Evaluation of the Efficacy of Strontium Chloride, Biodentine® and Biodentine® in Combination with Diode Laser in the Management of Dentinal Hypersensitivity- An *In vitro* SEM Study

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Abstract

Objective: To evaluate the efficacy of strontium chloride, Biodentine® alone and Biodentine® in combination with diode laser in the management of dentinal hypersensitivity using scanning electron microscope.

Materials and methods: This comparative *in vitro*, single blind study was carried out on 80 randomly selected extracted teeth. The selected 80 extracted teeth were categorized as: Group 1 - 20 teeth acid etched only; Group 2 - 20 teeth treated with strontium chloride; Group 3 - 20 teeth treated with Biodentine®; Group 4 - 20 treated with Biodentine® and diode laser. The samples underwent scanning electron microscope analysis.

Results: The qualitative analysis showed no occlusion of dentinal tubules in Group 1 (control group). Dentinal tubule occlusion was 91.2% in strontium chloride group, 81.3% in Biodentine® group and 80.0% in Biodentine® with diode laser group.

Conclusion: Within the limitations of this study it was concluded that Biodentine® alone showed better results than Biodentine® in combination with diode laser for dentinal tubule occlusion. However, when laser was used over Biodentine® in contact mode, the results showed Biodentine® to have a melted appearance, rather than the irregular fibrillar structures usually seen. This *in vitro* study was performed on extracted teeth which do not mimic the natural biological environment. Therefore, *in vivo* studies should be carried out to assess the potency of Biodentine® in occluding and sealing dentinal tubules and its potential for treatment of dentinal hypersensitivity.

Keywords: Dentinal hypersensitivity, Biodentine®, diode laser, scanning electron microscope

Introduction

Dentin hypersensitivity is a common condition characterised by transient tooth pain elicited by a variety of exogenous stimuli. There is substantial variation in the response to such stimuli from one person to another. Dentinal hypersensitivity is a painful clinical condition that affects 8-57% of the adult population, associated with the exposure of dentin to the oral environment. Clinical studies and questionnaires on dentinal hypersensitivity indicate a prevalence of 4-74% (Von Troil *et al.*, 2002).

Excluding sensitivity associated with tooth bleaching or other tooth pathology, the clinical cause of dentin hypersensitivity is exposed dentinal tubules as a result of gingival recession and subsequent loss of cementum on root surfaces (Dilsiz *et al.*, 2010). Clinical management of dentinal hypersensitivity is based on proper diagnosis,taking into account its severity, localized or generalized condition, elimination of other possible causes of pain, elimination or prevention of the causes.

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These include using an exploratory probe or jets of air from a triple syringe on the exposed surface to provoke a response from the patient. The degree of severity of pain can be quantified by means of a descriptive scale: slight, moderate or intense pain, or a visual analogue scale (VAS) from 0-10 (Gillamand Orchardson, 2006).

Treatment can be invasive in nature or non-invasive. Invasive procedures may include gingival surgery, application of resins, or a pulpectomy. Non-invasive treatment options are topical agents and dentifrices that contain a desensitizing active ingredient (Dilsiz *et al*., 2010).

Calcium silicate cements are used for sealing communications between the root canal system and the periodontium. Mineral trioxide aggregate (MTA) was developed and recommended for endodontic procedures because it is nontoxic, non-carcinogenic, non-genotoxic, biocompatible, insoluble in tissue fluids and dimensionally stable. MTA generates a greater frequency of dentin bridge formation than earlier materials. MTA also creates a biocompatible environment in periodontal tissues and can stimulate cementogenesis when used in the perforation area (Toptanci *et al*., 2013). To overcome the drawbacks of MTA, Biodentine® (Septodont, Saint-Maur-des Fossés, France) was introduced. In a case report by Firla (2012), Biodentine® induced mineralization after application. Mineralization occurs in the form of osteodentine by expressing markers of odontoblasts and increases TGF-β1 secretion from pulpal cells enabling early mineralization. The micromechanical adhesion of Biodentine® is caused by the alkaline effect during the setting reaction. The high pH causes organic tissues to dissolve out of the dentin tubules. The alkaline environment at the boundary area of contact between Biodentine® and hard tooth surface opens a path through which the dentin substitute mass can enter the exposed opening of the dentin canaliculi. This enables Biodentine® to be keyed to the dentine by means of innumerable microscopic cones, creating a stable anchorage with a sealing, bacteria-tight effect.

