



Effect of Diode Laser versus a Combination of Sodium Trimetaphosphate with Polyacrylic Acid on Obliteration of Dentinal Tubules: An *In Vitro* Study

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Abstract

Edited by: Filip Koneski Citation: Darwish MAE, Abo-Elezz AF, Safy RK. Effect of Diode Laser versus a Combination of Sodium Trimetaphosphate with Polyacrylic Acid on Obliteration of Dentinal Tubules: An *In Viro* Study. Open Access Maced J Med Sci. 2022 Mar 25; 10(D):160-165. https://doi.org/10.3889/oam/s.2022.8792 Keywords: Dentin hypersensitivity; Diode laser; Sodium trimetaphosphate; Polyacrylic acid and biomimetic remineralization *Correspondence: Mostafa Abd El-hakim Darwish, Department of Operative Dentistry; Sinai University, Egypt. E-mail: mostafaemail50@gmail.com Received: 28-Jan-2022 Revised: 19-Mar-2022 Accepted: 21-Mar-2022 Accepted: 21-Mar-2022 Accepted: 21-Mar-2022 Accepted: 21-Mar-2022 Accepted: 21-Mar-2022 Copyright: © 2022 Mostafa Abd El-hakim Darwish, Ahmed Fawzy Abo-Elezz, Rehab Khalil Safy Funding: This research did not receive any financial support

Competing interests: The adultions have declared that in to competing interests exist Open Access: This is an open-access article distributed under the terms of the Creative Commons Attribution-NonCommercial 4.0 International License (CC BY-NC 4.0) **BACKGROUND:** Irradiation with diode laser and biomimetic remineralization are important methods in the treatment of hypersensitivity and dentin remineralization.

AIM: The aim of the study was evaluation of the effect of diode laser versus the effect of sodium trimetaphosphate (STMP) with polyacrylic acid (PAA) on obliteration of dentinal tubules (DT).

MATERIALS AND METHODS: Sixty dentin discs with a thickness of 2 mm were prepared and conditioned with EDTA for 15 s. Then, all dentin discs were divided into three main groups (20 discs each) according to the treatment method; control, diode laser treated, and STMP with PAA group (biomimetic group). Each group was subdivided into four subgroups (five discs each) according to the storage time; 2 h (T_o), 1 month (T_1), 2 months (T_2), and 3 months (T_3), respectively. All samples were prepared to be analyzed after each time interval using environmental scanning electron microscope (ESEM). Comparison of differences of DT obliteration percentage made on each group before and after the treatment were performed using computer-assisted digital image analysis.

RESULTS: Control group showed the least DT obliteration percentage, the samples of laser group recorded statistically significant increase in DT obliteration percentage at T_0 in comparison to the biomimetic group. Meanwhile, at T_1 , there was no statistically significant difference between both laser and biomimetic groups. However, statistically significant decrease was recorded in laser group at T_2 and T_3 , respectively.

CONCLUSION: Irradiation with diode laser and biomimetic remineralization using PAA with STMP are a promising methods to obliterate opened DT effectively.

Introduction

Dentin hypersensitivity (DH) is a common clinical situation that is experienced by 10–20% of the general population. It is characterized by sharp, short pain arising from exposed dentin in response to stimuli typically osmotic, tactile, evaporative, thermal, or chemical and which cannot be associated with any other form of dental pathology or defect [1]. The etiological factors related to DH include poor oral hygiene, faulty tooth brushing, gingival recession, and premature contacts due to periodontal therapy or physiological reasons [2].

Different theories have been discussed on the DH of which direct innervation theory and odontoblast receptor theory have encountered some challenges. However, Brannstorm theory deals with the flow of tubular fluid which is more realistic [3]. At present, many methods and materials were launched to remove or reduce DH, they involve the use of toothpastes containing arginine, fluoride, oxalates, potassium salts, bioglass, varnishes, and different other materials. These materials usually exhibit their actions by disturbing nerve impulse transmissions or sealing dentinal tubules. The long-term effect of such treatment is uncertain, it is expected that promising therapies will emerge and produce more effective relief for patients with DH [4]. Therefore, long-lasting and effective treatment is of great interest to both patients and dental professionals.

