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To compare the remenaralizing potential of fluoridated calcium sodium phosphosilicate 1450 ppm NAF and 5000 ppm NAF by measuring the Vickers microhardness number of enamel in artificially induced caries like lesions in deciduous teeth – An in vitro study.

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Abstract

The present in vitro study was conducted to evaluate and compare the remineralization potential of Controlrx, Nupro, and Theramed on artificial early enamel lesions in primary teeth. The study sample was 90 deciduous teeth. Micro hardness was measured with Vickers Micro-hardness Number (VHN) tester. The hardness test was first determined by using load of 1 Newton (100 gm) applied for 15 second at four points along the cavity. The distance between two consecutive indentations was around 34µm. After measuring the hardness values of pretreated, remineralized and demineralized samples; VHN values were compared to determine the Remineralizing potential of 1450 ppm NaF (Theramed) and fluoridated calcium sodium phosphosilicate (NovaMin). The recorded values were statistically analyzed using Student's Paired t test, ANOVA and Post-Hoc Tukey's test. In conclusion it was observed that there was a significant difference in the micro hardness values of enamel specimens following demineralization and after treatment of specimens with the remineralizing agents.

Introduction

The tooth after eruption into the oral cavity undergoes a long process known as post-eruptive maturation characterized by multiple demineralization - remineralization cycles[1]. Enamel surface crystal composition at eruption time has large amounts of carbonate, water, and magnesium, among other elements, and it is porous. After undergoing many periods of demineralization and remineralization, the chemical composition and structure of surface enamel becomes more amorphous; less porous; contains less water, carbonates, and magnesium; and has increased amounts of fluoride and organic material[2].

Tooth surfaces within the oral cavity are colonized by a variety of bacterial species such as Streptococci mutans and Lactobacillus acidophilus capable of producing organic acids through the metabolism of dietary carbohydrates. These oral bacteria are the primary

constituents of dental plaque. Clearly, the formation of dental caries is associated with the presence of dental plaque and with the nature of the chemical events that occur at the tooth-plaque interface. The exchange of mineral (i.e. loss or gain) continues at the surface of enamel as long as the biofilm at the surface creates an environment favoring demineralization or remineralization. The pH of biofilm, levels of calcium, phosphate and hydroxyl ions in the saliva determines the shift of the balance towards demineralization or remineralization[3].

Demineralization starts only after the pH drops below the critical pH of 5.5. Below this pH there is an acidic environment, which results in loss of hydroxyl and phosphate ions by reacting with excess of hydrogen ions .This results in conditions of under saturation. In an individual with high caries activity, there is high frequency of exposure to fermentable carbohydrates and subsequent drop in pH, resulting in a prolonged periods of under saturation. This favors demineralization and over a period of time can form cavitation on the surface due to subsurface loss of minerals[4].

White-spot lesions are the earliest macroscopic evidence of enamel caries. White spots are evident when the amount of subsurface minerals loss (demineralization) exceeds the amount of minerals gained (remineralization) for a long time[5]. In such situation a visible change in the optical properties of enamel is evident in form of white opaque patches. The majority of demineralization in white-spot lesions occurs in the subsurface region of enamel. This subsurface demineralization increases porosity and changes the optical properties of the enamel. Typically, the enamel surface layer stays intact during subsurface demineralization, but without treatment the subsurface loss will continue and eventually the surface layer will collapse and lead to a cavity formation[6].

Remineralization is nothing but the net gain in minerals at the surface of enamel, which were lost due to demineralization. However if the mineral loss in active caries lesion produces a surface defect (cavity), remineralization cannot occur in such cases. Thus early intervention of incipient caries lesion aims at remineralization of an active non cavitated subsurface lesion and thereby preventing cavitation's by further mineral loss. Remineralization is dependent on the bioavailability of calcium, phosphate and hydroxyl ions. It is however enhanced by presence of sub levels of fluoride. As mentioned earlier, remineralization occurs once the pH of saliva returns to neutral. In such situation the extent of remineralization depends greatly on saliva composition and flow rate[7, 8]. In case of topical fluoride supplementation, the remineralizing potential of fluoride ions greatly depends on the calcium- phosphate concentration in the saliva[9].

In last decade, various remineralizing agents such as Fluoride varnish, CPP-ACP, Tricalcium phosphate, Novamin, etc. have been introduced, most of which contain fluoride, calcium, phosphate ions in varied forms andconcentrations. These agents tend to remineralize the subsurface caries lesion by providing calcium-phosphate with or without fluoride and control the surrounding micro-environment.

Recently the evolution of a new nana active technology i.e. Theramed was compared with the prospective calcium system which is prepared by reacting tricalcium phosphate (TCP) with Sodium Lauryl Sulphate (SLS) and shows the formation of a functionalized calcium phosphate when combined with fluoride. This prospective material may also interact with weekend enamel to help boost fluoride's remineralization potential. The results of this study showed statistically equivalent remineralization in Theramed and Prospective system; which was relatively

greater in comparison with both the 1000 ppm F test dentifrice and MI Paste Plus[10].

In the case of Nupro (Novamin), the active ingredient is a calcium sodium phosphosilicate that reacts when exposed to aqueous media and provides calcium and phosphate ions that form a hydroxyl-carbonate apatite (HCA) with time and binds to the tooth surface in order to initiate the remineralization process on the tooth enamel[11].

Many investigators have studied the demineralization and remineralization of enamel lesions in permanent teeth by using Nupro, but only a few studies have tested it in primary teeth. Also the effectiveness of Theramed in primary teeth is unclear,as the literature on the assessment of it appears to be scanty. Thus the present study was undertaken to determine and compare the remineralizing potential of the Nupro and Theramed with Controlrx i.e. 1.1%NaF

Keywords: NovaMin, Remineralization, Theramed, Vickers hardness number, White spot lesion,

Aim

1)To assess and compare the remineralizing potential of fluoridated calcium sodium phosphosilicate (NovaMin) and 1450 ppm NaF (Theramed) on artificial early enamel lesions in primary teeth.