Previous studies have suggested that Biodentine® is bioactive because it increases proliferation of the pulp cell line OD21 and that it can be considered as a suitable material for clinical indications of dentine-pulp complex regeneration (Zanihi *et al*., 2012).

Lasers, through their ability to melt peritubular dentin, can occlude dentinal tubules partially or totally, and therefore reduce hypersensitivity symptoms. A 810nm diode laser was found to seal dentinal tubules to a far lesser degree, with negligible effects on desensitization (Gholami *et al*., 2011). Another study evaluating the effect of diode lasers (810 and 980 nm) at different parameters on dentinal surfaces found that these lasers used at 0.8 and 1 W for 10 seconds in continuous mode were able to seal the dentin tubules. These parameters can be considered safe for pulp vitality, and may be effective in the treatment of dentinal hypersensitivity (Monica *et al*., 2013). The application of a diode laser, with a wavelength between 655 and 980 nm, can accelerate wound healing through the facilitation of collagen synthesis, promotion of angiogenesis, and augmentation of growth factor release. Furthermore, diode lasers display *in vitro* bactericidal and detoxification effects (Harris and Yessik, 2004) and can prevent ablation of the root surface, which theoretically reduces the risk of normal root tissue removal (Caruso *et al*., 2008).

The present study was carried out to evaluate and compare the efficacy of strontium chloride, Biodentine,® and Biodentine® in combination with diode laser in the management of dentinal hypersensitivity and also to observe the effect of diode laser on Biodentine® under scanning electron microscope. According to our knowledge this is the first study to report the potency of Biodentine® to treat dentinal hypersensitivity with or without laser.

Material and methods

This comparative, *in vitro* single blind study was carried out on 80 randomly selected extracted teeth. Extracted teeth were randomly collected from the Outpatient section of Department of Oral and Maxillofacial Surgery, Rajarajeswari Dental College and Hospital, Bangalore, India. Premolar and molar teeth were included in the study. Teeth with root caries, fractured roots, and teeth with root surface caries or external resorption were excluded from the study.

The selected 80 extracted teeth were categorized as; 1) 20 teeth acid etched only, 2) 20 teeth receiving strontium chloride, 3) 20 teeth receiving Biodentine® and 4) 20 teeth receiving Biodentine® with diode laser. Extracted teeth were collected and then stored in 4°C distilled water containing 0.2% thymol to inhibit microbial growth until use. Whilst hydrated, a low-speed diamond wafering blade was used to remove a portion of root surface to expose the dentinal tubules. These tooth specimens were then divided into 4 groups with 20 in each group. All groups underwent acid etching with 5% citric acid. The control group was acid etched with 5% citric acid and was left without any application of desensitizing agents. The second specimen group was brushed with strontium chloride toothpaste (Sensoform, Indoco Remedies, Mumbai, India) for 14 days. After each application the toothpaste was left on the root surface for 2 minutes and then rinsed with distilled water (Kulal *et al.,* 2016). Biodentine® (Bioactive Dentin Substitute, Septodont, Saint-Maur-des Fossés, France) was applied to the root surface in the third specimen group. In the fouth group Biodentine® and diode laser (Soft Tissue Diode Laser, Zolar Photon Technology and Manufacturing, Mississauga Ontario, Canada) were used in combination on the

dentin blocks. After the Biodentine® was applied on the root surface, the diode laser (810nm) was used in contact mode at 90 joules for 1 minute on the root surface of tooth specimens. The Biodentine® was left on the tooth and removed immediately prior to analysis. The samples were stored in distilled water.

