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"A COMPARATIVE EVALUATION OF REMINERALISATION POTENTIAL OF SILVER DIAMINE FLUORIDE, BIOACTIVE VARNISH AND NANO-HYDROXYAPATITE GEL IN ENAMEL OF PRIMARY TEETH USING ENERGY DISPERSIVE X-RAY SPECTROSCOPY: AN IN-VITRO STUDY"

Paediatric Dentistry

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ABSTRACT

Background: Demineralization is process of removal of mineral ions from hydroxyapatite crystals of hard tissue by regular exposure of tooth enamel to acids produced by bacterial plaque.1 When bacterial colony in biofilm continues to produce acids with sugar consumption, pH falls to 4.5–5 from normal pH (i.e. 7) and results in formation of pores between mineral structures. The most prevalent therapy for several years was use of fluoride, which can rebuild partially dissolved crystals, supplied in form of topical gel or varnish and was applied to arrest active dental caries by depositing calcium fluoride that is insoluble and act as a fluoride reservoir to improve enamel remineralization, by increasing amount of mineral content of early carious lesions.2 **Materials And Method:** Forty naturally exfoliated primary molars with intact buccal surface, free from caries, developmental defects or cracks were included. Teeth were cleaned, polished, decoronated, embedded in self-cured acrylic resin mold with buccal surface facing upwards and window was created. Sub surface enamel lesions were created using demineralizing solution for 4 days and allocated into 4 groups: group A (n=10)- 38% SDF, group B (n=10)- Bioactive Varnish, group C (n=10)- 20% Nano- Hydroxyapatite Gel (custom made) and group D (n=10)- Untreated. All specimens were then subjected to pH cycle for 9 days, sectioned and examined under EDX Spectroscope. Data was analysed by using SPSS (21.0 version). p-value < 0.05 was considered significant. **Results:** Significant difference was seen in percentage of calcium, phosphorus, silica and fluoride indicating highest mineral deposition with 20% nanohydroxyapatite gel when compared to 38% SDF and Bioactive varnish. **Conclusion:** Among all remineralizing agents used in the study custom made 20% nanohydroxyapatite gel had better remineralising efficacy when compared to 38% SDF and Bioactive Varnish.

KEYWORDS

38% SDF, Bioactive Glass varnish, Nano-hydroxyapatite

INTRODUCTION:

White spot lesion (WSL) is considered the first sign of early enamel demineralization. Clinically it appears as a chalky white opacity and is considered the earliest macroscopic evidence of enamel caries. WSLs are characterized by a porous subsurface enamel and the outermost enamel layer covering the lesion remains relatively intact which appears radiopaque. Control of WSLs includes prevention of demineralization and enhancement of remineralization to arrest lesion activity and improve esthetics.[3]

The topical application of fluoride varnishes provides a non-invasive method for caries prevention and control. Therefore, fluoride varnishes have been widely used to treat dentine hypersensitivity, prevent caries and remineralize early caries.[4]

Silver (Ag) has an extended record of use in both the medical and dental fields since the 1800s because of its antimicrobial and anti-caries properties. The combination of silver and fluoride was primarily advocated for the synergistic beneficial effects of being anti-microbial and potentially aiding remineralization of teeth affected by dental caries. In the late 1960s, silver diamine fluoride (SDF) was introduced in Japan. It showed relatively good chemical stability compared with previous silver fluoride compounds. Recently, the United States Food and Drug Administration approved the clinical use of SDF as it matches the goals for 21st century medical care of the World Health Organization.[12]

Silver diamine fluoride (SDF) is an anti-cariogenic agent that can be used for caries prevention as well as a standalone treatment in the control of dental caries. It is applied until the child is old enough to cooperate during dental treatment.[5]

