Heliyon 10 (2024) e35224

Contents lists available at ScienceDirect

Heliyon

journal homepage: www.cell.com/heliyon

Research article

Evaluation of the cumulative effect of photodynamic therapy and local fluoride on the microhardness and topography of demineralized enamel and cementum surfaces

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ARTICLE INFO

Keywords:
Caries
Fluoride varnish
Microhardness
Photodynamic therapy
Silver diamine fluoride

ABSTRACT

Background: The present study aimed to determine the cumulative effect of two photodynamic therapy methods with methylene blue and indocyanine green and two topical fluoride therapy methods with fluoride varnish and silver diamine fluoride alone and in combination on the microhardness and topography of demineralized enamel and cementum surfaces.

Materials and methods: Seventy-two sound human teeth were selected, and their buccal and lingual surfaces were assigned to two main groups of enamel and cementum using simple randomization. The initial surface hardness (SH) of the enamel and cementum in each sample was determined using a micro-Vickers hardness tester using a 200-g force in 10 s. Then artificial caries was induced by immersion in a demineralizing/remineralizing solution (i.e., each tooth provided two samples, one on the buccal aspect and the other on the lingual aspect). Each enamel/cementum main group was divided into two subgroups using simple randomization based on the local fluoride type (fluoride varnish and silver diamine fluoride) and the type of the photosensitizer agent (methylene blue and indocyanine green). Finally, 16 groups were achieved ($n = 9$). The final surface hardness of the enamel and cementum samples was determined as described above. Finally, the sample surfaces were prepared for the surface topography evaluation under a scanning electron microscope. The baseline microhardness was compared between the 16 study groups in the first step using one-way ANOVA. Then, three-way ANOVA was used to evaluate the effect of fluoride, laser, and surface (enamel and cementum) on microhardness.

Results: All the groups exhibited decreased microhardness due to the induction of artificial caries. In both main groups of enamel and cementum, the lowest decrease in microhardness was recorded with combined photodynamic therapy and methylene blue photosensitizer material and fluoride varnish (15.1 % for cementum and 16.7 % for enamel), and the highest decrease in microhardness was recorded in the methylene blue group (35.7 % for cementum and 34.9 % for enamel).

Conclusion: The combination of photodynamic therapy with the photosensitizer substance methylene blue or indocyanine green together with fluoride varnish or silver diamine fluoride is

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https://doi.org/10.1016/j.heliyon.2024.e35224
Received 30 December 2023; Received in revised form 16 July 2024; Accepted 24 July 2024
Available online 26 July 2024

effective on the remineralization of demineralized enamel and cementum. Although there is no difference between the combination of photodynamic therapy with fluoride varnish compared to fluoride varnish alone, both of these treatments are more effective than using photodynamic therapy alone.

1. Introduction

Dental caries is a progressive, local, and prevalent bacterial disease of the oral cavity with a multifactorial etiology, including cariogenic bacteria [1]. These microorganisms produce acids through a glycolytic pathway, demineralizing the tooth enamel and dentin [2]. Controlling biofilms and equilibrium between demineralization and remineralization processes are two methods to prevent recurrent caries [3]. Fluoride is naturally found in many water sources and is widely used as an anticaries agent [4]. Topical fluoride therapy is a nonsurgical approach to treat dental caries in its early stages [3], and its use as varnishes, mouthwashes, restorative materials, and in combination with silver has shown promise in preventing, inhibiting, and arresting dental caries [1]. In addition, the evidence from previous studies has shown promise regarding the disinfecting and anticaries effects of silver diamine [5]. The only reported disadvantage of silver diamine is the staining of restorative materials due to the silver content of this material, and its poor adhesion to different restorative materials disrupts the integrity of the bond [1]. However, success in using fluoride depends on the early diagnosis of caries, controlling the intake of carbohydrates, and dentists' knowledge of the use of different forms of fluoride [3]. Fluoride prevents demineralization by forming fluorapatite crystals which are more resistant to aid attacks than hydroxyapatite crystals. In addition, fluoride increases the formation and growth of new fluorapatite crystals, inhibits acid production by cariogenic bacteria, and is considered the gold standard in regenerating the tooth structure (remineralization) [6]. Eliminating biofilms decreases the caries rate, and several methods are available to remove dental biofilms, including mechanical removal and using disinfectants and chemical agents [7]. However, attempts to find treatment modalities that prevent biofilm formation have led to considerable research to discover new treatment options, such as photodynamic therapy that can eliminate biofilms, with more widespread use in cariology to control dental caries [8]. Photodynamic therapy is a non-invasive, repeatable, and low-cost method that can easily be applied without pain; in addition, it does not affect the patient's sense of taste [5]. In this method, a photosensitizer material is activated by a special light at a specific wavelength for maximum absorption by the material, resulting in the production of free radicals, singlet oxygen, and other reactive oxygen species with destructive effects on bacterial cells without damaging the host, finally leading to bacterial cell death.

