 4 : **Inter group comparison of all the groups (Confocal)**

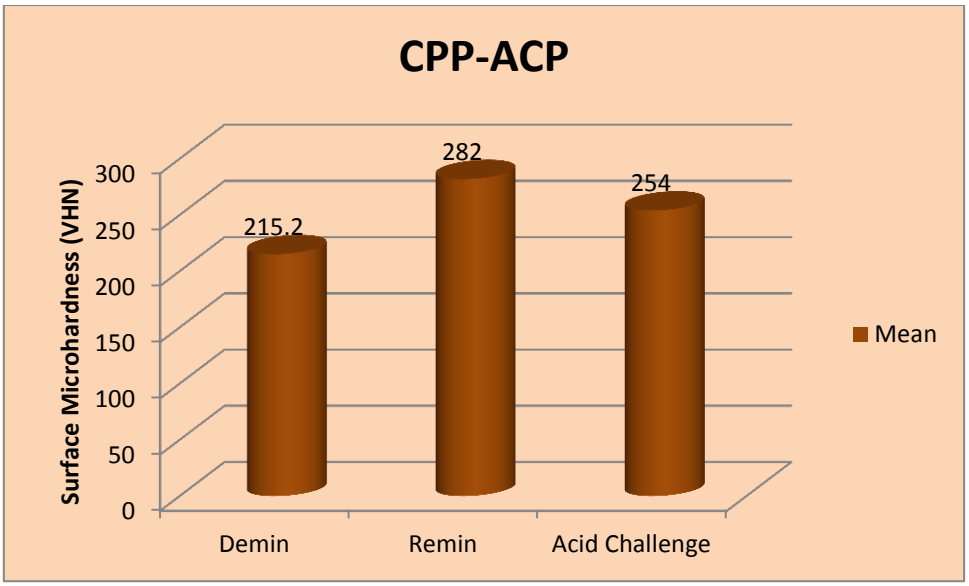


II.Graphs for microhardness group

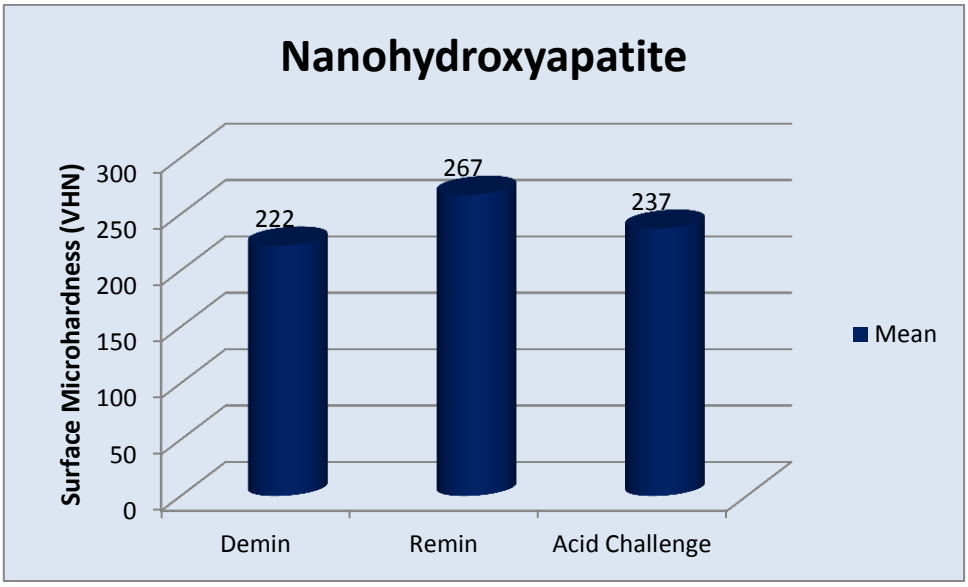
Graph 5: Intra group comparison for Group I (Resin infiltrant)



