



"A COMPARATIVE EVALUATION OF REMINERALISATION POTENTIAL OF 38% SDF AND BIOACTIVE VARNISH IN ENAMEL OF PRIMARY TEETH USING STEREOMICROSCOPE- AN IN-VITRO STUDY"

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ABSTRACT

Background: When pH falls to 4.5–5 from normal pH 7, minerals are dissolved and results in formation of pores between mineral structures. Clinically it appears as chalky white opacity known as white spot lesion and considered as earliest macroscopic evidence of enamel caries. The most prevalent therapy of such lesions is use of fluoride, which can rebuild partially dissolved crystals, supplied in form of varnish that is applied to arrest active caries by depositing calcium fluoride which act as a fluoride reservoir to enhance enamel remineralization. **Objective:** To evaluate and compare remineralisation potential of 38% SDF and Bioactive Varnish in enamel of primary teeth using stereomicroscope. **Methods:** Thirty naturally exfoliated primary molars with intact buccal surface, free from caries, developmental defects or cracks will be included. Teeth will be cleaned with 3% NaOCl solution, polished with non-fluoride containing paste, decoronated, embedded in self-cured acrylic resin mold with buccal surface facing upwards and window will be created. Specimens will be kept in demineralizing solution for 4 days to develop subsurface enamel lesion and sectioned for stereomicroscope. Specimens are allocated into 3 groups: group A (n=10)- 38% SDF, group B (n=10)- Bioactive Varnish and group C (n=10)- Untreated. All specimens are then subjected to pH cycle for 9 days, sectioned and visualized under stereomicroscope. Data will be analysed using SPSS version 21, Shapiro Wilk test is used to check normal distribution and level of statistical significance is set at $p < 0.05$. **Result:** Significant difference was seen in the remineralisation potential of control group, SDF group and bioactive varnish group **Conclusion:** Bioactive Varnish has higher remineralization potential as compared to 38% SDF

KEYWORDS : White spot lesion, SDF, Bioactive Varnish

INTRODUCTION:

Demineralization is the process of removing mineral ions from the hydroxyapatite crystals of hard tissue by a regular exposure of tooth enamel to acids produced by bacterial plaque. It occurs due to complex chemistry between bacteria, diet, and salivary components. A drop in the pH of oral cavity results in demineralization.¹

When the bacterial colony in the biofilm continues to produce acids with sugar consumption, pH falls to 4.5–5 from the normal pH (i.e 7) and the minerals are dissolved. This results in formation of pores between mineral structures. The most prevalent therapy for many years was the use of flouride, which can rebuild partially dissolved crystals, supplied in the form of topical gel or varnish that was applied to arrest active dental caries by depositing calcium fluoride that is not readily soluble and can act as a flouride reservoir to enhance enamel remineralization, increasing the speed of the remineralization process and the mineral content of early carious lesions. This therapy is cost effective and can be easily performed.²

White spot lesion (WSL) is considered the first sign of dental caries. It clinically appears as a chalky white opacity and is considered the earliest macroscopic evidence of enamel caries. WSLs are characterized by a porous subsurface enamel while the outermost enamel layer covering the lesion remains relatively intact and 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 control and prevention. Therefore, fluoride varnishes have been widely used to treat dentine hypersensitivity, prevent caries and remineralize early caries.⁴ This In-Vitro study is aimed to compare and evaluate remineralisation potential of 38% SDF and Bioactive Varnish in enamel of primary teeth using stereomicroscope.

Aim: To evaluate and compare the remineralisation potential of 38% Silver Diamine Fluoride and Bioactive Varnish in primary teeth using Stereomicroscope.

Objectives:

1. To evaluate the remineralisation potential of 38% Silver Diamine Fluoride.
2. To evaluate the remineralisation potential of Bioactive varnish.
3. To compare the remineralisation potential of 38% Silver Diamine Fluoride and Bioactive Varnish and in enamel of primary teeth using Stereomicroscope

Study Design And Location:

The study is an in-vitro comparative study carried out in the Department of Pediatric and Preventive Dentistry, Darshan Dental College and Hospital, Udaipur.

Source Of Data:

Thirty extracted human primary molars are collected from Department of Pediatric and Preventive Dentistry, Darshan Dental College and Hospital, Udaipur.

Materials:

Extracted human primary molars, 3% Sodium Hypochlorite, Normal saline, 38% SDF (e-SDF), FlouroDip Bioactive Varnish, Distilled water, Non-Flouridated Polishing paste, Self Cure Acrylic Resin, Acid resistant nail varnish, Acetone, Cotton swab, Adhesive paper, Artificial Saliva, 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)

Armamentarium: -

Scaler, Rubber cup for polishing, Double faced diamond disc, Tweezer, Microbrush, Syringe.

Equipment:-

Straight handpiece with micromotor, Contra angled handpiece with micromotor, Stereomicroscope.

