ORIGINAL RESEARCH

A SEM Evaluation of the Permeability of Different Desensitizing Methods on Occlusion of Dentinal Tubules: An *In-vitro* Study

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Abstract

Aim: The purpose of the current research was to assess the permeability of three various desensitizing techniques on dentinal tubule occlusion using a scanning electron microscope (SEM).

Materials and methods: For this research, 100 human sound premolar teeth that were extracted for orthodontic purposes were gathered. With the aid of a water-cooled diamond saw, the teeth were divided in a mesiodistal (vertically) orientation. A sectioned sample (5 mm long by 5 mm wide by 3.5 mm deep), including the cervical region, was taken from each buccal side. To fully open the dentinal tubules, these samples were then kept in 17% ethylenediaminetetraacetic acid (EDTA) for 40 minutes. The samples were divided into four groups ($n = 25$), each receiving the following dentin surface treatments: Group I: Control, Group II: Samples received NaF 5% varnish treatment, Group III: Samples received diode laser treatment, and Group IV: Samples received CPP–ACP treatment. An SEM was used to inspect each specimen at a magnification of about × 3000 and photomicrographs was assessed.

Results: The maximum occlusion of dentinal tubules was found in samples were treated with Diode laser (2.96 ± 0.14) followed by samples treated with NaF 5% varnish (3.38 ± 0.10), samples were treated with CPP–ACP (3.42 ± 0.06) and control group (4.26 ± 0.19). There was a statistically significant difference found between the groups.

Conclusion: In conclusion, all three desensitizing methods used in the present study were successful in the occlusion of dentin tubules. But the application of the Diode laser was effective in the reduction of dentin permeability compared to NaF 5% varnish and CPP–ACP.

Clinical significance: Dentin hypersensitivity (DH) is characterized by a brief period of intense discomfort. One approach to managing DH is to obstruct dentin tubules in order to decrease dentin permeability. There are many substances that can reduce hypersensitivity, but the finest commercially available substance for treating the condition by occluding the tubes should be acknowledged.

Keywords: Dentinal hypersensitivity, Dentinal tubules, Desensitizing methods scanning electron microscope.

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Introduction

Dentine sensitivity affects from 8 to 57% of adults and is a prevalent issue. People who have exposed and permeable dentine because of loss of tooth surface typically feel pain. Normal pain is caused by different exterior stimuli like mechanical, thermal chemical, mechanical, tactile, osmotic, or evaporative forces stimulating exposure of dentine and its tubules via pulpal nerves; this type of pain is not caused by any type of tooth pathology or defect.[1]

Dentinal hypersensitivity (DH) is defined as "Pain derived from exposed dentin in reaction to chemical, thermal, tactile, or osmotic stimuli that cannot be explained as originating from any other dental defect or disease". This is one of the most typical dental problems a dentist encounters consistently. They all communicate the same clinical representation and can be used interchangeably: Dentinal hypersensitivity, dentin hypersensitivity/cervical hypersensitivity/root hypersensitivity/cemental hypersensitivity/sensitivity.[2]

As per the hydrodynamic theory, dental hypersensitivity is believed to be caused by the fluid flow within the dentinal tubules, which is initiated by a stimulus and results in the activation of nociceptors in the region where the dentin and pulp meet. The hydrodynamic mechanism can therefore occur when dentinal tissue is exposed and the dentinal tubules are unobstructed, which is the primary cause of DH.[3]

There are two main approaches to treating dentin hypersensitivity: decreasing dentin permeability and reducing the inter-dental nerve's response to fluid shifts. There is a wide range of products available in the market that is designed to reduce DH by decreasing dentin permeability. Some of these products include laser therapy, the use of materials such as fluoride, hydroxyapatite, zinc chloride, potassium chloride, and strontium chloride, as well as dental adhesives, oxalate, glass ionomer cement, Portland cement, casein phosphopeptide-amorphous calcium phosphate, and bioglass.[4]

Treatment of hypersensitivity through tubule occlusion using chemical compounds is a widely recognized and effective method,

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and therefore it is important to identify the best available material for this purpose.[5] Therefore, the aim of this research was to contrast the effectiveness of three distinct desensitizing techniques on the occlusion of dentinal tubules using a scanning electron microscope.

Materials and Methods

The current research was conducted in the department of Periodontology, Kalinga Institute of Dental Sciences, Bhubaneswar, India. For this research, 100 human sound premolar teeth that were extracted for orthodontic purposes were gathered. The teeth were carefully cleaned, disinfected for one hour in a solution of 5% sodium hypochlorite, and kept in artificial saliva.