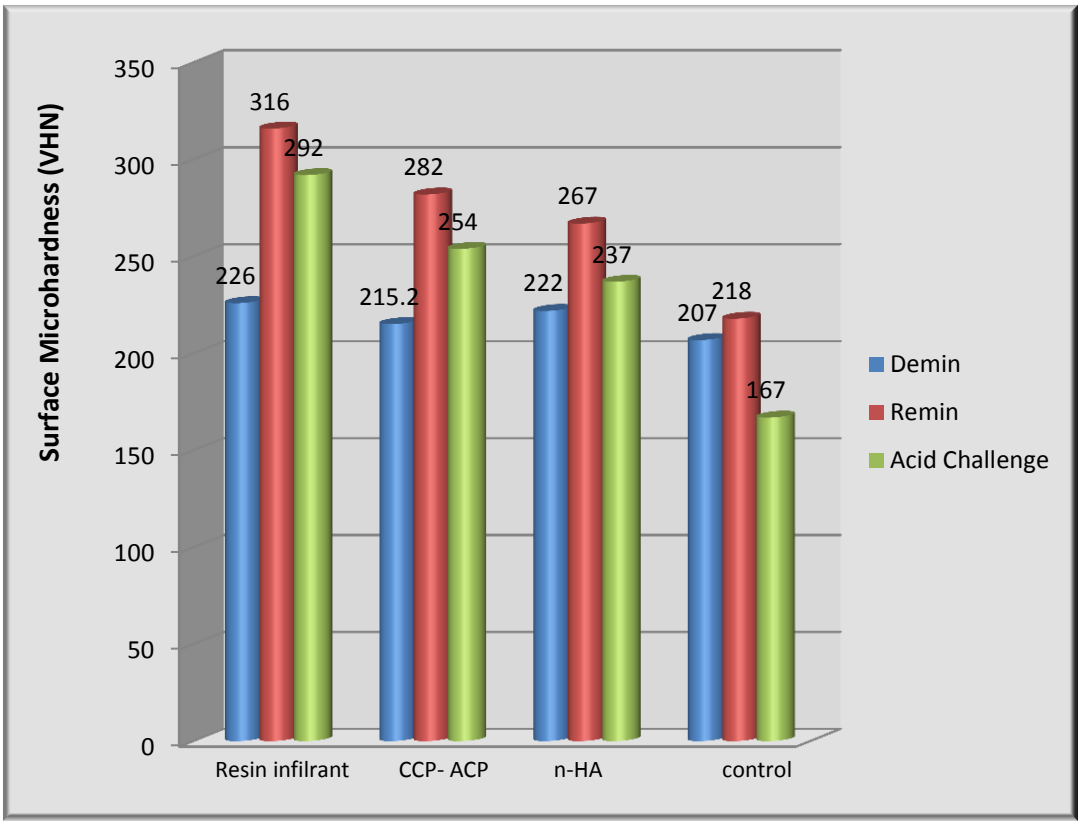
Graph 6: Intra group comparison for Group II (CPP-ACP)



Graph 7: Intra group comparison for Group III (nano-HA)



Graph 8 :Inter group comparison for all the groups



Carious lesions formed on the enamel surface are unique in that the enamel is both acellular and avascular. Thus, in contrast to other tissues enamel cannot heal by the cellular repair mechanism[58]. It is now a well established fact that the formation of incipient enamel white spot lesions is a reversible process, where periods of demineralization alternates with periods of remineralization[59]. A favourable environment in the oral cavity leads to remineralization and helps in the repair of the carious lesion[60]. In early carious lesions, the enamel surface remains relatively unaltered, whereas the mineral loss associated with the underlying lesion body can be substantial. Clinically such enamel lesions appear as whitish discoloured areas commonly referred to as White Spot lesions[61].

The process of demineralization and remineralization is governed by the degree of saturation of oral fluids with respect to apatite minerals[62].It has been concluded that there is an increase in rate and percentage of remineralization as the pH values decreased from 9.0 to 5.5[13]. The remineralization of enamel subsurface lesions involves the diffusion of ions through the surface layer of the lesion and then deposition of the ions into the crystal voids which is seen on the demineralized enamel. The ions will best penetrate the enamel in an uncharged form. The neutral ions will be in equilibrium with the charged ions. The uncharged ion penetrate the lesion and will dissociate in the form of charged ions along the diffusion path and ultimately in the lesion and promote the crystal growth[37].

