Comparative evaluation of use of diode laser and electrode with and without two dentinal tubule occluding agents in the management of dentinal hypersensitivity: An experimental in vitro study

Chitra Laxmikant Patil, Rajesh Prabhakar Gaikwad

Abstract:
Background: The present study aims to assess area of open dentinal tubules by use of diode laser and electrode alone and in combination with hydroxyapatite powder and strontium chloride powder by using the scanning electron microscope (SEM). Materials and Methods: A double-blinded study was carried out with 30 extracted teeth with no carious lesion or restored teeth were selected and grouped randomly as Group A - diode laser, Group B - electrode application, Group C - hydroxyapatite powder plus diode laser, Group D - hydroxyapatite powder plus electrode application, Group E - strontium chloride powder plus diode laser, and Group F - strontium chloride powder plus electrode application. All the samples were made by preparing a notch of 2 mm measured with UNC-15 probe at cementoenamel junction using a diamond cylindrical bur at high speed. The teeth were then washed with distilled water and stored in solution containing 2.5% glutaraldehyde solution in 0.1 M sodium phosphate buffer for 24 h. The samples were treated as mentioned in above said groups and were viewed under SEM the degree of occlusion of the dentinal tubules were quantified using an image analyzer. Results: Highest number of open dentinal tubules was found with Group B, that is, electrode application, whereas Group C, that is, hydroxyapatite powder plus diode laser showed highest occluded tubules. One-way ANOVA showed statistically significant difference across all the groups (P = 0.000); further, intergroup comparison with Tukey’s test showed better tubular occlusion with Group C. Conclusion: The SEM results showed highest tubular occlusion with hydroxyapatite powder plus diode laser and least with electrode.

Key words:
Dentinal tubule, diode laser, electrode, hydroxyapatite, hypersensitivity, strontium chloride

INTRODUCTION

The enigmatic problem of dentin hypersensitivity (DH) has long bewildered the dental profession. It is one domain where man has not yet achieved the elusive "gold standards" for its management. Dentine hypersensitivity is characterized by short, sharp pain arising from exposed dentine in response to stimuli typically thermal, evaporative, tactile, osmotic or chemical and which cannot be ascribed to any other form of dental defect or pathology (Holland et al. 1997). The incidence ranges from 4% to 74% mostly affecting female patients and the buccal aspect of the cervical area is the most prevalent site. DH can affect the patient of any age, most affected patients are in the age group of 20–50 years, with a peak between 30 and 40 years of age. An accurate diagnosis is important before starting the management of dentinal hypersensitivity. A number of theories have been proposed over the years to explain the pain mechanism such as the neural theory, the odontoblastic transduction theory, the modulation theory, the gate control theory, and presently, the most widely accepted hydrodynamic theory. Most of the desensitizing methods till date attempt to inhibit the pain by either sealing the...
dentinal tubules with coating mechanism or by altering the tubule content through coagulation, protein precipitation, or creation of an insoluble calcium complex. Dentinal tubule occlusion methods by use of calcium compounds and protein precipitates such as hydroxyapatite and strontium chloride have proven to have a beneficial effect in hypersensitivity treatment. Hydroxyapatite[8] remained a focus of interest as it possesses exceptional biocompatibility and unique bioactivity. Hydroxyapatite has close similarities with inorganic mineral component of bone and teeth.[9] Alternative therapies such as laser and electrode application have been widely researched. Among these lasers like neodymium-doped yttrium aluminum garnet, CO₂, and diode lasers have been used in dentinal hypersensitivity treatment. The aim of the study was to use different agents along with these lasers and electrodes and assess area of open dentinal tubules by using the scanning electron microscope (SEM). A diagram for complete methodology is presented in the chart 1

MATERIALS AND METHODS

All participants gave informed consent. Ethical clearance was obtained from institutional ethical committee. A total of 30 extracted teeth from patients of age group 20–50 years were selected for the study, of which 17 extracted teeth were of female and remaining were from males. The inclusion criteria for the study was (1) premolars with 2 mm cervical abrasion cavities on the buccal aspect. The exclusion criteria were (1) teeth having caries or pulpal pathosis and (2) teeth with restoration or which have had any therapeutic intervention performed on them in the last 6 months.

