

RESEARCH PAPER

Intelligent Nano Polyvinylidene Fluoride impact On Initial Carious Lesions (An *in vitro* study)

Athraa Sarhan Awadh *, Maha Jamal Abbas, Hayder Hamed Abed

College of dentistry, Mustansiriyah University, Baghdad, Iraq

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ABSTRACT

The objective of this investigation was to evaluate the efficacy of nano polyvinylidene fluoride at three concentrations (1.26%, 2.26%, and 3.26%) in comparison to 2.26% sodium fluoride varnish, in enhancing enamel microhardness and surface morphology. Fifty-seven extracted permanent teeth were sampled. Vickers microhardness testing measured enamel hardness, while FE-SEM examined surface morphology after treatment with different materials. The statistical analysis involved repeated measures ANOVA to assess differences among the study groups, followed by pairwise post hoc tests to compare surface microhardness means and recovery percentages (SMHR%) along with their standard deviations. In terms of enamel microhardness enhancement, all evaluated Nano PVDF concentrations performed similarly to or better than NaF varnish. The 3.26% nano-PVDF concentration was the most effective, generating a dense, homogeneous crystalline layer as demonstrated by FE-SEM. Lower concentrations of nano-PVDF (1.26% and 2.26%) showed significant remineralization effects, similar to NaF. Nano-PVDF, especially at 3.26%, offers improved remineralization with lower toxicity than fluoride. At lower concentrations, nano-PVDF was equally effective as sodium fluoride varnish, suggesting it could be a safer and more effective preventive dental solution. Assessing its long-term clinical performance requires further research.

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INTRODUCTION

Dental caries is a diverse microbial illness affecting the mineralized dental tissues and is characterized by a progressive demineralization process [1]. White spot lesions are the initial visible signs of enamel demineralization (DEM) that occur within the outermost layer of the enamel. The structural integrity of the lesion remains intact without any localized deterioration. However, if left untreated, and with ongoing mineral loss, it might result in cavitation [2, 3]. Cavities in the teeth and the early extraction of

primary teeth cause malocclusion and space loss for the bicuspid and cuspid that erupt during tooth development [4, 5]. Additionally, dental decay is highly preventable and reversible when detected early, and the inhibition of biofilm formation and the presence of salivary protective factors can halt the demineralization of enamel and dentin [6]. Recently, caries research has shifted its focus towards developing methods for non-invasive treatment of early carious lesions by remineralization, with the goal of preserving tooth structure [7]. Indeed, remineralization can

* Corresponding Author Email: athraa.sarhan@uomustansiriyah.edu.iq



be either naturally occurring through the saliva buffering system or be biologically induced by remineralizing agents [8]. Calcium, the primary constituent of bones and teeth, is essential during enamel demineralization. Fluoride ions serve as the principal mechanism for preventing enamel demineralization in the presence of calcium and phosphate ions produced by plaque bacterial organic acids, as they promote the development of fluorapatite in enamel. Fluoride solutions exist in two concentrations: low concentrations for daily use, including toothpastes and mouthwashes, and higher concentrations for professional application, such as dental gels and varnishes [9] [6]. Based on preventive dentistry, topical fluoride may be a beneficial technique for the arresting of caries lesions, as fluoride has been shown to be effective in the prevention of dental caries in various forms [10]. Although fluoride-based remineralization technologies have dominated preventive dentistry for the past century, researchers are now exploring the potential of alternative materials to enhance the resistance of teeth to dental caries, either in conjunction with or as a replacement for fluoride [11]. Nanotechnology has recently become the primary driver behind numerous new devices with diverse applications in the fields of medicine, electronics, and energy production [12]. In recent decades, novel, advanced synthetic polymeric biomaterials have garnered attention due to their processability, morphology, surface chemistry, and wide range of potential applications in electronic devices and biomedical science (sensors, biosensors, membranes, etc.), among these materials, nano-polyvinylidene fluoride (PVDF) is a fluoropolymer that possesses a variety of properties that render it a useful biomaterial: insolubility, low processing temperature, chemical resistance, biological stability, and both in vitro and in vivo nontoxicity [13, 14]. PVDF fibers are employed in bone regeneration, and their surface potential is responsible for regulating specific biological functions, including the anchoring and proliferation of cells. Additionally, they stimulate the formation of collagen, which is crucial for future remineralization processes [15]. Due to the absence of studies on the impact of nano-polyvinylidene fluoride on the remineralization of tooth enamel, this laboratory investigation was conducted to enhance our understanding of the application of nano-polyvinylidene fluoride in preventing dental caries and remineralizing early-

stage carious lesions.

MATERIALS AND METHODS

Samples

The tooth samples used in this study consisted of 57 premolars that were taken from Iraqi patients who were seeking orthodontic treatment. They were obtained from private clinics located in Baghdad and Maysan. The teeth were cleaned of tissue and debris [16]. Any tooth exhibiting a noticeable fracture or crack was discarded [17]. Subsequently, they were placed in a plastic screw cup filled with deionized water. To prevent bacterial growth, thymol crystals (0.1%) were added to the water [18].

