Scanning electron microscopic (SEM) comparison of dentinal tubule occlusion effected by a dentifrice containing NovaMin to that of diode laser (810nm)

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Abstract

Introduction: Dentin hypersensitivity (DH) due to exposed dentine is common among periodontitis patients. The present study compares & evaluates the dentinal tubule occlusion achieved with NovaMin® containing dentifrice & 810 nm diode laser by means of SEM.

Materials and Methods: Sixty human premolar dentin discs of 2mm thickness were sanded with wet 600-grit carborundum paper and treated with 17% EDTA. Specimens were allotted to three groups of twenty each: Group 1 - Control group, Group 2 –NovaMin® containing dentifrice application, Group 3 - lased with 810 nm diode laser. The proportion of completely occluded tubules and of partially occluded tubules to the total tubules (CO/TT & PO/TT) in the three groups were calculated, and analyzed.

Results: Both NovaMin® and Laser demonstrated more complete and partial occlusion of tubules than control group. The incidence of complete occlusion was more with NovaMin®, but that of partial occlusion was similar to diode laser group.

Conclusions: Although dentinal tubules occlusion was effective by both NovaMin®& diode laser (810 nm), NovaMin® produced a more complete occlusion of dentinal tubules.

Keywords: Dentin hypersensitivity, Diode laser, Dentinal tubule occlusion, Scanning electron microscope.

Introduction

Tooth hypersensitivity is a common problem that affects many adults worldwide. Dentin hypersensitivity (DH) prevalence ranges from 4 to 74%, subject to population, study setting and study design. In periodontal patients, DH has been stated to be between 72.5-98%. Periodontal diseases & periodontal treatment cause DH which leads to decreased plaque control, especially during post-operative period which in turn affect treatment outcome/results.

According to hydrodynamic theory, dentin hypersensitivity is due to fluid movement in dentinal tubules. In hypersensitive dentin, tubules will be more in number & also wide open which facilitates more fluid movement. So, by occluding the dentinal tubules, this hydrodynamic mechanism can be blocked to reduce DH.

NovaMin® is a bioactive glass-ceramic material containing calcium sodium phosphosilicate that deposit a hydroxy carbonate apatite (HCA) layer on dentin surface over a period of time. This layer along with residual NovaMin® particles block the dentinal tubules and relieves DH. The diode laser achieves desensitization by melting the dentin tissue. Applying an 810 nm diode laser caused melting and narrowing of tubules at 0.8-1.6 W, with total occlusion occurring at 2 W output.

This study aims to evaluate and compare using SEM, the effectiveness of a dentifrice containing NovaMin® and diode laser (810 nm) in dentinal tubule occlusion.

Materials and Methods

The protocol was approved by the author’s institutional review committee (IEC/M/10/2015/DCK). In the present invitro study, teeth collection & preparation were carried out at Government dental college, Kottayam & SEM study was done at department of Chemical Science, M.G. University, Kottayam. Clinically sound premolars [orthodontic extractions], were collected and kept in 10% formalin at normal temperature. Teeth with caries, restorations, or fractures were discarded.

The crown at the cemento-enamel junction (CEJ) and the apical 2/3 of all teeth were trimmed perpendicular to the long axis using a dental model trimmer with carborundum disc, under continuous water flow. A total of 60 dentin discs were made of approximately 2-mm thickness. The exposed dentin surfaces were sanded for 30 seconds with 600-grit wet carborundum paper to create a standard smear layer. This smear layer was then removed by a two minute application of 17% Ethylenediaminetetraacetic Acid (EDTA) followed by ultrasonic cleaning in distilled water for 1 min. The specimens were randomly allotted to three groups of 20 each.

Group 1: Surface treatment using distilled water was done for control group
Group 2: The specimens were swabbed with NovaMin® containing dentifrice [undiluted form weighing about 1gm], and after two minutes were lightly rinsed off.
Group 3: The specimens were lased with the 810 nm diode laser.

A 400-µm optical fiber was applied tangentially at a 45-degree angle at 1mm/sec speed and a power output of 2 W for 10 seconds in a non-contact mode [1 mm gap between laser tip and surface].

Scanning Electron Microscopy

After air-drying the specimens at room temperature for one day, they were mounted and placed in an ion sputtering device. A thin film of gold was sputter coated superficially on specimens in vacuum for 5 minutes to ensure a acceptable conductive surface for the specimens for the
SEM. Specimen surfaces were scanned and noted on the fluorescent screen at 3000 times magnification (at 6 kV), and the snapshot were taken (Fig. 1). The counts of completely occluded (CO) tubules, partially occluded (PO) tubules & total number of tubules (TT) were done in each of the photograph.

The type of dentinal tubule occlusion was assessed as follows:
1. Those showing complete penetration or canal obliteration with the crystal or resultant products were noted as completely occluded
2. Those showing a reduction in half of the diameter or a circumferential closure but with a patent canal opening were considered partially occluded.