The first premolars are the most affected teeth reaching more than half of the cases, and the most affected region is the cervical area of the buccal surface.[7]

Grouping and treatment methods

The teeth were divided randomly using computer generated random numbers[8] into six groups which were assigned as Group A, Group B, Group C, Group D, Group E, and Group F. The teeth under the six groups were treated by the specific agents as follows:

1. Group A – Diode laser
2. Group B – Electrode application
3. Group C – Hydroxyapatite powder plus diode laser
4. Group D – Hydroxyapatite powder plus electrode application
5. Group E – Strontium chloride powder plus diode laser
6. Group F – Strontium chloride powder plus electrode application.

Laser protocol

The teeth were irradiated with GaAlAs Diode Laser (Ezlase 940; biolase technology) with 400 μm fiberoptic handpiece at wavelength of 940 nm at a power setting of 0.1 W with a pulse length of 200 μm in noncontact mode for 30 s.

Electrode application protocol

Mega surgical low frequency radio surgery unit was used. Current generator with following specifications Input: 220–240 vac: 50–60 Hz, Frequency: 1 MHz. The electrode was inserted into the hand piece with a push motion. The active electrode was moved very quickly with no pressure and contact time of 0.2 s at the site. The electrode was cleaned with 3% hydrogen peroxide solution and subsequently wiped.

Procedure

The surfaces of all the teeth were treated with 37% phosphoric acid so as to remove the smear layer. The agents (extra fine hydroxyapatite powder of particle size 63 μ and strontium chloride powder of particle size 45 μ) were applied on the notch and then burnished for 30 s with a ball burnisher followed by diode laser and electrode application as specified in groups. The prepared tooth samples were then again stored in 2.5% glutaraldehyde solution in 0.1 M sodium phosphate buffer for 24 h until ready for SEM analysis. The conventional sputtering with thin gold palladium 25 nm film was done and the samples were viewed under SEM (JEOL JSM-7600F) under high-vacuum mode. Photomicrographs were taken at ×2000 and were analyzed by a single examiner.

Statistical analysis

Statistical analysis was done using one-way ANOVA test to compare the 6 groups. Pairwise comparison of the study groups with respect to the total scores was done by Tukey’s multiple post hoc procedure, and statistical significance was calculated. The degree of occlusion of the dentinal tubules (open area of dentinal tubules) was quantified using an image analyzer (image pro plus, version 4.5, media Cybernetics, USA). Each image was calibrated using the 1 μm scale bar in the SEM image to quantify the measured area. The polygon tool in the major tool bar was used to measure the area of dentinal tubules. The software can automatically draw the highlighted outline of the dentinal tubule from the difference in gray pixels between the dentinal tubule and the outer area, and then calculate the mean open total tubules area. The results were analyzed by one-way anova and Tukey’s test using the SPSS software version 19 (SPSS inc, Mumbai, Interchemie, India) statistical package program.

RESULTS

The micro photographic images from the SEM were quantitatively analyzed. Descriptive statistics for experimental groups was done [Table 1]. The highest number of open dentinal tubules were found with Group B [Figure 1; electrode application] 90.4 ± 2.55 followed by Group F [Figure 2; strontium chloride + electrode application] 78.4 ± 5.16, Group D [Figure 3; hydroxyapatite + electrode application] 70.9 ± 8.74, Group A [Figure 4; diode laser] 55.6 ± 5.34, Group E [Figure 5; strontium chloride + diode laser] 44.6 ± 6.09, and Group C [Figure 6; hydroxyapatite + diode laser] 37.2 ± 6.64 which showed the least number of open dentinal tubular area thus indicating that electrode application had no much of beneficial effect whereas burnishing of hydroxyapatite powder and diode laser combination occluded the majority of tubules. One-way ANOVA showed statistically significant difference across all the groups (P = 0.000) [Table 2].

Further intergroup comparison with Tukey’s test was carried out [Table 3 and Graph 1]. The SEM results showed
highest tubule occlusion with hydroxyapatite powder plus diode laser group and least in the electrode application group.