Sample preparation

To prepare the flat surface for Vickers microhardness testing, the buccal surface of each tooth was polished using Sof-Lex Disks (3M ESPE, USA) in a progressive manner. The polishing process started with the coarse disk, followed by the medium and fine disks, and ended with the superfine disk. A contra-angle slow-speed handpiece was used for polishing [19]. Then, the buccal and lingual surfaces of each tooth were covered with an acid-resistant nail varnish, leaving a circular opening of roughly six millimeters in diameter. The position of each window on the tooth surface was standardized using an orthodontic ruler. An imaginary line was drawn from the tip of the buccal cusps to the cervical line, and another line was drawn between the most apparent curvature of the mesial and distal surfaces. Thus, the central region of each surface was identified [20].

Caries-Like Lesion Induction in Enamel Specimens

The pH-cycling procedure was implemented in order to induce caries lesions on the surface of the enamel [21]. The demineralized and remineralized solutions were prepared by combining the following substances and adjusting the pH.

Demineralizing solution

This solution consisted of 0.075 M/L acetic acid, 1.0 M/L calcium chloride and 2.0 mM/L phosphate chloride. The pH was adjusted to 4.3 at 37 °C.

Remineralizing solution

This solution consisted of: 150 m M/L potassium

chloride, 0.9 m M/L potassium phosphate and 1.5 m M/L calcium nitrate. The pH was adjusted to 7 at 37 °C.

The Cycling Step

Each tooth was immersed in 20 ml of demineralizing solution and incubated at a temperature of 37 °C for a duration of 6 hours. Then, each tooth was withdrawn and washed for two minutes with running deionized water. Following that, individual tooth samples were submerged in 20 ml of remineralizing solution in an incubator set at 37 °C for a duration of 17 hours. The tooth samples were subsequently withdrawn and rinsed under running deionized water for a duration of two minutes, prior to the cycle being repeated. The aforementioned technique was conducted on a daily basis for a duration of ten consecutive days. The exterior enamel surface of each sample was analyzed under a polarized light microscope to identify any microscopic alterations.

Preparation of Nano PVDF mixture by different concentration

To prepare the 1.26% Nano-PVDF concentration, 0.315 grams of nano polyvinylidene fluoride (PVDF) powder (supplied by Nanochemazone, Canada) were carefully dissolved in 25 ml of triethyl citrate (Sigma-Aldrich, Germany) under continuous magnetic stirring at 80°C for two hours. Following that, 0.0945 grams of methyl cellulose (30%) were incorporated into the mixture and stirring was maintained for another two hours at the same temperature until a homogeneous and transparent solution was achieved. The same procedure was applied using 0.565 g PVDF with 0.169 g methyl cellulose and 0.815 g PVDF with 0.244 g methyl cellulose to obtain the 2.26% and 3.26% concentrations, respectively [22, 23].

Sample grouping and study design

The study design is shown in Fig. 1. The total sample of 57 sound teeth was divided as follows: two teeth were used for Field Emission Scanning Electron Microscope Examination (FESEM) of sound and demineralized enamel (after the creation of a white spot lesion), while the other 55 teeth were randomly assigned based on the type of treatment agent into five groups. Each group consisted of 11 teeth, one for the scanning electron microscope examination (FESEM), and the remaining teeth were used for the surface

microhardness test.

The groups will be allocated as follows:

Group A: untreated (demineralization followed by immersion in deionized

water, as the negative control group).

Group B: demineralization, followed by the application of 1.26% nano PVDF.

Group C: demineralization, followed by the application of 2.26% nano PVDF.

Group D: demineralization, followed by the application of 3.26% nano PVDF.

using a microbrush (application time: 4 minutes), the material was applied and the samples were stored in artificial saliva for 6 hours. Then, it was delicately removed from the enamel surface using cotton tips immersed in deionized water, without rubbing. To ensure complete removal of the agent, all specimens were examined under a stereomicroscope [24].

Group E: demineralization followed by the application of 2.26% sodium fluoride varnish. A thin layer of fluoride varnish was applied using a microbrush (application time: 4 min), left to act on the enamel surface for 6 hours in artificial saliva, and then delicately removed from the enamel surface using cotton tips immersed in deionized water without rubbing. To ensure complete removal of varnish, all specimens were examined under a stereomicroscope.

Surface microhardness testing

The microhardness of the material was determined using a Vickers hardness tester at the Department of Materials Engineering, University of Technology, on 50 teeth, ten for each treatment group: baseline (sound enamel surface), after demineralization, and then after remineralization. The measurement was conducted with a 500-gram load for a duration of 30 seconds. The mean hardness was determined by using three Vickers indentations per specimen [25, 26]. The Vickers microhardness test was conducted via an optical microscope employing a square-based diamond indenter with an included angle of 136° between the opposing faces. The magnification employed was 50 x. All the readings were conducted using the same calibrated equipment and inspector. The percentage of surface microhardness recovery (%SMHR) was used to calculate the extent of remineralization using the formula: $\%SHR = (SH2 - SH1)/(SH0 - SH1) \times 100$ where SH0 represents

baseline surface hardness, SH1 represents demineralization, and SH2 represents treatment results.

Scanning electron microscope

A total of seven teeth were employed for FESEM to investigate morphological alterations on the enamel surface. One representative tooth was selected from each of the five treatment groups, along with one tooth examined after demineralization and one sound, untreated tooth as a control. Field Emission Scanning Electron Microscopy (FESEM) was conducted

using a TESCAN MIRA III (Czech Republic) at a magnification of x1000, with an accelerating voltage of 15 kV. The aim was to observe surface morphology and detect any elemental changes without applying a conductive coating. Before imaging, the enamel specimens were disinfected using acetone and dehydrated through a graded ethanol series (70%, 80%, 90%, and 100%), each for 20 minutes. The samples were then left to air-dry at room temperature for 24 hours [27].