This conservative method is effective against resistant bacteria and rapidly affects the target organisms [2]. This treatment modality is an efficacious tool for treating various diseases and is an alternative to mechanical and pharmaceutical treatments. It can destroy bacterial plaque on teeth and be a treatment option for dental caries and biofilm-induced periodontitis. Data from recent studies indicate that photodynamic therapy is a potential treatment option to prevent the formation of cariogenic biofilms and treat dental caries [7,9,10]. Two photosensitizer materials, methylene blue and indocyanine, are used in this study. Methylene blue is a cationic phenothiazine dye with a low molecular weight and maximum light absorption at 660 nm. It is effective against gram-positive and gram-negative microorganisms [11].

Indocyanine green is the only material sensitive to light with a near-infra-red wavelength approved for clinical applications by the FDA. It absorbs light at 75–900 nm wavelength. It is an anionic tricarbocyanine dye with both hydrophilic and hydrophobic sections. It has low toxicity because it is easily metabolized by the liver and is not absorbed by the intestinal mucosa. Given these favorable immune properties, it is currently used in medicine and dentistry. Since the photodynamic reactions with indocyanine can proceeed without oxygen [12], research in dentistry to use indocyanine green has mainly focused on controlling periodontal and root canal infection by anaerobic bacteria and has proved effective [13]. The local use of fluoride before or after laser irradiation increases fluoride absorption and decreases solubility in acidic solutions [14]. Studies have shown that increasing the fluoride concentration will increase the resistance of dental tissues to acids; however, the amount of absorbed fluoride is low and limited to the surface layer [15]. Adequate absorption of fluoride is necessary to prevent dental caries, re-strengthen the tooth enamel, and prevent the demineralization of the enamel and root surfaces. However, excessive exposure to fluoride might lead to skeletal fluorosis and renal problems. Therefore, it is vital to provide accurate tools to monitor fluoride [4]. Applying photodynamic therapy and the laser beams used in this method can increase the absorption of fluoride, affecting the tooth's hard structures, increasing fluoride absorption into tooth pits and fissures, and depositing it in the enamel and dentin crystal structure. In addition, this affects the dental hard structures and their topography [15].

Studies have shown that laser irradiation might lead to the melting and re-crystalization of enamel, dentin, and root surface, increasing fluoride absorption into the enamel. If the root surface is irradiated, it can more easily react with fluoride. All these changes increase resistance to acids in these tissues, which might be an effective method to prevent enamel and root surface caries. Laser irradiation with or without fluoride can cause changes on enamel and root surfaces, including cracks, cavities, craters, porosities, and surface irregularities that can be observed in the topography of enamel and cementum surfaces, indicating the importance of evaluating surface topography [16]. However, no specific study is available on structural changes (topography) of the enamel and cementum after fluoride therapy in association with photodynamic therapy, with no data on the extent of changes in hardness after these treatments. Therefore, the present study evaluated the effects of two different types of photodynamic therapy and two fluoride therapy modalities alone or in combination on the microhardness and topography of demineralized enamel and cementum surfaces.

2. Materials and methods

2.1. Samples selection

The protocol of the present in vitro study was approved by the Ethics Committee of the Faculty of Dentistry, Tehran University of Medical Sciences (IR.TUMS.DENTISTRY.REC.1400.067). Seventy-two sound human teeth extracted for therapeutic reasons were selected. Teeth with cracks, caries, hypomineralization, and hypoplasia were excluded. To decrease the number of required teeth, the buccal and lingual surfaces of each tooth were prepared as samples.

2.2. Sample preparation

The teeth were disinfected in an 0.5 % chloramine solution for 24 h after removing all the calculi and soft tissue remnants, followed by storage in distilled water at 4 °C for two weeks. The buccal and lingual surfaces of the teeth were assigned to two principal enamel and cementum groups using the simple randomization method and mounted in self-cured acrylic resin. A 5 × 5-mm area was demarcated on the mounted samples (below the CEJ in the cementum group and above the CEJ in the enamel group).

2.3. Recording the initial microhardness

The samples' initial surface hardness was determined and reported after being mounted in acrylic resin before demineralization and remineralization on both the enamel and cementum surfaces by a micro-Vickers hardness tester, with a 200-gr force in 10 s, using the formula below:

$$VH = 2F\sin(136.2^{\circ})/d^2 = 1.854\ F/d$$

where VH is the Vickers hardness value, F is the applied force in kg, and d is the diameter of the square-shaped dent in mm.

2.4. pH-cycling to induce incipient caries

The caries induction processes were different on the enamel and cementum surfaces. The enamel samples were immersed in a demineralizing solution [30 % acetic acid, 2.2 mM $CaCl_2$, 2.2 mM $NaH(PO_2)_4$] for 6 h (30 mL for each sample) at a pH of 4.8). Then they were immersed in a remineralizing solution (0.9 mM potassium dihydrogen phosphate, 130 mM potassium chloride, 1.5 mM calcium chloride, and 20 mM HEPES solution) at a pH of 7 for 18 h. Finally, the samples were rinsed with deionized water for 5 s. The solutions were changed at the end of each cycle. This process was repeated for 6 days [17]. The cementum samples were immersed in 1000 mL of a demineralizing solution (0.05 M acetic acid, 2.2 mM calcium, and 2.2 mM phosphate ion, pH = 4.8) at 37 °C for 18 h, followed by rinsing in deionized water for 5 s. Then the samples were immersed in a remineralizing solution (1.5 M potassium chloride, 1.5 mM calcium, and 0.9 mM phosphate ion solutions, pH = 7) at 37 °C for 6 h. Finally, the samples were rinsed with deionized water. The solutions were changed at the end of each cycle. This procedure was repeated for 2 days [18].