**DISCUSSION**

At present, no universally accepted or highly predictable
Table 1: Descriptive analysis showing the mean area of the open dentinal tubules

| Groups | n  | Mean±SD     | SE    | 95% CI for mean | Minimum | Maximum |
|--------|----|-------------|-------|----------------|---------|---------|
|        |    |             |       | Lower bound    |         |         |
|        |    |             |       | Upper bound    |         |         |
| 1      | 5  | 55.6500±5.34193 | 2.38899 | 49.0171        | 62.2829 | 46.35   | 59.95   |
| 2      | 5  | 90.4620±2.55861 | 1.14424 | 87.2851        | 93.6389 | 86.75   | 93.15   |
| 3      | 5  | 37.2220±6.64630 | 2.97231 | 28.9675        | 45.4725 | 30.00   | 44.85   |
| 4      | 5  | 70.9400±8.74889 | 3.91262 | 60.0788        | 81.8052 | 62.18   | 84.50   |
| 5      | 5  | 44.6400±6.09512 | 2.72582 | 37.0719        | 52.2081 | 39.55   | 54.30   |
| 6      | 5  | 78.4140±5.16265 | 2.30861 | 72.0037        | 84.8243 | 73.42   | 85.20   |
| Total  | 30 | 62.8880±19.85358 | 3.62475 | 55.4746        | 70.3014 | 30.00   | 93.15   |

n – Number of samples; SD – Standard deviation; CI – Confidence interval; SE – Standard error

Table 2: ANOVA test showing significant difference between all the groups

| Sum of squares | Difference | Mean square | F   | Significance |
|----------------|------------|-------------|-----|--------------|
| Between groups | 10552.367  | 5           | 2110.473 | 57.663 | 0.000       |
| Within groups  | 878.410    | 24          | 36.600  |        |             |
| Total          | 11430.777  | 29          |         |        |             |

F value in the ANOVA test determines the P value, P<0.05 is considered statistically significant. F – Statistical ratio of two variances

Graph 1: Scanning electron microscope showing mean area of open dentinal tubule

Diagram 1: Flow chart showing the study design with scanning electron microscope

Desensitizing agent or any other mode of treatment has been identified. To prevent and to treat DH more effectively, attention must be given to the etiology, mechanism, and methods for the assessment of hypersensitivity. Dentinal tubule occlusion methods by use of calcium compounds and protein precipitates such as hydroxyapatite and strontium chloride have been proven to be beneficial in hypersensitivity treatment. Alternative therapies such as laser and electrosurgery have also been researched. Low output power laser therapy has been utilized in humans since the early 1970s. In the last 15 years, the introduction of lasers gave further possibilities to DH therapy. A study has been reported on the irradiation of 980 nm diode laser in dentin at different output powers and delivery modes which produce changes that ranged from smear layer removal to dentin fusion. In the present study, diode laser showed 55.6 mean area of open dentinal tubules which may be attributed to the melting of the dentin. The laser power used in the present study was 1 W which is safer to the pulp. A study conducted by Rimaneepong V et al. found showed that laser when used at a power setting of 0.8, 1, 1.6 and 2 W caused occlusion of the dentinal tubules without any adverse effect. However, of all the wave lengths, the diode lasers are the least researched as of yet in treatment of dentinal hypersensitivity. Electro surgery utilizes thermal energy for various dental applications and is used clinically for surgical procedures. However, it is minimally researched for effects on dentine and hence used only as a part of experiment in the study. In the present study new methodology was used to combine an image analysis system and SEM for a precise quantitative assessment of open dentinal tubular area for all samples using the image analyzer. Open dentinal tubule was
greater with electrode application which wasrationally found by Matsumoto et al. which showed that in hypersensitive dentine most dental tubules appear open when visualized by SEM. The tubular occlusion was highest in Group C, i.e., hydroxyapatite plus diode laser which may be in accordance with studies who have confirmed that the dentinal tubules were occluded predominantly with apatite mineral not only on the dentin surface but also deep inside the dentinal tubules to a depth of 10-15 mm from the dentin surface. Chemically, hydroxyapatite agents are composed of calcium and phosphate, and the saliva in the oral cavity is supersaturated with respect to hydroxyapatite, thus the chances of dissolution of these compounds by saliva is limited. Our results showed that strontium chloride powder plus diode laser shows more tubular occlusion than diode laser alone. These may be attributed to melting of the peritubular dentin as was also observed in the in vitro study conducted Gholami et al.\[12\] His study aimed at evaluating the occluding effect of erbium, chromium: yttrium-scandium-gallium-garnet (E: 0.25W, F: 20 Hz, Pd: 140 mS), Nd:YAG (P: 1W, F: 20 Hz), 30 CO (2) (P: 1W, Pd: 50 ms), and 810-nm diode (P: 2 W, Pd: 30 ms) lasers on dentinal tubules where melting of the peritubular dentin was observed as a dominant phenomenon. They concluded that the 810-nm diode laser sealed tubules to a far lesser degree, with negligible effects on desensitization. In fact, just minimal research has been done on application of the diode laser as an adjunct to strontium chloride and hydroxyapatite powders. In our present study strontium chloride plus diode laser showed more tubular occlusion than diode laser alone which may have been due to the additional benefit of strontium chloride which is an established desensitising agent. An in vitro study by Pol and Dalvi concluded that there were favorable results as regards the efficacy and reliability of hydroxyapatite and strontium chloride as a desensitizing agent.\[13\] Several studies have shown reduction in dentinal hypersensitivity by widely using strontium chloride and other strontium salts which includes those conducted by Pawlowska\[14\] who found that strontium chloride binds strongly to dentin and Jenson and Doering proposed that the mode of action of strontium was by binding to the matrix of the tubules thus reducing its radius. However, finding regarding the application of diode laser and diode laser in combination could not be corroborated with any such similar study as the detailed perusal of the available literature failed to show any such similar study. However, the study of Group D and F could not be corroborated with other relevant studies; hence further research may be required. The results obtained in the present study may be attributed to the occluding effects of hydroxyapatite and strontium chloride powders.