Objectives

1) To measure the hardness (VHN) values of the -a) Pre-treated samples (samples from distilled water)

b) Demineralized groups

c) Remineralized groups

2) Compare the hardness (VHN) values and determine the -

Remineralizing potential of 1450 ppm NaF (Theramed) and fluoridated calcium sodium phosphosilicate (NovaMin)

Material and Methodology

Type of study

The present study is an analytical type of study conducted in the Department of Pedodontics and Preventive Dentistry, Sinhgad Dental College and Hospital; Pune.

Source of data

Collection of 90 deciduous teeth from the department of pedodontics and immersed in the distilled water at room temperature immediately in order to prevent the desiccation of samples.

Criteria for tooth selection

- **Inclusion criteria**

1. Extracted deciduous teeth with at least one surface not undermined by caries.

- **Exclusion criteria**

1. Teeth having developmental defects.
2. Teeth showing extensive loss of coronal structure either due to caries or trauma.

Materials to be used

1. NaF 1.1% paste (CONTROLRX by CM Storm)
2. Fluoridated Novamin (NUPRO by Sensodyne)
3. NaF (1450ppm F) (Theramed by Henkal)
4. Nail varnish (Lakme- India)
5. Deionized water (Indigenously prepared)
6. Stirrer
7. Containers.
8. L shaped moulds.

- **Armamentarium**

1. Diamond disc and high speed straight handpiece (NSK)
2. 800, 1000, 1200 grit silicon carbide paper
3. Kidney tray (API)
4. Metal tweezer (API)
5. Glove (Surgi-safe Corp)
6. Self-cure acrylic resin. (DPI)

- **Demineralizing solution**:

2.2 mM CaCl2, 2.2 mM KH2PO4, 0.05M acetic acid at the pH adjusted to 4.4 with

1 M KOH. (Indigenously prepared)

- **Equipment's**:

1. pH meter.(BOSMERE Germany)
2. Vickers hardness machine (Vickers hardness tester-by REICHART Austria)

5. Storage of samples

The samples will be stored in a distilled water at room temperature.

6. Preparation of the samples

a) Debridement of samples and inspection for cracks, hypoplasia and white spot lesions.

b) Embedment of the samples in the acrylic, for stable placement of the sample on the Vickers Hardness Machine.

c) Coating the window of 2mm x 2mm on sound, intact surface of the buccal or lingual enamel of teeth, by using the acid resistant nail varnish.

d) Then subject the samples for VHN testing.

e) Demineralization of all the samples.

7. Process of demineralization

a) Immersion of the samples in the demineralizing solution for 96 hours to

Produce lesions.

b) Subjecting the sample's for Vickers Micro-Hardness testing to obtain the

hardness number

8. Remineralization of all the samples

a) Toothpaste supernatant preparation:

Toothpaste supernatants in Groups A, B and C will be achieved by suspending

15 g of the respective toothpaste in 45 ml of deionized water in order to achieve

1:3 (toothpaste: deionized water) ratio. The suspensions will be thoroughly Stirred. (Group A= n1, Group B= n2, Group C= n3)

- **Preparation of the samples:-**

1. Debridement of samples and inspection for cracks, hypoplasia and white spot lesions.
2. The radicular part of each tooth was removed using a high speed diamond disc. Custom made L shaped molds were made and self-cured acrylic resin was poured on it; then each enamel block was embedded in, on top of partially set, and allowed to set. An acid resistant nail varnish was applied around the exposed enamel surface leaving a window of 2 mm X 2 mm of enamel exposed at the center.
3. Bosmere Germany, Vickers micro hardness tester was used to evaluate micro hardness. A load of 25 grams was applied, for five seconds, for all the specimens. The micro hardness numbers (VHN) of five indentations at spacing of 100 microns were taken and the average value was considered the mean base line micro hardness (SMH) of the corresponding specimen. The objective of base line surface micro-hardness determination is to compare and calculate the changes that occur after induction of enamel lesions and after pH cycling.
4. Carious lesions representing preliminary stage of subsurface enamel demineralization were produced by suspending each tooth into glass beakers containing demineralization solution, for 96 hours, in an incubator at a temperature of 35 degree[12]. After induction of enamel lesions, all the specimens were evaluated for surface micro hardness measurements under 25 gram loads for five seconds duration. Each tooth was subjected to the following surface treatment.

- **Group A (n1)**

NaF 1.1% Paste 15 gm. +45ml of de-ionized water =

Supernatant Preparation of Controrx

- **Group B (n2)**

Fluoridated Novamin 15gm +45ml of de-ionized water = Supernatant Preparation of Nupro

- **Group C (n3)**

NaF (1450ppm F) System 15gm +45ml of de-ionized water = Supernatant preparation of Theramed

- **The pH cycling model**

The specimens will then be placed in the pH cycling system for 10 days. Each cycle Will be involving three hours of Demineralization twice a day with two hours of Remineralization in between. Specimens in Groups A, B and C will be treated for 60 seconds with toothpaste supernatant before and after the Demineralization cycle.

Remineralization of the samples of Group A, B and C is carried out with respective Supernatant preparations.

1) Measure the Vickers micro-hardness number values of each group after Remineralization.

2) Comparison of the micro-hardness values of demineralized and remineralised Samples.

- **Evaluation of Micro-hardness values**

1) VHN of pretreated samples (N=90)

2) VHN of demineralized samples (N=90)

3) VHN of remineralized samples (n1=30, n2=30, n3=30)

- **Evaluation technique**

After demineralization and remineralization of the specimens, Vicker's Hardness Test (VHN) will be performed to quantitatively evaluate the micro-hardness of the teeth samples. The (VHN) is expected to show the difference in the remineralization potential of the specimens, quantitatively.

Percentage of Surface Micro Hardness Recovery[10] was calculated as:

$$\% \text{ SMHR} = \frac{\text{Initial Enamel IE} - \text{Demineralized Enamel DE}}{\text{Treated enamel TE} - \text{Demineralized Enamel DE}} \times 100$$

Statistical Analysis

Data obtained was compiled on a Microsoft excel sheet (version 2010) and grouped as per three groups.

The mean, standard deviation, standard error of mean were calculated and subjected to statistical analysis using statistical package for social sciences (SPSS, v 22.0, IBM). Since data is numerical, following a normal curve and not having outliers, parametric tests are used for hypothesis testing.

