Comparison and Evaluation of Caries preventive efficacy of Resin infiltrant, Casein phosphopeptide amorphous calcium phosphate and nanohydroxyapatite using Vickers microhardness tester- An in vitro study.

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Abstract-

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

Materials and methods: 60 maxillary incisors extracted for periodontal reasons were sectioned at the middle third region of the crown 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 samples were divided into four groups which is Resin infiltrant (Group I), CPP-ACP (Group II), nano-HA (Group III) and control (Group IV) (n= 15) enamel samples in each group. The caries preventive efficacy of each group was evaluated using Vickers microhardness tester and the results were statistically analyzed.

Results: The mean microhardness value after demineralization of enamel samples was 226VHN, 222VHN, 207 VHN and 215VHN in Groups I, II, III, IV respectively. The mean microhardness value after Remineralization of enamel samples was Group I :316VHN > Group II : 282VHN > Group III : 267VHN > Group IV : 218 VHN. The mean microhardness after acid challenge of enamel samples for 14 days was 292VHN, 254VHN, 237VHN, 167 VHN in groups I,II,III, IV respectively.

Conclusion: The resin infiltrant showed higher caries inhibition potential than CPP-ACP and nanohydroxyapatite. In addition, resin infiltrant showed superior acid resistance and increased surface microhardness compared to CPP-ACP and nanohydroxyapatite.

Keywords: Casein phosphopeptide-amorphous calcium phosphate, Surface microhardness tester, nanohydroxyapatite, Resin infiltrant.

Introduction

White spot lesions are the early signs of demineralization occurring under intact enamel which may or may not lead to cavitation. It is initiated by pathogenic bacteria that have breached the enamel layer and by organic acids which are produced by them. This causes the removal of a certain amount of calcium and phosphate ions which fail to be replaced naturally during the remineralization process.(1) White spot lesions are commonly reversed by the process of remineralization mainly through the application of fluorides. (2) Remineralization is the process whereby calcium and phosphate ions are supplied from an external source 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 goal of caries management is to arrest the progression of the lesion. But, remineralization brought about by the topical application of fluoride requires multiple treatment sessions and long term follow up which requires motivation and cooperation from the patient which is difficult to achieve. 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 by occluding the micro porosities. 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). (3) Nanohydroxy apatite has been widely used as an effective anti-cariogenic agent mainly because of its unique remineralization potential. (4) 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. (5) 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 Vickers microhardness analysis.

MATERIALS AND METHODS:

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.

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. The samples were de-coronated and sectioned 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 of the crown for 60 samples with approximate dimensions of (5x5x5mm) and then stored in 10% formalin at room temperature of 37^0C 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 then stored in artificial saliva at 37^0C.

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 (6) for a period of 14 days, maintaining a pH of 5.0 and at 37^OC 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.

Distribution of 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 to 350 were selected for the study. 60 enamel samples (15 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.

Remineralization of enamel samples

GROUP I (Resin infiltrant)

Samples were then subjected to 15% HCl-etching for 2 min, rinsing for 30sec, ethanol dessication for 30 sec, infiltrant applied and light cured for 40sec.

GROUP II (CPP-ACP)

Enamel samples were then brushed with GC Tooth mousse toothpaste twice daily for 1 min and stored in artificial saliva for 30 days.

GROUP III (Nanohydroxyapatite)

Enamel samples were brushed with aclaim toothpaste twice daily for 1min and stored in artificial saliva for 30 days.

Group IV (Control)

Untreated enamel samples were stored in artificial saliva for 30 days .

Statistical analysis

Data were analyzed using Statistical Package for Social Sciences (SPSS) version 20.0. ANOVA and post hoc bonferroni test were used for intragroup comparisons and Tukey test was used for Intergroup comparisons.

RESULTS

Table 1 shows the inter group comparison of surface microhardness for all the four groups after demineralization. The mean microhardness value after demineralization of enamel samples was Group I (Resin infiltrant) 226 VHN, Group II (CPP-ACP) 222 VHN, Group III (nano-HA) 207 VHN, Group IV (control) 215 VHN [Figure 1 & 2] . The P value was 0.143 which was not statistically significant.

Table 2 shows the inter group comparison of surface microhardness for all the four groups after Remineralization. The mean microhardness value after Remineralization of enamel samples was Group I (Resin infiltrant) 316 VHN > Group II (CPP-ACP) 282 VHN > Group III (nano-HA) 267 VHN > Group IV (control) 218 VHN [Figure 1 & 2]. The P value was < 0.001 when comparing all the four groups. After Post hoc Tukey test there was statistically significant difference between Group I (Resin infiltrant) and the other 3 groups. Group II (CPP-ACP) and Group III (nano-HA) showed no significant difference.