Statistical analysis

The data analyzed using Statistical Package

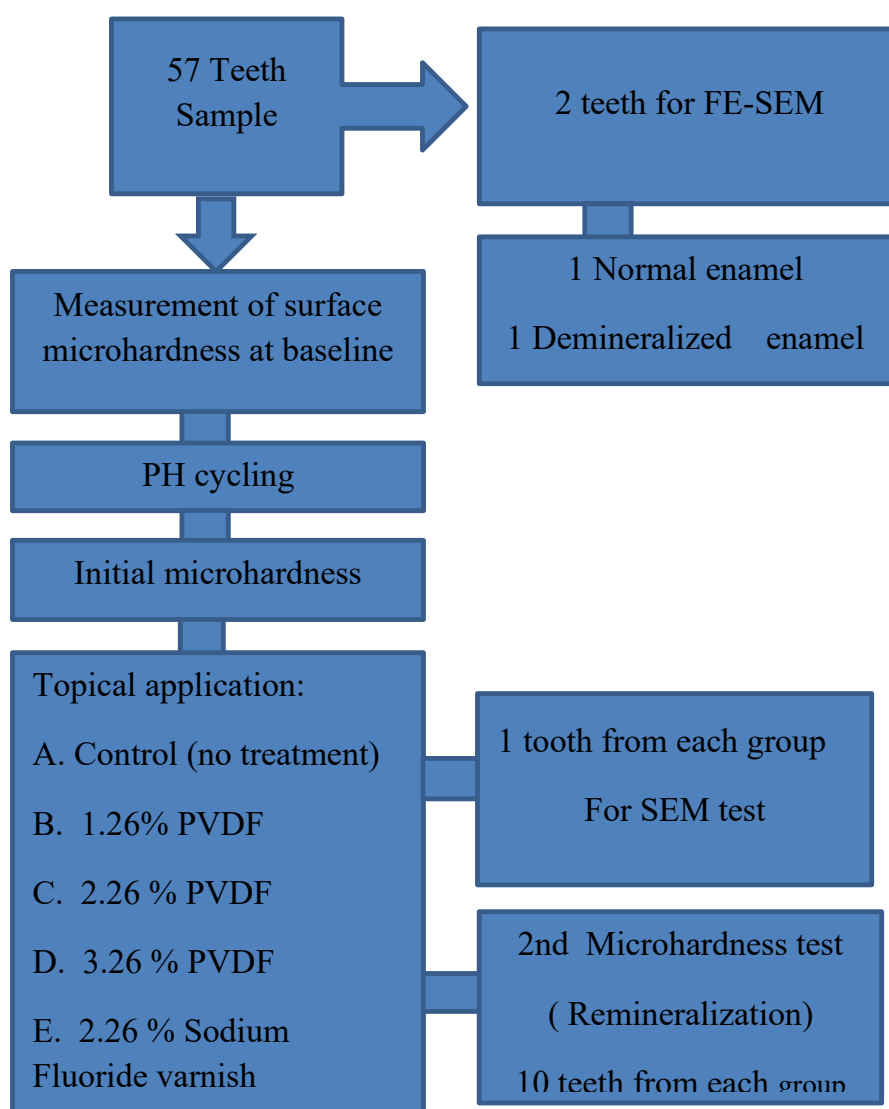


Fig. 1. Illustration of the study design

for Social Sciences (SPSS) version 28. The data presented as: 1) Descriptive Analysis: Mean, standard deviation of quantitative variables. 2) Inferential analysis: A) Shapiro-wilk test: to test the normality distribution of quantitative variables. B) Levene's test: test the equality of variances among groups. C) Repeated measure one way ANOVA test: to detect any overall differences between K related means (Baseline T0, Demineralization T1 and Remineralization T2). D) One Way ANOVA test: to test the hypothesis for a quantitative dependent variable by an independent variable followed by post hoc test (Games-Howell). A level of p-value less than 0.05 was considered significant.

RESULTS AND DISCUSSION

Enamel Microhardness

The microhardness means of the study groups are presented in Table 1 and Fig. 2. The data indicate that the microhardness values were highest at the baseline. Following pH cycling, there was a significant decrease in these values. Subsequently, the values exhibited an increase, with the exception of the negative control group, which did not show an upward trend.

The Normality Distribution Test of Microhardness

The normality distribution test (Shapiro-Wilk) results showed that the values of all phases were