Statistical Analysis
The ratio of completely occluded to total tubules [CO/TT] and partially occluded to total tubules [PO/TT] were calculated. Since the data was not having normal distribution, non-parametric tests like Kruskal-Wallis test [comparing all three groups] and the Wilcoxon rank-sum test [intergroup comparison] were used; under (i) CO/TT ratio and (ii) PO/TT ratio; and statistical significances were calculated (Table 1). The mean of CO/TT ratio as well as PO/TT ratio for each group were plotted (Figure 2 & 3). All analyses were done using SPSS 16.0 software.

Results
Mean of completely occluded to tubules (CO/TT) ratio was higher for NovaMin® compared to other groups, which was statistically significant (p-value < 0.05). Laser group showed (CO/TT) ratio lesser than NovaMin®, while control group demonstrated the least value [Fig. 2].

Mean of Partially occluded to total tubules (PO/TT) ratio showed no statistical difference (p-value 1.00) between laser group & NovaMin® group, while both groups showed higher mean than control group with significant statistical difference (p-value < 0.05). [Fig. 3, Table 1]

Table 1: Inter and multiple group comparison of Control, NovaMin® and Laser.

<table>
<thead>
<tr>
<th>Group</th>
<th>No: of Specimen</th>
<th>Mean ± SD</th>
<th>Mean Rank</th>
<th>Kruskal-Wallis Test</th>
<th>Wilcoxon Rank Sum Test</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control (1)</td>
<td>20</td>
<td>0.00 ±0.00&lt;sup&gt;A&lt;/sup&gt; 0.01 ± 0.02&lt;sup&gt;B&lt;/sup&gt;</td>
<td>12.00&lt;sup&gt;A&lt;/sup&gt; 10.60&lt;sup&gt;B&lt;/sup&gt;</td>
<td>Group 1, 2 &amp; 3&lt;sup&gt;*&lt;/sup&gt;</td>
<td>Group 1 &amp; 2&lt;sup&gt;<em>&lt;/sup&gt; Group 1 &amp; 3&lt;sup&gt;</em>&lt;/sup&gt; Group 2 &amp; 3&lt;sup&gt;NS&lt;/sup&gt;</td>
</tr>
<tr>
<td>Novamin (2)</td>
<td>20</td>
<td>0.25 ± 0.17&lt;sup&gt;A&lt;/sup&gt; 0.17 ± 0.07&lt;sup&gt;B&lt;/sup&gt;</td>
<td>46.38&lt;sup&gt;A&lt;/sup&gt; 40.62&lt;sup&gt;B&lt;/sup&gt;</td>
<td></td>
<td></td>
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<tr>
<td>Laser (3)</td>
<td>20</td>
<td>0.09 ± 0.10&lt;sup&gt;A&lt;/sup&gt; 0.18 ± 0.09&lt;sup&gt;B&lt;/sup&gt;</td>
<td>33.12&lt;sup&gt;A&lt;/sup&gt; 40.28&lt;sup&gt;B&lt;/sup&gt;</td>
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</table>

As the data was not normally distributed non-parametric Kruskal-Wallis test was used to compare all three groups while another non-parametric Wilcoxon rank-sum test was used for comparison of each group with the other.

SD: Standard Deviation.
<sup>A</sup> Ratio of completely occluded and total tubules,
<sup>B</sup> Ratio of partially occluded and total tubules.
* Significant difference (p-value ≤ 0.05)
NS Not Significant (p-value is 1.00)

Fig. 1: SEM micrograph (X 3000) of control, NovaMin® & Diode laser groups respectively
Abhilash Antony et al.

Scanning electron microscopic (SEM) comparison of dentinal tubule occlusion effected...

**Fig. 2:** Comparison of tubular occlusion (CO/TT ratio)

The bar graph depicts the mean ratio of the number of completely occluded tubules to the total number of tubules. Control group, NovaMin group and laser group showed Mean ± SD: 0.00 ± 0.00, 0.25 ± 0.17, 0.09 ± 0.10 respectively. The mean value is highest for the NovaMin group, which indicates more completely occluded tubules than the other groups.

**Fig. 3:** Comparison of tubular occlusion (PO/TT ratio)

The bar graph depicts the mean ratio of the number of partially occluded tubules to the total number of tubules. Control group, NovaMin group and laser group showed Mean ± SD: 0.01 ± 0.02, 0.17 ± 0.07, 0.18 ± 0.09 respectively. The mean value is highest for the Laser group, which is almost similar to NovaMin group; indicates both had more partially occluded tubules than the controlled group.

**Discussion**

There are two principal methods of treating dentin hypersensitivity, a) to occlude dentinal tubule, thereby blocking the hydrodynamic mechanism, b) to block neural transmission at the pulp. The majority of treatments aim at occluding the dentinal tubules to relieve hypersensitivity.