The caries preventive effect of fluoride is mainly due to the formation of calcium fluoride like precipitates hampering demineralization. Fluoride has been the most widely used remineralizing agent for caries prevention. But, there is some concern associated with the wide array of prescription and over the counter fluoride products marketed. This has led to the total fluoride intake increasing to harmful levels[63].Recently many newer remineralizing agents have been introduced and been concluded that a successful outcome can be achieved for noninvasive management of early enamel carious lesions.

Infiltration of the natural enamel lesions seems to be a promising treatment alternative or supplement to the commonly accepted concept of remineralization and restorative treatment. With the advent of the resin infiltrant, the mechanical stabilization of demineralized enamel, preservation of sound tooth structure, permanent occlusion of superficial micropores and cavities, sealing of the porous deeply demineralized areas, arrest the progression of lesion, minimized risk of secondary caries, delay of restorative intervention for longer periods, no risk of postoperative sensitivity and pulpal inflammation, reduced risk of gingivitis and periodontitis, improved esthetic outcome when used as a masking resin on demineralized labial surfaces and high patient acceptance have been attained[64].

The current trend in caries prevention focus on the non invasive approaches with the remineralizing agents by hampering the demineralization and promoting the remineralization. Hence in the present study the resin infiltrant (ICON), casein

phosphopeptide amorphous calcium phosphate (GC Tooth mousse) and nanohydroxyapatite (Aclaim) were chosen and compared to evaluate the caries preventive efficacy.

Softened enamel when exposed to saliva or to a remineralizing solution for an adequate time has potential to regain mineral and thus re-acquire mechanical strength[65].Natural saliva and its synthetic substitutes (artificial saliva) reduce enamel mineral loss, enhance enamel rehardening and decrease lesion depth in various in vitro and in vivo studies[66].The artificial saliva used in this present study was aimed at simulating natural saliva relevant for remineralization processes. Therefore, the pH value of the saliva (6.2 to 7.4) was adjusted to natural salivary pH under stimulation conditions and thus, demonstrates improved salivary buffer capacity.

It is desirable that the resin infiltrant should have the following characteristics. It should be hydrophilic, highly surface active and bacteriostatic. It should be non-toxic to the oral tissues, should be poylmerizable to a solid state, should be resistant against the chemical and mechanical challenges which are associated with the oral cavity and finally should be cosmetically pleasing[67].

The infiltration of enamel lesions is mainly driven by capillary forces. Hence, both the pore volume and capillary radius of the solid to be penetrated strongly influence the resin infiltration. Therefore the surface layer might hamper resin penetration because of its relatively low pore volume. Acid etching might remove the

highly mineralized surface layer and thus enhance resin infiltration into the more porous lesion body[68]. Deep penetration cannot be achieved in active early caries using the same treatment protocols as for artificial caries like lesions. This discrepancy might be explained by the difference in lesion structure particularly with regard to the surface layer. Because of alternating demineralization and remineralization cycles in the oral cavity the surface layers of natural carious lesions obviously are inhomogeneous and may show higher mineral contents compared to artificial lesions. For artificial carious lesions, the mean thickness of the surface layer and mineral content was about 15-30um, 63-76vol% respectively[69]. In contrast, it is seen that in natural carious lesions a mean thickness of 40µm and mineral content of 83% on the surface layer were observed[70]. An effective reduction in the surface layer of natural enamel caries can be achieved with 15% hydrochloric acid gel for 90-120s[71].However without pretreatment of the frequently impermeable surface layer, resin penetration is limited[72].

The relatively high viscosity of dental resins and short treatment time for the resin to penetrate require relatively large pores to open access to the lesion body. Therefore, the complete erosion of the surface layer and exposure of the lesion body should be the aim of a conditioning procedure prior to infiltration of low viscosity resins[73]. A controlled reduction of the surface layer can be accomplished by acid etching.

