Journal of Oral Biology and Craniofacial Research 13 (2023) 224–229

Contents lists available at ScienceDirect

Journal of Oral Biology and Craniofacial Research

journal homepage: www.elsevier.com/locate/jobcr

Comparative evaluation of diode laser alone and in combination with desensitizing toothpaste in occlusion of dentinal tubules - A SEM study

Hemalatha Doppalapudi *, Anil Kumar Kancharla, Anwesh Reddy Nandigam, Mohammad Sheema Tasneem, Shiva Shankar Gummaluri, Swatantrata Dey

Department of Periodontology and Oral Implantology, Sree Sai Dental College and Research Institute, Srikakulam, Andhra Pradesh, India

ARTICLE INFO

Keywords:
Dentin sensitivity
Scanning electron microscopy
Laser therapy

ABSTRACT

Background: In regular dental practice, dentinal hypersensitivity is a common oral condition affecting the adult population. Although a variety of treatment procedures are available, there is no single method that proved to be potent in eliminating dentine hypersensitivity and providing patients with complete relief. Hence the present study performed to estimate the occlusion of dentinal tubules using a diode laser alone and in combination with various desensitizing toothpaste, using the scanning electron microscope.

Materials and methods: This in-vitro study consisted of five groups wherein cervical cavities were prepared on the extracted teeth followed by the application of 17% EDTA. The cavities were then treated with Novamin toothpaste, pro-arginine toothpaste, diode laser, and in combinations respectively. Then SEM analysis was done and the results obtained were statistically analyzed using tukey's multiple post hoc analysis for intra and inter group comparisons.

Results: The groups which were treated with a combination of diode laser and the desensitizing agent showed statistically significant ($P < 0.05$) better dentinal tubule occlusion than other groups.

Conclusion: The use of a diode laser has an added benefit in treating dentinal tubule occlusion when compared to desensitizing kinds of toothpaste.

1. Introduction

Dentin Hypersensitivity(DH) is a common oral condition encountered in regular practice with a large prevalence range of 1.3%–92.1% and peak within the cohort of 20–40 years[1] It can be defined as a short, sharp "bright" style of pain that is rapid in onset, followed by a dull, aching pain in response to stimuli like thermal, evaporative, tactile, osmotic, or chemical, arising from exposed dentin and cannot be ascribed to the other type of dental pathology or dental defect or disease. (Holland et al., 1997, Canadian board on Dentine Hypersensitivity 2003).[2] The pain is transient and characterized by its brief and shooting nature with a response that is felt immediately. The interventions to treat DH are classified into 2 types by the mode of delivery: in-office or professionally applied agents by the dentist and over-the-counter (OTC) or at-home therapy which can be used by patients themselves.[3] The desensitizing agent is considered ideal when it is relatively painless, simple to apply, rapid in action with long-term effectiveness, with no discoloration of teeth and permitting proper occlusion of dentinal tubules without endangering the pulp.[4] Agents like novamin which are Bioactive glass release calcium and phosphate, thereby occluding dentinal tubules.[5] Cummins et al. stated that a combination of arginine and carbonate, when deposited on exposed root surfaces can physically block and seal the open tubules.[6] Recently irradiation with low-output lasers on affected teeth has been proposed due to their significant anti-inflammatory actions. Diode laser works by generating continuous waves without overheating.[7] Only a few studies are conducted on diode laser application to treat DH. Hence, the present study aims to evaluate the clinical efficacy of diode laser alone and together with desensitizing agents containing Novamin and Pro argin on the dentinal tubule occlusion (percentage of dentinal tubules) under a scanning microscope (SEM).

Abbreviations: DH, Dentinal Hypersensitivity; SEM, Scanning Electron Microscope; LT, Laser Therapy.