One of the drawbacks related to the most commonly used materials that can be applied by the patients themselves which include potassium compound, that it takes a long time to experience symptomatic relief typically up to 3 months [5]. Hence, provision of immediate treatment is mandatory and important. Furthermore, one of the most important aspects of the treatment is longevity, this suggests that further long-term studies in different materials and techniques should be achieved.

Recently, diode laser was introduced as one of rapid method of DH treatment [6]. It is a high-energy laser, with low cost for purchasing and maintenance, also greater versatility given from its compact size [7]. At present, few studies have reported on the interaction of diode laser energy with the structural alterations on dentin surface [8], [9]. Laser appears more satisfied than traditional methods because of its fast and innovative effect with minimal side effects to patients [10].

In addition, diode laser can treat DH effectively [11], but like other types of lasers, it requires more studies to make more evaluations. Searching for other treatment methods of DH lead to launching a biomimetic approach through using of STMP and PAA in non-classical pathway for dentin remineralization [12], PAA was used as an analog for sequestering calcium ions released [13], which mimic the role of non-collagenous protein during natural remineralization [14].

Despite the fact that several studies have shown the effect of desensitizing agent on the occlusion of dentinal tubules, further researches are needed to compare between many types of lasers like the diode laser and biomimetic remineralization methods on obliteration of DT. This study compared the effect of diode laser and biomimetic remineralization on obliteration of DT.

Materials and Methods

The materials used in this study are described in Table 1.

Table 1: Materials, description, composition, manufacturers, and batch numbers

Materials	Description	Composition	Manufacturers	Patch number
Sinai white	Type 1 white	3CaO·SiO ₂ ,	Sinai White	
Portland cement [®]	Portland cement	2CaO·SiO ₂ ,	Portland Cement	
		3CaO·Al2Õ₃,	Co®	
		and	http://www.	
		CaSO ₄ ·2H ₂ O	sinaiwhitecement.	
			com/	
Polyacrylic acid [®]	Polyacrylic acid	$(C_3H_4O_2)_n$	SIGMA-ALDRICH®	1002418633
	powder	MW 1800	http://www.	
			sigmaaldrich.com/	
Sodium	Sodium	Na ₃ P ₃ O ₉	SIGMA-ALDRICH®	1002627536
trimetaphosphate®	trimetaphosphate	MW 305.89	http://www.	
	powder		sigmaaldrich.com/	

Selection of teeth

After the approval of Ethical Committee of "The Faculty of Dentistry of Suez Canal University" (Number 104/2018), this study was carried out on 60 recently extracted human permanent molars which were extracted for periodontal reasons from patients aged 20–40 years old [15]. They were checked and thoroughly washed with water, scaled to remove any blood, calculus, plaque, and attached periodontal tissues. Teeth were examined for freedom of cracks using a magnifying lens. Any tooth showing any signs of caries, micro-cracks, or other defects like attrition was discarded, followed by storage of the teeth in distilled water, having 0.5% chloramine-T antiseptic solution at room temperature, and used within 1 month from extraction [16].

Specimens preparation

After fixation of each tooth in acrylic resin block, an automated diamond saw (Isomet 4000, Buehler Ltd., Lake Bluff, USA) [17], [18] was used to prepare 60 samples. Sixty teeth were used for preparation of samples by removal of the occlusal enamel and exposing of dentine, then dentin discs of 2 mm thickness were cut perpendicular to the long axis of each tooth using a low-speed Isomet saw under water cooling then dentin surface of each sample was polished with 1200-grit silicon carbide paper under running water to create a smooth surface layer [19]. The occlusal surface of each prepared dentin disc was marked using nail varnish (KIKO Milano, Italy) and then conditioned by EDTA for 15 s. Pre-operative photomicrograph using ESEM (Quanta FEG-250) at 3000X was taken for each conditioned dentin surface for evaluation of patency of DT. Any sample photomicrograph showing any blockage of DT was discarded and replaced.