The specimens were dried and prepared for analysis by scanning electron microscopy (SEM). After drying, the tooth blocks were mounted onto aluminum stubs and subsequently coated with a thin layer of gold/palladium in a sputter coater. Photographs of the samples were obtained from the camera which was fixed to the SEM. The surfaces of the samples were scanned and examined using SEM at 3000X (Carl Zeiss Neon 40 Crossbeam, Germany). Standardized scanning electron micrographs were taken and converted into binary black and white images. The black pixels which represented the open dentinal tubules were counted and statistically evaluated.

Statistical Analyses

The following methods of statistical analysis were used in this study. The data were entered in Microsoft Excel and statistical analyses were performed using the Statistical Package for Social Science (SPSS ver 10.5) software. The normality of the data was accessed using Shapiro Wilk test. The null hypothesis for this test was that the data were normally distributed. One-way analyses of variance were used to test the difference between groups. Analysis of variance is a technique by which the total variation is split into two parts one between groups and the other within the groups. In the above test, p value less than 0.05 was taken to be statistically significant.

Results

Figure 1 shows the study design. *Figure 2* shows the mean dentinal tubules seen under SEM. *Table 1* shows the number of closed dentinal tubules in all groups. All three desensitising agents were able to achieve dentinal tubule occlusion, however in Group 1 (control group) there was no occlusion of the dentinal tubules (*Figure 2*). Dentinal tubule occlusion was found to be 91.2% in Group 2, 81.3% in Group 3 and 80.0% in Group 4 (*Table 2*). On comparison, statistically significant differences were seen between Group 2 and 3, and between Group 2 and 4. However there was no statistical difference between Group 3 and 4. *Figure 3* shows mean percentage of completely occluded tubules. When comparing desensitizing agents, it was observed that there was a statistically significant difference among the 3 agents used (F=5194.3, $p< 0.001$; *Table 3*). There was a statistical significant difference between Group 2 and 4 ($p= 0.001$). Furthermore, as the mean difference between Group 2 and 4 was 0.13, Group 2 was more efficacious compared to Group 4 (*Table 3*). There was no statistical significant difference between Group 3 and 4 ($p=0.753$) in dentinal tubule occlusion (*Figure 3*) and in mean percentage of completely occluded tubules (*Table 3*; *Figure 4*).

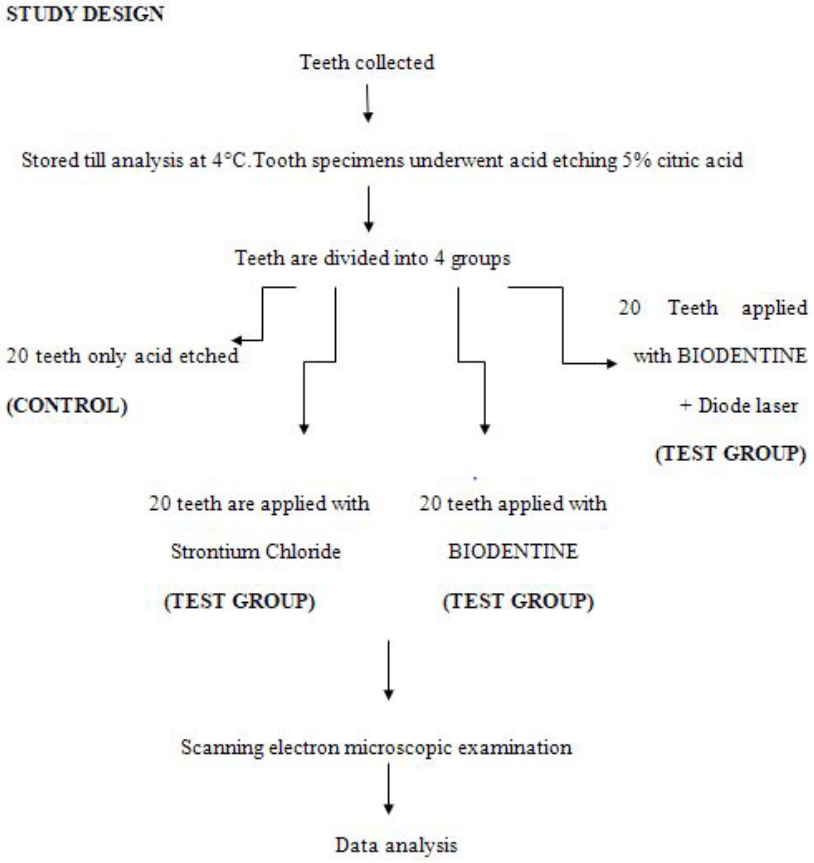

Figure 1. Study design.