Bioactive glass (BAG) is a remineralizing agent developed by Larry Hench in 1969. BAG has unique characteristics as a scaffold material for bone tissue engineering. It binds both soft and hard tissues thereby stimulating tissue mineralization owing to its superior bioactivity. It has been widely used in medical field for bone regeneration and recent research has focused on its use in dental applications. Several previous studies have indicated that BAG can remineralize enamel and dentin and has antimicrobial effect on oral bacteria.[6]

Hydroxyapatite is the main mineral composition which is found in the bone and tooth of human beings. It is the utmost stable calcium-phosphate compound under physiologic conditions. Upon acidic circumstances, nano-HA can considerably enhance the remineralization potential by increasing the dissemination of mineral ions to the centre of the lesion. It also adheres to the demineralized porosities and establishes a constant apatite structure. Nano-Hydroxyapatite showed an extreme affinity to enamel and was considered to be one of the potent biologically compatible. There are few studies related to nano-HA gel and enamel remineralization.[7]

Therefore, the aim of the present study was to compare the mineral content of artificial enamel caries in primary teeth treated 38% SDF, Bioactive Varnish and Nano hydroxyapatite gel after being subjected to pH cycling. The null hypothesis stated here was that there is no difference in the remineralization potential among treatment groups.

MATERIALS AND METHODS:

The study was undertaken to evaluate and compare the remineralisation potential of 38% Silver Diamine Fluoride, Bioactive Varnish and Nano-Hydroxyapatite Gel in primary teeth using Energy Dispersive X-Ray Spectroscopy.

Selection Of Teeth:

Forty extracted human primary molars with sound tooth structure, free from caries, white spot lesions, developmental defects and cracks with intact buccal surface were included in the study. Teeth were stored in 3% sodium hypochlorite solution, cleaned and polished with non-fluoride containing polishing paste and washed with distilled water before being used in the study.

Specimen Preparation:

The Teeth were examined under a stereomicroscope to exclude teeth with developmental defects, cracks or enamel demineralization in the buccal surface and stored in distilled water until used and then decoronated using a double-faced diamond disc with crown embedded in self-cured acrylic resin mold with buccal surface facing upwards.

4x4 mm^2 square adhesive tape was placed on the centre of buccal surface of each specimen. The entire specimen was then coated with acid resistant nail varnish. Adhesive paper was removed and 4x4 mm^2 window was created in all the specimens. The specimens were washed and immersed in distilled water at 37^0C for 1 day.

Creation of White Spot Lesion:
Subsurface enamel lesions were created by submerging each specimen in 10 ml of a demineralization solution (pH 4.4) for 4 days inside a light resistant container until a distinct visual change in enamel in both dry and wet conditions is evident (ICDAS score 2).

After induction of white spot lesions, each specimen was rinsed with 10 ml distilled water and dried with a stream of compressed air and 4x4x3 cu mm enamel sections were made for quantitative elemental analysis (Ca, P, F, Si and Ca/P) using energy dispersive X-ray spectroscopy (EDX).

Application of Remineralizing agents and pH Cycle:
The teeth in each group were treated with their respective remineralizing agents and subjected into pH cycle for 9 days. After pH cycling, specimens were washed with distilled water and 4x4x3 cu mm enamel sections were made for quantitative elemental analysis (Ca, P, F, Si and Ca/P) using energy dispersive X-ray spectroscopy(EDX).