All samples were randomly divided into three main groups according to the remineralizing method (20 samples each). Where, each sample of the control group (R_o) inserted on the top surface of Portland cement block with water-to-powder ratio of 0.35:1, [20] which prepared and allowed to set for 1 week before using [21]. The complex of both sample and Portland cement block was immersed in 15 ml SBF [22]. Preparation of laser group (R₄) occurred through irradiation of the top surface of each sample by diode laser with 980 nm wavelength (2.0W/CW, 166 J/ cm²) twice for 10 s (5 s each) at 1 mm distance from dentin with perpendicular positioning of the optical fiber in relation to the dentin surface [11], and then samples were immersed in SBF. Finally, preparation of biomimetic group (R₂) was achieved through immersion of each sample in 2.5 wt% STMP solution for 5 min [22], then each sample was inserted on top of Portland cement block (as mentioned before in control group) then all samples were immersed in mixture of 15 ml of the SBF and 500 µg/mL polyacrylic acid (PAA, Mw 1800; Sigma-Aldrich) and buffered into pH 7.4 [22] to act, as a biomimetic group. The remineralizing medium was changed every month [23].

Each group was subdivided into four subgroups according to the storage time (n = 5) where all samples of the subgroups (T_0) were photomicrographed by ESEM after 2 h, (T_1) after 1 month, (T_2) after 2 months, and (T_3) after 3 months.

ESEM evaluation and analysis

The extent of dentinal tubule occlusion was assessed using ESEM and average of three ESEM images was taken for each sample then the degree of dentinal tubules occlusion percentage was made through using of computer-assisted digital image analysis (Image J 1.60, Scion, and Frederick, MD, USA) [24].

Statistical analysis

The data were collected, tabulated, and checked for normality test, then analyzed by using IBM SPSS Corp. Released 2013. IBM SPSS Statistics for Windows, Version 22.0. Armonk, NY: IBM Corp. Qualitative data were described using number and percent. Quantitative data were described using median (minimum and maximum) for non-parametric data after normality test (Table 2) using Shapiro–Wilk test. Significance of the results was judged at the (0.05) level.

Table 2: Test of normality

Dentinal tubule occlusion Shapiro–Wilk					
Statistic	Df	р			
0.625	45	0.000**			
0.670	15	0.00**			
0.750	15	0.001**			
0.796	15	0.003**			

Results

For the first group Ro (control group)

There was a statistically significant increase in DT obliteration percentage from 2 h (.323) to 1 month (1.33) then there was no statistically significant increase DT obliteration percentage up 3 months (1.25).

For the second group, R₁ (laser group)

There was no statistically significant difference between 2 h (79.7) and 1 month (78.7), then there was statistically significant decrease in DT obliteration percentage from 1 month to 2 months (67.75) and finally there was no statistically significant between 2 and 3 months (64.25).

For the third group R, (biomimetic group)

There was a statistically significant increase in DT obliteration percentage in all time intervals from 2 h up to 3 months. At two hours it was .654, 1 month 78.38, 2 months 87.77 with the most significant median value at 3 months 93.06.

ESEM photomicrograph and Image J estimations

ESEM photomicrograph of conditioned dentin showed widely opened DT creating spongylike appearance with different shapes of opened DT, some had oval shape and others were circular. The ESEM photomicrograph of control group in all time intervals showed homogeneous surface with very little DT obliteration, dentin surfaces were smooth and DT was visible and exposed, as showed in (Tables 3) and (Figure 1). There was a statistically significant increase in DT obliteration percentage from 2 h to 1 month then there was no statistically significant increase DT obliteration percentage up 3 months.

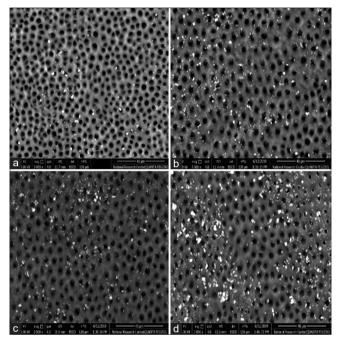


Figure 1: Control group ESEM photos at 3000× magnification (a) After 2 h, (b) After 1 month, (c) After 2 months, and (d) After 3 months

The ESEM photomicrograph of laser group showed that dentin surfaces were covered by melted dentin and the majority of DT were sealed with little exposed DT in T_0 and T_1 by time anon significant decrease in DT obliteration percentage was observed in T_2 followed by T_3 , as showed in (Tables 3) and (Figure 2), according to the time, there was no statistically significant difference between 2 h and 1 month, then there was statistically significant decrease in DT obliteration percentage from 1 month to 2 months and finally there was no statistically significant between 2 and 3 months.