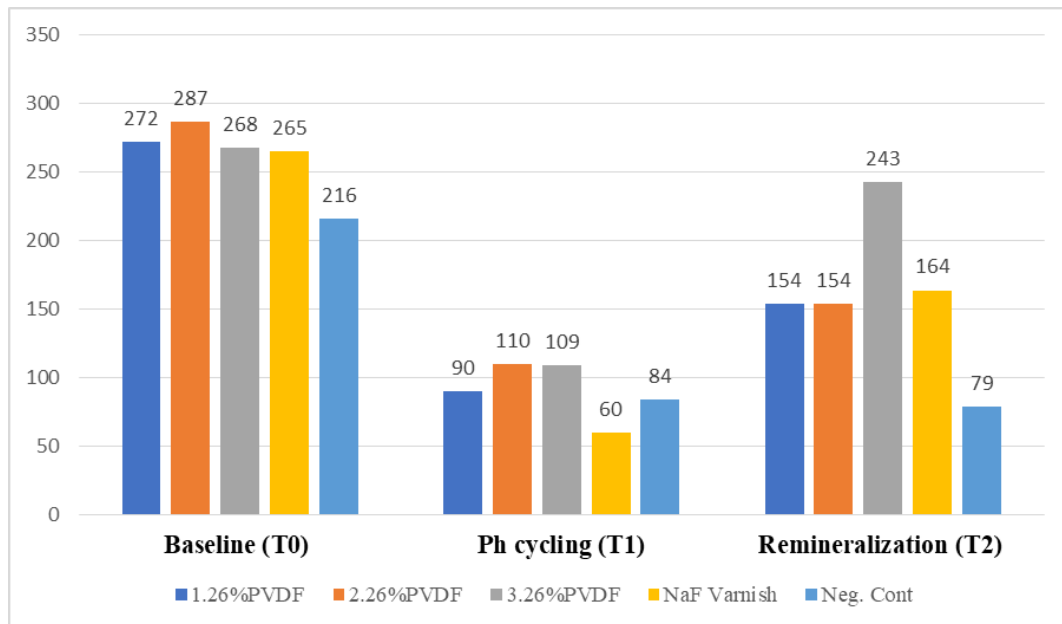


Fig. 2. The microhardness means of all groups in different phases

Table 1. The microhardness means and standard deviations of all groups in different phases

Groups	N	Baseline (T0)		Ph- cycling (T1)		Treatment (T2)	
		Mean	SD	Mean	SD	Mean	SD
1.26% PVDF	10	271.8	49	89.7	35	153.5	23
2.26% PVDF	10	286.7	31	110.4	51	154.2	35
3.26% PVDF	10	268.7	20	109	18	243.8	23
NaF Varnish	10	265	16	59.6	35	164.6	37
Negative Control	10	216.4	26	83.7	36	79.2	30

normally distributed (Table 2).

The Statistical Test for Comparisons of Phases in Each Group

The Repeated Measures ANOVA test showed a significant difference between the phases of all groups ($P = < 0.001$) Table 3.

Descriptive Measures of SMHR% Among Groups

The Surface Microhardness Recovery Percentage [SMHR% = $100 * ((T2 - T1) / (T0 - T1))$] of the study groups is presented in Table 4 and Fig. 3. The data demonstrate that the 3.26% nano PVDF

group exhibited the highest SMHR%, whereas the negative control group had the lowest.

The normality distribution test of SMHR%

The normality distribution test (Shapiro Wilk test) results showed that all of the surface microhardness recovery percentage SMHR% were normally distributed (Table 5).

The Statistical Test for Comparing SMHR% Between Groups

The Welsh test demonstrates a significant difference among the study groups ($p < 0.001$)

Table 2. Normality distribution test of the surface microhardness according to different phases.

Phases	Groups	Shapiro Wilk		
		Statistic	Df	P value
Baseline	1.26% PVDF	0.9	10	0.8
	2.26% PVDF	0.9	10	0.8
	3.26% PVDF	0.9	10	0.9
	NaF Varnish	0.8	10	0.6
	Neg. Cont.	0.9	10	0.06
PH- cycling	1.26% PVDF	0.9	10	0.6
	2.26% PVDF	0.9	10	0.5
	3.26% PVDF	0.9	10	0.5
	NaF Varnish	0.8	10	0.01
	Neg. Cont.	0.9	10	0.1
Remineralization	1.26% PVDF	0.9	10	0.3
	2.26% PVDF	0.6	10	0.4
	3.26% PVDF	0.9	10	0.9
	NaF Varnish	0.9	10	0.07
	Neg. Cont.	0.8	10	0.3

Table 3. The Repeated Measures ANOVA test of microhardness for the study groups

Repeated Measures ANOVA								
Phases								
Groups	N	Baseline (T0)		Ph cycling (T1)		Treatment (T2)		P-value
		Mean	SD	Mean	SD	Mean	SD	
1.26% PVDF	10	271.8	49	89.7	35	153.5	23	< 0.001
2.26% PVDF	10	286.7	31	110.4	51	154.2	35	< 0.001
3.26% PVDF	10	268.7	20	109	18	243.8	23	< 0.001
NaF Varnish	10	265	16	59.6	35	164.6	37	< 0.001
Negative Control	10	216.4	26	83.7	36	79.2	30	< 0.001

Table 4. The Surface Microhardness Recovery Percentage SMHR of the study groups

SMHR: Surface Microhardness Recovery Percentage			
Groups	N	Mean	SD
1.26% PVDF	10	34.6	14
2.26% PVDF	10	23.9	10
3.26% PVDF	10	81.9	26
NaF Varnish	10	48	27
Negative Control	10	-4	2

according to their SMHR% as shown in Table 6.

Pairwise post-hoc testing (Games-Howell) shows a significant difference only between the following groups: (1.26% PVDF and 3.26% PVDF), (1.26% PVDF and Cont. Neg), (2.26% PVDF and 3.26% PVDF), (2.26% PVDF and Cont-Neg), (3.26% PVDF and Cont-Neg), and (NaF Varnish and Cont-Neg) groups. These results are detailed in Table 7.