2.5. Study groups

The samples were randomly assigned to 16 groups based on the type of local fluoride, the type of the photosensitizer material, and the enamel or cementum sample (n = 9:1): 1) silver diamine fluoride on the enamel surface, 2) silver diamine fluoride on the cementum surface, 3) fluoride varnish on the enamel surface, 4) fluoride varnish on the cementum surface, 5) silver diamine fluoride + photodynamic therapy with methylene blue at a concentration of 100 μg/mL on the enamel surface, 6) silver diamine fluoride + photodynamic therapy with methylene blue at a concentration of 100 μg/mL on the cementum surface, 7) silver diamine fluoride + photodynamic therapy with indocyanine green at a concentration of 1000 μg/mL on the enamel surface, 8) silver diamine fluoride + photodynamic therapy with indocyanine green at a concentration of 100 μg/mL on the cementum surface, 9) fluoride varnish + photodynamic therapy with methylene blue at a concentration of 100 μg/mL on the enamel surface, 10) fluoride varnish + photodynamic therapy with methylene blue at a concentration of 100 μg/mL on the cementum surface, 11) fluoride varnish + photodynamic therapy with indocyanine green at a concentration of 1000 μg/mL on the enamel surface, 12) fluoride varnish + photodynamic therapy with indocyanine green at a concentration of 1000 μg/mL on the cementum surface, 13) photodynamic therapy with methylene blue at a concentration of 100 μg/mL on the enamel surface, 14) photodynamic therapy with methylene blue at a concentration of 100 μg/mL on the cementum surface, 15) photodynamic therapy with indocyanine green at a concentration of 1000 μg/mL on the enamel surface, 16) photodynamic therapy with indocyanine green at a concentration of 1000 μg/mL on the cementum surface.

2.6. Photodynamic therapy and fluoride therapy

The samples were applied with fluoride varnish and silver diamine fluoride according to the test groups and according to the factory instructions by microbrush on the desired surface, then the photosentisizer substance methylene blue with a concentration of 100 (μg)/ml by a 30-gauge syringe on the enamel surface and Demineralized cementum was placed for 5 min and irradiated by a 660 nm red diode laser with a power of 150 mW for 1 min.The photosensitizer indocyanine green material (1000 μg/mL) was placed on the demineralized enamel and cementum surfaces with a 30-g syringe for 5 min and irradiated with 250-mW 880-nm laser for 1 min. Finally, the samples were brushed with fluoride-containing toothpaste for 7 min once. Then they were rinsed with distilled water and stored in 0.9 % saline solution for 24 h.

2.7. Determining the final microhardness

The final surface hardness of the enamel and centum surfaces was determined after photodynamic therapy and fluoride therapy by a micro-Vickers hardness tester, similar to that before treatment.

2.8. Evaluation of the surface topography

Three samples from each enamel and cementum group after treatments and one sample as a base from enamel and cementum before any treatment and immersion in the demineralizing/remineralizing solutions were prepared for surface topography evaluations under a scanning electron microscope (SEM) at 3000*5*0*100 magnification(Figures [1(a-i), 2(a-i)]). This procedure involved placing the samples in a vacuum for 200 s, followed by gold-sputtering, after which the samples were ready to be placed in the vacuum