Table 3 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 was Group I (Resin infiltrant) 292 VHN > Group II (CPP-ACP) 254 VHN > Group III (nano-HA) 237 VHN > Group IV (control) 167 VHN [Figure 1 & 2]. 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.

DISCUSSION

The current trends 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.[6] Natural saliva and its synthetic substitutes (artificial saliva) reduce enamel mineral loss, enhance enamel rehardening and decrease lesion depth.[7] The artificial saliva used in this study 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.

The 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.[8] A controlled reduction of the surface layer can be accomplished by acid etching. In the present study to create the artificial enamel lesions on the enamel samples, the specimens were exposed to demineralizing solution composed of 6µm methyl hydroxydiphosphonate, 3mMCaCl2.2H2O, 3mM KH2PO4, 50Mm acetic acid and traces of thymol for 14 days (pH 5.0 at 37^0C). [9]

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. A 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.[8] 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[9-12] and also correlates with the normal micro hardness of enamel (322 to 353 VHN). [13] Microhardness values decreases from the outer enamel surface towards the dentinoenamel junction, which may explain the range of baseline values obtained.

In the present study the mean hardness value after demineralization for 14 days was in the range from 207 to 226 VHN [Table1][Figure 1] for all the four groups. According to a study by Elkassas et al in 2014 [13] the mean hardness value after demineralization was in the range from 206 to 230VHN and Gaspersic et al in 1995 [9] reported the hardness value was 200 to 250 VHN which is in accordance with the current study.

The surface microhardness values of resin infiltrant (Group I) was significantly higher than CPP-ACP (Group II), nano-HA (Group III) and control (Group IV). 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.[14,15]

CPP-ACP had a mean hardness of 282 VHN [Table 2] [Figure 1] after remineralizing the enamel samples for 30days. Reynolds [15] 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 254 VHN [Table 3] [Figure 1]. This hardness after acid challenge was significantly greater than the untreated control group. This was in accordance with a study by Elkassas et al [13] which was in the range of 250 VHN to 285 VHN.

Huang et al [16] 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.
The 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 nanohydroxyapatite showed similar caries inhibiting potential. However, 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).
- ✓ 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 and can be used as an alternative in micro invasive approach.

CONFLICT OF INTEREST: Nil

SOURCE OF FUNDING: Nil

ETHICAL CLEARANCE: Not required for invitro study

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TABLES

Table 1: Comparison of the microhardness for all the 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	
CPP-ACP	5	222.00	15.24795	203.0672	240.9328	200.00	240.00	0.143 (NS)
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	

Table 2: 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		
CPP-ACP	5	282.60	8.59069	271.9332	293.2668	270.00	290.00	<0.001	Resin infiltrant*> (CPP-ACP=nano-HA)*>control
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		

Table 3: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		

FIGURES

Figure 1: Inter group comparison of all the groups after Demineralization, Remineralization and acid challenge