Table 3: Pairwise comparison of the six study groups with respect to the dentinal tubular area by Tukey’s multiple post hoc procedures

| Group   | Variance | Mean difference | SE  | Significance | 95% CI               |
|---------|----------|-----------------|-----|--------------|----------------------|
| Tukey HSD |          |                 |     |              |                      |
| Group 1 | 1.00     | −34.81200*      | 3.82625 | 000          | −46.6425 to −22.9815 |
|         | 3.00     | 18.43000*       | 3.82625 | 0.001        | 6.5995 to 30.2605   |
|         | 4.00     | −15.29200*      | 3.82625 | 0.006        | −27.1225 to −3.4615 |
|         | 5.00     | 11.01000        | 3.82625 | 0.079        | −0.8205 to 22.8405  |
|         | 6.00     | −22.76400       | 3.82625 | 0.000        | −34.5945 to −10.9335 |
| Group 2 | 1.00     | 34.81200*       | 3.82625 | 0.000        | 46.6425 to 46.6425  |
|         | 3.00     | 53.24200*       | 3.82625 | 0.000        | 41.4115 to 65.0725  |
|         | 4.00     | 19.52000*       | 3.82625 | 0.000        | 7.68953 to 31.3505  |
|         | 5.00     | 45.82200*       | 3.82625 | 0.000        | 3.9915 to 57.6525   |
|         | 6.00     | 12.04800*       | 3.82625 | 0.044        | 0.2175 to 23.8785   |
| Group 3 | 1.00     | −18.43000*      | 3.82625 | 0.001        | −30.2605 to −6.5995 |
|         | 2.00     | −53.24200*      | 3.82625 | 0.000        | −65.0725 to −41.115 |
|         | 4.00     | −33.72200*      | 3.82625 | 0.000        | −45.5525 to −21.8915 |
|         | 5.00     | −7.42000        | 3.82625 | 0.404        | −19.2505 to 4.4105  |
|         | 6.00     | −33.72200*      | 3.82625 | 0.000        | −53.0245 to −29.3635 |
| Group 4 | 1.00     | −7.42000        | 3.82625 | 0.006        | 3.6165 to 27.1225   |
|         | 2.00     | −41.19400*      | 3.82625 | 0.000        | −31.3505 to −7.6895 |
|         | 3.00     | 33.72200*       | 3.82625 | 0.000        | 21.8915 to 45.5525  |
|         | 5.00     | 26.30200*       | 3.82625 | 0.000        | 14.4715 to 38.1325  |
|         | 6.00     | −7.47200        | 3.82625 | 0.397        | −19.3025 to 4.3585  |
| Group 5 | 1.00     | −11.01000       | 3.82625 | 0.079        | −22.8405 to 0.8205  |
|         | 2.00     | −45.82200*      | 3.82625 | 0.000        | −57.6525 to −33.9115 |
|         | 3.00     | 7.42000         | 3.82625 | 0.404        | −4.4105 to 19.2505  |
|         | 4.00     | −26.30200*      | 3.82625 | 0.000        | −38.1325 to −14.4715 |
|         | 6.00     | −33.77400*      | 3.82625 | 0.000        | −45.6045 to −21.9435 |
| Group 6 | 1.00     | 22.76400*       | 3.82625 | 0.000        | 10.9335 to 34.5945  |
|         | 2.00     | −12.04800*      | 3.82625 | 0.044        | −23.8785 to −0.2175 |
|         | 3.00     | 41.19400*       | 3.82625 | 0.000        | 29.3635 to 53.0245  |
|         | 4.00     | 7.47200         | 3.82625 | 0.397        | −4.3585 to 19.3025  |
|         | 5.00     | 33.77400*       | 3.82625 | 0.000        | 21.9435 to 45.6045  |