Occlusion of pores by penetration of the resin into the body of the lesion is

probably responsible for the retention of the material, allowing an expectation of a stable result over time. The durability of the result is dependent on the lesion's environment. Caries inhibition is being maintained in a weakly demineralizing environment, but it is likely that in a patient at uncontrolled risk of caries demineralization will continue to recur at the periphery of the resin infiltrated area[74].

Resin infiltration seems suited particularly for proximal lesions where, when invasive treatment is chosen, the ratio of normal tissue to carious tissue leads to a significant loss of healthy tissue in order to gain access to the lesion, even when applying micro-invasive methods of preparation such as sono-abrasion. Resin infiltration technique is indicated for non cavitated lesions of ICDAS codes 2 and 3[75].

Hydrochloric acid has been applied in aesthetic dentistry to remove superficial discolourations by enamel microabrasion[76]. It has been shown that a short term contact of this strong acid with mucosa has been shown to be harmless, safety precautions such as rubberdam should be used in clinical practice[77].

The anticariogenic potential of CPP-ACP has been attributed to the ability of CPP to localize ACP at the tooth surface increasing the level of calcium phosphate in plaque. In this way the CPP-ACP may act as a calcium phosphate reservoir, buffering the free calcium and phosphate ion activities, thereby helping to maintain a state of supersaturation with respect to tooth mineral reducing enamel demineralization and enhancing remineralization[78].

CPP-ACP reduces caries activity by a dose dependent mechanism and the subsequently formed mineral is more resistant to acid attack. The CPP can stabilize over 100 times more calcium phosphate than is normally possible in aqueous solution. This occurs at neutral and alkaline pH before spontaneous precipitation. In the due process of mineralization ACP and the crystalline phases namely dicalcium phosphate dihydrate and octacalcium phosphate have been implicated as intermediates during the formation of hydroxyapatite depending upon the pH and the degree of saturation[80].

In the present study to create the artificial enamel lesions on the enamel samples, the specimens were exposed to demineralizing solution composed of 6µm methylhydroxydiphosphonate, 3mM CaCl2.2H2O, 3mM KH2PO4, 50Mm acetic acid and traces of thymol for 14 days (pH 5.0 at 37 ^{0}C) (Buskes et al in 1985[57]) .The pH was checked daily with the help of a pH meter and when necessary it was corrected by adding small amounts of either glacial acetic acid or potassium hydroxide solution and pH was maintained at 5.5.The demineralizing solution was shown to be effective in creating artificial caries like lesions. The mean lesion depth after demineralizing the enamel samples for all the four groups was 247µm which is in accordance with the results of previous studies by Meyer lueckal et al in 2007[36] who reported the mean lesion depth was 357µm, Mueller et al in 2006[30] the mean lesion depth was 237 µm.

In the present study, confocal laser scanning microscope was used to visualize the penetration of the material. In a study by Pioch et al in 1996[18] it was proved that confocal laser scanning microscope had the following advantages of nondestructive examination, since the layer visualized can be situated up to 100µm below the surface. Moreover drying of the samples which is required for conventional scanning electron microscopy or transmission electron microscopy is not necessary leading to decreased risk of shrinkage or other artifacts[18].

Microhardness testing is considered to be a relatively simple and reasonably reliable method for the provision of indirect information about the mineral content changes of hard dental tissues. Vickers surface microhardness technique has been utilized as an indirect mineral content assessment method in several laboratory models simulating the effect of application of various commercial products in vitro[81].

Surface micro hardness indentation provides a rapid and nondestructive method in demineralization and remineralization studies. Indentation hardness testing with either Vickers or Knoop indenter have been used for the measurement of initial enamel hardness, enamel softening as an initial manifestation of the erosive process, as well as enamel hardening after remineralization. Both indenters are suitable for hardness testing of non-metallic materials. The load of 100 g was chosen for this study for hardness indentation because they created longer Vickers diagonals, which were recommended to prevent errors in optical measurement[82]. The hardness values

obtained for enamel samples in this study were in the range of 330.0 to 349.6 VHN, which were in agreement with the studies by Gaspersic and Reyes-Gasga et al [83,84] and also correlates with the normal micro hardness of enamel (322 to 353 VHN [85]). Microhardness values decreases from the outer enamel surface towards the dentinoenamel junction, which may explain the range of baseline values obtained.

