



Comparative Evaluation of Efficacy of Diode Laser, Bonding Agent and Desensitising Agent on Occlusion of Dentinal Tubules: A Scanning Electron Microscopic Study

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Abstract

Aim: To compare the efficacy of diode laser (Zolar laser, 810nm, Canada), bonding agent (Tetric N bond universal, Ivoclar Vivadent, USA) and a desensitising agent (Gluma Desensitizer, Kulzer, Japan) on the occlusion of dentinal tubules.

Methods: 68 human premolars were sectioned & dentin discs of 1mm thickness was made. The dentinal tubules were opened by etching the dentine discs in a Petri dish with 37% phosphoric acid for 30 seconds. The dentine discs were randomly divided into four groups, each

containing 17 discs: Group 1: diode laser, Group 2: Tetric N bond universal, Group 3: Gluma Desensitizer, & Group 4: control group (no treatment done).

For laser application, the specimen was subjected to 810 nm diode laser irradiation with 1W power in a continuous mode for 20 seconds. The irradiation was done in sweeping motion with 1 mm distance from the surface. Tetric N-Bond self-etch adhesive was applied in a thick layer, for at least 30 s, using a light brushing motion. Then, it was dried with a steady stream of air for 3 s and light-cured for 10s. In the Gluma group, a small amount of Gluma desensitizer, was applied on the

specimen using small cotton pellets for 30-60 seconds. The surface is then dried by applying a stream of compressed air until the fluid film was disappeared and the surface was no longer shiny, and then rinsed thoroughly with water.

All the specimens were sputter-coated with a thin gold layer & were examined under Scanning electron microscope-SEM (JSM 6490LA-JEOL Ltd, Japan) at a magnification of 1500x. The images were then be assessed independently by three blinded reviewers to score the tubule occlusion & the SEM images were categorized as follows: Occluded (100% of tubules occluded), Mostly occluded (50% to <100% of tubules occluded), Partially occluded (25% to <50% of tubules occluded), Mostly unoccluded (<25% of tubules occluded) & Unoccluded (0%, no tubule occlusion). The mean score of tubule occlusion as assessed by three blinded reviewers was taken and used for analysis.

All statistical procedures were performed using Statistical Package for Social Sciences –SPSS Version 22 Software, IBM Statistics, USA. All quantitative variables expressed in mean and standard Deviation. P-value based on Analysis of Variance (ANOVA) followed by post-hoc analysis using Bonferroni test after adjusting for multiple comparisons. Overall score is measured by p - value based on Kruskal Wallis Test. Probability value ($p < 0.05$) was considered statistically significant.

Result: The mean and standard deviation of the control group was 1.78 ± 3.38 . For specimens treated with laser the mean and standard deviation turned out to 61.27 ± 12.85 , whereas for those treated with gluma it was 46.69 ± 15.01 . Specimens treated with bonding agent showed mean and standard deviation 24.39 ± 5.09 . The result was found to be statistically highly significant as p

value obtained was less than 0.001. It was found that dentin discs treated with diode laser showed more dentinal tubule occlusion compared to gluma and dentin bonding agent groups.

Conclusion: The study concluded that the maximum dentinal tubule occlusion was obtained by diode laser.

Keywords: Dentinal hypersensitivity, diode lasers, Gluma desensitizer, Tetric N bond universal bonding agent.

Introduction

Dentinal hypersensitivity (DH) has been defined as “pain derived from exposed dentin in response to chemical, thermal, tactile, or osmotic stimuli which cannot be explained as arising from any other dental defect or disease.”¹ Dentinal hypersensitivity, also known as dentin hypersensitivity, cervical hypersensitivity, root hypersensitivity, cemental hypersensitivity, or simply sensitivity, is one of the most common problems a dentist encounters in daily practice. These terms all convey the same clinical condition and can be used interchangeably.²

Several theories have been proposed to explain the mechanism of dentinal hypersensitivity (DH): (I) direct innervation of the dentin, (II) the odontoblast receptor theory, and (III) the hydrodynamic theory, which is the most widely accepted.³