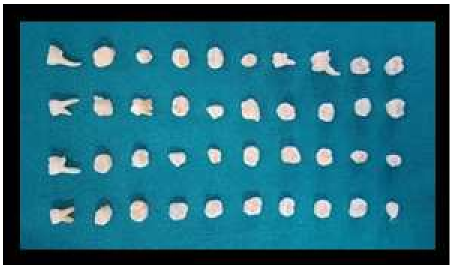


Figure 1: Forty extracted human primary molars

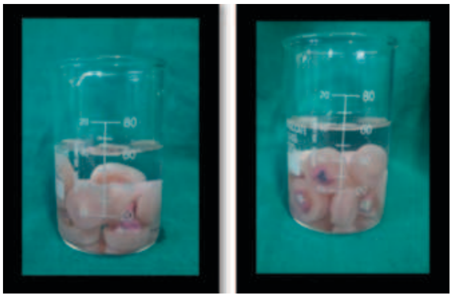


Figure 2: pH Cycle for 9 days

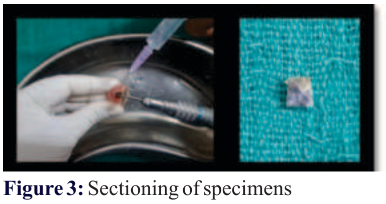


Figure 3: Sectioning of specimens
Figure 4: Gold sputtered specimens

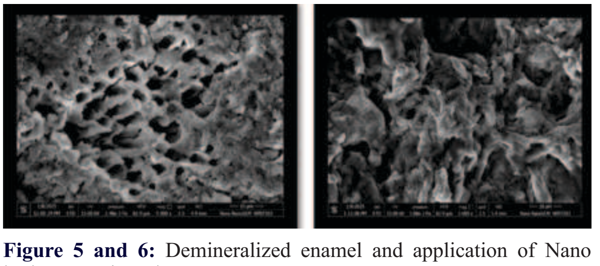


Figure 5 and 6: Demineralized enamel and application of Nano hydroxyapatite gel

Statistical Analysis:
Data was entered into Microsoft Excel spreadsheet and was checked for any discrepancies. Summarized data was presented using Tables and Graphs. The data was analysed by SPSS (21.0 version). Shapiro Wilk test was used to check which all variables were following normal distribution. Data was normally distributed therefore, inferential statistics were performed using the parametric test.

For intergroup comparison ONE WAY ANOVA followed by post hoc Tukey's test were used. For intragroup comparison, Paired t test was used. Level of statistical significance was set at p-value less than 0.05

RESULTS:

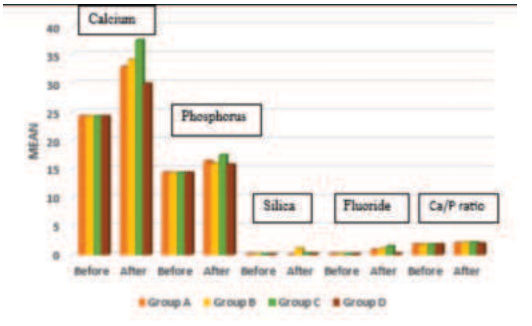
Table I: Materials used in the study

NAME	COMPOSITION
38% SDF (Kids- e-dental)	25% silver, 8% ammonia, 5% fluoride, and 62% water.
FlouroDip Bioactive Varnish (Prevest Denspro Pvt. Ltd)	Sodium Fluoride, Bio-glass, Rosin Gum, Ethyl Alcohol and Flavouring Matter
20% Nano-Hydroxyapatite Gel	Hydroxyapatite Powder and Sodium Carboxy Methyl Cellulose Powder mixed with distilled water in ratio of 1:1 having 20% concentration and pH 9.86.
Demineralization solution	2.20mmol/L calcium chloride, 2.20mmol/L monosodium phosphate, 1mol/L potassium hydroxide and 0.05 mol/L acetic acid; pH 4.4
Remineralization solution	1.5 mmol/L calcium chloride, 0.9 mmol/L monosodium- phosphate, 150 mmol/L potassium chloride; pH 7.0