Microscopic features of the outer enamel surface using Field Emission Scanning Electron Microscope (FESEM)

The goal of the FE-SEM investigation was to assess the topographic surface alterations of one representative sample from each study group in addition to one sound tooth and one tooth after demineralization. Figs. 4 – 10 show the FE-SEM

patterns of the groups.

Sound enamel surface

Fig. 4 shows the surface of sound enamel, where it can be observed that the surface has a homogenous smooth appearance with no visible irregularities. This FE-SEM image reflects the crystalline structural nature of the enamel.

Demineralized enamel surface

Fig. 5 shows the enamel surface after the demineralization process, where minerals are lost from the enamel surface, leading to changes in its microscopic structure. The demineralization process led to the exposure of enamel crystallites, which were visible on the surface, showing pitting and crystal dissolution. The demineralized surface

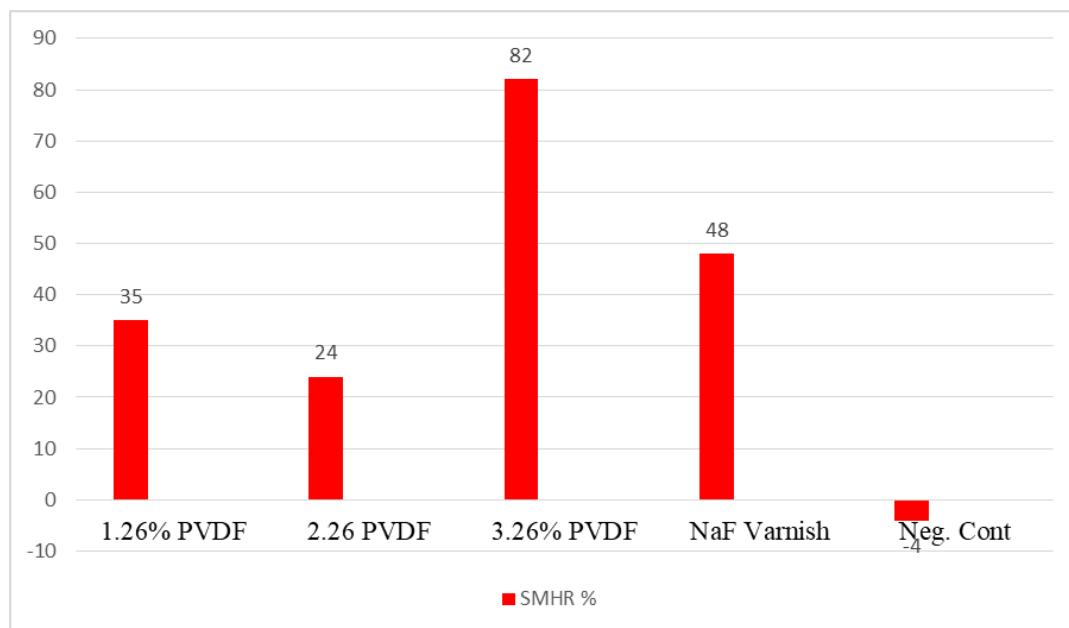


Fig. 3. The Surface Microhardness Recovery Percentage SMHR of the study groups

Table 5. Normality distribution test of the surface microhardness recovery percentage

	Groups	Shapiro Wilk		
		Statistic	Df	P value
SMHR%	1.26% PVDF	0.9	10	0.7
	2.26% PVDF	0.8	10	0.1
	3.26% PVDF	0.8	10	0.1
	NaF Varnish	0.1	10	0.3
	Neg. Cont.	0.7	10	0.08

was rough and eroded, with visible areas of pits and voids due to mineral loss.

Negative Control group

Fig. 6 refers to the tooth surface in the negative

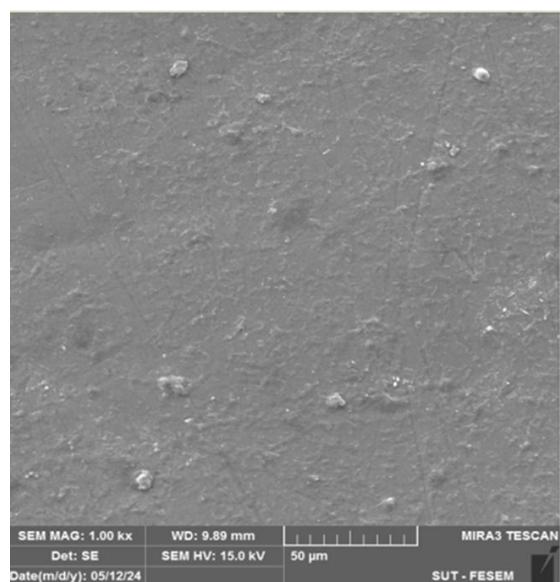


Fig. 4. FESEM image of sound enamel surface (x1000 magnification)

Table 6. The comparison of the SMHR% (mean $\pm$ SD) between all the study groups

SMHR: Surface Microhardness Recovery Percentage						
Groups	N	Mean (SD)	SD	Statistics	df	*P-Value
1.26% PVDF	10	34.6	14	28.5	16.7	< 0.001
2.26% PVDF	10	23.9	10			
3.26% PVDF	10	81.9	26			
NaF Varnish	10	48	27			
Co2nt. Neg.	10	-4	2			