Preparation of Samples

With the aid of a water-cooled diamond saw, the teeth were divided in a mesiodistal (vertically) orientation (Buehler Ltd.). A sectioned sample (5 mm long by 5 mm wide by 3.5 mm deep), including the cervical region, was taken from each buccal side. Each piece was polished flat (600-grit) to remove enamel and reveal the cervical dentin region beneath. Diamond pastes (6 mm, 3 mm, 1 mm, and 14 mm) and 1000- and 1200-grit aluminum oxide abrasive paper were used to wet polish the exposed dentin surfaces in order to simulate dentin hypersensitivity in cervical areas. To fully open the dentinal tubules, these samples were then kept in 17% ethylenediaminetetraacetic acid (EDTA) for 40 minutes. The sample teeth were prepared, and the dentinal tubular opening that would duplicate hypersensitive dentin was scrutinized under a scanning electron microscope, then these samples were ultrasonically treated in distilled water for 12 minutes.

Samples Distribution to Different Desensitizing Methods

The samples were divided into four groups ($n = 25$) at random based on the dentin surface treatments that were used.

Group I: Control—A cotton brush was used to apply distilled water for 20 seconds.

Group II: Samples were treated with NaF 5% varnish—A fine layer of 5% NaF varnish was painted on the sensitive surface using a disposable micro brush for 60 seconds, following the manufacturer's recommendations. Varnish application was done on dentin for three consecutive days.

Group III: Samples were treated with a diode laser—GaAlAs Diode Laser (DENLASE, the Diode Laser Treatment System, from China Daheng Group, Inc.) was used to treat teeth. The laser was used with a 400 m fiber-optic handpiece, operating at 1 W, 200 m pulse lengths, and 200 m pulse intervals in a noncontact mode for 60 s. Dentin was exposed to a diode laser for three consecutive days.

Group IV: Samples were treated with CPP–ACP—The CPP-ACP paste (GC, Melbourne, Australia) agent was applied to the samples in this group twice daily (with a 12-hour delay of 3 minutes) for a week. All the samples were then stored in artificial saliva after each therapy session.

Evaluation of Occlusion of Dentinal Tubules using SEM

The samples were dried, affixed on metal stubs, and placed in a sputter coating machine after three days. For 10 minutes, gold at a 25 nm thickness was sputtered onto the samples. An SEM (Zeiss Sigma, Germany) was used to inspect all of the specimens at a magnification of about × 3000, and photomicrographs were analyzed to determine whether dentinal tubules were open in the control group and blocked in the desensitizing agent groups. The mean score of tubule occlusion by the two-blinded examiners was taken and used for statistical analysis, and the following criteria were applied to SEM images:[6]

- All tubules are occluded (100%)
- Most tubules are occluded (between 50 and 100% occluded)
- Tubules that are partially occluded (from 25 to 50%)
- Only 25% of the tubules are occluded
- Unoccluded (0 percent; no tubule occluded)

Statistical Analysis

SPSS Statistics for Windows Software, version 22 (IBM Corp., Armonk, NY, USA) was used to analyze the data. The average values of the parameters were determined based on the various stimuli. Analysis of variance (ANOVA) repeated measure test was used to assess the scores that were obtained. The level of significance was considered at 5%, i.e., $p < 0.05$ was deemed statistically significant in this research.

Results

Table 1 shows the mean exposed dentinal tubules before intervention. The mean dentinal tubular exposure of the control group was 4.32 ± 1.20, NaF 5% varnish was 4.63 ± 0.06, Diode laser was 4.68 ± 0.03 and CPP–ACP group was 4.46 ± 1.12. There was no statistically significant differences ($p < 0.001$) were noted between the groups.

Table 2 reveals the mean occlusion of dentinal tubules after the intervention. The highest dentinal tubule occlusion was found in samples that were treated with Diode laser (2.96 ± 0.14) followed by samples that were treated with NaF 5% varnish (3.38 ± 0.10), samples were treated with CPP–ACP (3.42 ± 0.06) and control group (4.26 ± 0.19). There was a statistically significant difference found between the groups.

Table 3 depicted the multiple comparisons of mean occlusion of dentinal tubules after intervention using Tukey's *post hoc* test. There was a statistically significant difference found between all the groups, except NaF 5% varnish and CPP–ACP group ($p > 0.001$).

The inference of the present study indicates that the application of the Diode laser was effective in the reduction of dentin permeability compared to NaF 5% varnish and CPP–ACP.

Table 1: Evaluation of the mean exposed dentinal tubules before intervention

Desensitizing methods	*n*	*Mean ± SD (μ)*	*Standard error*	*F-value*	*p-value*
Group I: Control	25	4.32 ± 1.20	0.0216	22.318	0.942
Group II: Samples were treated with NaF 5% varnish	25	4.63 ± 0.06	0.0459		
Group III: Samples were treated with Diode laser	25	4.68 ± 0.03	0.01011		
Group IV: Samples were treated with CPP–ACP	25	4.46 ± 1.12	0.0524		

Table 2: Evaluation of the mean occlusion of dentinal tubules after intervention

Desensitizing methods	*n*	*Mean ± SD (μ)*	*Standard error*	*F-value*	*p-value*
Group I: Control	25	4.26 ± 0.19	0.0334	**24.316**	**0.001***
Group II: Samples were treated with NaF 5% varnish	25	3.38 ± 0.10	0.0108		
Group III: Samples were treated with Diode laser	25	2.96 ± 0.14	0.0249		
Group IV: Samples were treated with CPP–ACP	25	3.42 ± 0.06	0.0618		