Hydrodynamic Theory for sensitive dentine was first proposed by Brannstorm.⁴ The hydrodynamic theory suggests that when stimulants are applied to dentin, they cause the movement of intratubular fluid. This movement induces a mechanical change in the nerve endings located at the pulp-dentin interface, where the pain is experienced. Therefore, techniques that reduce the movement of intratubular fluid or decrease dentin

permeability can alleviate dentinal hypersensitivity (DH).^{5,6}

Various products on the market aim to reduce dentinal hypersensitivity by decreasing permeability. These include laser therapy and the application of materials such as fluoride, hydroxyapatite, strontium chloride, zinc chloride, potassium chloride, dental adhesives, glass ionomer cement, oxalate, bioglass, Portland cement, and casein phosphopeptide-amorphous calcium phosphate.⁷

The laser reduces dentin hypersensitivity through two mechanisms: (1) it directly influences the electrical conduction of nerve fibers within the dental pulp, and (2) it blocks dentinal tubules by causing them to melt.^{8,9}

The Gluma desensitizer, produced by Heraeus Kulzer GmbH in Wehreim, Germany, is a desensitizing agent composed of a 5% aqueous solution of glutaraldehyde and 35% 2-hydroxyethyl methacrylate.^{10,11} The application of glutaraldehyde to hypersensitive dentin causes protein coagulation within the dentinal tubules, leading to their occlusion and a subsequent reduction in dentin hypersensitivity.¹²

A well-established method for managing dentin hypersensitivity is the topical application of dental adhesives.¹³ Dentin desensitizers and adhesives are used on exposed dentin surfaces to occlude the open dentinal tubules. Recently, one-bottle self-etching adhesives have been developed to streamline the application process into a single step. These adhesives can potentially reduce dentin hypersensitivity by decreasing dentin permeability.^{14,15}

The aim of this study is to compare the efficacy of diode laser (Zolar laser, 810nm, Canada), bonding agent (Tetric N bond universal, Ivoclar Vivadent, USA) and a desensitising agent (Gluma Desensitizer, Kulzer, Japan) on the occlusion of dentinal tubules.

In this study, 68 non-diseased single-rooted human premolar teeth, extracted for orthodontic purposes, were selected. The teeth were meticulously cleaned and then disinfected by immersing them in a 5% sodium hypochlorite solution for one hour. The crowns were sectioned perpendicular to the long axis of the teeth using a diamond disc to obtain dentine discs, each with a thickness of 1.0 mm from the mid-coronal dentine. This procedure removed the occlusal enamel, thereby exposing the middle dentine and ensuring that the surfaces of the discs were devoid of enamel and pulp horns. Next, the dentine discs were polished with 600-grit silicon carbide paper to achieve a uniform surface. These polished samples were then placed in a jar of distilled water and subjected to sonication for 10 minutes to remove any remaining abrasive particles from the polishing process. After sonication, the samples were rinsed with saline solution. To expose the dentinal tubules, the dentine discs were etched with 37% phosphoric acid in a Petri dish for 30 seconds. Following etching, the discs were rinsed with distilled water and then sonicated for an additional five minutes. The etched and sonicated dentine discs were subsequently stored in phosphate-buffered solution (pH 7) in preparation for analysis via scanning electron microscopy.

The dentine discs were randomly divided into four groups, each containing 17 discs:

Group 1: Specimens to be treated with diode laser.

Group 2: Specimens to be treated with Tetric N bond universal.

Group 3: Specimens to be treated with Gluma Desensitizer.

Group 4: control group (no treatment will be done).

For the laser application phase of the study, the specimens were irradiated using an 810 nm diode laser

set to 1W power in continuous mode. The irradiation was performed for 20 seconds per specimen, utilizing a sweeping motion while maintaining a distance of 1 mm from the surface of each sample.

Tetric N-Bond self-etch adhesive was applied as per the manufacturer's guidelines. A thick layer of the adhesive was brushed onto the surface for a minimum of 30 seconds using a light brushing technique. Following this, the adhesive was dried with a steady stream of air for 3seconds and then light-cured for 10 seconds.