Table II- Intragroup comparison of elements

Group	Calcium wt %		Phosphorus wt %		Silica wt %		Fluoride wt %		Ca/P ratio	
	Before	After	Before	After	Before	After	Before	After	Before	After
A-38% SDF	24.16 ± 2.97	32.74 ± 5.55	14.31 ± 4.27	16.33 ± 1.62	0.113 ±0.108	0.031 ± 0.023	0.124 ±0.138	0.841 ± 0.628	1.804 ± 0.472	2.036 ± 0.498
B-Bioactive Varnish	24.16 ± 2.97	33.98 ± 1.72	14.31 ± 4.27	15.96 ± 1.45	0.113± 0.108	1.107 ± 0.817	0.124 ±0.138	1.067 ± 0.606	1.804 ± 0.472	2.143 ± 0.194
C-20% Nano-hydroxyapatite gel	24.16 ± 2.97	37.47 ± 1.35	14.31 ± 4.27	17.41 ± 2.12	0.113± 0.108	0.235 ± 0.341	0.124 ± 0.138	1.486 ± 0.547	1.804 ± 0.472	2.180 ± 0.277
D - Control	24.16 ± 2.97	29.82 ± 6.35	14.31 ± 4.27	15.72 ± 3.73	0.113± 0.108	0.168 ± 0.182	0.124 ± 0.138	0.205 ± 0.200	1.804 ± 0.472	1.965 ± 0.463
p value	0.001		0.267		0.001		0.001		0.001	

Note: p-values > 0.05 indicate no significant difference in phosphorus levels after application.



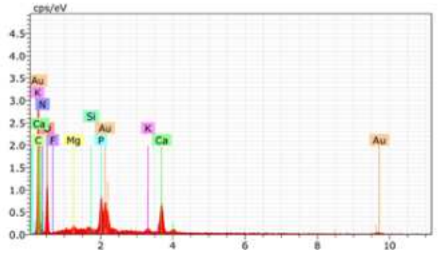
Graph 1- Intragroup comparison of elements

Table II and Graph 1 –
It represents intragroup comparison between before and after application of remineralizing agents.

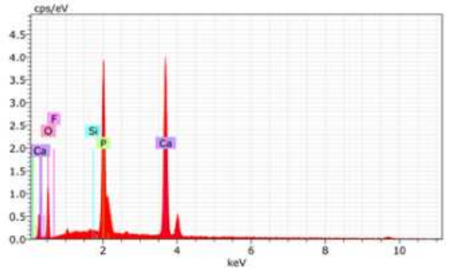
- Calcium levels significantly increased in all groups, with the highest gain in Group C (24.16 ± 2.97 to 37.47 ± 1.35 wt%), followed by Group B, Group A, and the least in Group D.
- Phosphorus levels showed no statistically significant difference post-treatment ($p = 0.267$), indicating stable phosphate levels across groups.
- Silica content varied significantly ($p = 0.001$), with the highest increase in Group B (1.107 ± 0.817 wt%), possibly due to bioactive varnish components.
- Fluoride levels significantly increased in all treated groups, especially Group C (1.486 ± 0.547 wt%) and Group B (1.067 ± 0.606 wt%), confirming their superior fluoride uptake.
- Ca/P ratio improvements were significant ($p = 0.001$) and highest in Group C (2.180 ± 0.277), followed by Group B, A, and the least in the control group.

Significant difference was seen in the mean difference in percentage of calcium, phosphorus, silica and fluoride when compared among four study groups with $p<0.05$.

As the percentage of deposition of calcium, phosphorus, silica and fluoride ions were more, remineralisation potential was seen to be highest with 20% nanohydroxyapatite gel when compared to 38% SDF and Bioactive varnish.



Graph 2- EDX SPECTRUM -- Demineralized enamel



Graph 3- EDX SPECTRUM – After application of 20% nanohydroxyapatite gel

DISCUSSION:

Several studies have been carried out to evaluate the efficacy of remineralizing potential of agents that are available to remineralize white spot lesions; however, there is scarcity of data on the use of gel comprising of Nano-hydroxyapatite as a main composition.

Hence, the purpose of current study was to evaluate and compare remineralisation potential of 38% Silver Diamine Fluoride, Bioactive Varnish and Nano-Hydroxyapatite Gel in primary teeth using Energy Dispersive X-Ray Spectroscopy.