In present study acid conditioning with Icon (2 min with 15% hydrochloric acid) could have led to deeper resin penetration than etching with 37% phosphoric acid gel (Paris et al., 2007[33]). It could be argued that removal of surface layer by 15% HCl could additionally weaken the lesion structure. According to a study done by Meyer-lueckel et al in 2008[36] it was proved that no cavitation occurred after acid etching even if the complete surface layer was completely eroded and subsequent resin infiltration could ensure restrengthening of the lesion structure.

In the present study the Icon-dry (which contains 99% ethanol) was applied for 30s prior to application of the infiltrant. In a study by Paris et al in 2007[32] it was proved that addition of ethanol are associated with higher penetration coefficient by decreasing the viscosity and contact angle, hence they can be used as promising tools for rapid penetration. It was also proved that mixtures containing large amounts of HEMA, TEGDMA, and ethanol are associated with higher penetration coefficients and satisfactory hardening therefore, they might be promising tools for rapid caries penetration.

In the present study the mean penetration of the treated enamel samples for the resin infiltrant group (Group 1) which was observed using confocal laser scanning microscope is 158µm. In previous studies by Paris et al in 2007[31] and Meyer – lueckel et al in 2006[29] the mean penetration of resin infiltrants was 58µm and 104µm respectively. It was proved in a study by Kielbassa et al in 2005[31] that because of alternating demineralization and remineralization cycles in the oral cavity, the surface layers of natural lesions are inhomogeneous and may show higher mineral content compared to artificial lesions. Natural caries lesions might be contaminated with organic materials such as proteins and carbohydrates that might hamper resin penetration compared to artificial lesions created in this study. 15% HCl in previous studies had shown to completely erode the surface layer in artificial lesions compared to the natural lesions. This could have led to deeper resin penetration in this study.

In the present study the mean hardness value after demineralization for 14 days was in the range from 207 to 226 VHN for all the four groups. According to a study by Elkassas et al in 2014[55] the mean hardness value after demineralization was in the range from 206 to 230VHN and Gaspersic et al in 1995[83] reported the hardness value was 200 to 250 VHN which is in accordance with the current study.

In the present study, surface microhardness values of resin infiltrant (group1) was significantly higher than CPP-ACP (Group2), nano-HA (Group3) and control (Group 4). The reason for higher surface hardness of resin infiltrant could be

due to TEGDMA which is the main ingredient has a lower viscosity that result in higher penetration depth, thus increasing the penetration coefficient of the resin. Furthermore, an increase of surface microhardness for resin infiltrant (Group I) might be due to higher conversion associated with TEGDMA. Higher initiator concentrations within the resin may increase the conversion, and thereby increase hardness[20].

In the present study, the mean hardness value after acid challenge of the treated enamel samples was Group I (Resin infiltrant) > Group II (CPP-ACP) ⩾ Group III (nano-HA) > Group IV (control). The Group I (resin infiltrant) showed significantly higher surface hardness than Group II, Group III and Group IV after acid challenge. A study by Paris et al in 2013[53] concluded that the twice application of infiltrants was shown to increase the microhardness for lesions infiltrated with most resins. It is therefore possible that twice applied resins might compensate polymerization shrinkage and fill porosities and crevices within the infiltrated lesion body and reducing mineral loss when challenged again by demineralization. In a study by Schwendicke et al in 2013[53] resin infiltration was shown to significantly increase microhardness and reduce mineral loss after a demineralization challenge compared with untreated control lesions.