Collection Of Samples And Groups:

A total of 30 samples will be collected for the study with samples per group:

Group A (n=10): 38% SDF (Kids-E-Dental, Mumbai, India)

Group B (n=10): FluoroDip Bioactive (Prevest DenPro, India)

Group C (n=10): Control Group

Inclusion Criteria-

Human primary molars freshly extracted for orthodontic reasons or naturally exfoliated and free from caries, white spot lesions, developmental defects and cracks with intact buccal surface are included in the study

Exclusion Criteria-

Extracted primary molars with Carious lesions, White spot lesions, Developmental defects, Cracks on buccal surface are excluded from the study.

Methodology:**Preparation Of Specimens:**

Thirty extracted human primary molars are utilized in the study. Extracted human primary molars (n= 40) are stored in 3% sodium hypochlorite solution and washed with distilled water before being used in the study. Specimens are cleaned and polished with non-fluoride containing polishing paste and are examined under a stereomicroscope to exclude teeth with developmental defects, cracks, or enamel demineralization in the buccal surface and stored in distilled water until use. The teeth are decoronated using a double-faced diamond disc, and the crown is embedded in self-cured acrylic resin mold with buccal surface facing upwards.

Creation Of Window:

4x4 mm² square adhesive tape is placed on the centre of buccal surface of each specimen. The entire specimen is then be coated with acid resistant nail varnish. Adhesive paper will is removed and 4x4 mm² window is created in all the specimens. The specimens are washed and immersed in distilled water at 37°C for 1 day.

Creation Of White Spot Lesion (ICDAS II):

Demineralization solution is made by mixing 2.20 mmol/L calcium chloride, 2.20 mmol/L monosodium phosphate, 1 mol/L potassium hydroxide and 0.05 mol/L acetic acid with distilled water having pH 4.4. Subsurface enamel lesion is 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 specimens are rinsed with 10 ml distilled water and dried with a stream of compressed air.



Fig 1:creation Of White Spot Lesion

Preparation of Specimen to view under Stereomicroscope (Before application of Remineralizing Agents)

Buccolingual sections are made to view specimen under stereomicroscope

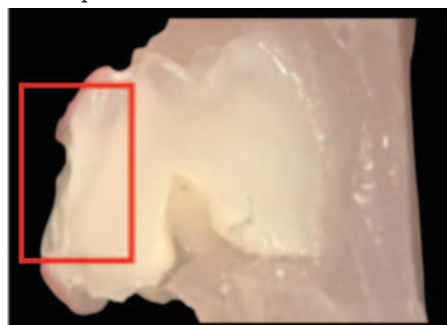


Fig 2: Stereomicroscopic Image of white spot lesion

The specimens are divided into three groups for the study.

Group A (n=10) : 38% SDF is applied to window and allowed to dry for 2 mins. Specimens are washed with 10 ml distilled water for 30 seconds, dried with compressed air and stored in artificial saliva for 24 h.

Group B (n=10) : Thin coat of Bioactive Varnish is applied to window and allowed it to dry for 5 mins. Varnish is carefully removed from all specimens using a cotton swab dipped in acetone to avoid harming the enamel surface. Specimens are placed in artificial saliva for 24hrs.

Group C (n=10): Specimens are left untreated and stored in artificial saliva for 24 hrs.

All Specimens: Are rinsed with distilled water for 1 min before being subjected to pH cycling.

Remineralization solution is made by mixing 1.5 mmol/L calcium chloride, 0.9 mmol/L monosodium- phosphate, 150 mmol/L potassium chloride with distilled water having pH 7.0

Ph Cycle:

Demineralization/Remineralization cycles consist of specimens immersion in 60 ml of demineralization solution for 4 hrs followed by immersion in 60 ml of remineralization solution for 20 hrs. All specimens are subjected to 9 days of pH cycling (8 days de/remineralization + 1-day remineralization). This cycle is repeated for 8 days consecutively, and new solutions are used daily. On 9th day, the specimens are immersed in the remineralization solution for 24 hrs.

Preparation of Specimen to view under Stereomicroscope (After application of Remineralizing Agents)

After pH cycling, specimens are washed with distilled water and buccolingual sections are made to view under Stereomicroscope.



A: SDF

B: Bioactive Varnish

C: Control

Fig 3: Stereomicroscopic Image of after application of remineralizing agents

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 non parametric test. For comparison of

continuous quantitative data among more than two groups, Kruskal Wallis test were used. For intragroup comparison, Wilcoxon sign rank test were used. Level of statistical significance was set at p-value less < 0.05.

RESULTS:

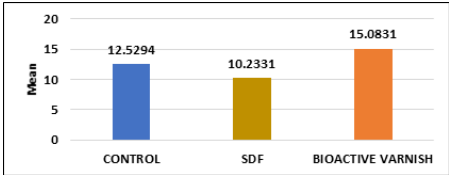
Significant difference was seen in the remineralisation potential of control group, sdf group and bioactive varnish as p<0.05.