Intra group comparison of means of various time intervals like baseline, after demineralization and after pH cycling was done by repeated measures ANOVA followed by Tukey's post hoc test. Inter group comparison between 3 groups at baseline, after demineralization and after pH cycling was done by ANOVA followed by Tukey's post hoc test.

$p<0.05$ was considered to be statistically significant, keeping α error at 5 % and β error at 20%, giving the power to study as 80%.

Formulae used for analysis:

Mean, $X = \sum Xi / n$ $\quad i = 1, 2....n$

$$\text{Standard deviation (SD)} = \sqrt{\sum (X_i - \bar{X})^2 / n-1}$$

Standard error, SE = SD/ n

Variance = $SD^2 = \sum (x_i - x)\ 2n - 1$

Paired t test, t = Mean difference / Standard error of difference

One way ANOVA,F = Between sample variance / Within sample variance

Post-hoc Tukey's test: highest significance difference (HSD) =

tuk= s2n

tuk- table value,

s2 = within group variance;

n= sample size

Results

The present in vitro study was conducted to evaluate and compare the remineralization potential ofControlrx, Nupro, and Theramed

The recorded values are presented as mean±SD, range and percentage changes. The recorded values were statistically analyzed using Student's Paired t test, ANOVA and Post-Hoc Tukey's test.

The results of the present study were evaluated under the following headings;

Master chart I: Compiled data representing the mean depth of demineralization and remineralisation in the various groups.

Table I: Comparison of the mean and standard deviation and the significance (p) value of difference in demineralization and remineralisation for the various groups.

Table II: Intergroup comparison of the significance (p) value of difference in remineralization among the various groups.

Observation from master chart I

Master chart I shows the compiled data representing the mean depth of demineralization and remineralisation of the groups.

Observation from Table I and graph I

Table I and graph I shows the intergroup comparison of the mean and standard deviation and the significance (p) value of difference in demineralization and remineralisation among the various groups.

Group I showed a mean demineralization depth of 216.97, remineralisation depth of 237.60 and a mean difference of de and remineralisation of -20.633. The difference in de and remineralisation were statistically significant (p<0.00).

Group II showed a mean demineralization depth of 220.37, remineralisation depth of 272.27 and a mean difference of de and remineralisation of -51.90. The difference in de and remineralisation were statistically significant (p<0.00).

Group III showed a mean demineralization depth of 217.73, remineralisation depth of 259.67 and a mean difference of de and remineralisation of -41.93. The difference in de and remineralisation were statistically significant (p<0.00).

Observation from table II

Table II shows intergroup comparison of the significance (p) value of the difference of remineralisation among the various groups.

On comparison of group I and II a mean difference of -34.667 was observed in percentage of remineralisation, which was statistically not significant (p>0.00).

On comparison of group I and III a mean difference of -22.067 was observed in percentage of remineralisation, which was statistically significant (p<0.015).

On comparison of group II and III a mean difference of 12.600 was observed in percentage of remineralisation, which was statistically significant (p<0.240).

The remineralisation potential was statistically significant for all groups except for group III (P=0.24), where they were not significant.

Table I

Groups	Demineralization		Remineralisation		Difference		T value	p value
	Mean	SD	Mean	SD	Mean diff	SD		
Group I	216.97	28.181	237.60	30.30	-20.633	11.208	-10.083	0.000
Group II	220.37	33.447	272.27	33.898	-51.900	17.109	-16.615	0.000
Group III	217.73	25.131	259.67	25.148	-41.933	21.635	-10.616	0.000

Table II

Comparison of remineralisation in various groups

Groups	Mean diff	p value
1&2	-34.667	0.000**
1&3	-22.067	0.015*
2&3	12.600	0.240#

Discussion

In spite of growing awareness towards oral healthcare, prevalence of dental caries still remains high. A number of factors like increased consumption of cariogenic food,

poor oral hygiene, lack of knowledge about initiation and maintenance of oral hygiene, all these factors make it difficult for the dentist to intervene at early stages of caries process, which often result in progress of caries process and ultimately lead to loss of tooth structure through formation of cavities[13].

Clinically, the early enamel lesion appears white because the normal translucency of the enamel is lost. The surface becomes fragile and is susceptible to damage from probing. The most important feature of white spot lesion is the presence of relatively intact surface layer overlying subsurface demineralization (40-70%). Even though initial enamel lesions have intact surfaces, still they have a low mineral content at the surface layer when compared to sound enamel; thus showing a lower hardness value at the surface than for sound enamel tissue[13,14]. Organic acids are produced by the metabolic activity of microorganisms in the bacterial plaque. The chemical changes in plaque brought about by microbial activity and the presence of carbohydrate are reflected in changes in the ionic composition of the extracellular aqueous phase of dental plaque, referred to as plaque fluid. Because the caries process is a dynamic one involving periods of demineralization and remineralization, the chemical conditions that support both these processes should be reflected in the composition and properties of plaque fluid[15]

These acids diffuse through the pellicle into the surface enamel. These acids attack the apatite crystals, particularly at the vulnerable lattice points where carbonate ions are present. This causes Ca^{++}, OH^{-} , PO_4^{--} , F^{-} , CO_3^{-} , Na^{+} and Mg^{++} to be removed from the crystal lattice and to diffuse into the solution phase between the crystals. The dissolving calcium ions and phosphate ions form various calcium phosphate salts that either diffuse to the exterior or provide an environment that facilitates the repair of the faulty crystallites beneath the surface of enamel facilitating remineralization[16]. Mineral losses or demineralization proceeds as long as sufficient acid is available. As more enamel dissolves, concentration of the Ca^{++} ion and PO_4^{--} ion increases. As calcium and phosphate ions diffuse outwards, remineralization at the surface becomes more and more likely. This leads to the formation of an apparently intact enamel surface layer about 20-40 microns where the mineral content is higher than the body of the lesion.It is becoming accepted that the decreasing trend in caries prevalence and incidence is due, at least in part, to the increased availability of fluoride in water supplies and toothpaste, and/or by clinical applications. The fact that fluoride can be incorporated readily into the crystalline lattice of tooth mineral, resulting in a tissue less soluble in an acid environment, has been the scientific cornerstone for caries prevention. The major concept prevailing in the past was that the caries-inhibitory effect of fluoride was because of its incorporation in tooth mineral during the development stages or of the tooth prior to eruption. This led to the extensive systemic use of fluorides in caries prevention[17].

