

# An *in vitro* Analysis of Different Desensitizing Agents applied on Human Dentin

<sup>1</sup>Poorva Chouksey, <sup>2</sup>Adarsha M Shankaregowda, <sup>3</sup>Usha H Lingareddy, <sup>4</sup>Ashwini Paramashivaiah  
<sup>5</sup>Vijayalakshmi Lakshminarasimhaiah, <sup>6</sup>Upasana Lingaiah

## ABSTRACT

The aim of the study was to evaluate the ability of four topical desensitizing agents on dentinal tubule impediment utilizing confocal laser scanning microscopy (CLSM). Buccal cervical areas of 45 extracted human molars were smoothed and wet-cleaned with SiC paper, trialed by utilization of 17% ethylenediaminetetraacetic acid (EDTA) so as to mimic the clinical aspect of overly sensitive dentin of cervical surfaces.

The teeth were randomly divided into one control group (n = 5) and four experimental groups, as indicated by the dentin surface treatments: group I: GLU; group II: MSC; group III: NAN; group IV: TMD; group V: control. The samples were analyzed under the confocal laser examining magnifying lens. The extents of totally blocked, halfway impeded, and open tubules inside each group were calculated.

The ratios of totally and incompletely blocked tubules to the total tubules for all the groups were determined, and the data were statistically analyzed utilizing nonparametric tests and statistical significance was calculated. The depth of penetration was greatest for MS Coat (1.35), while it was least for Gluma (1.07). The difference among all the groups was not statistically significant for depth of penetration. Gluma desensitizer demonstrated all the more totally impeded tubules (0.44) as well as partially blocked tubules (0.37). The distinctions among all the groups were statistically significant for tubule impediment ( $p \leq 0.05$ ).

**Keywords:** Confocal laser microscopy, Dentinal hypersensitivity, Topical desensitizing agents.

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<sup>1</sup>Postgraduate Student, <sup>2</sup>Professor, <sup>3</sup>Principal, Professor and Head, <sup>4</sup>Associate Professor, <sup>5,6</sup>Reader and Associate Professor

<sup>1-5</sup>Department of Conservative Dentistry and Endodontics Vokkaligara Sangha Dental College & Hospital, Bengaluru Karnataka, India

<sup>6</sup>Department of Oral Medicine and Radiology, V S Dental College & Hospital, Bengaluru, Karnataka, India

**Corresponding Author:** Adarsha M Shankaregowda, Professor Department of Conservative Dentistry and Endodontics Vokkaligara Sangha Dental College & Hospital, Bengaluru Karnataka, India, e-mail: adarshgowda11@gmail.com

## INTRODUCTION

Dentin hypersensitivity (DH) is neither an uncommon issue nor a recent one. However, it remains an ineffectively understood area and consequently, there appears to be no effective or everlasting treatment for this painful clinical condition. Albeit different hypotheses have been proposed to explain the mechanism of DH. It is as yet indistinct how stimuli applied to the external dentin surface may stimulate nerve fibers.<sup>1</sup>

Dentin hypersensitivity has been defined as a short, sharp pain arising from exposed dentin in response to stimuli—typically thermal, evaporative, tactile, osmotic, or chemical—and which cannot be ascribed to any other dental defect or disease.<sup>2</sup> Dentin sensitivity (DS) is observed frequently. Its predominance has been accounted for to be between 4 and 69% among adults.<sup>3</sup>

The existing literature on the prevalence of DH is highly varied as a result of the use of largely different methods of evaluation. The DH varies from 15 to 70 years of age and the peak incidence is between 20 and 40 years.<sup>4</sup> The highest incidence of DH has been recorded on the buccal cervical zone of teeth.

The most commonly affected teeth are canines > premolars > incisors > molars. Chronic trauma from toothbrushing, acid erosion from the environment, gastric regurgitation or dietary substances, anatomical factors, the gingival recession caused by periodontitis, or periodontal surgery are some of the factors that have been implicated.<sup>5</sup>

Strikingly, an essentially higher extent of left *vs* right contralateral teeth was reported in right-handed patients with DH. Clinical trials have demonstrated that everyday utilization of desensitizing toothpaste twice daily requires 2 to 4 weeks to show any significant desensitization. If after using desensitizing toothpaste, the patient's DS remains a concern, clinicians should reassess the differential diagnosis and consider in-office treatments beginning with topically applied desensitizing agents.<sup>4</sup>

For the most part, as a predisposing factor to DH, the dentin needs to become exposed, as a result of the loss of enamel and/or gingival recession.<sup>6</sup> Addy et al<sup>7</sup> revealed that all sensitive teeth have low plaque scores, recommending that toothbrushing with dentifrice may encourage the improvement of DH. Others state that there is a positive correlation between DH and plaque scores.