Specimen Preparation

In our study, the teeth were decoronated with the help of double-sided diamond disc and embedded in acrylic mold with buccal surface facing upwards. 4x4 mm of adhesive tape was applied on the buccal surface and acid-resistant nail varnish was applied over it, resulting into 4x4 mm^2 window on the buccal surface. One benefit of this design was that it allowed for uniform and standardized area of enamel undergoing demineralizing and remineralizing process.

The enamel of primary molar consists of tightly packed and intertwined mineral rods that are bound by proteins, which belong to an interfacial layer system. These rods or prisms in turn consist of protein-bound hydroxyapatite (HA) fibres that run parallel and at certain angles to the direction of the prisms giving uniform mineral quantity and thickness. As the enamel in the central portion of the primary molar is uniform and sufficiently thick, it minimizes sampling errors related to measurement in specific area for EDX analysis. Hence, it was standardized and brought on by insufficient enamel area undergoing specific acidic changes.[14]

In contrast to our study, Kamath P et al (2017) used 3x3 mm sticking plaster which was placed on the exposed tooth surface and acid-resistant nail varnish was applied around it to delineate the area to be studied.[9]

38% SDF as a Remineralizing agent

SDF promotes absorption of calcium, leading to inhibition of calcium dissolution from enamel. 38% SDF mainly comprises of 25% silver, 8% ammonia, 5% fluoride, and 62% water. Silver ions in the SDF that are responsible for the antimicrobial action, reacts with hydroxyapatite crystals and result in the precipitation of silver phosphate that forms an insoluble hard layer on the enamel surface. The remineralizing capacity of SDF is also promoted by the presence of fluoride ions found in the solution. One ml of 38% SDF contains approximately 205.2 mg of fluoride (44,800 ppm).[11] In the presence of calcium and phosphate ions, fluoride forms fluorapatite crystals and calcium fluoride hence there is increase in the mineral density and hardness of the enamel.[2]

In our study, according to table II and Graph 1 in the given results, demineralized primary enamel showed 24.16% calcium, 14.31% phosphorous and 0.124% fluoride before and 32.74% calcium, 16.33% phosphorous and 0.841% fluoride after application of 38% SDF. The possible reason for this was high affinity of demineralized enamel for calcium, phosphorous and fluoride ions to deposit into the porous structure of hydroxyapatite and convert it into more acid resistant and remineralized fluorapatite crystal. In addition to this, another reason could be the flowability of SDF that allowed full contact of SDF on enamel surface in a relatively short duration of time by containing more fluoride which slows down the diffusion of fluoride ions into the surface of the enamel.[4]

Similarly, in the study conducted by **Mohamed Y et al (2023),** 38.4% of calcium, 18.3% phosphorus and 0.9% fluoride was seen after application of 38% SDF. **This was due to** the presence of calcium, phosphate and fluoride ions that forms fluorapatite crystals and calcium fluoride that increases the mineral density and hardness of porous enamel structure.[2]

Bioactive Varnish As A Remineralizing Agent

A study by Kim HJ (2021) stated that BAG (Bioactive glass) can react with saliva, inducing the release of Ca2+, PO4+ and Si4+ at the glass surface, and the subsequent precipitation of a polycondensated silica-rich layer serves as a template for the formation of calcium phosphate. As the reaction and the deposition of Ca-P complexes continue, this layer crystallizes into hydroxycarbonate apatite, which is chemically and structurally similar to biological apatite.[6]

However, the application of BAG (Bioactive glass) as a remineralization agent has been limited, owing to its relatively long reaction time. According to Hench (1969), the release of calcium and phosphate ions from BAG (Bioactive glass) requires at least 2 hours. Moreover, newly formed calcium and phosphate compounds from BAG were detectable after 24 hrs.[7]

According to table I, the Composition of Bioactive varnish used in our study includes

- **5% Sodium Fluoride:** Provides fluoride ions essential for remineralization
- **Bio-glass:** Enhances the remineralization process by releasing calcium and phosphate ions.
- **Rosin Gum:** Acts as a natural resin to form a protective film over the treated area.
- **10 -20% Ethyl Alcohol:** Serves as a solvent to dissolve the active ingredients and aids in the varnish's application.
- **Spearmint Oil:** Adds flavour to the varnish.