However, it is now realized that the primary caries preventive mechanisms of the action of fluoride are post eruptive through topical effects that can interfere with the dynamic equilibrium at the interface between mineral surface and oral fluid. The frequent delivery of fluoride to the tooth surface is currently the most efficient measure leading to caries arrestment and reversal. The paradigm shift that the incipient non-cavitated enamel caries can be healed[17]. In our study the experimental groups included were Theramed (1450 ppm), Nupro (5000 ppm) and Controlrx (5000 ppm)

Invitro Caries Progression

In human teeth, the enamel comprises of 96% calcium apatite, either as hydroxyapatite (HAP) (Ca_{10}

$(PO_4)6(OH)_2$) or fluorapatite (Ca_{10} (PO_4)$6F_2$). Such a high mineral content ensures that teeth are the hardest and probably the strongest biological material within the human body. In both the adult and deciduous tooth, enamel is the outer structure that envelops the crown. It is almost fully mineralized with highly organized HAP crystallites, making it mechanically hard and highly resistant to wear. In general, the deciduous teeth are whiter, softer, smaller, and weaker compared to their permanent counterparts. In addition, their enamel is thinner and has a higher organic content. The microstructure of enamel is highly textured with aligned prisms or rods that run approximately perpendicular from the dentin-enamel-junction (DEJ) towards the tooth surface. Each rod consists of tightly packed carbonated hydroxyapatite crystals that are covered by a nanometre-thin layer of enamelin and oriented along the rod axis. However, in deciduous/primary teeth, the outer-most layer is generally devoid of the usual prism structure[18].

Primary teeth were chosen in our study for the induction of artificial caries lesion. Only samples free of caries, developmental defects and enamel fractures and micro-fractures were included as previous studies[19,20]have revealed any pre-existing alteration of surface morphology of the tooth directly influenced the caries progression.In the ISO report it is recommended that the extracted teeth should be stored in distilled water or in a 0.5% chloramines bacteriostatic/bactericidal solution. Hence in our study the extracted teeth were stored in distilled water[21].

The labial third of each of the primary tooth was painted with nail varnish by leaving the window of 1mm by 1mm.

The complexity of the development of caries lesion has led to the development of model systems to improve our understanding of the mechanism of de and remineralization processes[22].In vitro chemical models provide information about the effects of caries preventive agents on the de-/remineralization dynamics at the surface and in the subsurface of the teeth[23]. Hence in such models, techniques involving the microbial production of acid have now generally been displaced by the direct use of acid to produce artificial caries like lesions. There is however considerable controversy as to additional conditions required. However, recent studies have reported that a closed agitated, acidic, aqueous solution is able to develop artificial caries like solutions almost irrespective of the conditions that exist[24].Similar chemical caries model for the production of artificial caries lesion was used in our study to, which consisted of 2.2 mM $CaCl_2$, 2.2 mM KH_2PO_4, 0.05M acetic acid at the pH adjusted to 4.4 with 1 M KOH. The solution was kept at a temperature of 37°C. The teeth were suspended in the solution for 4 days which created a subsurface demineralization of approximately 150 microns width with an intact surface simulating an early enamel lesion[25].

The concentration of both calcium and phosphates, in the demineralization solution, was at 50% of saturation level, causing dissolution of only enamel subsurface. Addition of fluoride prevented surface demineralization by forming Fluor apatite at the surface, which simulated the naturally occurring early enamel lesions having intact surface layer.

Considering the importance of the surface layer in caries progression, the evaluation of changes in this region is relevant. Surface micro hardness (SMH) measurement is a suitable technique for this purpose. Micro hardness measurement is appropriate for a material having fine microstructure, non-homogenous or prone to cracking like enamel. Surface micro hardness indentation provides a relatively simple, non-destructive and rapid method in demineralization and remineralization studies. Therefore, in the present study, the micro hardness values for each specimen were measured in three steps; the base line

micro hardness, after induction of carious lesion (demineralization) and after pH cycling[26].

Enamel microhardness was tested by a Vicker's Microhardness Tester,Reichert Austria ,Sr.No.363798. The mean of five hardness measurements made at 35 µm intervals was used as representative Vickers Hardness Number (VHN). The diagonal length of the indentation was measured and converted to VHN; which is in accordance with FerdaDogans study.[27]

Testing was performed with diamond pyramid indenters, which have a square-based diamond indenter with a 136º angle. Measurements were taken using a microscope of 100x magnification since identification was too small to be seen and measured with the naked eye. The test was determined using a load of 1 Newton (100 gm) applied to the specimens for 15 seconds as recommended in the pilot study (a pilot study was conducted to select the most appropriate preparation of the samples, The hardness test was first determined by using load of 0.5 Newton (50 gm) applied for 15 second at four points along the cavity. The indentation was too small and its boundaries neither clear nor sharp under the microscope. The load was thus increased to 1 Newton (100 gm) which gave a clear indentation). This load and time were constant for all samples throughout the study. The distance between two consecutive indentations was around 34µm and the indentation was never close to any edge of the specimen nor to any other indentation. The criteria for indentation acceptance were sharpness of diagonal edges, uniformity of diagonal shape (geometry) and absence of irregularities in the testing area.[28]

The values (VHN) obtained during the initial base line micro hardness measurements in the present study were in the range of VHN 340 - 437, which satisfies the VHN range of normal enamel tissue[29]. The surface micro hardness values for each group of the enamel specimens were decreased to 193- 271 at the end of 96 hours of demineralization which is in accordance with the study conducted by LataS et al[30]

There are different methods for evaluation ofdemineralization and remineralization of enamel whichmay be direct or indirect. Direct techniques are longitudinalmicroradiography, transverse microradiography andwavelength independent X-ray microradiography.Indirect techniques include polarized light microscopy, quantitative energy dispersive X-ray analysis, microhardness measurement methods and iodide permeability. Indirect methods are nevertheless quantitative and canmeasure changes in the real physical parameters. In case of polarized light they can detect the general porosity of the enamel substrate. The use of surface micro hardness test scan measure the change in surface structural strength. Surface micro hardness is a physical property which assesses the effect of chemical and physical agents on hard tissues of teeth. This is a useful way to examine the resistance of fluoride treated enamel. It is an appropriate test for enamel due to its fine microstructure, non-homogenous and brittle nature. Micro hardness indentation provides a relatively simple, rapid and non-destructive method in demineralization and remineralization studies. Microhardness tests are of different type which includes: Knoop, Vickers and Brinnel. In the present study, VHN was adoptedas the basis for investigation over Knoop's because thesquare shape of indent obtained in VHN is more accurateto measure. Even the minute changes in the square shapeindent obtained after the test can be easily detected[31].