*Statistically significant

Table 3: Multiple comparative evaluation of mean occlusion of dentinal tubules after intervention using Tukey's *post hoc* test

Groups	*Compared with*	*Mean difference*	*Significance*
Control	NaF 5% varnish	0.88	**0.001*****
	Diode laser	1.3	**0.001***
	CPP–ACP	0.84	**0.001***
NaF 5% varnish	Control	–0.88	**0.001***
	Diode laser	0.42	**0.001***
	CPP–ACP	–0.04	0.794
Diode laser	Control	–1.3	**0.001***
	NaF 5% varnish	–0.42	**0.001***
	CPP–ACP	–0.46	**0.001***
CPP–ACP	Control	–0.84	**0.001***
	NaF 5% varnish	0.04	0.794
	Diode laser	0.46	**0.001***

*Statistically significant

Discussion

Dental hypersensitivity is a common clinical condition where the patient experiences exaggerated pain in response to certain stimuli. The location of the pain (buccal, lingual, palatal, or occlusal) does not change the diagnosis of dental hypersensitivity. Clinical signs of irreversible pulpitis, such as intense and persistent pain, are indicative of true hypersensitivity that can arise from pulpal inflammation, as opposed to the short, sharp pain associated with dentinal hypersensitivity.[7]

The permeable, liquid-filled, mineralized tissue known as dentin contains dentinal tubules that aid in penetrability. Loss of enamel and cementum due to attrition, erosion, abfraction, and gingival recession exposes dentinal tubules to the mouth environment, resulting in hypersensitivity.[8] The long-standing difficulty in treating painful teeth issues like dental hypersensitivity has led to a significant issue. It is necessary to develop effective therapy plans that can obstruct tubules quickly, permanently, and with resistance to the difficulties of the oral environment. Dentin hypersensitivity is presently treated with great success by occluding dentinal tubules.[9]

The samples treated with the Diode laser in the current research had the highest degree of dentinal tubule occlusion, followed by samples treated with NaF 5% varnish, samples treated with CPP-ACP, and samples from the control group. According to Alfredo et al.,[10] Hubbezoglu et al.,[11] and Secilmis et al.[12] studies stated that, the energy of the 980-nm diode laser absorbed by the dentin's mineral content increases when the power inputs are raised from 2 to 4 W in the continuous mode, leading to melting and crystalline arrangement. Due to the rapid sealing of exposed dentinal tubules and its safety for odontoblasts and pulp tissue, recent research advocates the use of a 980-nm diode laser at 1.0 W power parameter as an effective treatment option for DH in routine clinical practice. A power of 1.0 W is the recommended power parameter. Mehl et al.[13] recommended cautious application of the laser energy due to its damaging thermal effects on the pulp and surrounding structures. Pressure and temperature in the pulpal area can rise as laser energy density increases.

The reaction between NaF and the calcium ions in dentinal fluid, which results in the creation of calcium fluoride crystals, which are then deposited on the dentinal tubule openings, may be responsible for the gradually decreasing stimuli in NaF 5% varnish. Additionally, the number and width of dentinal tubules in hypersensitive-exposed areas have been shown to be higher than in normal dentin, which was in accordance with the study conducted by Liu and Lan.[14] This means that the patency of sensitive dentin hinders the action of therapeutic tubule-occluding agents and necessitates a longer course of treatment. The decrease in stimulus caused by NaF varnish is also consistent with research conducted by Ritter et al.[15] who noted a noticeable drop in DH between baseline measurements and 1, 2, 3, and 4-week NaF varnish examination intervals.

Kanaparthy and Aruna[16] used CPP-ACP in comparison to a placebo to treat tooth sensitivity. The results showed a substantial decrease in pain in the GC tooth mousse group. Similar outcomes were also observed in studies conducted by Saraf et al.[17] and Wang et al.,[18] which assessed the effectiveness of desensitizing toothpaste for the treatment of dental hypersensitivity using dentine tubule occlusion. They discovered that these agents could be helpful and used a method of evaluation similar to our research, the mean tubular diameter measurement.

One limitation of the present study is that it did not measure the depth of penetration of the agents into the dentinal tubules, which is an important factor for longer-lasting relief from DH. Also, the evaluation of the occlusion of dentinal tubules by the laser was not investigated comprehensively. The study only focused on the surface examination of the dentinal tubules after the application of the diode laser. Additionally, the study did not analyze the resistance of the occluded tubules to acid challenge. Further clinical research is necessary to evaluate each of these variables.

Conclusion

Despite having different chemical compositions and application methods, it can be concluded that all three desensitizing methods used in the present study were successful in the occlusion of dentin tubules. But the application of the Diode laser was effective in the reduction of dentin permeability compared to NaF 5% varnish and CPP–ACP.

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