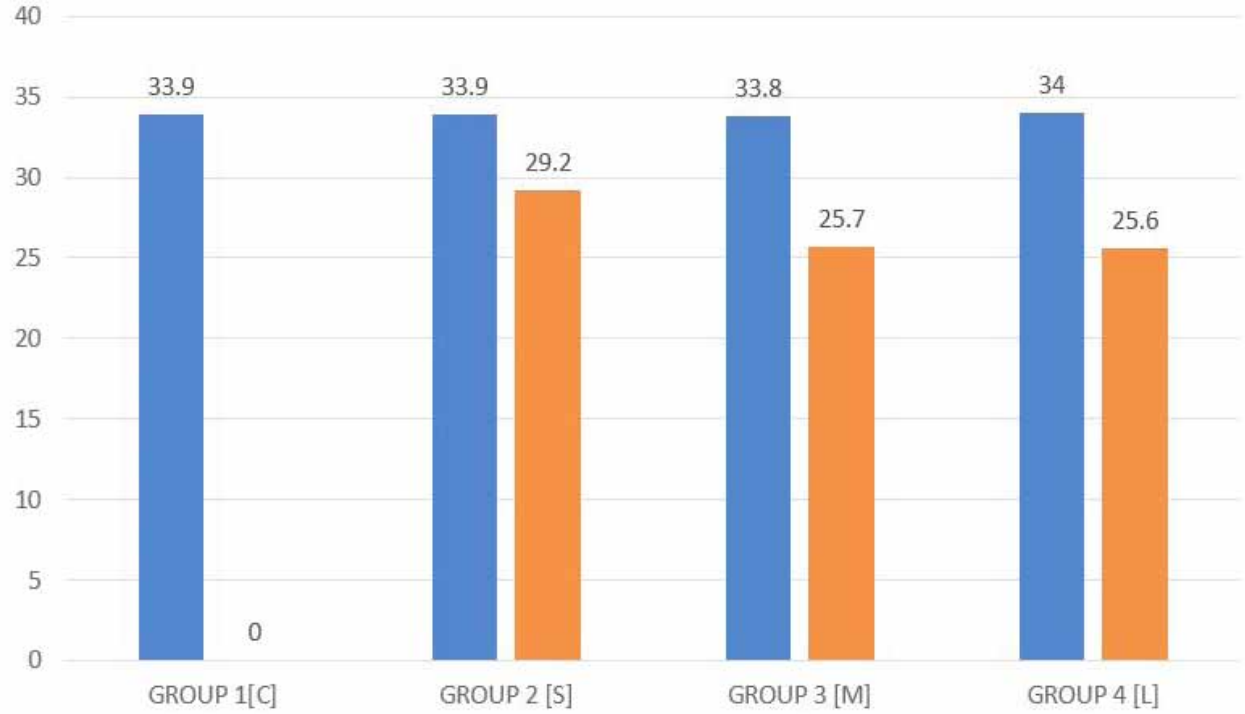

Figure 2. Comparison of mean open and closed dentinal tubules among the study groups.
The blue bars represent the number of open dentinal tubules after the removal of smear layer and red bars represent the closed number of closed dentinal tubules after treating the tooth sections with different agents.