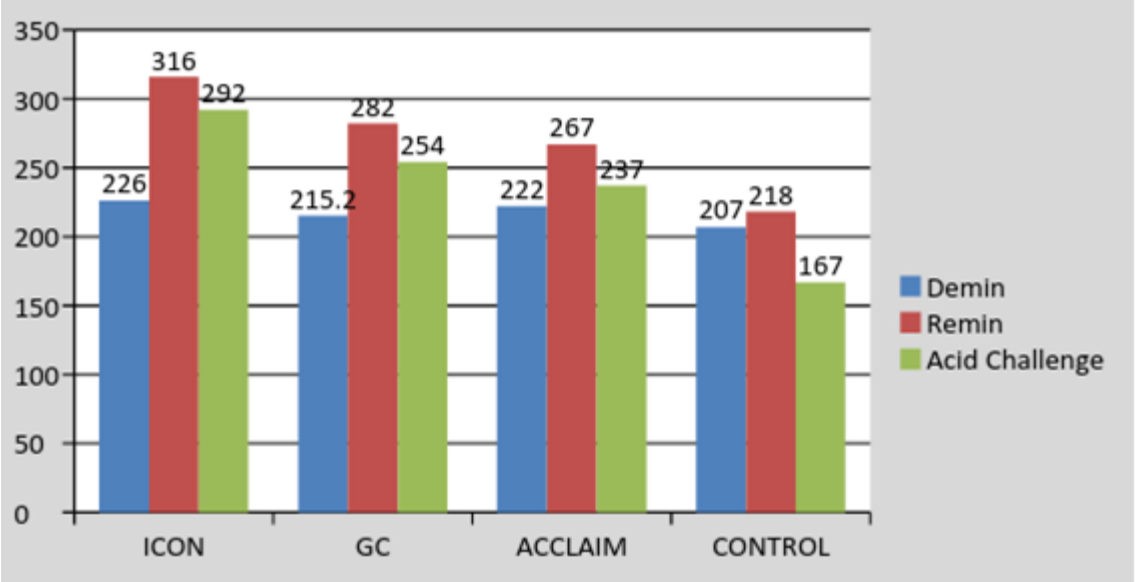
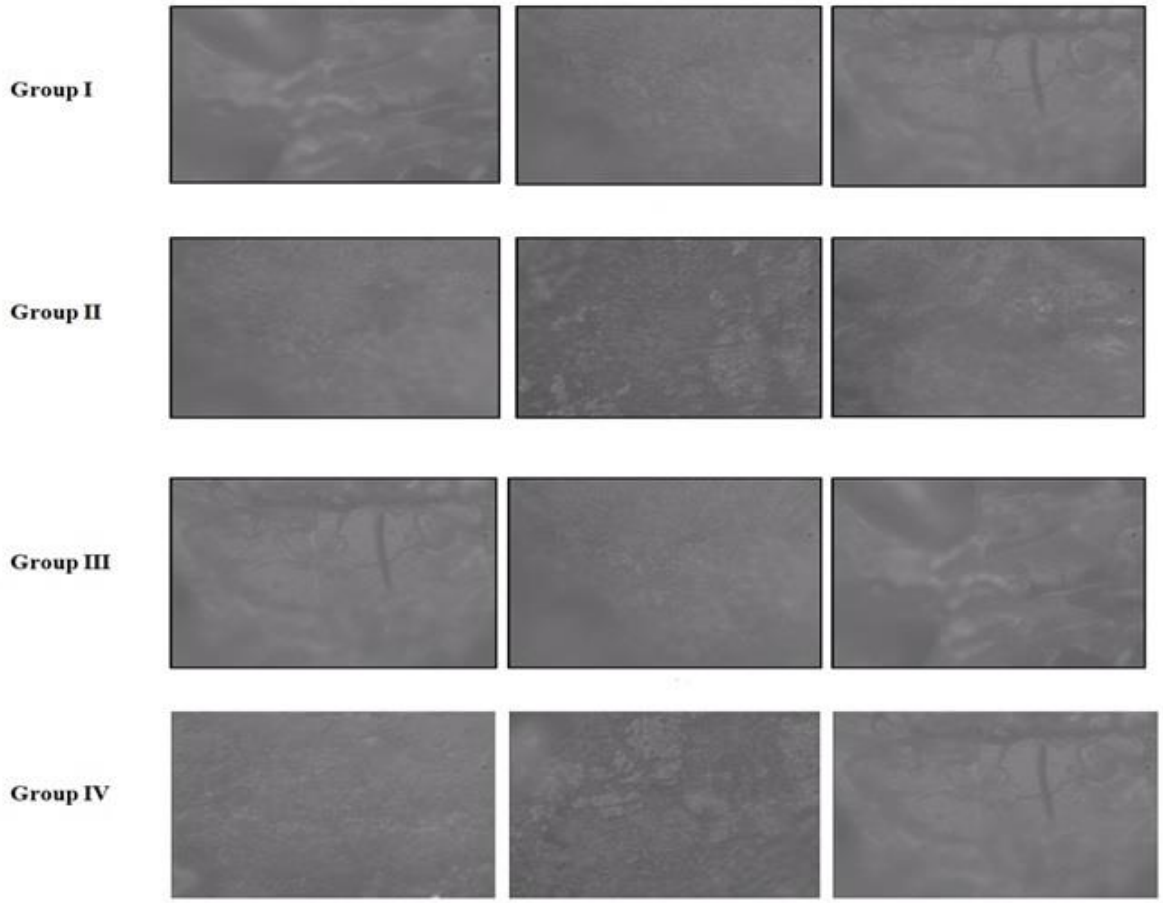


Figure 2: Microhardness images for all the experimental groups


Group I
Group II
Group III
Group IV