The Vickers hardness values obtained during the baselinemean micro hardness measurements in the present study were in the range of 367.47-372.83 VHN. The surface mean micro hardness values for each group of the enamel specimens reduced to 216.97-220.37 VHN after

thedemineralization process for 96 hrs. Demineralized enamel specimens were then subjected to a remineralizing cycle of 10 days and treated with the remineralizing agent twice daily.The present study was designed to determine the period of the expected remineralization under continuous pH conditions. Simulation of the natural mouth environment forces the researchers to use pH-cycling techniques. Different modifications of this technique have been applied for investigating caries processes and effect of caries preventive agents. Therefore, pH-cycles creating models can be accepted as a good evaluating method of the caries process and also provide standard study conditions. Because of these reasons, the present research was designed on a pH cycle.[27]

The period for demineralization in the pH cycling phase was for three hours twice a day, which was to simulate the duration of demineralization (low cariogenic challenge) that occurs in the oral cavity[32]. The test material was applied on enamel blocks twice a day to simulate the normal recommended daily oral prophylaxis.

After remineralization ; Group A increased to 237.60 VHN, in Group B it was 272.27 VHN and in Group C it was 259.67 The mean increase in microhardness of enamel is in accordance with the study conducted by Lata S et al

The results of the present study revealed greater increasein mean micro hardness following remineralization with Nupro than with Theramed and Controlrx, which was statistically significant. Thismay be attributed to the beneficial properties of Nupro (Novamin).

Several studies have reported the caries inhibitory effectsof Novamin (bioactive glass) whenit comes in contact with saliva, it rapidly releases sodium, calcium, and phosphorous ions into the saliva that are available for remineralization of the tooth surface. The ions released forms hydroxycarbonate apatite (HCA) directly. They also attach to the tooth surface and continue to release ions and remineralize the tooth surface after the initial application. These particles have been shown to release ions and transform into HCA for up to 2 weeks. Ultimately, these particles will completely transform into HCA[33].

Recently Novamin (bioactive glass) materials have been introduced into many fields of dentistry. This unique material has many novel features, most important of which are its ability to act as a biomimetic mineralizer, matching the body's own mineralizing traits, while also affecting the cell signals in a way that benefits the restoration of tissue structure and function. Bioactive glass is considered as a break through advance in the remineralization technology. This is because the current standard treatment for tooth remineralization and prevention of decay is rather slow acting and is dependent on adequate saliva as a source of calcium and phosphorus[34].

Bioactive glasses have been used as substitutes for reconstruction of facial bones, rehabilitation of dento-alveolar complex, regeneration of periodontal pockets and recently for the treatment of hypersensitive teeth. The surface reactive bioactive glass contains SiO2, Na2O, CaO, and P2O5.38

Bioactive glass in aqueous environment immediately begins surface reaction in three phases, leaching and exchange of cations, network dissolution of SiO2, and precipitation of calcium and phosphate to form an apatite layer.[35]

Clark and Hench first proposed a well-detailed sequence of reactions occurring at the surface of silica-based bioactive glasses. These involved the following steps[36]:

- Rapid exchange of Na+ in the glass with H+ in solution;

- Loss of soluble silica as Si(OH)4 by breaking of Si-O-Si bridges and subsequent formation of surface silanol groups in the process;
- Condensation and repolymerization of surface silanols to form an SiO^{2-}rich surface layer;
- Migration of Ca^{++} and PO_4^{3-} to the surface through the silica-rich layer and formation of a Ca-P rich layer on the surface of the glass;
- Incorporation of OH-, CO_3^{2-} from the solution and subsequent crystallization of the Ca-P layer to form HCA.

Within 3-6 hr in vitro, the calcium phosphate layer will crystallize into the carbonated hydroxyapatite (CAP) layer which is essentially the bonding layer. Chemically and structurally, this apatite is nearly identical to bone and tooth mineral, thus allowing the body to attach directly to it. These Bioglass® surface reactions from implantation to 100-150 μm CAP layer formation takes 12 to 24 hr[35]. The standard for Bioglass® formulation is commonly known as 45S5 which has been used extensively in research studies. It contains 45 wt% SiO_2, 24.5 wt% Na_2O and CaO, and 6 wt% P_2O_5. Bioactive glasses have traditionally kept the P_2O_5 fraction constant while varying the SiO_2 content. In fact, the network breakdown of silica by OH^- was found to be time dependent upon the concentration of SiO_2. It is now understood that keeping the silica below 60 wt% and maintaining a high CaO/P_2O_5 ratio guarantees a highly reactive surface[35].

Bioglass® is also marketed worldwide under the trade names PerioGlas® and NovaBone®. Recently, it has been demonstrated that fine particulate bioactive glasses (<90 μm) incorporated into an aqueous dentifrice have the ability to clinically reduce the tooth hypersensitivity through the occlusion of dentinal tubules by the formation of the CAP layer. Investigators using bioactive glass compositions have demonstrated a significant anti-microbial effect towards caries pathogens (S. mutans, S. sanguis) upon exposure to bioactive glass powders as well as solutions and extracts[35].

A study on the reminerlization potential of Bio-active glass on artificially carious enamel and dentin using Raman Spectroscopy was conducted by Olfat E. Hassanein and T.A. El-Brolossy. The results of this study indicate that Bioactive glass has the potential for remineralizing artificially carious enamel and dentin[34].

M.VahidGolpayegani et al (2012)[37]stated that, Novamin has a higher capability to enhance enamel resistance against caries development by altering its surface micro-hardness when compared to 1.1% NaF (Topex) tooth paste.