Table 1. Number of closed dentinal tubules in all the groups

	N	Mean	SD	Min.	Max.	F value	*p* value
Group 1 (C)	20	0.0	0.000	0	0	3922.367	***<0.001***
Group 2 (S)	20	29.2	0.894	28	31		
Group 3 (M)	20	25.7	1.089	23	27		
Group 4 (L)	20	25.6	1.317	23	28		

C = Control group
S = Strontium chloride group
M = Biodentine® group
L =Biodentine® and diode laser group
SD = Standard deviation
$p<0.05$ is significant

Table 2. Percentage of completely occluded tubules in test the groups

	N	Mean	SD	Min.	Max.	F value	*p* value
Group 2 (S)	20	86.4	3.184	80.6	91.2	5194.333	***<0.001***
Group 3 (M)	20	76.0	2.524	71.4	81.3		
Group 4 (L)	20	75.2	2.834	71.4	80.0		

S = Strontium chloride group
M = Biodentine® group
L =Biodentine® and diode laser group
SD = Standard deviation
$p<0.05$ is significant

Table 3. Percentage of probit in the test groups

	N	Mean	SD	Min.	Max.	F value	*p* value
Group 2 (S)	20	1.224	0.543	0.2	2.0	95.636	***<0.001***
Group 3 (M)	20	-0.552	0.430	-1.3	0.3		
Group 4 (L)	20	-0.685	0.483	-1.3	0.1		

S = Strontium chloride group
M = Biodentine® group
L =Biodentine® and diode laser group
SD = Standard deviation
$p<0.05$ is significant

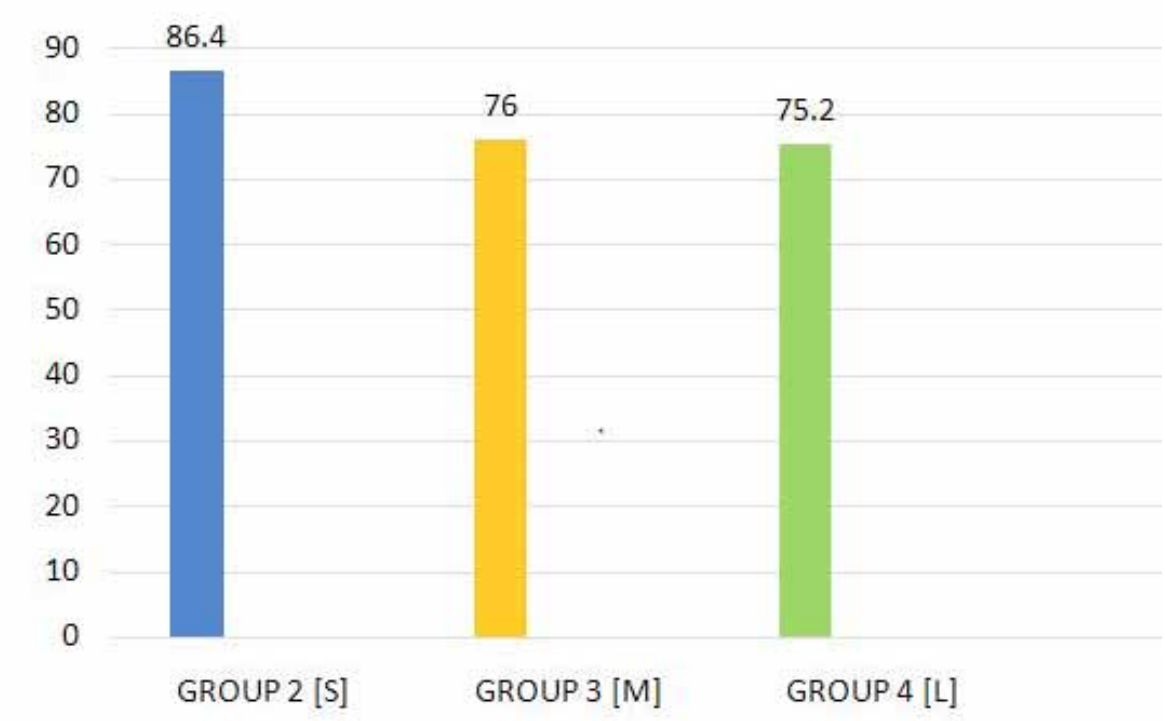

Figure 3. Comparison of mean percentage of completely occluded tubules in the test group.