* Corresponding author.
E-mail addresses: hemadbds@gmail.com (H. Doppalapudi), anilkanc@gmail.com (A.K. Kancharla), anweshperio@gmail.com (A.R. Nandigam), sheema.tasneem@gmail.com (M. Sheema Tasneem), sivashankar.gummaluri@gmail.com (S.S. Gummaluri), dona.ultimate@gmail.com (S. Dey).

https://doi.org/10.1016/j.jobcr.2023.01.010
Received 26 April 2022; Received in revised form 10 January 2023; Accepted 12 January 2023

2. Methods

This study was conducted from January 2021 to July 2021 in the Department of Periodontology, Sree Sai dental college and research institute, Srikakulam, after obtaining approval from the ethical committee of the institute - SSDC&RI/IRB/IEC/2019–20/408/4/1.

3. Sample size calculation

Sample was estimated using the formula: N = 2 [Z1-α+ Z1-β]2 X σ2 / S - SO

σ = standard deviation (pooled)
S = real difference between 2 methods
SO = clinically acceptable margin

Based on this formula, the total sample size estimated was 70
and the final sample size taken in the present study was 75 with 15 samples per tooth.

3.1. Inclusion criteria

Healthy teeth without any abnormalities, extracted for orthodontic purposes were included in the present study.

3.2. Exclusion criteria

Teeth with decay, restorations, malformations, and fractures were eliminated.

Experimental Procedure: Before the initiation of the study protocol, all the natural teeth were color-coded based on groups. The complete armamentarium used for the in-vitro study can be seen in Fig. 1. Cervical cavities of 3mm length x 3mm width x 2mm depth were prepared using no. 2 round bur on the buccocervical region of extracted teeth and etched using 17% ethylenediaminetetraacetic acid for 2 mins and sonicated in water for 2 mins.

3.3. Randomization & study design

Randomization was done using computer-generated random numbers, for extracted teeth and divided into five treatment groups. The materials used for dentinal tubule occlusion were Sensodyne repair & protect dentifrice (Glaxo SmithKline, India), Colgate Pro-relief dentifrice (Colgate-Palmolive India Ltd.) and Zolar photon plus diode laser (Zolar technology and MFG Canada).

The current study includes five experimental groups, namely,

Group I (N) – NovaMin group
Group II (P) –Pro-Argin™ group

Fig. 1. Armamentarium used.

Group III (DL) – Diode laser group
Group IV (N + DL) – NovaMin + laser group
Group V (P + DL) –Pro-Argin™ + Laser Group

Control groups 1,2, were treated with their respective tooth paste, using cotton pellet with gentle firm rubbing motion, for 6 min a day for seven days and group 3 treated by diode laser in a noncontact mode for 60 s for seven days. The test groups (Groups 4& 5) were treated with their respective toothpaste followed by diode laser application. All specimens from each group were stored in artificial saliva which acts as a substitute for natural saliva, which was replaced after treatment of specimens every 24 h for 7 days.

3.4. Scanning electron microscope analysis

In the present study, after gold sputtering the scanning electron microscope evaluation was conducted in an analytical laboratory, at the University College of Technology, Osmania University, Hyderabad using SEM HITACHI S–3700N. All the specimens were observed under the SEM at 1000x, and 2000x magnification, with Extra High Tension (EHT) −10.00 kV.

3.5. Parameters evaluated

Two well-trained blinded reviewers, who were in complete agreement, assessed the scores of occluded dentinal tubules on a scale of 1–5, based on Davies et al., West et al., 2011 scoring system.[8,9]

Score 1: Completely Occluded (100%)
Score 2: Mostly but not completely occluded (50–<100%)
Score 3: Partially occluded/un-occluded (25–<50%)
Score 4: Mostly but not completely un-occluded (<25%)
Score 5: Completely un-occluded (0%).

The quantitative analysis of the SEM images (Fig. 2) was done at 1000x and 2000x magnification for the calculation of partial and complete mean occlusion of the dentinal tubules. The total occluded tubules percentage for each micrograph was measured using the following simple formula:

Percentage of partially or fully occluded tubules = the total number of occluded tubules (partially + fully occluded) x 100, divided by the total number of tubules.