J.F.Weffel (2009)[38] made a statement that, “NovaMin—Likely Clinical Success” can also be taken as a question which can be answered in one word— PROMISING. for claims of caries prevention and remineralization.

Studiessuggest that in addition to fluoride, other minerals may berequired to extend levels of enamel protection and remineralization from cavitations to sound surfaces. This view is supportedwith several studies and perspectives that claimsynergistic behavior among various minerals (e.g. calcium,strontium, phosphate and fluoride) leads to improved remineralization efficacy. Due to the clinically proven benefits of fluoride, it isdesirable that a promising calcium agent should not interferewith the action of fluoride and should enhance fluoride’s activity in remineralizing weakened enamel. Two examples ofcommercially available dental products containing promisingcalcium agents plus NaF are MI Paste Plus and Theramed SOS, with each containing labeled amounts of 900 ppm and1450 ppm F, respectively. MI Paste Plus contains a promising calcium technology (casein phosphopeptide-amorphous calcium-phosphate, CPP-ACP, Recaldentc) while Theramed SOS contains another

(a hydroxyapatite-protein composite, Nanitactived). Recently the evolution of a new prospective calciumsystem that is prepared by reacting tricalcium phosphate (TCP)with sodium lauryl sulfate (SLS) to form a functionalizedcalcium phosphate was reported. Since SLS bears a strong affinity to enamel; and the fact that calcium and phosphateare manifested in the dentition, when combined with fluoride,this prospective material may also interact with weakenedenamel to help boost fluoride's remineralizing benefits[10].

Fluoride ions promote the formation of fluorapatite in enamel in the presence of calcium and phosphate ions produced during enamel demineralization.[39]

Fluoride ions can also drive the remineralization of previously demineralized enamel if enough salivary or plaque calcium and phosphate ions are available when the fluoride is applied. Thus the f TCP + 900 ppm of NaF containing dentifrice have an added advantage of fluoride in addition to novel functionalized tricalcium phosphate.

The f-TCP contains tri-calcium phosphate is specially milled with a simple organic ingredient known as "Sodium Lauryl Sulfate" (SLS). During the milling process, they functionalise the TCP resulting in an organic-calcium phosphate hybrid being formed. This importantly ensures that the Calcium oxides are protected from the undesirable interactions with fluoride, which could render both calcium and fluoride inactive which prevents calcium phosphate reaction with fluoride and formation of calcium fluoride. As a result fluoride, calcium and phosphate are available in aqueous form for remineralization process[40].

In a study conducted by Karlinsey et al in 2011[41]compared effect of f-TCp + 500ppm of fluoride with combination of three other remineralizing agents and measured changes in surface microhardness of the enamel. At the end of 10 days of treatment the enamel microhardness increased by 106.2 ± 7.4 VHN.

Robert L. Karlinsey et al(2009)summarized that, an in vitro remineralization/demineralization pH cycling model evaluated the reversal of "white-spot"(i.e. noncavitated) lesion in bovine enamel treated with MI Paste Plus, Theramed SOS, and Naf test dentifrice with and without a calcium phosphate technology. Based on the collected data, it was proposed that; Theramed SOS containing 1450 ppm F was found to generate the highest levels of remineralization amongst the three.

NaF is a preferred agent for caries investigations[42]. Fluoride has been recognized as a valuable therapeutic agent to provide partial protection against dental caries for approximately one-half century. Delivered in optimal amounts in the community water supply, fluoride provides a caries reduction of 60%. [43,44].In case of Group A, there was a slight increase in surfacehardness following remineralization, as compared to other two groups because NaF which is inorganic in naturereacts with hydroxyapatite of enamel forming a thick layerof calcium fluoride. This thick layer of calcium fluorideinterferes with further diffusion of fluoride from the topicalfluoride thus providing a relatively lower bioavailability of fluoride ions[45]. Another limitation is that the sodiumcation does not have any independent caries prophylacticproperty.Thus, in terms of bioavailability this translates into a significantly lower salivary fluoride level being availablefrom NaF. In contrast to our findings, a study by Lippert et al; compared the anticaries potential of two new commercial dentrifices containing amine fluoride and NaF by Vickers hardness testing. They concluded that NaF showed superior anticaries potential when compared to AmF. They attributed this to the presentation of the fluoride compound and formulation excipients on deciding the anti-caries potential in vitro.

Enamel primarily made of calcium-phosphate based crystalline mineral called Hydroxyapatite (HAP). The HAP crystals are packed tightly together to forms millions of microscopic prisms and lattices. Fluoride is incorporated in to solid crystalline lattice by iso-ionic exchange to form fluoro-hydroxyapatite (FAP). This form of enamel is harder than naturally occurring enamel[46].

When outer surface of enamel is exposed to fluoride (> 50 ppm), calcium fluoride can be formed. This precipitates on the enamel surface and acts as a source of enamel ions and may also acts as a barrier to demineralization. The calcium fluoride releases fluoride ions under acidic conditions which diffuse rapidly into the underlying enamel, resulting in formation of FAP and subsequent enamel hardening or increase in fluoride level in saliva[46]. The concentration of fluoride ions and ph of saliva affects the extent and rate of benefit associated with fluoride by altering the degree of saturation of FAP.Even at very low level in saliva fluoride drives the thermodynamically equilibrium for remineralisation by calcium and phosphate from saliva. In addition to improving the balance between demineralization and remineralisation, fluoride also have antimicrobial activity[47]. Fluoride inhibits the step in glycolysis inhibiting the plaque metabolism. Rate of microbial growth decreases hence reduction in rate of plaque growth and acid production.

Conclusion

The following conclusions were drawn from the present study:

It was observed that there was a significant difference in the microhardness values of enamel specimens following demineralization and after treatment of specimens with the remineralizing agents.

1. It was observed that all the three remineralizing agents used in the study significantly increased the microhardness of the enamel specimens after a period of 10 days
2. Nupro (Novamin, Bioactive glass) can be used as an alternative to fluoride as a remineralizing agent. As it has shown higher remineralising potential than the other two groups.