Present study compared ratio of dentinal tubule occlusion among dentinal disc samples treated with NovaMin®, diode laser & control groups. Results of present study can be summarized as: Both NovaMin® & 810 nm diode laser demonstrated more CO & PO tubules than control group (p-value <0.05). NovaMin® showed significant difference with Laser group when CO/TT ratio was compared (p-value is 0.041, which is < 0.05). But no significant difference was observed between NovaMin® & Laser group in comparison of PO/TT ratio (p-value = 1.00). This indicates NovaMin® is superior to diode laser in terms of dentinal tubule occlusion.

In-vitro studies by Joshi S et al.,13 & Gupta AK et al.,14 & Bakri MM et al.,10 are in accordance with present study. This is also in accordance with the findings of clinical studies by Litkowski & Greenspan15 and Du Min Q et al.,5 who found NovaMin® to be a more effective desensitizer.
Based on the meta-analysis results by Mengjiao Zhu\textsuperscript{16} in 2015, NovaMin\textsuperscript{®} was more effective than negative controls in relieving DH, used either as a toothpaste or as a prophylaxis paste. 810 nm diode laser application is an in-office treatment. Expensive equipment & accessories (e.g., protective goggles) and experienced personnel are required. Usually in-office treatments are complicated and expensive and can be used for treating a limited number of teeth. In-office desensitizing therapy are supposed to provide an immediate relief for DH symptoms. Although biostimulatory action of Diode laser is well confirmed, its action on occlusion of dentinal tubules is still controversial. Present study attempts to find scope of diode laser application to achieve tubular occlusion.

The present study indicates 810 nm diode laser could occlude dentinal tubules partially comparable to NovaMin\textsuperscript{®}. But complete occlusion of dentinal tubules is relatively rarer. This result is in accordance with Asnaashari M’s review article,\textsuperscript{17} which states that, for a laser in order to actually alter the dentin surface, it has to melt and resolidify the surface which should effectively close the dentinal tubules. But our study shows that this does not occur.

Parameters for 810-nm diode laser in present study is almost similar to study by Gholami GA et al.,\textsuperscript{18} except that pulsed mode was used in his study. Mean ± SD value of tubular diameter (µ) for 810nm diode laser (3.28 ± 1.05) compared to before laser irradiation (3.53 ± 1.04) showed diode laser caused a lesser degree of tubular occlusion. According to Kumazaki et al.,\textsuperscript{19} diode laser group demonstrated 69.2% improvement while placebo group showed only 20%.

In a study by Yilmaz HG et al.,\textsuperscript{20} comparing the desensitizing effects of a gallium-aluminum-arsenide (GaAlAs) laser (8.5 J/cm\textsuperscript{2} energy density) and sodium fluoride (NaF) varnish, the authors found diode laser irradiation to be more effective, faster & more comfortable in office procedure than traditional DH treatment. Another study\textsuperscript{17} indicated that the reduction in DH could be the result of alteration of physically occluding the dentinal tubules.

An in vitro study by Umana et al.,\textsuperscript{11} showed a narrowing of the dentinal tubules when using a 810 nm diode laser at 0.8–1.6 W and a diode laser (980 nm) at 0.8–1 W power. The dentin showed melting areas and a total occlusion of tubules with 810nm diode laser at 2 W and 980 nm diode laser at 1.6 – 2 W power. The authors reported that diode lasers at a wavelength of 810 and 980 nm when used in a continuous mode at power of 0.8W and 1W occluded the dentin tubules with no relevant pulpal damage. Present study (in vitro), analysed the effect of NovaMin\textsuperscript{®} & Diode laser (810nm) in dentin tubule occlusion, using a reproducible model such as the dentin disc, which helped to understand about the potential occluding, and thus desensitizing properties of 810 nm diode laser & NovaMin\textsuperscript{®} containing dentifrice. Some limitations of this in-vitro study were, it cannot simulate oral environment, didn’t measured thermal effect of laser on pulp, smaller sample size etc.

**Conclusion**

Both NovaMin\textsuperscript{®} & 810 nm laser occluded dentinal tubules, but NovaMin\textsuperscript{®} was found to be superior to 810 nm diode laser in causing complete tubular occlusion. Further randomized clinical trials are required to confirm these results in general population.

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**Conflict of Interest:** Nil.

**Footnotes**

1. * Vantej\textsuperscript{®} toothpaste, Dr. Reddy’s Laboratories Ltd; Hyderabad, India.
2. † Picasso diode laser unit, AMD Lasers, Inc.(AMD); West Jordan, UT.
3. ‡ JEF JFC-1600 autofine coater, JEOL Ltd; Tokyo, Japan.
4. § Scanning Electron Microscope JEOL JSM-6390, JEOL Ltd; Tokyo, Japan.
5. kSPSS Inc., Chicago, IL,USA

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