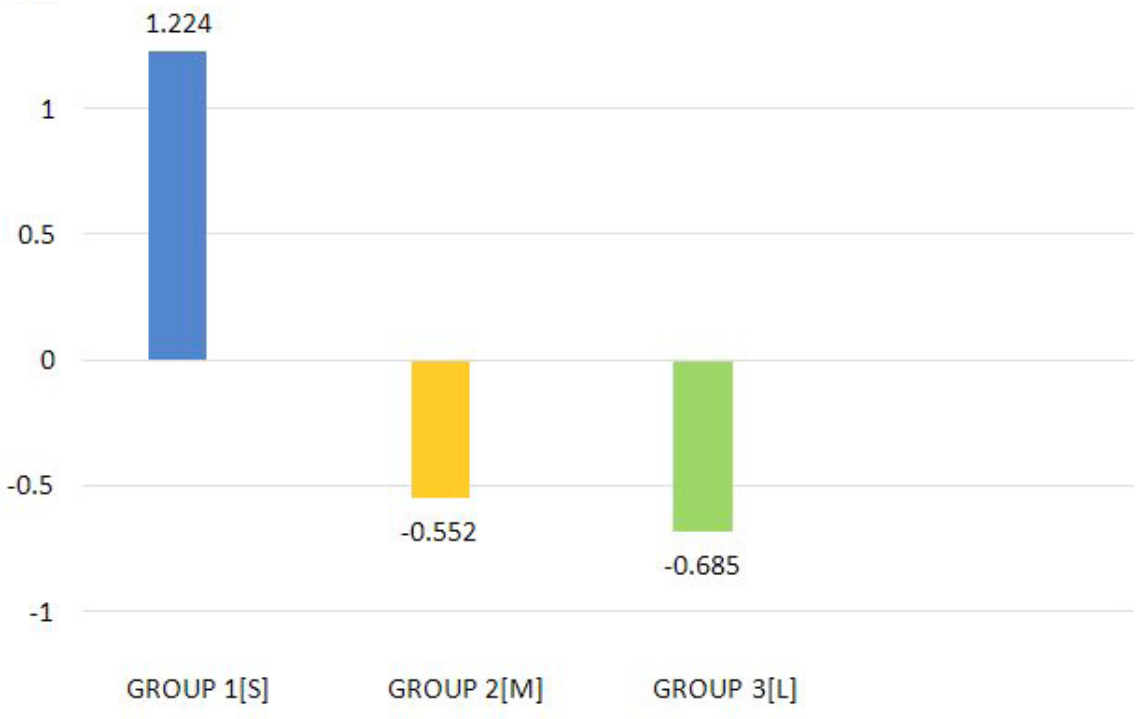

Figure 4: Comparison of mean percentage of completely occluded tubules in the test group

The SEM micrographs showed both open and closed dentinal tubules. In *Figure 5* (control group), only open dentinal tubules are visible. In *Figure 6*, the strontium chloride group, most dentinal tubules appeared to be partially and completely closed. In *Figure 7*, the Biodentine® group, many dentinal tubules appeared to be covered by Biodentine® crystals, however a few tubules appeared open. In *Figure 8*, the micrograph of Biodentine® with diode laser in contact mode, we can see a melted like appearance of the crystals of Biodentine.® Many open dentinal tubules were seen and their numbers was greater than compared with the Biodentine® group.

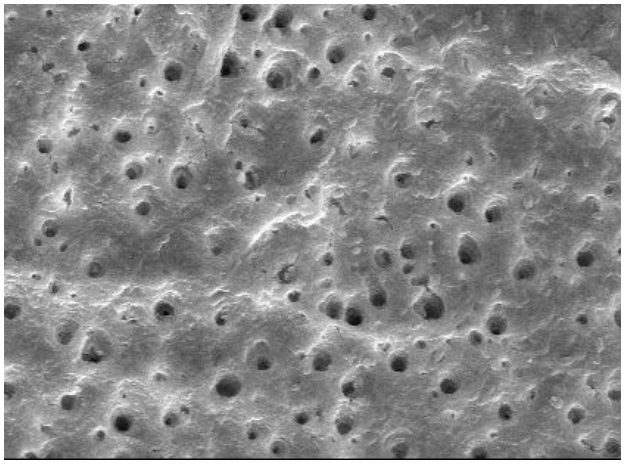

Figure 5. SEM image of control group.