1 ml of FlouroDip Bioactive varnish contains 50mg of sodium fluoride equivalent to 22.6 mg of fluoride.(22,600 ppm)

Hence in our study as shown in table II and Graph 1, we have applied bioactive glass varnish 5 minutes daily for seven days and EDX analysis showed 33.98% of calcium, 15.96% phosphorus, 1.107% silica and 1.067% Fluoride, with highest percentage of silica among all remineralizing agents used in the study. The reason for this could be the longer duration and higher substantivity of varnish that leads to retention of calcium and phosphorous ions into the porous crystalline structure of demineralized hydroxyapatite. Silica present in the varnish forms a protecting layer for calcium phosphate hence, prevents further dissolution of minerals after any acidic changes.

In accordance to our study, similar results were seen in study conducted by Morad YO et al (2024) using bioactive glass gel in thirty deciduous molars and concluded that highest percentage of remineralization was found in Bioactive glass group, which almost returned the mineral content to base line followed by fluoride varnish group, while the lowest percentage was found in diode laser group.[8]

In contrast, to our study, a study by Zhang R (2021) showed Ca/P ratio in bioactive glass group of 1.55, which was similar to our results i.e 2.14. The reason explained was co-precipitation of calcium carbonate and silicon phosphate ions which speculated that the co-precipitations formed by bioactive glass exert poorer acid resistance than normal deciduous teeth.[13]

There are many studies that have included bioactive glass either in the form of toothpaste, gel or varnish, but no study clearly determines the duration of application of varnish, hence our study was carried out to evaluate the remineralizing potential of bioactive varnish after application for seven days.

Nano-hydroxyapatite as a Remineralizing agent
Composition of nanohydroxyapatite gel

In our study, according to table I, 20% Nano Hydroxyapatite Gel was made by mixing Nano-Hydroxyapatite Powder and Sodium Carboxy Methyl Cellulose Powder with Distilled Water in ratio of 1:1 having 20% concentration and pH 9.86.

Similarly, **Juntavee A et al (2021) carried out in-vitro study using 20% and 30%** nano-hydroxyapatite gel, and stated that 20% nano-hydroxyapatite gel was equally capable of remineralization compared to 30%. This indicate that increase in the concentration of NHG might not be the prime factor indicating the remineralization potential for carious lesions.[7]

In our study, 10 gms of SCMC (Sodium Carboxy Methyl Cellulose) powder was mixed with 10gms of nanohydroxyapatite powder in a ratio of 1:1. This mixed powder was then dissolved in 100 ml of distilled water to obtain 20% concentration of gel.

SCMC (Sodium Carboxy Methyl Cellulose) was used to increase the viscosity of the gel to provide a slower rate of diffusion of minerals into the carious surface of enamel. The higher concentration of nano-HA together with SCMC compensates for the slow rate of diffusion of nano-HA. Additionally, it was assumed that pH values higher than 5.5 would arrest caries progression and might promote remineralization, hence pH of the gel used in our study was kept 9.86.[7]

Duration Of Application Of Nanohydroxyapatite Gel:

To our knowledge, there is scarcity of literature using 20% nano-hydroxyapatite gel in primary enamel along with its frequency of application, thus we have applied this agent twice daily to evaluate its remineralizing potential.

A study by P. Hemalata et al (2020)[1] stated that seven-day remineralization failed to remineralize artificial enamel caries completely. So, we extended the time of application of Nano-hydroxyapatite gel for fourteen days.