However, brushing without dentifrice brings down DH scores while brushing with toothpaste increases them, and argues in favor of toothpaste adding to DH, presumably because of their abrasiveness.<sup>7</sup> The hydrodynamic hypothesis is the most commonly accepted mechanism to explain DH. As indicated by this hypothesis, painful stimuli coming from the oral environment act on the surface of the uncovered dentin and cause a rapid fluid movement within the dentinal tubules. According to this speculation, proposed by Brännstrom et al,<sup>8</sup> clinical research has been stimulated by proposing two strategies for desensitizing dentin: (1) minimize the capacity of the intradental nerves to respond to fluid movements and (2) decrease stimuli-evoked fluid shifts in the dentinal tubules by reducing dentine permeability.<sup>9</sup> The agents that impede dentinal tubules may cause protein precipitation, crystal precipitation on or in dentinal tubules, or conventional restorative techniques may block the tubules.<sup>10</sup> Dentinal tubules can be obliterated on the surface and/or occluded with the tubule orifices.

However, superficial occlusion of the tubules can be removed by daily toothbrushing, dissolution of the precipitate facilitated by saliva, or consumption of acidic beverages, causing transient desensitizing effects. Successful treatment with durable outcome has been related to intratubular deposition, which reduced the fluid flow or totally seals the tubule lumen.<sup>11</sup> The mechanism of action of various chemical desensitizing agents is still not well understood.

Therefore, the purpose of this study was to evaluate the ability of four topical desensitizing agents on the dentinal tubule occlusion using CLSM. Although different speculations have been proposed to explain the mechanism of DH, it is still unsure how stimuli applied to the outer dentin surface may stimulate nerve fibers.<sup>1</sup> Dentin hypersensitivity has been defined as a short, sharp pain arising from exposed dentin in response to stimuli, typically thermal, evaporative, tactile, osmotic, or chemical, and which cannot be ascribed to any other dental defect or disease.<sup>2</sup> Dentin sensitivity is observed frequently.

Its prevalence has been accounted for to be between 4 and 69% among adults.<sup>3</sup> The existing literature on the prevalence of DH is highly varied as a result of the use of widely different methods of evaluation. The DH varies from 15 to 70 years of age and the peak incidence is between 20 and 40 years.<sup>4</sup>

The most astounding occurrence of DH has been accounted for on the buccal cervical region of teeth. The teeth most commonly influenced are canines > premolars > incisors > molars. Chronic trauma from toothbrushing, acid erosion from the environment, gastric regurgitation or dietary substances, anatomical factors, the gingival

recession caused by periodontitis, or periodontal surgery are some of the factors that have been implicated.<sup>5</sup>

However, brushing without dentifrice brings down DH scores, while brushing with toothpaste increases them, and contends for toothpaste adding to DH, probably on account of their abrasiveness.<sup>7</sup> Therefore, the purpose of this study was to assess the capacity of four topical desensitizing agents on the dentinal tubule impediment utilizing CLSM. The DH is related to the fluid flow inside the dentinal tubules, and as indicated by Poiseuille's law, this movement of fluid is directly proportional to the fourth power of the radius.<sup>12</sup>

As a consequence, any diminution in the radius of the tubule opening would be required to lessen dentin permeability and as such should be effective in treating DH.<sup>13</sup> The hypersensitive dentin surfaces uncover that they have more patent tubules per unit area than nonsensitive dentin.

Dentin will only be sensitive if the tubules are patent from the pulp to the oral environment, and this patency will alter with formation and elimination of the smear, hence, resulting in an episode condition.<sup>14</sup> The present investigation has demonstrated that the four desensitizing agents tested all impeded dentinal tubules, though to various levels. Absi et al<sup>15</sup> and Yoshiyama et al<sup>16</sup> reported that in naturally desensitized dentin, the majority of the tubules were blocked.

Based on transmission electron microscopy (TEM), Yoshiyama et al<sup>16</sup> revealed that tubular impediments could be because of the extension of the intratubular dentin layer or deposition of substances in the tubules. A portion of the impediments in their investigation were crystals of inorganic salts, however, some might be organic in origin. However, the nature of the impeding layer is imperative.