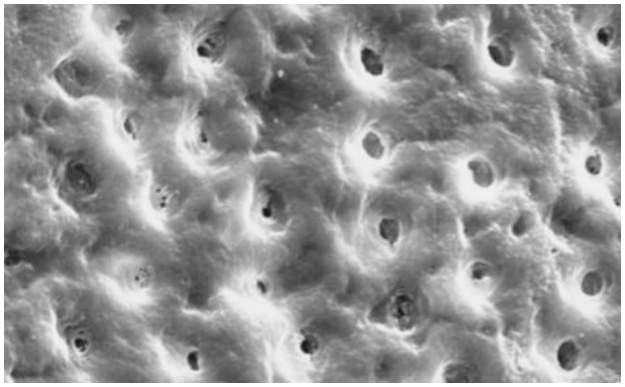

Figure 6. SEM image of strontium chloride group.

Figure 7. SEM image of Biodentine® group.

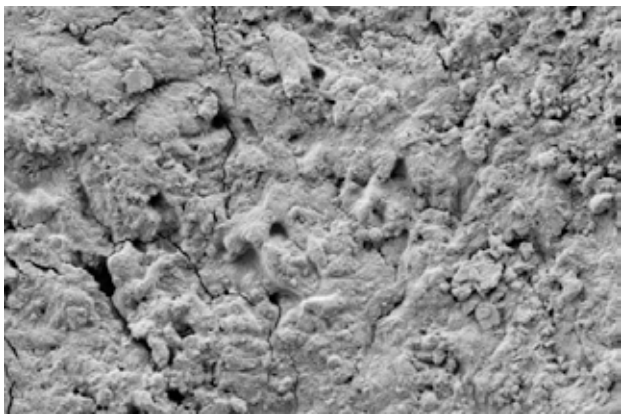

Figure 8. SEM image of Biodentine® and diode laser group.

Discussion

Dentin hypersensitivity (DH) is a sharp, localized and short pain in response to thermal, chemical, mechanical or osmotic stimuli, ceasing after the stimulus removal. The etiology is multifactorial and the factors involved are unclear (Addy *et al*., 1997).

In this study we compared the occlusion of dentinal tubules using strontium chloride, Biodentine® with and without laser. Strontium chloride was most effective in occluding dentinal tubules, followed by Biodentine® group and then Biodentine® with diode laser.

Mineral trioxide aggregate (MTA) is a bioactive material composed of Portland cement and bismuth oxide. Many investigations have shown that MTA can induce hard tissue formation,and previous studies have shown the effect of MTA on cementoblasts and odontoblasts (Kim *et al*., 2008; Paranjipe *et al*., 2010; Tani-Ishii *et al*., 2007).

MTA also creates a biocompatible environment in periodontal tissues and can stimulate cementogenesis when used in perforation areas. Previous studies have examined the effects of MTA *in vitro* on the proliferation of oral keratinocytes and cementoblasts, and compared White MTA (WMTA) with gray MTA (GMTA). It was found that cementoblast proliferation significantly increased when grown on the surface of WMTA, compared with cementoblasts grown on GMTA (Oviir *et al*., 2006). Cementoblasts can attach to the surface of MTA and proliferate. Cementoblasts also express mineralized matrix genes when cultured on MTA (Paranjipe *et al*., 2010; Thomson *et al*., 2003).

Biodentine® induces early mineralization by increasing the secretion of TGF-β1 from pulpal cells after application (Laurent *et al*., 2010). It also acts by stimulating odontoblasts and cell differentiation, thereby facilitating reactionary and tertiary dentin formation. In another study using Biodentine,® results showed that Biodentine® can induce immortalized murine pulp cell differentiation into odontoblast like cells and stimulate bio mineralization (Zanihi *et al*., 2012). Biodentine® formed a layer over the open dentinal tubules on root surfaces in our study.