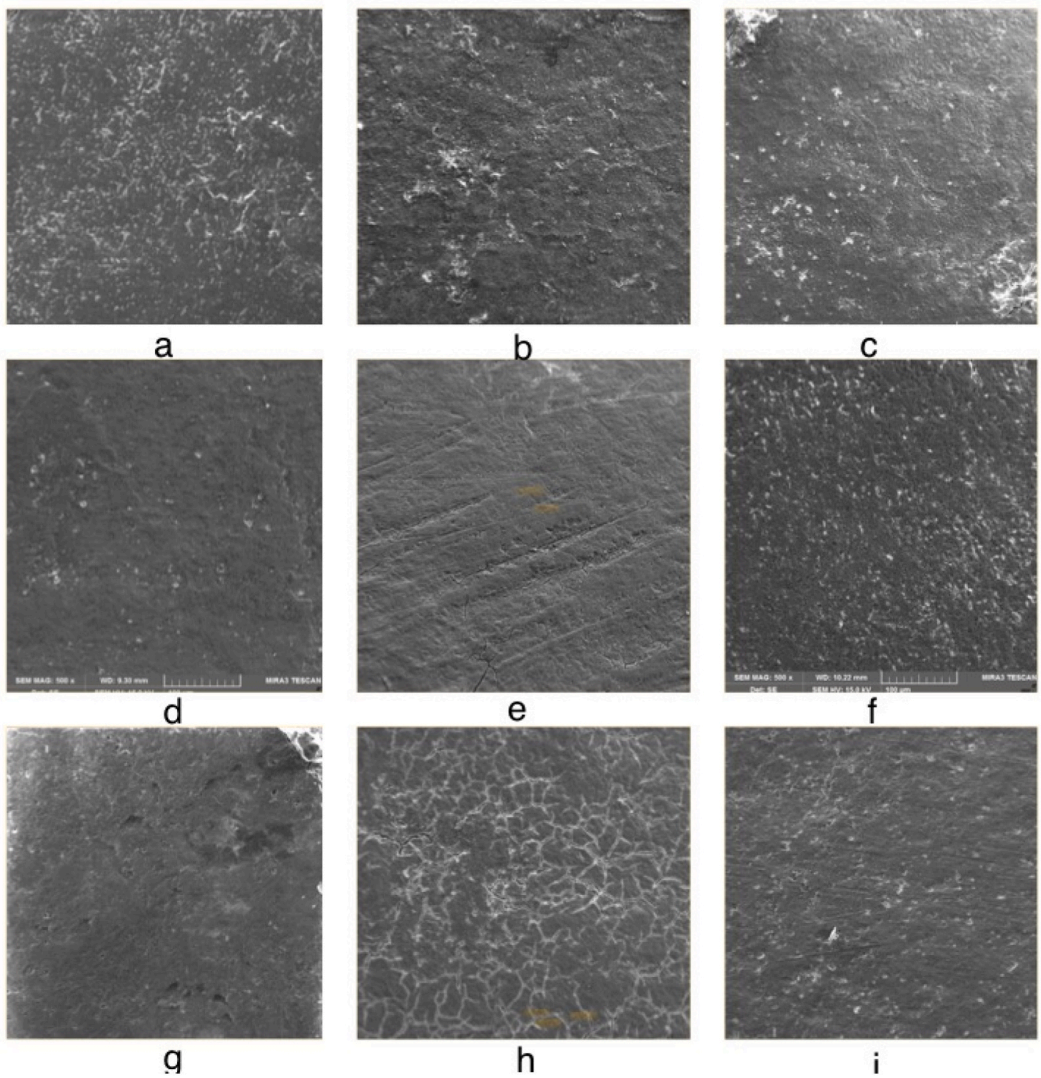


Fig. 1. The surface topography of enamel samples at × 500 magnification. a) Microhardness before treatment. b) Enamel after photodynamic therapy with methylene blue and fluoride varnish. c) Enamel after photodynamic therapy with methylene blue and silver diamine fluoride. d) Enamel after photodynamic therapy with indocyanine green and fluoride varnish. e) Enamel after photodynamic therapy with indocyanine green and silver diamine fluoride. f) Enamel after photodynamic therapy with methylene blue. g) Enamel after photodynamic therapy with indocyanine green. h) Enamel after treatment with fluoride varnish. i) Enamel after treatment with silver diamine fluoride.

chamber for imaging.

2.9. Statistical analysis

One-way ANOVA was used to compare the initial microhardness between the 16 study groups. Then three-way ANOVA was used to evaluate the effects of fluoride, laser, and surface (cementum or fluoride) on microhardness.

3. Results

3.1. Cementum samples

In all the cementum groups, the final microhardness after treatment was significantly different from the initial microhardness (before inducing incipient caries) ($P = 0.00$). The amount of final and initial microhardness in different groups is given separately in Table 1.

Post hoc Tukey tests did not reveal significant differences in initial microhardness between the study groups ($P > 0.05$). However, a