Table 1: Inter-group comparison of remineralisation potential

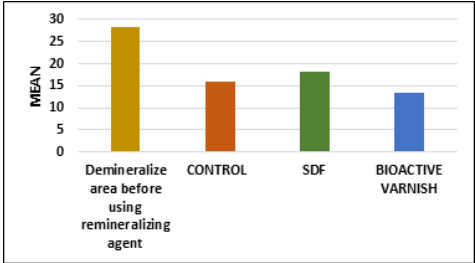
	N	Mean	Std. Deviation	Std. Error	95% Confidence Interval for Mean		Minimum	Maximum
					Lower Bound	Upper Bound		
CONTROL	10	12.5294	14.28543	3.96206	3.8968	21.1620	-6.79	37.33
SDF	10	10.2331	18.44404	5.11546	-.9125	21.3787	-25.00	44.26
BIOACTIVE VARNISH	10	15.0831	17.69763	4.90844	4.3885	25.7776	-14.00	46.00
P VALUE	0.045*							
Post hoc	Bioactive > Control > SDF							

Table 2: Intragroup comparison of remineralisation potential

		Mean	N	Std. Deviation	Std. Error Mean	P VALUE
CONTROL	Demineralize area before using remineralizing agent	28.39315	10	13.101584	3.633726	0.008*
	CONTROL	15.86377	10	5.827589	1.616282	
SDF	Demineralize area before using remineralizing agent	28.39315	10	13.101584	3.633726	0.049*
	SDF	18.16008	10	9.674570	2.683243	
BIOACTIVE VARNISH	Demineralize area before using remineralizing agent	28.39315	10	13.101584	3.633726	0.010*
	BIOACTIVE VARNISH	13.31008	10	7.146894	1.982192	



Graph 1: Remineralization potential of control, SDF and Bioactive varnish group



Graph 2: Comparison of demineralized area before and after use of remineralizing agents.

DISCUSSION:

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

The study carried out by Yu OY et al (2018) evaluated and compared the surface microhardness and showed the adjunctive function of ozone and SDF had a comparable remineralizing effect to SDF alone on carious enamel lesions.⁴The Systemic review by Rogalnikovaitė K et al (2023) stated that SDF increases the mineral density of hard dental tissues, enabling effective remineralisation of carious lesions in both enamel and dentine, which results in a measurable rise in microhardness.⁷ In vitro study conducted by Y. Mohamed and R. Ashraf (2023) evaluated SDF and Pchi using Vickers microhardness test, EDX and SEM and stated that SDF showed the prismatic pattern displayed thick lines of remineralization along the prismatic borders as well as Pchi revealed improvements of the enamel ultrastructure.²

Bioactive glass (BAG) is a remineralization agent developed by Larry Hench in 1969. BAG has appealing characteristics as a scaffold material for bone tissue engineering. It binds both soft and hard tissues and stimulates tissue mineralization owing to its superior bioactivity. It has been adopted for medical use, such as 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

In vitro study conducted by Hyun-Jung Kim , So-Yeon Mo and Duck-Su Kim (2021) evaluated light cure bioactive glass varnish as remineralizing agent using Microhardness tests, field emission scanning electron microscopy (FE-SEM) coupled with energy dispersive X-ray spectroscopy (EDS) and X-ray diffraction (XRD) analysis and concluded that BAG-containing varnish can help regain the microhardness of demineralized enamel and increase HAP crystal precipitation on the demineralized surface.⁶Another in vitro study conducted by Obeid AT,et al (2024) analysed fluoride varnish modified by nanostructures with the bioactive qualities of silica (SiO2) and niobium pentoxide (Nb2O5), using surface hardness and energy dispersive X-ray spectroscopy (EDX) showed that bioactive material has higher remineralizing potential due to increase in Ca/PO4 deposition in enamel.

A study by Morad YO et al (2024) included deciduous enamel specimens, experimented with Fluoride varnish and bioactive glass gel irradiated with gamma radiation, analysed using Energy Dispersive X-ray Analysis and SEM showed that fluoride content significantly increased after remineralizing agents application with the greatest increase in Bioactive glass group followed by fluoride varnish, which supports our study demonstrating high efficacy of Bioactive glass as a remineralizing agent in enamel of primary teeth.⁹In vitro study conducted by Gungor AS et al (2024) measured Lesion depths using stereomicroscope and measured using image analysis software (ImageJ) 58S5 BAG and EMD+PA (Enamel matrix derivative +Phosphoric Acid) have remineralization capacity as effective as fluoride varnish.¹⁰

Limitation:

Further evaluation of remineralizing potential of SDF and Bioactive glass Varnish for its enamel morphology and crystalline structure is needed. Our study being an in-vitro study, further research is needed in clinical trials to simulate the oral environment. Additionally, the compliance and

acceptance of these agents in children and parents along with long follow ups are needed to be evaluated.

CONCLUSION:

SDF showed the higher affinity towards the demineralized area of primary enamel compared to Bioactive varnish suggesting that SDF has potential effect of arresting caries rather than remineralisation. Interestingly, Bioactive Varnish has higher remineralization potential as compared to 38% SDF due to its bioactive components, higher fluoride concentration (22.6mg), potentially high Calcium and phosphate ion deposition and higher penetration into demineralized area, which makes it a suitable remineralisation agent to treat white spot lesions in primary enamel.

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