*By Welsh test

Table 7. Pairwise post-hoc comparisons of SMHR% of all groups

Post hoc Pairwise Comparisons (Games-Howell)				
(I) Group	(J) Group	Mean difference (I-J)	Std. Error	P-Value
1.26% PVDF	2.26% PVDF	10.7	6.2	0.4
	3.26% PVDF	-47.3	10.6	0.007
	NaF Varnish	-13	11	0.7
	Cont. Neg.	39	5.4	< 0.001
2.26% PVDF	3.26% PVDF	-58.0	10.0	0.002
	NaF Varnish	-24	10.4	0.2
	Cont. Neg.	28.1	4.1	< 0.001
3.26% PVDF	NaF Varnish	33.9	13.5	0.1
	Cont. Neg.	86.2	9.5	< 0.001
NaF Varnish	Cont. Neg.	52.2	9.9	< 0.001

control group, where the tooth was stored in deionized water and was not treated with any remineralizing agent. Numerous micro-pores and cracks were visible on the enamel surface.

Sodium Fluoride Varnish Group

Fig. 7 shows the enamel surface treated with sodium fluoride after demineralization. The porosities were coated with NaF varnish; however,

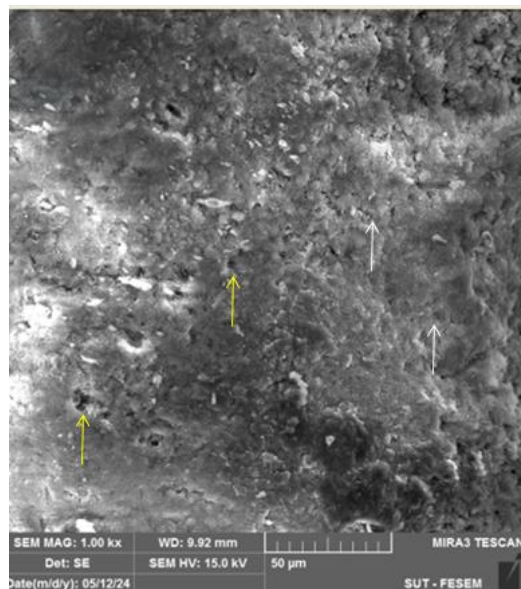


Fig. 5. FESEM image of demineralized enamel surface, (x1000 magnification), yellow arrows show voids, white arrows show pits

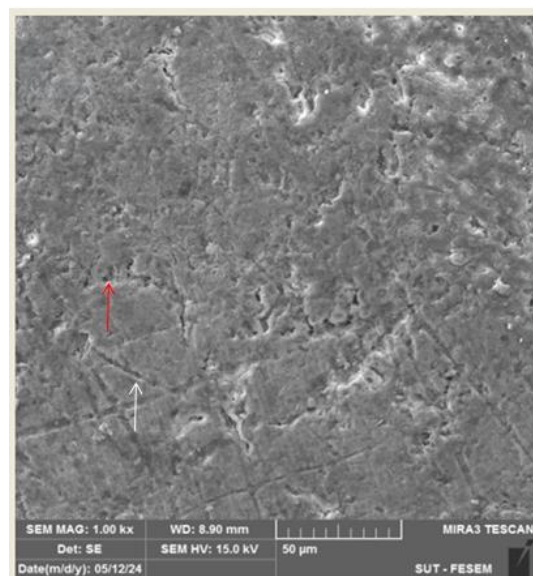


Fig. 6. FESEM image of enamel surface of negative control group, (x1000 magnification), red arrow shows pore and white arrow shows crack

the underlying pattern was still visible in many areas. The morphological surface appeared smooth, interlaced with some grooves and micro porosities, as the NaF coating seemed to have filled some of the voids and smoothed parts of the

enamel surface.

1.26% Nano PVDF Group

Fig. 8 refers to the enamel surface after treatment with 1.26% nano PVDF. It can be

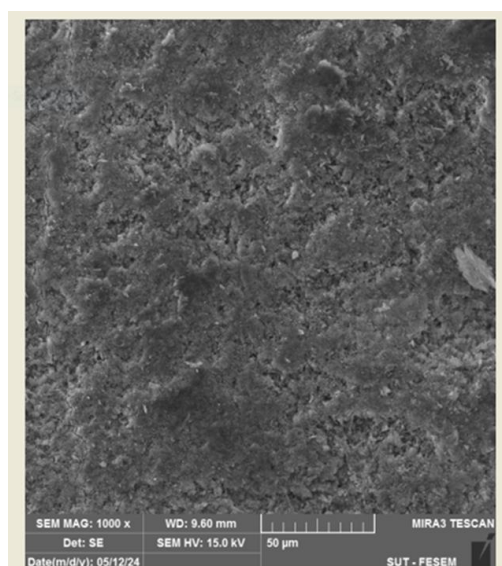


Fig. 7. FESEM image of treated enamel surface with NaF Group (x1000 magnification)