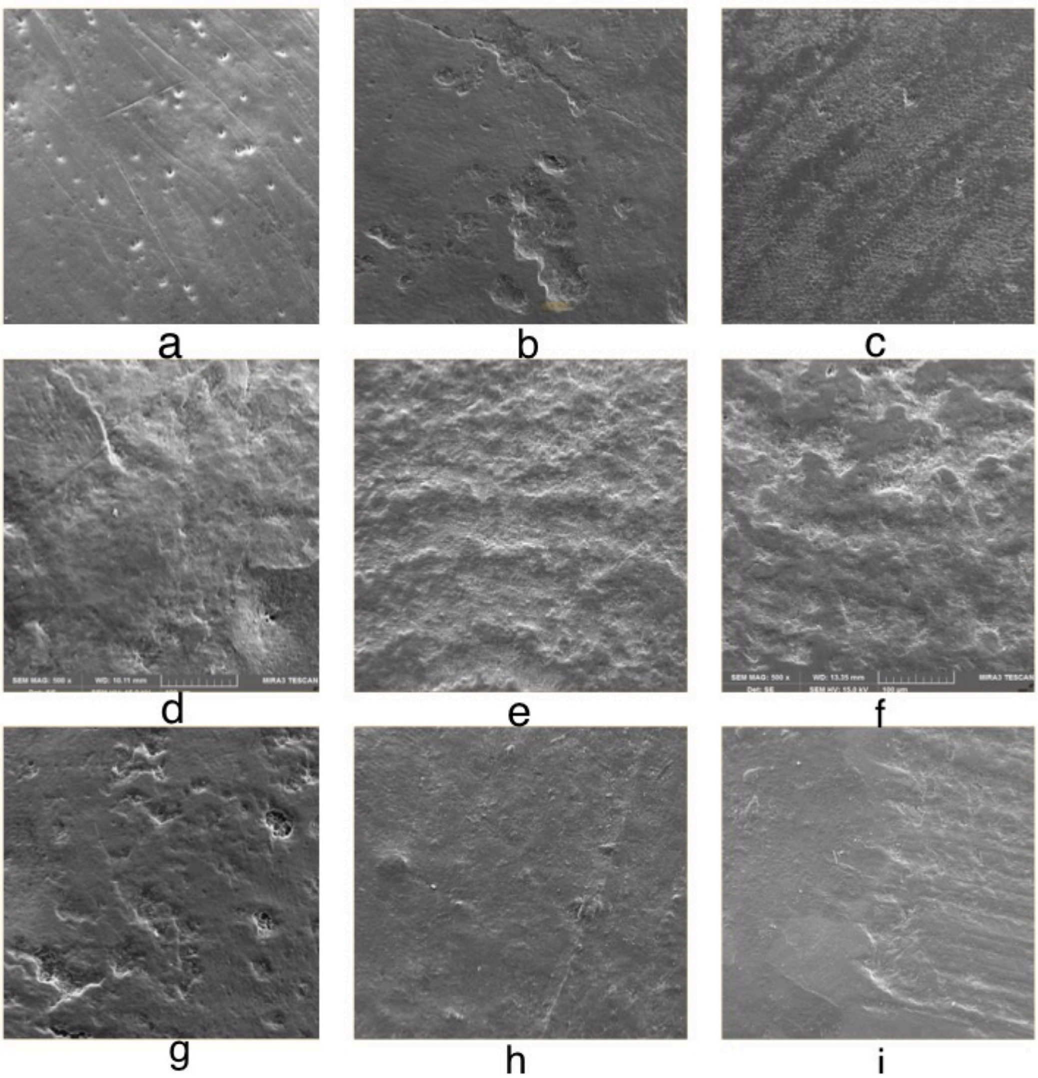


Fig. 2. The surface topography of cementum samples at × 500 magnification. a) Microhardness before treatment. b) Cementum after photodynamic therapy with methylene blue and fluoride varnish. c) Cementum after photodynamic therapy with methylene blue and silver diamine fluoride. d) Cementum after photodynamic therapy with indocyanine green and fluoride varnish. e) Cementum after photodynamic therapy with indocyanine green and silver diamine fluoride. f) Cementum after photodynamic therapy with methylene blue. g) Cementum after photodynamic therapy with indocyanine green. h) Cementum after treatment with fluoride varnish. i) Cementum after treatment with silver diamine fluoride.

Table 1
Comparison of initial and final microhardness in terms of the study variables in the cementum group.

Variable	Microhardness	Mean	SD	Mean difference between the initial and final microhardness
Silver diamine fluoride	Initial	48.27	2.68	12.81
	Final	35.46	3.97	
Fluoride varnish	Initial	49.01	2.46	12.90
	Final	36.11	2.20	
Photodynamic therapy with methylene blue	Initial	47.98	3.49	17.26
	Final	30.72	2.57	
Photodynamic therapy with indocyanine green	Initial	47.33	3.36	16.01
	Final	31.32	3.37	
Silver diamine fluoride + photodynamic therapy with methylene blue	Initial	46.50	2.71	7.98
	Final	38.51	3.00	
Silver diamine fluoride + photodynamic therapy with indocyanine green	Initial	48.31	3.87	11
	Final	37.31	2.97	
Fluoride varnish + photodynamic therapy with methylene blue	Initial	47.92	3.61	7.31
	Final	40.61	3.72	
Fluoride varnish + photodynamic therapy with indocyanine green	Initial	48.97	1.97	10.35
	Final	38.62	3.25	

significant difference was seen in the final microhardness in some groups ($p < 0.05$), the results of which are given in Tables 2 and 3.

The least decrease in cementum microhardness was observed in the fluoride varnish + methylone blue group (15.1 %) (Table 4).

The highest decrease in cementum microhardness was observed in the methylene blue group (35.7 %)(Table 4)

3.2. Enamel samples

In all the enamel groups, the final microhardness after treatment was significantly different from the initial microhardness (before inducing incipient caries) ($P = 0.00$). The amount of final and initial microhardness in different groups is given separately in Table 5.

Post hoc Tukey tests did not reveal significant differences in initial microhardness between the study groups ($P > 0.05$). However, a significant difference was seen in the final microhardness in some groups ($p < 0.05$), the results of which are given in Tables 6, 7 and 9.

The least decrease in enamel microhardness was recorded in the fluoride varnish + methylene blue group (16.7 %)(Table 8).

The highest decrease in enamel microhardness was observed in the methylene blue group (34.9 %)(Table 8).

4. Discussion

Considering the importance of treating dental caries and using a practical and less invasive method with the highest yield, the present study was designed to evaluate the combined effect of two photodynamic therapy methods and two local fluoride therapy methods alone or in combination on the surface topography of demineralized enamel and cementum. according to the results, the greatest impact on the amount of remineralization of enamel and cementum was found when combining photodynamic therapy with methylene blue and fluoride varnish, although the combination of photodynamic therapy with photosensitizer substance methylene blue or indocyanine green with fluoride varnish or silver diamine fluoride has no significant difference And they are effective in the remineralization of demineralized enamel and cementum.The present study compared the microhardness of enamel and cementum before demineralization and remineralization compared to that after the treatment procedures. The reason for decreased microhardness in all the groups was the demineralization of the samples. The efficacy of different materials in remineralization was evaluated by comparing the changes in the microhardness of the samples. The least decrease in cementum and enamel microhardness was related to the combined effect of fluoride varnish and methylene blue, indicating the potential effect of this combination on remineralization compared to other materials. The highest decrease in microhardness was observed in the methylene blue group, which might be attributed to its acidic nature and no use of a remineralizing agent.