3.6. Statistical analysis

Intra Class Correlation (ICC) estimation was done using statistical package for social sciences version 25 (SPSS Inc, Chicago, IL), a value is 0.840 was indicative of strong reliability among the examiners. Tukey's multiple Post hoc test was done for intragroup and intergroup comparison. $P < 0.05$ was considered to be statistically significant.

4. Results

Intergroup comparison of dentinal tubule occlusion(score wise) at 1000X can be seen in Table 1,3 and at 2000X in Table 2,3. Both Groups 1 and 2 demonstrated occlusion of the dentinal tubules with a smear layer and irregular deposits on dentin. The percentage of un-occluded tubules is high in these groups compared to diode laser groups. Group 3 showed significant occlusion of the dentinal tubules which was mostly partial. The percentage of partially occluded tubules is more in these groups due to the melting of dentin. The combination groups i.e. Groups 4, 5 showed statistically significant dentinal tubule occlusion compared to other groups. This may be due to crystalline re-arrangement of dentine in addition to occlusion by desensitizing agents. At both 1000X and 2000X magnification, laser groups showed better dentinal tubule occlusion than non laser groups. Compared to other groups, Group 4,5

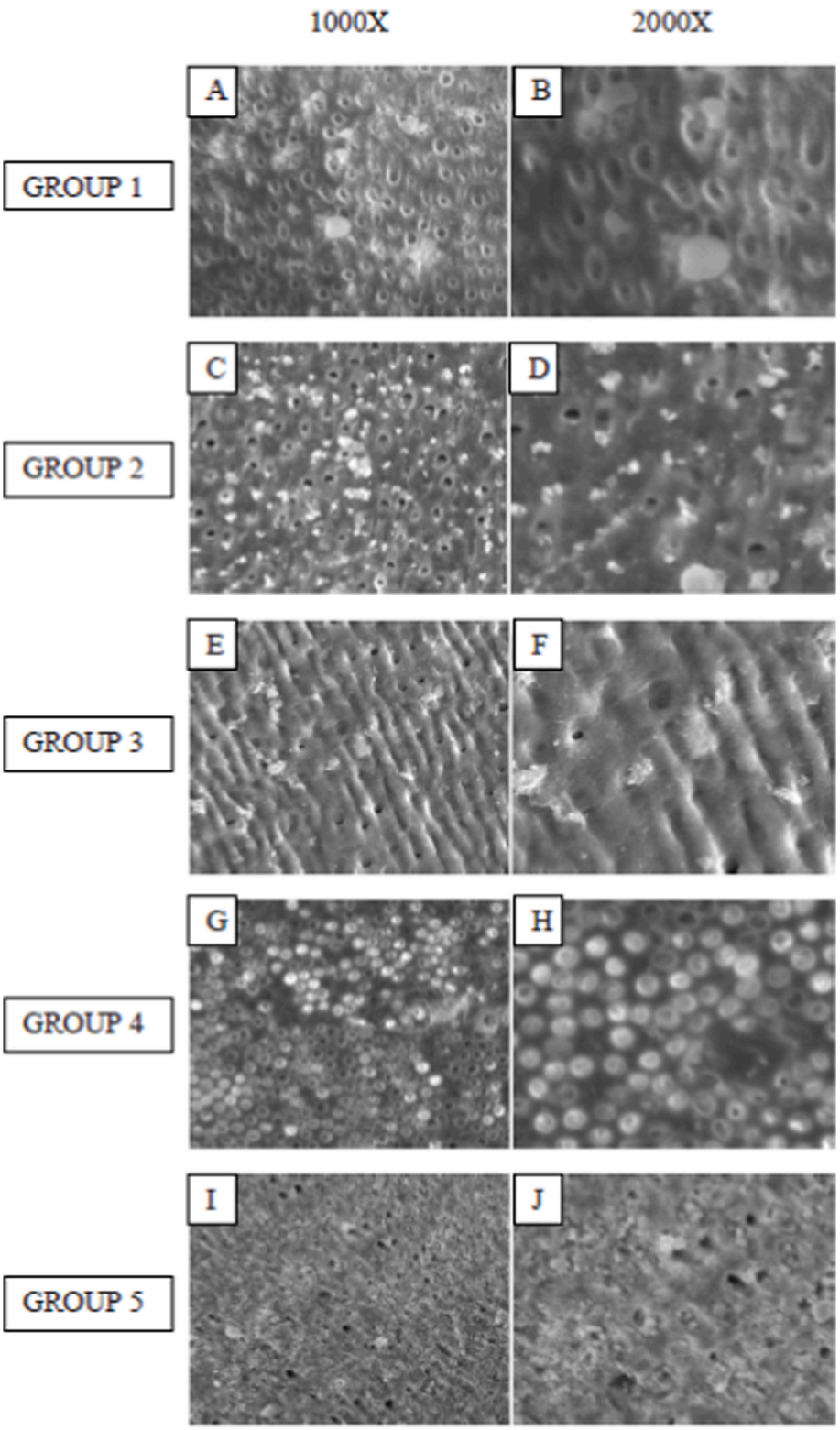

Fig. 2. Scanning electron microscopic images of Group 1,2,3,4,5 at 1000X, 2000X respectively
SEM images of Tooth samples. **A-B)** Samples of Group 1 showing occluded dentinal tubules due to deposition of toothpaste particles (×1000 and ×2000 respectively). **C-D)** Samples of group 2 dentinal tubules are not visible due to the deposition of minerals from the toothpaste (×1000 and ×2,000, respectively). **E-F)** Group 3 diode laser-treated dentin surface, melting leading to crystalline rearrangement of dentin, thereby occluding the dentinal tubules are seen in F (×1000 and ×2000, respectively). **G- H)** Novamin and diode laser-treated samples showing mostly occluded tubules 1000 and ×2,000, respectively). **I-J)** Pro argin and laser-treated groups showed obvious total closure of most of the dentinal tubules with few open dentinal tubules(x1000 and ×2,000, respectively).