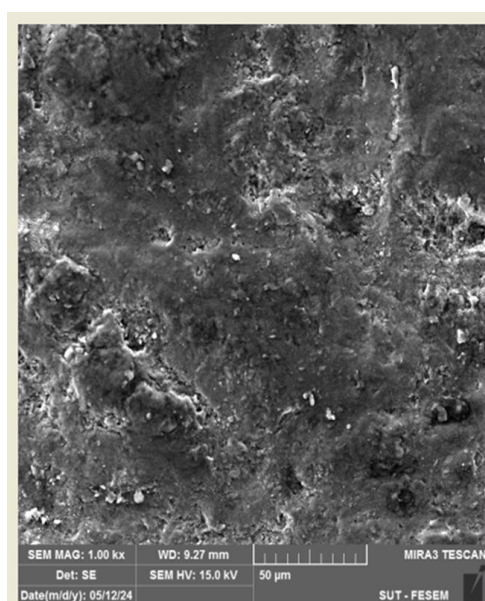


Fig. 8. FESEM image of enamel surface treated with 1.26% nano PVDF group

observed that some pores and cracks have been partially closed, but not entirely. The surface shows a regular structure and less roughness, with a reduction in cracks and porosity. There are some areas that may indicate the beginning of crystal formation.

2.26% Nano PVDF Group

Fig. 9 shows the enamel surface after treatment with 2.26% nano PVDF. The enamel surface is covered with relatively smooth and more homogeneous crystalline structure. Additionally, the underlying demineralized pattern was no longer visible.

3.26% Nano PVDF Group

Fig. 10 shows the enamel surface treated with 3.26% nano PVDF, displaying the formation of a dense crystalline layer on the surface. The enamel surface was entirely covered with a granular-shaped hydroxyapatite deposit.

The current work used Vickers microhardness testing and field emission scanning electron microscopy to explore the effect of three different concentrations of Nano PVDF (1.26%, 2.26%, and 3.26%) on enamel microhardness and surface morphology. These findings were compared to a commonly used varnish containing 2.26% sodium fluoride (NaF). The results show that Nano PVDF

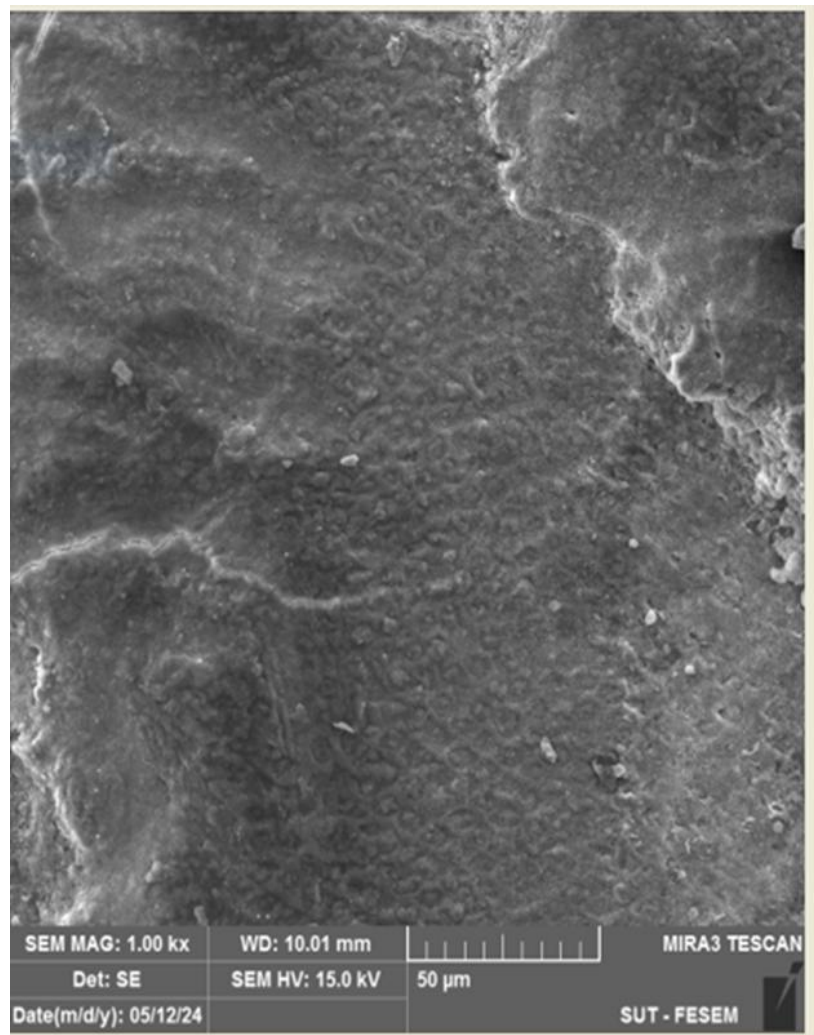


Fig. 9. FESEM image of enamel Surface treated with 2.26% nano PVDF

has substantial potential as a remineralizing agent, especially at the 3.26% concentration. The Vickers microhardness test indicated that the application of Nano PVDF at a concentration of 3.26% resulted in the most significant enhancement in enamel hardness compared to all other tested groups. This indicates a higher remineralization capacity, attributable to the formation of a dense crystalline layer on the enamel surface. This finding is consistent with earlier research highlighting the role of polymer-based nanoparticles in improving enamel resistance via mineral deposition and surface modification [28, 29]. The FESEM analysis confirmed these findings by demonstrating substantial morphological alterations. The 3.26%