Table 2
Comparisons of final microhardness of cementum groups.

Group	A	B	C	D
Methylene blue	+			
Indocyanine green	+	+		
Silver diamine fluoride		+	+	
Fluoride varnish			+	+
Indocyanine green + silver diamine fluoride			+	+
Silver diamine fluoride + methylene blue			+	+
Fluoride varnish + indocyanine green			+	+
Methylene blue + fluoride varnish				+

The marked cells in each column are not significantly different ($P > 0.05$). However, they are significantly different from the remaining unmarked cells ($P < 0.05$).

Table 3
Comparison of p value of final microhardness among cement test groups.

Group	P value
Silver diamine fluoride - Photodynamic therapy with methylene blue	0.046
Silver diamine fluoride - Fluoride varnish + photodynamic therapy with methylene blue	0.022
Fluoride varnish - Photodynamic therapy with methylene blue	0.014
Fluoride varnish - Photodynamic therapy with indocyanine green	0.043
Photodynamic therapy with methylene blue - Silver diamine fluoride + photodynamic therapy with methylene blue	0.000
Photodynamic therapy with methylene blue - Silver diamine fluoride + photodynamic therapy with indocyanine green	0.001
Photodynamic therapy with methylene blue - Fluoride varnish + photodynamic therapy with methylene blue	0.000
Photodynamic therapy with methylene blue - Fluoride varnish + photodynamic therapy with indocyanine green	0.000
Photodynamic therapy with indocyanine green - Silver diamine fluoride + photodynamic therapy with methylene blue	0.000
Photodynamic therapy with indocyanine green - Silver diamine fluoride + photodynamic therapy with indocyanine green	0.004
Photodynamic therapy with indocyanine green - Fluoride varnish + photodynamic therapy with methylene blue	0.000
Photodynamic therapy with indocyanine green - Fluoride varnish + photodynamic therapy with indocyanine green	0.000

$P > 0.05$: There is no significant difference between the enamel and cementum groups.
$P < 0.05$: There is a significant difference between the enamel and cementum groups.

Table 4
The percentage reduction of the final microhardness compared to the initial microhardness in cementum groups.

Group	Mean
Silver diamine fluoride	26.36
Varnish	26.21
Methylene blue	35.71
Indocyanine green	33.51
Silver diamine fluoride + methylene blue	17.00
Silver diamine fluoride + indocyanine green	22.21
Varnish + methylene blue	15.10
Varnish + indocyanine green	21.11
Total	24.65

Table 5
Comparison of initial and final microhardness in terms of the study variables in enamel groups.

Variable	Microhardness	Mean	SD	Mean difference between the initial and final microhardness
Silver diamine fluoride	Initial	283.76	4.02	68.30
	Final	215.46	5.64	
Fluoride varnish	Initial	287.45	6.98	68.88
	Final	218.53	8.30	
Photodynamic therapy with methylene blue	Initial	277.48	6.42	97.33
	Final	180.15	6.12	
Photodynamic therapy with indocyanine green	Initial	285.24	7.59	97.53
	Final	187.71	7.89	
Silver diamine fluoride + photodynamic therapy with methylene blue	Initial	291.74	5.48	53.15
	Final	238.58	6.55	
Silver diamine fluoride + photodynamic therapy with indocyanine green	Initial	287.01	3.75	70.30
	Final	216.71	3.93	
Fluoride varnish + photodynamic therapy with methylene blue	Initial	291.15	5.43	48.30
	Final	242.85	8.08	
Fluoride varnish + photodynamic therapy with indocyanine green	Initial	286.21	4.99	64.75
	Final	221.45	6.90	

In the cementum group, comparing the combination of photodynamic therapy with methylene blue or indocyanine green along with fluoride varnish, there is no significant difference compared to the use of fluoride varnish alone, also there is no significant difference between photodynamic therapy with methylene blue or indocyanine green alone, but there is a significant difference between combination of photodynamic therapy with fluoride varnish compared to photodynamic therapy alone, although the following sequence can be concluded by comparing the final microhardness percentage reduction compared to the initial one in the cementum group:

fluoride varnish + photodynamic therapy with methylene blue < silver diamine fluoride + photodynamic therapy with methylene blue < fluoride varnish + photodynamic therapy with indocyanine green < photodynamic therapy with indocyanine green + silver diamine fluoride < fluoride varnish < silver diamine fluoride < photodynamic therapy with indocyanine green < photodynamic

Table 6
Comparison of the final microhardness between the enamel groups.

Group	A	B	C
Methylene blue	+		
Indocyanine green	+	+	
Silver diamine fluoride		+	+
Fluoride varnish		+	+
Indocyanine green + silver diamine fluoride			+
Silver diamine fluoride + methylene blue			+
Fluoride varnish + indocyanine green			+
Methylene blue + fluoride varnish			+

The marked cells in each column are not significantly different ($P > 0.05$). However, they are significantly different from the remaining unmarked cells ($P < 0.05$).