Within the limitations of our study and among the various materials evaluated it appears that,Before extrapolating the results of our study into clinical practice it must be stated that our results were obtained in an ideal environment in vitro. It is therefore, premature to draw any conclusions regarding the remineralization potential of these materials in situ. Saliva, plaque and many other confounding factors may affect the efficacy of these remineralizing agents. We therefore recommend further controlled studies on in-vivo models to confirm our observations and ascertain the true clinical efficacy of these newer remineralising agents.

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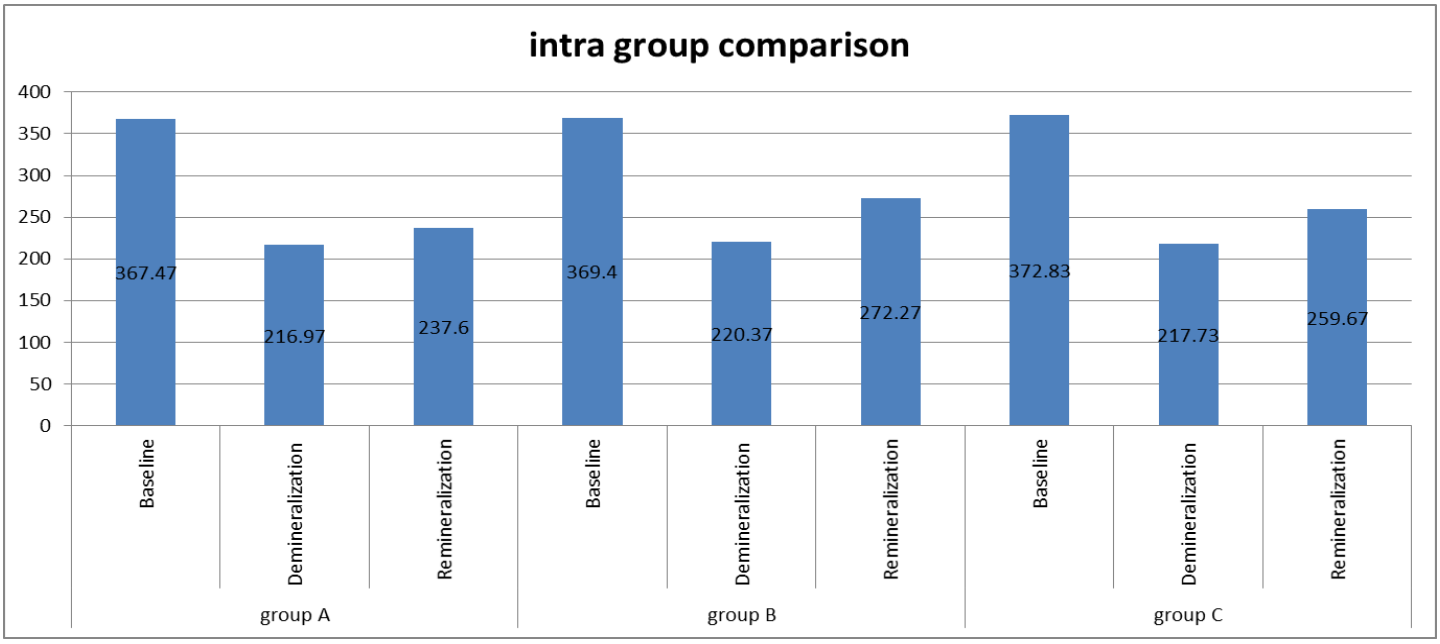
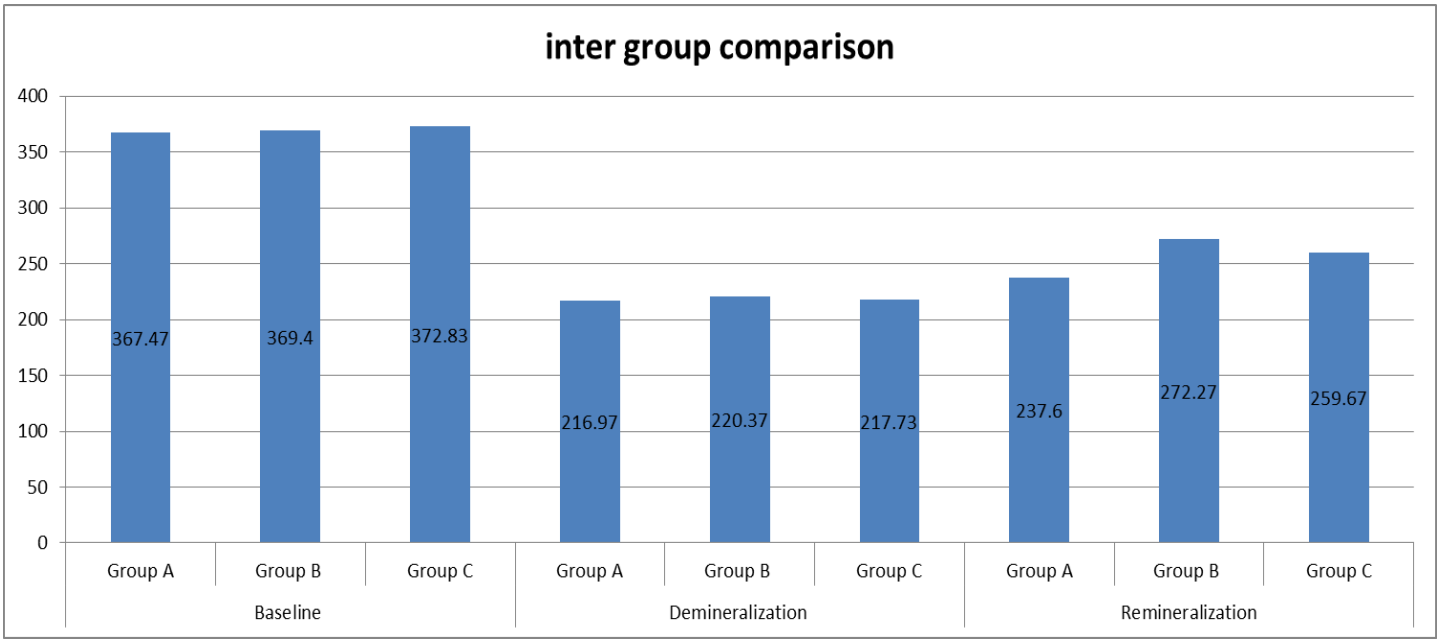
Legends Graph

Graph 1: Descriptive statistics showing the intergroup comparison of group A,B and C at Baseline, after Demineralisation and post Remineralization.