Nano PVDF group demonstrated a uniform, dense, and well-defined crystalline structure on the enamel surface, suggesting effective interaction with hydroxyapatite crystals. The lower concentrations of Nano PVDF (1.26% and 2.26%) exhibited minimal surface changes, whereas the NaF group presented distinct globular deposits characteristic of fluoride treatments. These observations indicate that whereas NaF is an excellent remineralizing agent, Nano PVDF, especially at 3.26%, offers a comparable and potentially superior surface effect [30]. A primary advantage of Nano PVDF compared to NaF varnish is its polymeric composition and nanostructure. The polymer matrix enhances adherence to the

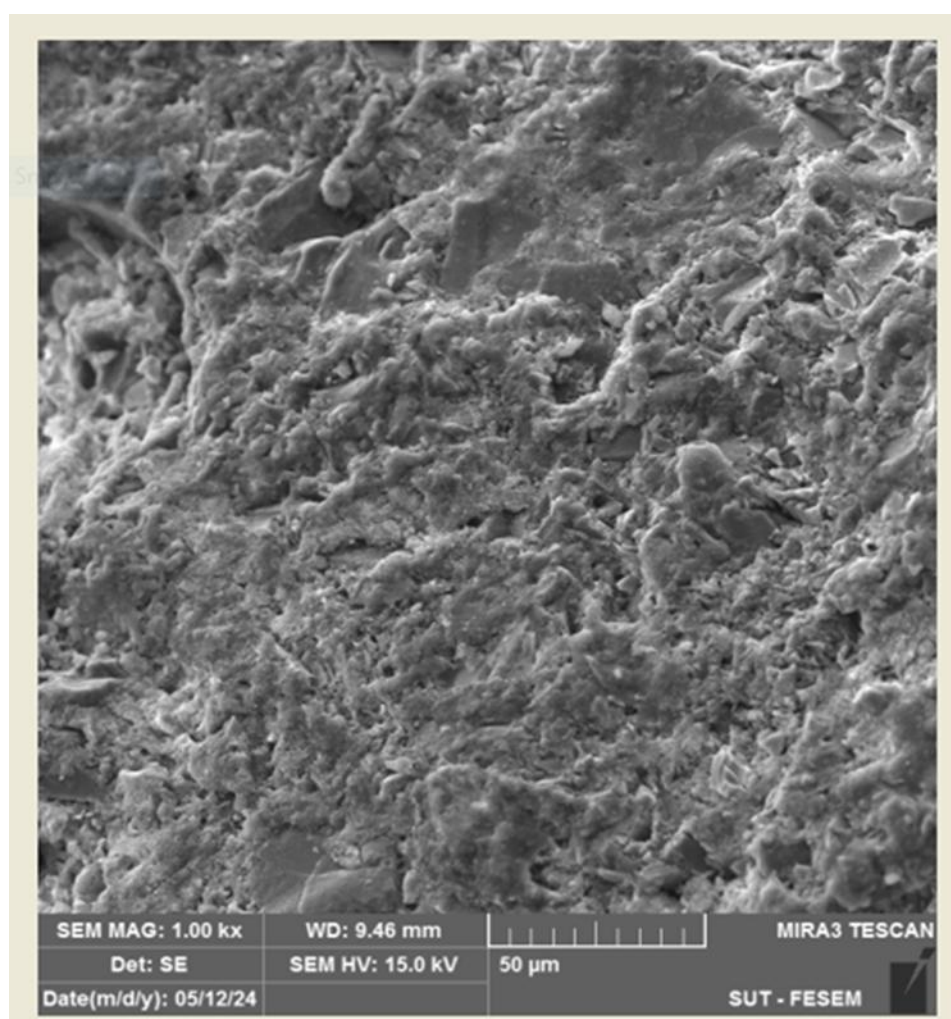


Fig. 10. FESEM image of enamel surface treated with 3.26% nano PVDF (x1000 magnification)

enamel surface, thereby improving retention and ensuring extended exposure to active components. Furthermore, the nanoscale dimensions improve surface infiltration, facilitating deeper interaction with enamel imperfections. These characteristics underscore the promise of Nano PVDF as a substitute for conventional fluoride-based therapies, mitigating apprehensions regarding fluoride toxicity [31]. According to the experimental findings of this study, the 2.26% Nano PVDF exhibited competitive remineralizing efficiency compared to the 2.26% NaF varnish, despite its relatively lower performance than the 3.26% concentration. This indicates a concentration-dependent effect, where the remineralization potential of Nano PVDF increases with concentration, with a threshold concentration required for optimal results. The findings of this study are significant for enamel remineralization therapy. Due to increasing apprehensions over fluoride toxicity, especially in children and prolonged therapies [32], nano PVDF presents a safer alternative with similar effectiveness. Further research is recommended to examine the long-term stability of the crystalline layer formed by Nano PVDF and its prospective use in preventive dentistry.

CONCLUSION

This study found that Nano PVDF, particularly at a 3.26% concentration, outperformed lesser concentrations (1.26% and 2.26%) and 2.26% NaF varnish in terms of enamel microhardness and the formation of a dense crystalline layer, as evidenced by Vickers microhardness tests and FESEM analysis. The results indicate that Nano PVDF is a promising alternative to fluoride-based therapies, providing equivalent or greater remineralization while posing fewer toxicity issues. Its polymeric structure and nanoscale features enhance its efficacy and its promise for long-term use in preventive dentistry. Further research is encouraged to assess its durability and therapeutic effectiveness.

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CONFLICT OF INTEREST

The authors declare that there is no conflict

of interests regarding the publication of this manuscript.

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