Table 7
Comparison of p value of final microhardness among enamel test groups.

Group	P value
Silver diamine fluoride - photodynamic therapy with methylene blue	0.011
Fluoride varnish - photodynamic therapy with methylene blue	0.004
Fluoride varnish - photodynamic therapy with indocyanine green	0.042
photodynamic therapy with methylene blue - Silver diamine fluoride + photodynamic therapy with methylene blue	0.000
photodynamic therapy with methylene blue - Silver diamine fluoride + photodynamic therapy with indocyanine green	0.008
photodynamic therapy with methylene blue - Fluoride varnish + photodynamic therapy with methylene blue	0.000
photodynamic therapy with methylene blue - Fluoride varnish + photodynamic therapy with indocyanine green	0.002
photodynamic therapy with indocyanine green - Silver diamine fluoride + photodynamic therapy with methylene blue	0.000
photodynamic therapy with indocyanine green - Fluoride varnish + photodynamic therapy with methylene blue	0.000
photodynamic therapy with indocyanine green - Fluoride varnish + photodynamic therapy with indocyanine green	0.018

$P > 0.05$: There is no significant difference between the enamel and cementum groups.
$P < 0.05$: There is a significant difference between the enamel and cementum groups.

Table 8
The percentage reduction of final microhardness compared to the initial microhardness in the enamel groups.

	Mean
Silver diamine fluoride	24.15
Varnish	24.03
Methylene blue	34.90
Indocyanine green	34.29
Silver diamine fluoride + methylene blue	18.24
Silver diamine fluoride + indocyanine green	24.52
Varnish + methylene blue	16.75
Varnish + indocyanine green	22.76
Total	24.95

Table 9
Percentage reductions in final microhardness compared to the initial microhardness in the enamel and cementum groups.

Group	P-value
Silver diamine fluoride	0.499
Varnish	0.390
Methylene blue	0.800
Indocyanine green	0.815
Silver diamine fluoride + methylene blue	0.657
Silver diamine fluoride + indocyanine green	0.483
Varnish + methylene blue	0.547
Varnish + indocyanine green	0.496

$P > 0.05$: There is no significant difference between the enamel and cementum groups.
$P < 0.05$: There is a significant difference between the enamel and cementum groups.

therapy with methylene blue.

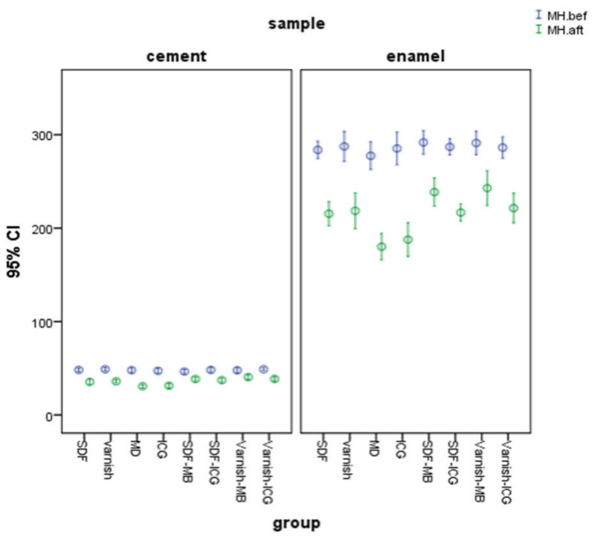
In the enamel group, there is no significant difference between combination of photodynamic therapy with methylene blue or indocyanine green along with fluoride varnish or silver diamine fluoride compared to the use of fluoride varnish or silver diamine fluoride alone, also there is no significant difference between photodynamic therapy with methylene blue or indocyanine green alone but there is a significant difference between the combination of photodynamic therapy with fluoride varnish or silver diamine fluoride compared to photodynamic therapy alone, although the following sequence can be concluded by comparing the percentage reduction of the final microhardness compared to the initial one in the enamel group.

fluoride varnish + photodynamic therapy with methylene blue < silver diamine fluoride + photodynamic therapy with methylene blue < fluoride varnish + photodynamic therapy with indocyanine green < fluoride varnish < silver diamine fluoride < photodynamic therapy with indocyanine green + silver diamine fluoride < photodynamic therapy with indocyanine green < photodynamic therapy with methylene blue.

In addition, a comparison of percentage decreases in final microhardness compared to the initial microhardness in the enamel and cementum groups showed no significant differences between the enamel and cementum, indicating that anticariogenic treatments, including fluoride varnishes and photodynamic therapy, can be as effective in managing root surface caries as incipient caries on the enamel surface.

Shihabi et al. confirmed the effect of fluoride varnish on enamel remineralization, reporting that fluoride varnish could change the microhardness of the enamel surface, making it effective in treating dental caries, consistent with the present study [6]. Oliciera et al., too, reported decreased microhardness in all the groups, indicating that CO_2 laser irradiation combined with different commercial fluoride products could increase microhardness in enamel carious lesions, consistent with the present study [3]. Mei et al. confirmed the effect of laser on fluoride absorption, reporting that CO_2 and Er:YAG lasers increased fluoride absorption higher than that by Nd:YAG and diode laser irradiation on all the surfaces; in addition, CO_2 and Er:YAG laser irradiation increased fluoride absorption in the dentin treated by SDF compared to the Nd:YAG and diode laser irradiation. In the present study, silver diamine fluoride alone resulted in a higher decrease in microhardness than silver diamine fluoride combined with photodynamic therapy, confirming the effect of light and laser irradiation on higher fluoride absorption, consistent with the study by Mei et al. [15].