showed the highest mean total dentinal tubule occlusion with statistically significance (see Table 3).

5. Discussion

Present study was the first of its kind conducted to evaluate dentinal tubule occlusion based on a scoring system in addition to total tubular occlusion by comparing desensitizing kinds of toothpaste to laser groups.

Dentin may be a porous, fluid-filled, mineralized tissue, but attrition, erosion, abfraction, and gingival recession contribute to loss of enamel and cementum causing hypersensitivity. Various theories have been proposed for explaining the etiology of dentinal hypersensitivity, among which hydrodynamic theory has been widely accepted. Wasim Bari in 2019 stated dentinal tubules are twice wider and more patent per unit area in hypersensitive dentin when compared to normal non-sensitive

Table 1
Mean Scores of dentinal tubule occl usion at 1000X magnification.

SCORES	Mean ± SD				
	N group	P Group	DL Group	N + DL group	P + DL group
Score 1	14.75 ± 1.72	10.13 ± 1.90	22.39 ± 6.23	36.80 ± 4.03	34.66 ± 5.21
P value					
N group	–				
P group	.030[a]	–			
L group	.000[a]	.000[a]	–		
L + N group	.000[a]	.000[a]	.00[a]	–	
L + P group	.000[a]	.000[a]	.00[a]	0.637	–
Score 2	27.10 ± 3.52	21.34 ± 3.24	48.46 ± 3.68	44.75 ± 3.47	40.52 ± 2.70
P value					
N group	–				
P group	.000[a]	–			
L group	.000[a]	.000[a]	–		
L + N group	.000[a]	.000[a]	.027[a]	–	
L + P group	.001[a]	.000[a]	0.000[a]	.008[a]	–
Score 3	31.88 ± 2.62	33.03 ± 7.32	17.46 ± 2.53	10.09 ± 1.60	14.94 ± 3.21
P value					
N group	–				
P group	0.932	–			
L group	.000[a]	.000[a]	–		
L + N group	.000[a]	.000[a]	.000[a]	–	
L + P group	.000[a]	.000[a]	0.426	.012[a]	–
Score 4	14.99 ± 5.80	27.02 ± 8.72	6.29 ± 2.39	5.14 ± 1.48	6.42 ± 1.65
P value					
N group	–				
P group	.000[a]	–			
L group	.000[a]	.000[a]	–		
L + N group	.000[a]	.000[a]	0.967	–	
L + P group	.000[a]	.000[a]	1.0	0.952	–
Score 5	10.66 ± 3.15	8.14 ± 1.80	6.03 ± 1.87	3.02 ± 0.90	3.36 ± 0.89
P value					
N group	–				
P group	.005[a]	–			
L group	.000[a]	.027[a]	–		
L + N group	.000[a]	.000[a]	.001[a]	–	
L + P group	.000[a]	.000[a]	.003[a]	0.988	–