In the present study the remineralized area for Group II (casein phosphopeptide-amorphous calcium phosphate group and Group III (nanohydroxyapatite) observed in confocal laser scanning microscope is 28.8μm and

26.3µm respectively. In the present study paste type formulation of CPP-ACP was used. The remineralization process of CPP-ACP involves diffusion of calcium and phosphate ions through the protein/water filled pores of the caries surface enamel into the body of the enamel lesion. Once in the body of the enamel lesion, these calcium and phosphate species increase the activities of Ca^{2+} and PO_4^{3-}, thereby increasing the degree of saturation with respect to hydroxyapatite[37].

In the present study CPP-ACP had a mean hardness of 282VHN after remineralizing the enamel samples for 30days. Reynolds (1998)[12] concluded that in CPP-ACP technology, ACP is stabilized by CPP casein –derived peptides. CPP contains the aminoacid cluster sequence –Ser(p)-Ser(p)-Ser(p)-Glu-Glu- and have been reported to bind amorphous calcium phosphate, forming small clusters of casein phosphopeptide-amorphous calcium phosphate(CPP-ACP).This helps to prevent these clusters from reaching critical size needed for precipitation, thereby stabilizing the calcium phosphate in solution. This close proximity makes it available to the tooth when needed. These nanocomplexes act as calcium phosphate reservoirs when incorporated into the dental plaque and onto the tooth surface.

After acid challenge the remineralized enamel samples resulted in mean surface microhardness 254VHN.This hardness after acid challenge was significantly greater than the untreated control group. This was in accordance with a study by Elkassas et al in 2014[55] which was in the range of 250VHN TO 285VHN.

Huang et al in 2009[39] reported that the nano-HA has been shown to remineralize initial enamel lesions in vitro. Since the surface area and proportion of atomicity increase with decreasing particle size, nanoHA has bioactive and biocompatible properties. The incipient enamel lesions being more porous than sound enamel structure allows for a greater penetration of solution of the ion constituents and allow for a larger surface area being made available for subsequent reaction of enamel mineral. These factors increased the potential of nano-HA to directly fill up defects and micropores on demineralized teeth. If nano-HA penetrates the enamel pores, the nano-HA will act as a template in the precipitation process and will continuously attract a large amount of Ca^{2+} and PO_4^{3-} from the remineralization solution to the enamel surface to fill the vacant positions of the enamel calcium crystals. This in turn will promote crystal growth and integrity. Recent studies have indicated the significant role that crystal size play in biological phenomena involving calcium phosphates, notably HA. The solubility of hydroxyapatites increases with increasing ionic substitution in the apatite lattice and decreasing crystallinity and particle size[87].

The surface microhardness values after remineralization for 30 days for Group III (nano-HA) is 267VHN. The mechanism for nano-HA remineralization is acicular crystals of nano-HA sedimented onto the enamel surfaces and directly filled up defects and micropores on demineralized teeth surfaces after demineralization. This resulted in the observed decreases in cavities and defects of enamel surface and increased surface hardness of the enamel surface[39].

After acid challenge the microhardness value of Group III (nanoHA) is 237VHN which is significantly greater than the initial demineralization which showed the acid resistant potential of nanoHA. This could be due to the solubility of nano-HA is increased in acidic condition, so nano-HA would still deposit onto the demineralized surface to biomimetically repair lesions. In addition, the surface of the artificial lesions will possess a negative electrostatic potential (Huang et al 1997[88]). The reduction in pH would decrease in the magnitude of the negative zeta potential of nano-HA as pH decreases towards so called zero point (Yin et al 2002, Skartsila and Spanos 2007[89]) thus the electrostatic repulsion force between nanoHA and tooth enamel would be reduced, leading to increased deposition of nanoHA onto the surfaces under acidic conditions.

After acid challenge for a period of 14 days the amount of remaining resin infiltrant which was resistant to acid attack was 114µm (72%), amount remaining for CPP-ACP was 16.4µm (57%), for nano-HA was 13.8µm (50%), increased progression of depth of lesion and no resistance for untreated control group.

Within the limitations of this invitro study, resin infiltrant showed greater caries inhibiting potential than other remineralizing agents like casein phosphopeptide-amorphous calcium phosphate and nanohydroxyapatite. In the present study CPP-ACP and n-HA showed similar caries inhibiting potential. However, there are few limitations in the present invitro study, hence further in vivo studies are needed to confirm the results of this study under simulated oral environment.