In addition, Belcheva et al. reported that CO_2 laser irradiation combined with fluoride was more effective in protecting the enamel surface and providing resistance against the loss of mineral agents than CO_2 laser irradiation or fluoride alone [14] The result of Belcheva et al. is consistent with the current study in terms of the effectiveness of laser radiation in combination with fluoride in protecting the enamel surface and resisting the removal of minerals, but it is inconsistent in terms of the effectiveness of CO2 laser combination with fluoride compared to fluoride alone And probably the reason can be due to the difference in the type of laser in the 2 studies. Zhang et al. reported that laser irradiation associated with fluoride could change the root shape and increase fluoride absorption by the root surface [16]. Gao et al. reported a significant synergistic effect of a combination of CO_2 laser and fluoride treatment on inhibiting root demineralization, which might be explained by higher fluoride absorption in the root [18]. Therefore, the present study results are consistent with those of Zhang et al., and Gao et al. Moghadam et al. studied the effect of diode laser irradiation with or without fluoride therapy on preventing the demineralization of primary tooth enamel. The results showed significant effects of laser, fluoride, and laser-fluoride combination on decreasing the final microhardness. In addition, the results showed that fluoride vanishes, diode laser, and their combination decreased the enamel's microhardness loss, potentially preventing primary tooth enamel demineralization, consistent with the present study [19]. Valizadeh et al. reported that CO_2 and Er:YAG laser alone did not significantly affect the tooth structure resistance against cariogenic solutions. However, they might have had synergistic effects when they were used with an NaF varnish. The fluoride varnish used before laser irradiation increased the tooth structure resistance,



Graph 1. Initial and final surface microhardness in the enamel and cementum groups.

positively affecting its hardness, consistent with the present study [20].

According to a general conclusion, despite the positive effect of fluoride varnish and silver diamond fluoride on increasing the mineralization and microhardness of the enamel and cementum, combining these materials with photodynamic therapy and laser increases these materials' penetration and effect. According to Graph 1, in the enamel and cementum groups, the final microhardness was very close to the initial microhardness compared to the enamel. SEM images indicated the filling of porosities, resulting in a surface appearance of the final samples that resemble the initial samples, which was less evident in enamel samples. Given the prevalence of root surface caries in elderly patients, the effects of the treatments applied in the present study, especially the combination of fluoride and photodynamic therapy, might prove promising in controlling incipient cementum caries on root surfaces. Over all the findings of this study have some limitations such as the in vitro nature of the study, which may limit the generalizability of the findings to clinical settings (see Gragh 2).

5. Conclusion

The combination of photodynamic therapy with the photosensitizer substance methylene blue or indocyanine green together with fluoride varnish or silver diamine fluoride is effective on the remineralization of demineralized enamel and cementum. Although there is no difference between the combination of photodynamic therapy with fluoride varnish compared to fluoride varnish alone, both of these treatments are more effective than using photodynamic therapy alone.

Data Availability statement

All data supporting the findings of this study are available within the tables and figures of this research.

Funding statement

No funding was obtained for this study from any organizations.

Ethical statement

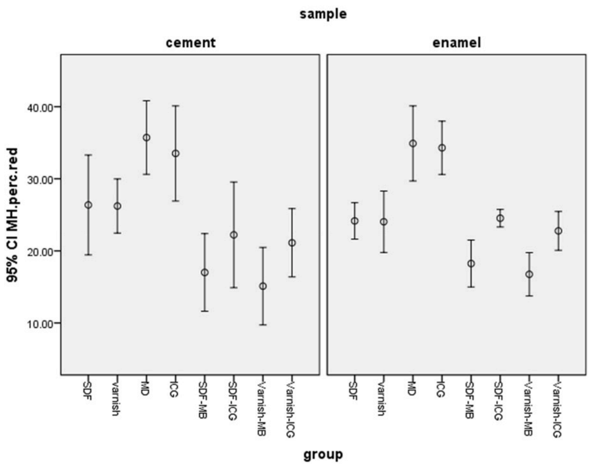
The ethical aspects of this study were reviewed and approved by Ethics Committee of the Faculty of Dentistry, Tehran University of Medical Sciences; with ethical code of: (IR.TUMS.DENTISTRY.REC.1400.067).

CRediT authorship contribution statement

Sedighe Sadat Hashemikamangar: Writing – original draft, Visualization, Project administration, Methodology, Investigation, Formal analysis, Conceptualization. **Mahtab Vahedi:** Investigation, Funding acquisition, Formal analysis, Data curation. **Mohammadreza Khadivi Moghadam:** Software, Resources, Data curation. **Behnaz Behniafar:** Writing – review & editing, Visualization, Validation, Supervision. **Nasim Chiniforush:** Project administration, Methodology.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to



Graph 2. Comparison of percentage reductions in microhardness in the enamel and cementum groups.

influence the work reported in this paper.

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