The biomimetic group photomicrograph showed that there was little DT obliteration in T

Table 3: Comparison of the effect of time and materials change on dentinal tubules occlusion

Groups		follow-up		Test of	
	2 hours	1 st month (T1)	2 nd month	3 rd month	significance
	(To)		(T2)	(T3)	(Freidman
					test)
Control (R _o)			1.106 ^{c, A}	1.251 ^{c, A}	0.02**
Median	0.323 ^{с, в}	1.33 ^{b, A}			
Minimum-	0.14–0.54	0.96-1.58	0.98–1.35	0.93–1.85	
maximum					
Laser (R ₁)					0.002**
Median	79.78 ^{a, A}	78.78 ^{a, A}	67.75 ^{ь, в}	64.25 ^{b, B}	
Minimum-	71.34–	71.97-80.48	65.69-80.4	60.28-72.89	
maximum	84.21				
STMP+PAA (R ₂)					0.003**
Median	0.654 ^{b, D}	78.38 ^{ª, C}	87.77 ^{a, B}	93.06 ^{ª, A}	
Minimum-	0.45-0.89	69.74–79.64	77.73–90.56	80.64–94.14	
maximum					
Test of					
significance	KW	KW	KW	KW	
between groups	p=0.001**	p=0.007**	p=0.003**	p=0.002**	
All parameters descri	bed as median	(minimum-maximu	m). ** ^{. ab.c} Similar lo	wer case superso	ripted letters

An parameter described as instant (immuni-maximum). Similar over dass superscripted letters in same column denote non-significant difference between groups, **^{A,B,C}Similar upper case subscripted letters in same row denote nonsignificant difference between times. KW: Kruskal–Wallis test, STMP: Sodium trimetaphosphate, PAA: Polyacrylic acid.

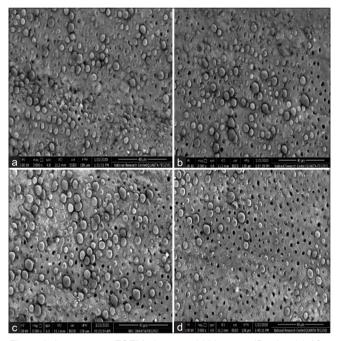


Figure 2: Laser group ESEM photos at 3000× magnification (a) After 2 h, (b) After 1 month, (c) After 2 months, and (d) After 3 months

with smooth dentin surface and visible exposed DT. Meanwhile, T_1 photomicrograph showed that the majority of DT were sealed and obliterated with little percentage of exposed DT, as showed in (Table 3) and (Figure 3). There was a statistically significant increase in DT obliteration percentage in all time intervals from 2 h up to 3 months, two hours, 1 month, 2 months with the most significant median value at 3 months.

In comparison to control group, the laser group showed a statistically significant increase of DT obliteration percentage with the most significant median value after 2 h followed by 1 month then followed 2 months with the least amount of obliteration

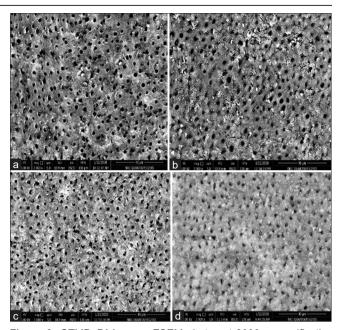


Figure 3: STMP+PAA group ESEM photos at 3000× magnification (a) After 2 h, (b) After 1 month, (c) After 2 months, and (d) After 3 months

percentage was at 3rd month. The biomimetic group revealed statistically significant increase in DT obliteration percentage with the most significant median value at 3 months (93.06) followed by 2 months than followed by 1 month with the least amount of obliteration percentage was at 2 h (0.654). Meanwhile laser group in comparison to biomimetic group record statistically significant increase in DT obliteration percentage after 2 h and then after 1 months, there was no statistically significant difference followed by statistically significant decrease in laser group after 2 and 3 months (Figure 4).