Conclusion

Within the limitations of this invitro study the following conclusions can be elucidated,

- ✓ The resin infiltrant (ICON) showed higher caries inhibition potential than CPP-ACP (GC tooth mousse) and nano-HA (Aclaim).
- ✓ In addition, resin infiltrant showed superior acid resistance compared to CPP-ACP and nano-HA.
- ✓ The resin infiltrant has a promising role in the management of early enamel carious lesion.
- ✓ The resin infiltrant can be used as an alternative micro invasive approach.

In view of the results presented the inhibition of caries progression by the resin infiltration technique should be considered as an alternative approach to the more invasive therapies and warrants a place in the range of minimally invasive dentistry techniques. Infiltrating resins (ICON) have opened up an innovative pathway in the management of initial carious lesions, compared with remineralization techniques that may require several follow up visits. It is recommended to investigate the effects of Resin infiltrant, CPP-ACP and nano-HA with invivo studies to firmly conclude the present results.

Summary

This invitro study was undertaken to evaluate the caries preventive efficacy of the resin infiltrant, casein phosphopeptide-amorphous calcium phosphate and nano-hydroxyapatite on non cavitated enamel lesions using the confocal laser scanning microscope and vickers microhardness test. 60 freshly extracted maxillary human incisors which were extracted for periodontal reasons were taken for the study. The collected teeth were then sectioned horizontally using a diamond disk (Axis dental, Texas) attached to a slow speed straight handpiece (NSK, Japan). Enamel samples with the dimension of (5×5× 5 mm) were prepared for all the four groups. Then the sectioning of the enamel samples in the middle third region of the crown portion was done. The obtained enamel portion was embedded in acrylic resin blocks (DPI) after which the embedded specimens were stored in artificial saliva at 37°c.

In order to create artificial enamel lesions, the samples were demineralized by placing in a beaker containing the demineralizing solution. The study samples were stored for a period of 14 days maintaining a pH of 5.0 and at 37°C temperature. The study samples were divided into four groups of containing 15 samples each

- ✓ Group I (Resin infiltrant)
- ✓ Group II (CPP-ACP)
- ✓ Group III (nano-HA)
- ✓ Group IV (control)

Ten samples from each of the study group were randomly selected and subjected for confocal laser scanning microscope evaluation to evaluate the depth of penetration. Five samples from each of the groups were randomly selected and subjected to microhardness evaluation by using the Vickers microhardness tester (Zwick Inc Germany) to determine the Vickers Hardness Number value (VHN).The VHN value was calculated 1)After demineralization of the enamel samples for 14 days 2) After the application of resin infiltrant, CPP-ACP, nano-HA for 30 days 3) Re exposing the treated enamel samples to the demineralizing solution for a period of 14 days.

Group1: 15 enamel samples after demineralization were infiltrated with the resin infiltrant (ICON,DMG) by first etching the enamel sample with 15% hydrochloric acid for 2minutes, after which rinsing with water was done for 30 seconds to remove the etchant. After drying the sample, ethanol was applied to dessicate the enamel sample for 30 seconds and finally infiltrated with resin infiltrant and it was allowed to remain for 3 minutes and the excess was removed and cured for 40 seconds (LED). **Group 2:** 15 enamel samples after demineralization were brushed twice daily for 1 minute with a powered tooth brush (Oral-B) using CPP-ACP for 30 days. **Group 3**: 15 Enamel samples after demineralization were brushed twice daily for 1 minute with powered tooth brush(Oral-B) using nano-HA tooth paste for 30 days. **Group 4:** 15 enamel samples after demineralization were placed in artificial saliva for 30 days and it was taken as control group.

The Data were analyzed using Statistical Package for Social Sciences (SPSS) version 20.0. The collected Data were statistically analyzed using **ANOVA** and **Post hoc Bonferroni test** was used for comparing intragroups and **Tukey** test was used to compare the intergroups at the level of significance $p<0.05$.