Mean ± SD - Mean with standard deviationN group - Novamin groupP group - Pro argin groupDL group - Diode Laser groupN + DL group - Laser + Novamin groupP + DL group - Laser + Pro argin group.
[a] The mean difference is statistically significant at the 0.05 level.

Table 2
Mean Scores of dentinal tubule occlusion at 2000X magnification.

SCORES	Mean ± SD				
	N group	P Group	DL Group	N + DL group	P + DL group
Score 1	13.28 ± 1.82	10.73 ± 1.57	25.53 ± 5.80	39.54 ± 9.37	31.77 ± 5.08
P value					
N group	–				
P group	0.714	–			
L group	.000[a]	.000[a]	–		
L + N group	.000[a]	.000[a]	.000[a]	–	
L + P group	.000[a]	.000[a]	.023[a]	.002[a]	–
Score 2	26.52 ± 4.12	24.45 ± 6.06	27.82 ± 4.98	37.20 ± 6.25	44.74 ± 5.03
P value					
N group	–				
P group	0.825	–			
L group	0.964	0.426	–		
L + N group	.000[a]	.000[a]	.000[a]	–	
L + P group	.000[a]	.000[a]	.000[a]	.002[a]	–
Score 3	29.76 ± 2.09	32.49 ± 6.31	33.62 ± 5.71	13.75 ± 5.05	15.77 ± 6.81
P value					
N group	-				
P group	0.648	-			
L group	0.308	0.980	-		
L + N group	.000[a]	.000[a]	.000[a]	-	
L + P group	.000[a]	.000[a]	.000[a]	0.849	-
Score 4	19.62 ± 5.83	23.66 ± 7.80	8.75 ± 3.68	5.92 ± 1.83	4.14 ± 0.90
P value					
N group	-				
P group	0.147	-			
L group	.000[a]	.000[a]	-		
L + N group	.000[a]	.000[a]	0.48	-	
L + P group	.000[a]	.000[a]	0.07	0.84	-
Score 5	9.74 ± 2.87	7.89 ± 1.55	5.86 ± 1.88	3.86 ± 1.11	3.56 ± 1.18
P value					
N group	-				
P group	0.056	–			
L group	.000[a]	.027[a]	–		
L + N group	.000[a]	.000[a]	.032[a]	–	
L + P group	.000[a]	.000[a]	.009[a]	0.99	–

Mean ± SD - Mean with standard deviation.
N group - Novamin group.
P group - Pro argin group.
DL group - Diode Laser group.
N + DL group - Laser + Novamin group.
P + DL group - Laser + Pro argin group.
[a] The mean difference is statistically significant at the 0.05 level.

dentin.[10] Arnold in 2015 concluded that by deposition of an occluding layer on the dentinal surface or by placing occluding material into the tubules, hypersensitivity can be minimized.[11]

Orchardson and Collins (1987), considered premolars (37.8%) as primary sensitive teeth for DH followed by incisors (25.9%) and canines (24.6%).[12]

Various in vitro studies were conducted to analyze the mode of actions and desensitizing agent properties that included hydrostatic fluid filtration systems; attenuated total reflection Fourier transform infrared spectroscopy; energy dispersive X-ray analysis; confocal laser scanning microscopy and electron spectroscopy analysis.[1,13,14] The use of SEM in the present study was done because of its numerous advantages like a non-destructive approach, high-resolution, three-dimensional images, and topographical information.[15,16]