P<0.05 is considered to be statistically significant; CI – Confidence interval; SE – Standard error; HSD – Honestly significant difference.
agents, long-term studies are required to have it established as a desensitizing agent. Also, further comparative studies with various agents are required to prove the superiority, if any, over hydroxyapatite, strontium chloride, electrode application, and diode lasers.

Financial support and sponsorship
Nil.

Conflicts of interest
There are no conflicts of interest.

REFERENCES

1. Holland G R, Narhi M N, Addy M. Guidelines for the design and conduct of clinical trials on dentin hypersensitivity. J Clin Periodontol 1997; 24: 808-813.
2. Taani DQ, Awartani F. Prevalence and distribution of dentin hypersensitivity and plaque in a dental hospital population. Quintessence Int 2001;32:372-6.
3. Flynn J, Galloway R, Orchardson R. The incidence of 'hypersensitive' teeth in the West of Scotland. J Dent 1985;13:230-6.
4. Irvine JH. Root surface sensitivity: A review of aetiology and management. J N Z Soc Periodontol 1988;66:15-8.
5. Jarcho M, Kay JF, Gumar KI, Doremus RH, Drobeck HP. Tissue, cellular and sub cellular events at a bone ceramic hydroxyapatite interface. J Biosci Bioeng 1977;1:79-92.
6. Wang M. Developing bioactive composite materials for tissue replacement. Biomaterials 2003;24:2133-51.
7. Lilja J. Innervation of different parts of the predentin and dentin in young human premolars. Acta Odontol Scand 1979;37:339-46.
8. Adriaens PA, Edwards CA, De Boever JA, Loesche WJ. Ultrastructural observations on bacterial invasion in cementum and radicular dentin of periodontally diseased human teeth. J Periodontol 1988;59:493-503.
9. Kreisler M, Al Haj H, Daubländler M, Götz H, Duschner H, Willershäuser B, et al. Effect of diode laser irradiation on root surfaces in vitro. J Clin Laser Med Surg 2002;20:63-9.
10. Marchesan MA, Brugnera-Junior A, Souza-Gabriel AE, Correa-Silva SR, Sousa-Neto MD. Ultrastructural analysis of root canal dentine irradiated with 980-nm diode laser energy at different parameters. Photomed Laser Surg 2008;26:235-40.
11. Rimaneepong V, Palamara JE, Wilson PR. Pulpal space pressure and temperature changes from nd:YAG laser irradiation of dentin. J Dent 2002;30:291-6.
12. Gholami GA, Fekrazad R, Esmaiel-Nejad A, Kalhori KA. An evaluation of the occluding effects of er; Cr:YSGG, nd:YAG, CO\textsubscript{2} and diode lasers on dentinal tubules: A scanning electron microscope in vitro study. Photomed Laser Surg 2011;29:115-21.
13. Pol D, Dalvi S. A comparative evaluation of the efficacy of hydroxyapatite vs. strontium chloride in the management of dentinal hypersensitivity – A clinical and SEM study. J Indian Dent Assoc 2010;4:152-4.
14. Pawlowska J. Strontium chloride its importance in dentistry and prophylaxis. Czas Stomatologica 1956;59:353-361.
15. Jensen ME, Doering JV. A comparative study of two clinical techniques for treatment of root surface hypersensitivity. Gen Dent 1987;35:128-32.