Results of this study for the confocal group showed that Group I (Resin infiltrant) showed the maximum depth of penetration followed by Group II (CPP-ACP) and Group III (nano-HA). After acid challenge Group I (Resin infiltrant) showed the maximum resistance to demineralization followed by Group II (CPP-ACP) and Group III (nano-HA) and least by control. Results of this study for microhardness showed that Group I (Resin infiltrant) had maximum surface hardness followed by Group II (CPP-ACP) and Group III (nano-HA) which showed no significant difference in hardness, least hardness by control. After acid challenge Group I (Resin infiltrant) showed the maximum hardness followed by Group II (CPP-ACP), Group III (nano-HA) and the least hardness for Group IV (control). From the results obtained in this invitro study the following conclusions can be elucidated, Resin infiltrant showed higher caries inhibition potential than CPP-ACP and nano-HA. In addition, resin infiltrant showed superior acid resistance compared to CPP-ACP and nano-HA. It has a promising role in the management of early enamel carious lesion. It can be used as an alternative micro invasive approach.

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ANNEXURE -1

Estd : 1987

INSTITUTIONAL ETHICS COMMITTEE AND REVIEW BOARD

Rajas Dental College & Hospital

Thirurajapuram, Kavalkinaru,Jn – 627 105, Tirunvelveli District.

DCI Recognition No : DE-3(44)-93/2246 Dated 09-11-1993

Affiliated to The Tamil Nadu Dr. M.G.R. Medical University, Chennai

This ethical committee has undergone the research protocol submitted by **Dr.Kingston**, Post Graduate Student, Dept of Conservative Dentistry and Endodontics under the title " **comparative evaluation of caries preventive efficacy of resin infiltrant, casein phosphopeptide –amorphous calcium phosphate and nano-hydroxyapatite-an in vitro study** " under the guidance of **Dr.R.Jonathan** for consideration of approval to proceed with the study.

This committee has discussed about the material being involved with the study, the qualification of the investigator, the present norms and recommendation from the Clinical Research scientific body and comes to a conclusion that this research protocol fulfills the specific requirements and the committee authorizes the proposal.

I.E.C. & R.B. RAJAS DENTAL COLLEGE & HOSPITAL ETHICAL COMMITTEE & R.B.

Dr. I. PACKIARAJ MDS
CHAIR PERSON
Ethical Committee

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ANNEXURE -2

STATISTICAL ANALYSIS

ANOVA [Analysis Of Variance]

Anova is a statistical test which analyzes variance. It is helpful in making comparison of two or more means which enables a researcher to draw various results and predictions about two or more sets of data.

F = MST/MSE

Where,
F = Anova Coefficient
MST = Mean sum of squares due to treatment
MSE = Mean sum of squares due to error.

Bonferroni correction

In statistics, the **Bonferroni correction** is a method used to counteract the problem of multiple comparisons. It is considered the simplest and most conservative method to control the familywise error rate.

The Bonferroni Correction states that choosing all $p_i \leq \frac{\alpha}{m}$ will control the $FWER \leq \alpha$. The proof follows from Boole's inequality:

$$FWER = Pr\left\{\bigcup_{I_o}\left(p_i \leq \frac{\alpha}{m}\right)\right\} \leq \sum_{I_o}\left\{Pr\left(p_i \leq \frac{\alpha}{m}\right)\right\} \leq m_0\frac{\alpha}{m} \leq m\frac{\alpha}{m} = \alpha$$

This result does not require that the tests be independent.

It was used as $\sum_{i=1}^{n} \frac{\alpha}{n} = \alpha$, but the correction can be generalized and applied to any $\sum_{i=1}^{n} a_i = \alpha$, as long as the weights are defined prior to the test.

Bonferroni correction can be used to adjust confidence intervals. If we are forming m confidence intervals, and wish to have overall confidence level of $1 - \alpha$, then adjusting each individual confidence interval to the level of $1 - \frac{\alpha}{m}$ will be the analog confidence interval correction

Tukey's HSD

$$\frac{M_1 - M_2}{\sqrt{MS_w\left(\frac{1}{n}\right)}}$$