Kobayashi in 2010 stated that NovaMin (bioactive glass) as an inorganic chemical amorphous calcium sodium phosphosilicate (CSPS) (CaNaO6Psi) contains 45% SiO, 24.5% NaO, 24.5% CaO, and 6% PO.[17] Novamin during this study has shown a tremendous effect on dentinal tubule occlusion which was proved by previous in-vitro studies[18] and in vivo studies.[16,19] Zhu in his meta-analysis concluded Novamin® to be a better agent in reducing DH than negative controls.[20]

Table 3
Comparison of percentage of total occluded tubules between the groups at 1000x and 2000x magnification.

Magnification	Mean ± SD				
	N group	P Group	DL Group	N + DL group	P + DL group
1000 X	89.02 ± 2.80	91.70 ± 1.79	94.60 ± 1.76	96.85 ± 0.84	96.56 ± 0.86
P value					
N group	–				
P group	001[a]	–			
L group	.000[a]	.000[a]	–		
L + N group	.000[a]	.000[a]	.008[a]	–	
L + P group	.000[a]	.000[a]	.028[a]	0.991.	–
2000 X	90.34 ± 18.49	91.60 ± 1.54	94.71 ± 1.58	96.03 ± 1.16	96.22 ± 1.19
P value					
N group	–				
P group	0.175	–			
L group	000[a]	000[a]	–		
L + N group	.000[a]	.000[a]	0.143	–	
L + P group	.000[a]	.000[a]	0.067	0.997	–

Mean ± SD - Mean with standard deviation.
N group - Novamin group.
P group - Pro argin group.
DL group - Diode Laser group.
N + DL group - Laser + Novamin group.
P + DL group - Laser + Pro argin group.
[a] The mean difference is statistically significant at the 0.05 level.

Colgate Sensitive Pro-Relief contains arginine with physiological pH of 6.5–7.5 and acts by forming a charged complex that readily binds to the charged dentine surface and within the dentinal tubules.[6] In the present study, Group II (91.70%) showed more occluded tubules than Group I (89.02%) contradicting the results of invitro study by Chen et al. in which Novamin group showed better dentinal tubule occlusion.[21]

The current study used a diode laser at 810 wavelengths because it is one of the foremost commonly used wavelengths in dentistry.[22] The diode laser at 1 W power was used, which is considered safe for the pulp and seals the dentinal tubules.[23] Basic mechanism of a laser is by disarranging the crystalline arrangement due to thermochemical ablation, which induces melting of the dentin tissue, which is more intense when used at higher parameters.[23],[24]

Study done by Wasim Bari et al. showed that sturdiness of Diode Laser over Colgate Pro-relief (Pro argin) and Aclaim (nanocrystals of Hydroxyapatite), can be attributed to photothermal effects, heating and melting the surface hard tissue.[10] Shamel et al., 2022 evaluated nano-hydroxyapatite (n-Hap) and diode laser in dentinal tubule occlusion and stated that the combination therapy group showed more significant dentinal tubule occlusion than other groups.[25]

In the present study, combination therapy (Group IV, V) showed a greater percentage of dentinal tubular occlusion, thus enhancing the treatment efficacy when compared to other groups. These findings are incomitant with other studies.[18],[23],[25]

The limitations of this study were the small sample size, other aspects that were not within the scope of this present study, including identification of the composition of the products formed on the dentin specimens, analysis of the extent of the product's penetration deep into the dentinal tubules, and evaluation of permeability of the dentin. Future in vivo studies is required to confirm the present results.

6. Conclusion

Within the parameters of the study design, a combination of diode laser and Novamin & Pro-argin desensitizing toothpaste reported better results for dentinal tubule occlusion compared to other groups. Further, the use of the laser group as a desensitizing agent had shown significant dentinal tubular occlusion compared to Novamin & Pro-argin groups.

Declaration of competing interest

Nil.

Acknowledgements

None.

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