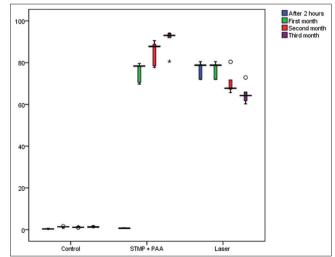


Figure 4: Box and Whisker plot for comparing median dentine tubule occlusion after 2 h, 1^{st} , 2^{nd} , and 3^{rd} months of follow-up between studied groups

Discussion

Although DH is one of the most common and disturbing disorders, the treatments which have been proposed for it are neither sufficient nor durable. Difficulties in the treatment of DH gave rise to many protocols and therapeutic operations which are currently being used for the improvement of the treatment of DH. Accordingly, in the present study the control group revealed little increase in percentage DT obliteration mainly after 1 month and this effect did not increase significantly by time up to 3 months. Therefore, longlasting and effective treatment is of great interest to both patients and dental professionals.

Although it would seem that the laser irradiation treatment reduces symptoms of pain related to DH effectively, further studies and more suitable followups are necessary. In the present study, laser group showed rapid effect on dentinal tubules obliteration which had maximum level of DT obliteration and record highest median value after 2 h and 1 month, then there was a significant decrease in obliteration percentage after 2 and 3 months. The ESEM photomicrograph of laser group showed that dentin surfaces were covered by melted dentin and the majority of DT were sealed with little exposed DT. This effect on DT may be due to the laser-induced thermal effects on dentin leading to narrowing or blocking of the dentinal tubules [11]. Consequently, stabilization of structure alteration of lased dentin is highly recommended by repeated irradiation with combination between laser and desensitizing material that could achieve the best prolonged results [10]. These results come in agreement with Liu et al. [11], who found that irradiation with diode laser could be effective for routine treatment of DH because of its rapid sealing of the exposed DT, and come in agreement with Oak [8], who evaluated the rapid effect of diode laser on sealing ability of DT in extracted teeth using ESEM.

In addition, our study was interested in changing concepts in biomimetic remineralization of fibrillar collagen, including the recently discovered, nonclassical particle-based crystallization concept through formation of polymer-induced liquid- precursors, experimental collagen models for remineralization, the need for using phosphate-containing and biomimetic analogs for biomimetic remineralization of collagen [13]. The biomimetic group in the present study has been found to be effective in DT obliteration but take a long time in comparison to laser group according to the storage time, after 2 h of storage, there was no or little effect on DT obliteration, it gradually increased by time up to 3 months, this results could be attributed to the effect of biomimetic remineralization effect of STMP with PAA in bioactive medium such SBF [14]. It worth nothing that the biomimetic group records the highest value of DT obliteration percentage after 3 months in comparison to other groups; however, laser group revealed faster effect on DT obliteration percentage through just after 2 h. This result could be explained on the basis of rapid sealing ability of laser on exposed dentinal tubules by melting of superficial layer of dentine and crystalline arrangement due to the thermal effects of the laser energy [11]. In the other side, the longterm remineralization of the biomimetic group may be attributed to step by step remineralization that occurs in biomimetic remineralization starting in the presence of a polyanionic analog of matrix proteins (PAA), which make pre-nucleation clusters further condense into larger particles of fluidic amorphous ACP precursors with sequestration mechanism of STMP that stabilize these clusters [13]. Therefore, addition of PAA to the Portland cement in SBF system has been shown to be essential for the stabilization of the amorphous calcium phosphate (ACP) precursors released by the interaction between set SBF and Portland cement [12]. These results come in agreement with Cao et al. [14], who examined the methods for biomimetic remineralization of dentin and discussed many studies reported in vitro success in biomimetic mineralization of dentin with different methods, including the use of NCP analogs and using bioactive materials.

From all mentioned results, this study showed that irradiation with diode laser or biomimetic remineralization of dentin using PAA with STMP obliterate the DT effectively. Thus, the null hypothesis of the present study was rejected.

Conclusion

Under the limitations of this *in vitro* study, the following could be concluded:

- 1. Irradiation with 980-nm diode laser and biomimetic remineralization using PAA with STMP are a promising methods to obliterate the opened dentinal tubules effectively.
- 2. Diode lasers have been found to have rapid effect in occluding dentinal tubules when compared with the biomimetic remineralization.

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