In the Gluma group, a small amount of Gluma desensitizer was applied to the specimens using small cotton pellets, following the manufacturer's instructions. The desensitizer was left on the surface for 30-60 seconds. Afterward, the surface was dried with a stream of compressed air until the fluid film disappeared and the surface was no longer shiny. Finally, the specimens were thoroughly rinsed with water.

All the specimens were sputter-coated with a thin gold layer & was examined under Scanning electron microscope-SEM (JSM 6490LA-JEOL Ltd, Japan) at a magnification of 1500x. The percentage of tubular occlusion in an SEM image was calculated as:

No. of dentinal tubules occluded *100

Total number of dentinal tubules

The images were then assessed independently by three blinded reviewers to score the tubule occlusion & the SEM images were categorized as follows:

Score 1 - Occluded (100% of tubules occluded)

Score 2 - Mostly occluded (50% to <100% of tubules occluded)

Score 3 - Partially occluded (25% to <50% of tubules occluded)

Score 4 - Mostly unoccluded (<25% of tubules occluded)

Score 5 - Unoccluded (0%, no tubule occlusion).

The mean score of tubule occlusion, as assessed by three blinded reviewers, was calculated and used for the analysis.

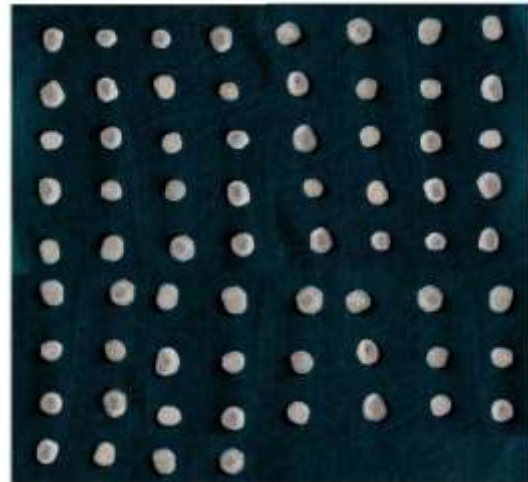


Figure 1: Dentin Discs Sample



Figure 2: Laser Irradiation



Figure 3: Bonding Agent Application



Figure 4: Gluma Application

Results

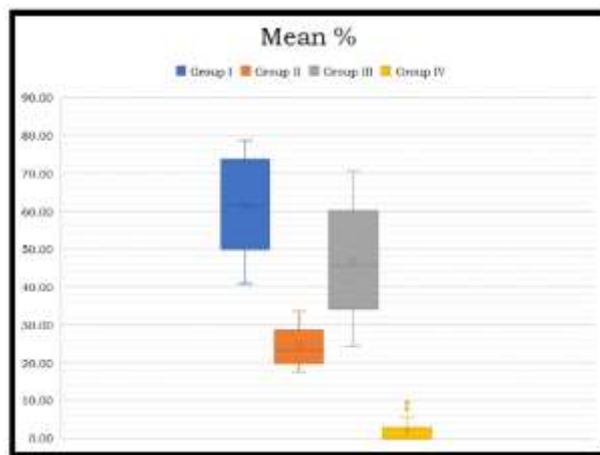
All statistical procedures were performed using Statistical Package for Social Sciences -SPSS Version 22 Software, IBM Statistics, USA. Calculations for power (80%) of study was performed before the commencement of the study. All quantitative variables expressed in mean and standard Deviation. P value based on Analysis of Variance (ANOVA) followed by post-hoc analysis using Bonferroni test after adjusting for multiple comparisons. Overall score is measured by p - value based on Kruskal Wallis Test. Probability value ($p < 0.05$) was considered statistically significant.