When Biodentine® was viewed under scanning electron microscope, needle-like crystals with an apatitic appearance were seen. In the group with Biodentine® some open dentinal tubules were seen during SEM analysis in a few samples. This may be due to faults during application or processing.

In our study, Biodentine® and diode laser were used in combination on the root surface where dentinal tubules had been exposed after the smear layer was removed. After the Biodentine® was applied onto the tooth, the diode laser was used in contact mode at 90 joules for 1 minute on each root block. Lasers, through their ability to melt peritubular dentin, can partially or totally occlude dentinal tubules, and therefore reduce patients' symptoms of hypersensitivity. Currently, several studies have reported that the application of 980nm diode laser could be used safely in endodontic treatment and in root canal disinfection (Faria *et al.*, 2013; Marchesan *et al.*, 2008). However, few studies have reported on the interaction of 980nm diode laser energy with the dentin surface and the ensuing structural alterations. More studies are required to determine whether the 980nm diode laser can treat dentine hypersensitivity effectively, similar to other types of lasers (Liu *et al.*, 2013).

In a similar study a galium: aluminum: arsenide (GaAlAs) diode laser used was a 970nm wavelength laser with an optical fiber (200 mm diameter). The specimens were irradiated with a frequency of 10 Hzincontactmode for 30 seconds (Fabio *et al.*, 2016). Results showed good dentinal tubule occlusion. In another study,specimens weretreated with a 980 nm GaAlAs diode laser (0.5W, non-contact mode with a distance of 2-4 mm and using a fiber of 320μm diameter) for 60 seconds (Rajeswari *et al.*, 2015). In a similar study using a diode laser in continuous, tangential or noncontact modes (the distance between the optical fiber and the irradiated surface was 1mm), delivered energy densities per second 2547, 3184, 5092, and 6366J/cm^2 for the following output power settings: 0.8, 1, 1.6, and 2 W. The optical fiber diameter was 200 μm. Irradiation speed in the study was 1mm/sec(Monica *et al.*, 2013).

Many investigations have been carried out focusing on the effectiveness of the use of diode lasers for dentinal hypersensitivity. Matsumoto *et al.* (1985) showed an 85% improvement in teeth treated with laser. Yamaguchi *et al.* (1990) noticed an effective improvement index of 60% in the group treated with laser compared to 22.2% in the control non-lased group. In another study results showed an improvement of 69.2% in the group treated with laser compared to 20% in the placebo group (Kumazaki *et al.*, 1990).

Several studies have described a synergistic action of lasers in association with desensitizing agents. These studies demonstrated that lasers can improve the permanence of the desensitizer for longer time than when they are used alone. For this reason, if a laser device is used in addition to a conventional desensitizing agent, the latter remains above the tooth surface for 60 seconds before the irradiation (Kumar and Mehta, 2005; Lan *et al.*, 1999; Pesevska *et al.*, 2010).

When a laser was used over Biodentine® in contact mode and the root blocks analyzed by SEM, our results showed a melted appearance of the Biodentine® rather than the irregular fibrillar structures usually seen. This melted appearance could possibly ensure better sealing and complete closure of the exposed dentinal tubules. However, in the group with laser and Biodentine® the results showed reduced dentinal tubule occlusion when compared with the Biodentine® group. This could be because a portion of Biodentine® might have been removed when the laser was used in contact mode, and also because of the surface alterations produced due to laser use because of which some dentinal tubules were seen open when viewed under SEM.

Conclusion

Within the limitations of this study it was concluded that Biodentine® alone showed better results than Biodentine® in combination with diode laser in dentinal tubule occlusion. However, when the laser was used over Biodentine® in contact mode, results showed a melted appearance of the Biodentine® rather than the irregular fibrillar structures usually seen. This *in vitro* study was performed on extracted teeth which do not mimic the natural biological environment. However, *in vivo* studies should be carried out to prove the potency of Biodentine® in occluding and sealing dentinal tubules so that it can be used in treating dentinal hypersensitivity.

Conflict of interest

Authors state that there is no conflict of interest.

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