Graph 2: Descriptive statistics showing the intergroup comparison of group A,B and C at Baseline, after Demineralisation and post Remineralization.



Graph 3: Descriptive statistics showing the comparison of Remineralization value of groups A,B and C.

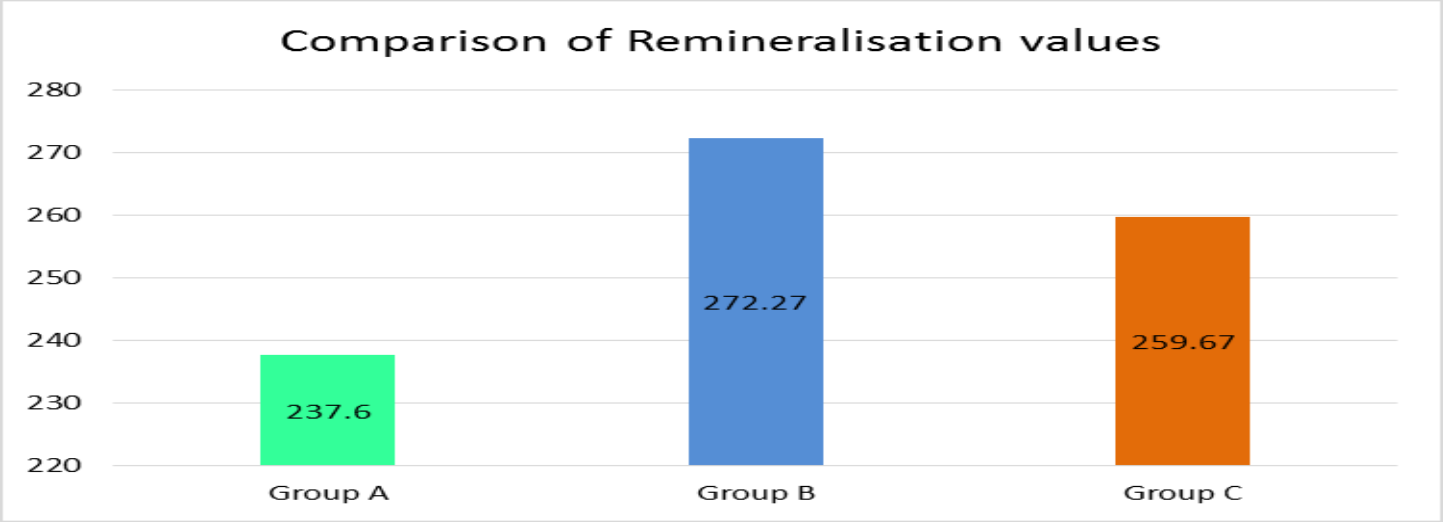


Table I: Descriptive statistics showing the intergroup comparison of mean, standard deviation and p value of ANOVA and Post hoc Tukey's test among the various groups.

Descriptive

	N	Mean	Std. Deviation	Std. Error	p value of ANOVA test	p value of post hoc Tukey's test
Baseline	30	367.47	35.510	6.483	0.000**	1&2 0.000**
Demineralization	30	216.97	28.181	5.145		1&3 0.000**
Remineralization	30	237.60	30.306	5.533		2&3 0.034*
Total GROUP A	90	274.01	73.870	7.787		
Baseline	30	369.40	33.925	6.194		1&2 0.000**
Demineralization	30	220.37	33.447	6.107	0.000**	1&3 0.000**
Remineralization	30	272.27	33.898	6.189		2&3 0.000**
Total GROUP B	90	287.34	70.515	7.433		
Baseline	30	372.83	35.498	6.481		1&2 0.000**
Demineralization	30	217.73	25.131	4.588	0.000**	1&3 0.000**
Remineralization	30	259.67	25.148	4.591		2&3 0.000**
Total GROUP C	90	283.41	71.846	7.573		

* = statistically significant ($p<0.05$)

**= statistically highly significant ($p<0.01$)

= non-significant difference ($p>0.05$)

Table II: Descriptive statistics showing the intergroup comparison of mean, standard deviation and p value of ANOVA and Post hoc Tukey's test among the various groups

Descriptive

		N	Mean	Std. Deviation	Std. Error	p value of ANOVA test	p value of post hoc Tukey's test
Baseline	Group 1	30	367.47	35.510	6.483	0.835#	1&2 0.975#
	Group 2	30	369.40	33.925	6.194		1&3 0.824#
	Group 3	30	372.83	35.498	6.481		2&3 0.924#
	Total	90	369.90	34.662	3.654		
Demineralization	Group 1	30	216.97	28.181	5.145		1&2 0.894#
	Group 2	30	220.37	33.447	6.107	0.894#	1&3 0.994#
	Group 3	30	217.73	25.131	4.588		2&3 0.935#
	Total	90	218.36	28.831	3.039		
Remineralization	Group 1	30	237.60	30.306	5.533		1&2 0.000**
	Group 2	30	272.27	33.898	6.189	0.000**	1&3 0.015*
	Group 3	30	259.67	25.148	4.591		2&3 0.240#
	Total	90	256.51	32.975	3.476		

* = statistically significant ($p<0.05$)

**= statistically highly significant ($p<0.01$)

= non-significant difference ($p>0.05$)

Table III: Descriptive analysis showing the mean and standard deviation for the demineralization and remineralization among the various groups

Groups	Demineralization		Remineralization		Difference		T value	p value
	Mean	SD	Mean	SD	Mean diff	SD		
Group I	216.97	28.181	237.60	30.30	-20.633	11.208	-10.083	0.000
Group II	220.37	33.447	272.27	33.898	-51.900	17.109	-16.615	0.000
Group III	217.73	25.131	259.67	25.148	-41.933	21.635	-10.616	0.000

Table IV: Descriptive statistics showing the intergroup comparison of remineralization among the various groups

Groups	Mean diff	p value
1&2	-34.667	0.000**
1&3	-22.067	0.015*
2&3	12.600	0.240#