According to table II and Graph 1, 37.4 % mean deposition of calcium and 17.4% phosphorus ion was seen after application of nanohydroxyapatite gel. The reason attributed to this could be that calcium phosphates are dissolved and remineralized locally as HA or FA and incorporated into voids of enamel prisms, which then fuse together with the prism crystals. The particle size of nano-hydroxyapatite powder is 20 to 80 nm and size of hydroxyapatite pore ranges between 20 to 40 nm, this makes nanohydroxyapatite particles to deposit into crystalline lattice and undergo remineralization. Hence, Nanoparticulate hydroxyapatite may act as a calcium and phosphate reservoir that maintains a vital state of supersaturation of these ions with respect to the tooth minerals and facilitates their deposition on the surface of demineralized enamel.[1]

Similar results were seen in a study conducted by P. Hemalata et al (2020) which stated that the deposition of calcium and phosphate is more in the nano-hydroxyapatite group as its composition mainly has calcium and phosphate.[1]

The study conducted by Kamath P. et al (2017) showed that the specimens treated with nano-HA exhibited surface Ca/P ratio close to that of the biological enamel and synthetic nano-HA, indicating an hydroxyapatitic coating deposition on the demineralized enamel surface which was similarly seen in our study suggesting highest Ca/P ratio (2.18) among all other remineralizing agents.[9]

A review article by Meyer F et al (2022) explains four mode of action of hydroxyapatite such as formation of a protective HAP Layer, calcium release and ph-buffering, deep remineralization of caries lesions and inhibition of bacterial plaque adhesion.[10]

While comparing the amount of fluoride uptake with the application time of remineralizing agents, single application of 38% SDF showed 0.841%, 7 days application of Bioactive varnish showed 1.067% and 14 days application 20% nano-hydroxyapatite gel showed 1.486% of mean fluoride concentration. This highlights that 38% SDF has remineralizing potential within shorter duration of time as compared to bioactive varnish and nano-hydroxyapatite gel which requires longer duration of application time for effective remineralization of white spot lesions in primary enamel.

The topical application of nano-HA gel indicates an attractive route to deliver the material in the oral cavity and can be more effective and less toxic than conventional formulations and provide its effectiveness directly at the site of action, especially for the noncooperative young child and the medically compromised patient who faced with trouble in using toothbrushes and toothpaste for hygiene control.[7]

Thus, we can justify nano-hydroxyapatite as an upcoming newer generation and potent remineralizing agent utilizing nano technology in the field of pediatric and preventive dentistry.

Limitations:
The Limitation In Our Study Includes:

- The inability to simulate the varying pH levels that naturally fluctuate in the oral cavity, which can influence microbial activity and oral health. Additionally, the diverse microbial environment present in the oral cavity cannot be fully replicated in in-vitro studies, which may limit the scope of the findings.
- Furthermore, there is a need for additional research using advanced techniques such as Polarized Light Microscopy, X-ray Diffraction (XRD), Fourier-Transform Infrared Spectroscopy (FTIR), and Atomic Force Microscopy (AFM) to gain a deeper understanding of materials.
- Finally, clinical studies with longer follow-up periods are required to obtaining more comprehensive and accurate data that reflect the long-term effects and dynamics of remineralizing agents to treat white spot lesions in primary teeth.

CONCLUSION:

Based on the results obtained from the present study it can be concluded that

1. All the agents used in the study i.e 38% SDF, Bioactive Varnish and 20% nanohydroxyapatite gel possesses remineralizing potential in enamel of primary teeth.
2. Custom made 20% nanohydroxyapatite gel had better remineralising efficacy when compared to 38% SDF and Bioactive Varnish.
3. Considering the application time, 20% nanohydroxyapatite gel requires longer duration to undergo remineralization for white spot lesions.

Additionally, more studies should be carried out to authenticate the remineralising potential of nanohydroxyapatite gel considering the role of nanotechnology in the field of pediatric dentistry.

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