Table 1: Overall & Individual pair-wise Comparison (Mean %) of dentinal tubule occlusion.

| | N | Mean | Standard Deviation | p-value |
|-----------|----|-------|--------------------|----------|
| Group I | 17 | 61.27 | 12.85 | |
| Group II | 17 | 24.39 | 5.09 | |
| Group III | 17 | 46.69 | 15.01 | |
| Group IV | 17 | 1.78 | 3.38 | |
| Group I | | | Group II | < 0.001* |
| | | | Group III | 0.001* |
| | | | Group IV | < 0.001* |
| Group II | | | Group III | < 0.001* |
| | | | Group IV | < 0.001* |
| Group III | | | Group IV | < 0.001* |

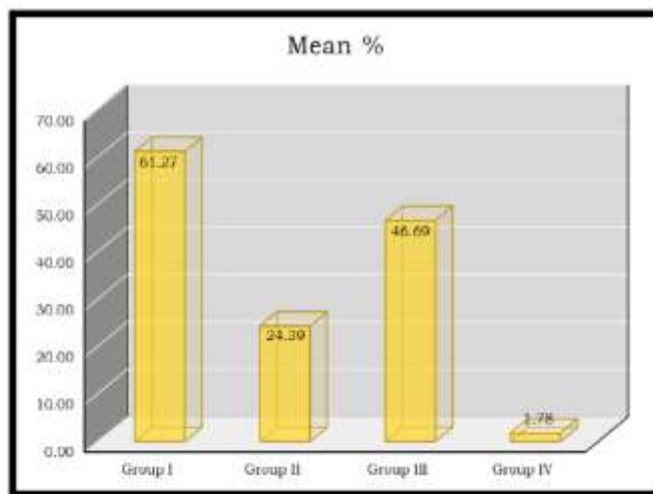
p-value based on Analysis of Variance (ANOVA) followed by post-hoc analysis using Bonferroni test after adjusting for multiple comparisons. * = Statistically Significant ($p < 0.05$)

Graph 1: Overall mean % of dentinal tubule occlusion.



X-Axis: Groups, Y-Axis: Mean % of Tubule Occlusion

Graph 2: Mean % of dentinal tubule occlusion by individual group.



X-Axis: Groups, Y-Axis: Mean % Of Tubule Occlusion

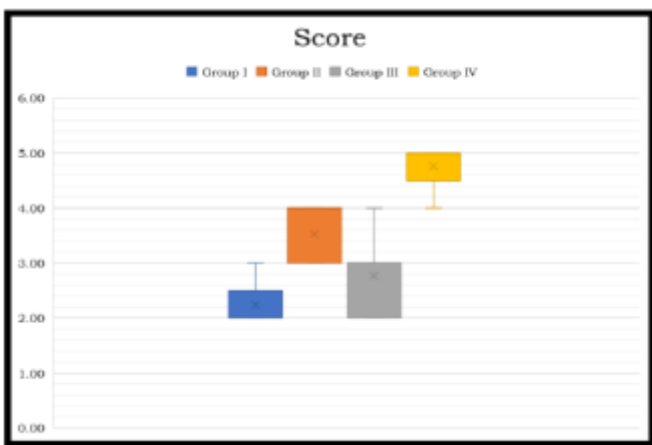
The mean and standard deviation of the control group was 1.78 ± 3.38 . For specimens treated with laser the mean and standard deviation turned out to 61.27 ± 12.85 , whereas for those treated with gluma it was 46.69 ± 15.01 . Specimens treated with bonding agent showed mean and standard deviation 24.39 ± 5.09 . The result was found to be statistically highly significant as p value obtained was less than 0.001.

Table 2: Overall Comparison (Score)

| | N | Mean Rank | p-value |
|-----------|----|-----------|----------|
| Group I | 17 | 14.71 | < 0.001* |
| Group II | 17 | 39.53 | |
| Group III | 17 | 25.06 | |
| Group IV | 17 | 58.71 | |

p-value based on Kruskal Wallis Test
 * = Statistically Significant (p < 0.05)

Graph 3: Overall score of individual groups.



X-Axis: Groups, Y-Axis: Overall Score

Overall score of individual groups shows that dentin discs treated with diode laser have more dentinal tubule occlusion compared to gluma and dentin bonding agent groups respectively. The study concluded that the maximum dentinal tubule occlusion was obtained by diode laser.

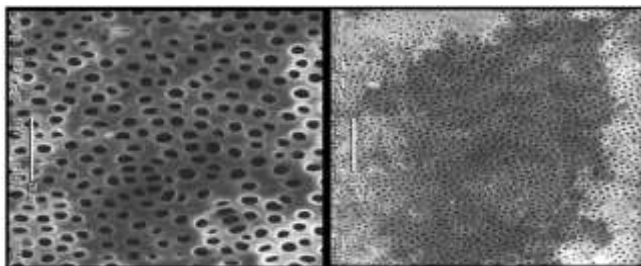


Figure 5: Morphology of dentinal tubules treated with distilled water (control), seen under scanning electron microscope (× 1,500).

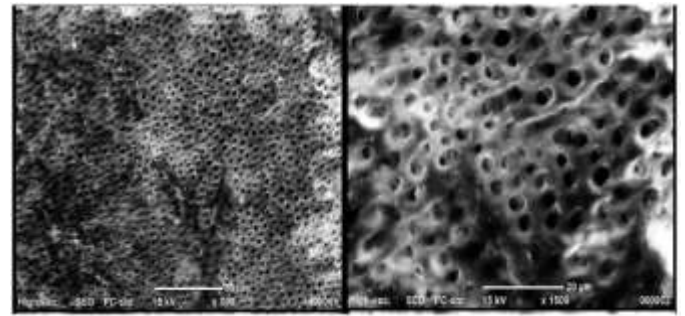


Figure 6: Morphology of dentinal tubules treated with diode laser, seen under scanning electron microscope (× 1,500 & 500). Melting of dentinal tubules resulting in dentinal tubule occlusion can be seen.

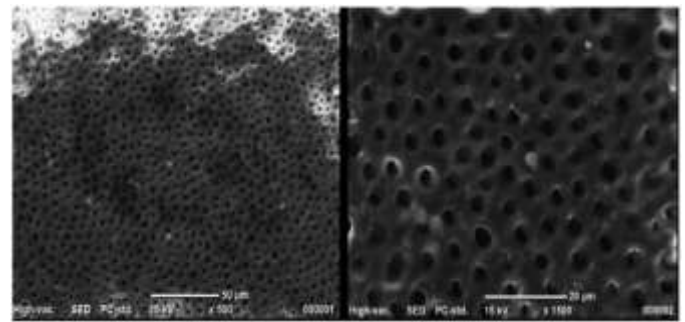


Figure 7: Morphology of dentinal tubules treated with Gluma desensitizer, seen under scanning electron microscope (× 1,500 & 500). Resinous layer occluding the surface of the tubules can be seen.

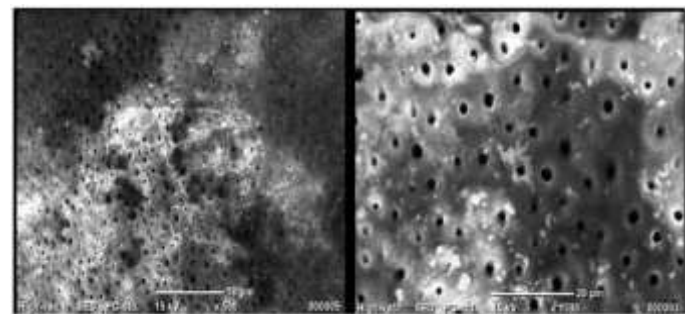


Figure 8: Morphology of dentinal tubules treated with Tetric N bond universal, seen under scanning electron microscope (× 1,500 & 500).

Discussion

Dentinal hypersensitivity is a dental condition of significant clinical concern, characterized by the onset of

painful symptoms that can affect any tooth and patients of all ages. The incidence is particularly higher in females aged 20 to 40 years. This condition is more common in individuals with periodontal disease and often occurs transiently in those undergoing scaling and root planing, periodontal surgery, dental whitening, and other conservative dental treatments.^{16,17}

Desensitizing agents function through two primary mechanisms: occlusion of dentinal tubules and nerve depolarization. Occlusion of dentinal tubules occurs by precipitating proteins or forming a superficial pellicle over the tubules. On the other hand, nerve depolarization involves the penetration and diffusion of ions into the dentinal structure, ultimately reaching the pulp.¹⁸

Diode lasers have emerged as one of the most extensively studied and effective treatments for dentinal hypersensitivity (DH), even in high-grade cases. These lasers operate through two primary mechanisms. Firstly, they induce a melting effect, leading to the crystallization of dentin's inorganic component and the coagulation of fluids within dentinal tubules. Secondly, diode lasers are thought to reduce the pain threshold of pulpal nerves, thereby alleviating discomfort associated with DH. Additionally, diode lasers are believed to create an amorphous sealed layer on the dentin surface, which results from partial melting of the surface. This sealed layer contributes to the reduction of dentinal tubule permeability, further reducing sensitivity.¹⁹

In the current study, the percentage of the obstruction of dentinal tubules in the diode laser group was found to be 61.27%. It caused partial or complete obstruction of several individual dentinal tubules. Similar results are seen in study by Corneli R et al in which the laser group showed complete occlusion of tubules.²⁰

Gholami et al. proposed that the mechanism of action of the diode laser involves inhibiting signal transfer. Despite its limited absorption in water, it's suggested that the energy emitted by the diode laser could lead to a significant temperature increase, facilitating the melting and closure of dentinal tubules.²¹

The Gluma desensitizer is formulated as an aqueous solution containing 5% glutaraldehyde and 35% hydroxyethyl methacrylate. Glutaraldehyde serves as a biological fixative, and it has been proposed that it occludes dentinal tubules by reacting with plasma proteins from dentinal fluid. This reaction leads to the coagulation and precipitation of proteins, resulting in the total or partial closure of the tubules.^{22,23} Hydroxyethyl methacrylate, a hydrophilic monomer present in dentin bonding agents, possesses the capability to infiltrate acid-etched and moist dental hard tissues. When combined with glutaraldehyde, hydroxyethylmethacrylate contributes to the occlusion of dentinal tubules, enhancing the desensitizing effect of Gluma.²²

Gluma desensitizer produced a greater number of partially occluded tubules in this study. Surabhi Joshi et al described similar results in their study with Gluma desensitizer producing a greater number of partially occluded tubules and fewer completely occluded tubules. Previous studies pointed that specimen treated with Gluma desensitizer showed a resinous layer of thickness 1–2 μm occluding the surface of the tubules which were visible in our reports also.²⁴

The topical application of self-etching adhesives has been employed to manage dentinal hypersensitivity (DH).^{26,26} Microscopic analyses have shown that these adhesives, when applied to sensitive dentinal areas, can occlude patent tubules and form an acid-base resistant hybrid layer on the dentin surface. This hybrid layer,

known as the "acid-base resistant zone," combines dentin with the adhesive, creating a structure that is more resistant mechanically, chemically, and biologically than normal dentin. This enhanced structure is often referred to as "Super Dentin."²⁷ One of the functional monomers capable of creating Super Dentin is 10-methacryloyloxydecyl dihydrogen phosphate (10-MDP) which is present in the present study.

Hashimoto and others²⁸ detected fluid movement across the resin-dentin interface during air drying and during/after polymerization. They also found that adhesive resin films per se are permeable to fluid.²⁹ This permeability may be attributed to the composition of single-bottle self-etching adhesives, which contain a mixture of hydrophilic and less hydrophobic monomers. Consequently, these adhesives retain some level of permeability to water after application to the dentinal surface.³⁰ However, despite this permeability, single-bottle self-etching adhesives can reduce dentin permeability to some degree.

However, this methodology still has several acknowledged limitations. Firstly, the depth of penetration of the agents into the dentinal tubules was not estimated. Additionally, aspects beyond the scope of the present study, such as the identification of the composition of the products formed on the dentin specimens, evaluation of dentin permeability, and assessing the resistance of occluded tubules to acid challenge, were not examined.

Conclusion

Within the limits of this study, after comparing the three desensitizing agents and control group, it was concluded that:

- All the three desensitizing agents were effective in the closure of dentinal tubules despite their different chemical compositions and application procedures.
- On intergroup comparison between Diode laser, Gluma desensitizer & Tetric N bond universal bonding agent, it was found that Diode laser had shown better results in the closure of the dentinal tubules, followed by Gluma desensitizer and then Tetric N bond universal bonding agent.
- All these agents can be effectively used for the treatment of dentinal hypersensitivity.
- Further clinical studies are required for the evaluation of efficacy of these agents & the duration of their efficiency.

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