Pashley and Carvalho<sup>17</sup> noticed that tubules apparently occluded with a smear plug are porous to both solvent and solute. In this way, the surface appearance alone may not correlate with sensitivity or permeability.<sup>18</sup> Research has demonstrated that the ideal DH treatment should imitate normal desensitizing procedures prompting spontaneous impediment of open dentin tubules.<sup>19</sup> Gluma desensitizer has the longest history of utilization as a desensitizing agent in clinical settings.

Since glutaraldehyde is a biologic fixative, it has been recommended that the dentinal tubules are impeded as an effect of reaction with plasma proteins from the dentinal fluid that diminishes the diameter of the dentinal tubules. Hydroxyethyl methacrylate (HEMA), a compound of dentin bonding agents, is a hydrophilic monomer with the capacity to penetrate into acid etched and moist dental hard tissue. Subsequently,<sup>20</sup> this precipitation advances HEMA polymerization, which additionally causes dentinal tubule impediment.

## MATERIALS AND METHODS

Forty-five human premolar teeth with no decays or previous restorations, extracted for periodontal reasons, were selected from a pool of freshly extracted teeth. Dental plaque, calculus, and external debris were removed using manual and ultrasonic scalers. The teeth were stored in 1% chloramine T solution at 37°C.

Teeth were decoronated and divided into four experimental groups containing 10 samples each and control group of five samples, namely group I: GLUMA power gel by Heraeus Kulzer; group II: MS Coat by Sun Medical; group III: Nanoseal by Nippon; group IV: Toothmate desensitizer by Kuraray Noritake; and group V: Control group. The teeth were sectioned below cemento-enamel junction using a water-cooled diamond saw (Struers minitom).

From each buccal surface of the tooth crown, a sectioned sample (5 mm length × 3 mm width) was obtained including the cervical area. To remove enamel and expose the underlying dentin cervical area, each fragment was ground (600-grit) flat on a polishing machine. The exposed dentin surfaces were wet-polished with 1000- and 1200-grit silicon carbide abrasive paper using Struers Labapol-5, simulating hypersensitive dentin in cervical regions. The exposed dentinal surface was cleaned with EDTA (pH 7.4) for removal of the smear layer using 17% EDTA liquid for 4 minutes (every 30 seconds replaced). The products were applied according to manufacturer's instructions.

Then the samples were examined by CLSM into two planes, x-y (treated surface) and x-z or y-z (optical sections perpendicular to the treated surface) at 50× magnification to clearly visualize the dentinal tubules and possibly precipitated salts, crystals, protein coagulation. The total number of tubules was counted from the various images captured by the CLSM.

Out of the total tubules, those that were completely impeded, incompletely occluded, and open tubules were counted. The ratio of completely impeded tubules to the total tubules as well as the ratio of incompletely occluded tubules to the total tubules was calculated. The results were statistically compared using one-way analysis of variance (ANOVA) followed by Tukey's *post hoc* analysis.

The desensitizing agents, composition, and manufacturer are given in Table 1. Desensitizing agents, mechanism, and application are given in Table 2.

## RESULTS

The depth of penetration was highest for MSC and lowest for Gluma. However, insignificant differences were detected regarding the depth of penetration of four desensitizing agents into the dentinal tubules.

**Table 1:** Desensitizing agents, composition, and manufacturer

Desensitizing agents	Composition	Manufacturer
Gluma desensitizer power gel (GLU)	Gluteraldehyde, hydroxyethylmethacrylate, pyrogenic silica, water, dye	Heraeus Kulzer, Hanau, Germany
MS Coat (MSC)	Polymethylmethacrylate, polystyrene sulfonic acid copolymer, oxalic acid, fluoride, water	Sun Medical Co., Shiga, Japan
Nanoseal (NAN)	Fluoride-calcium-aluminum-silicate glass in aqueous dispersion Phosphoric acid aqueous solution	Nippon Shika Yakuhin Co., Ltd., Shimonoseki, Japan
Toothmate desensitizer (TMD)	Powder: tetra-calcium phosphate, dicalcium phosphate anhydrous Liquid: water and preservative	Kuraray Noritake Dental Inc., Okayama, Japan

**Table 2:** Desensitizing agents, mechanism, and application

Desensitizing agents	Mechanism	Application
Gluma Desensitizer Power gel (GLU)	Blocks tubules by precipitation of multilayer protein in dentin fluid	Clean, rinse, application 60 s dwell, rinse, air-dry
MS Coat (MSC)	Reacts with tooth structure and forms precipitate (polymer film) that blocks dentin tubules	Clean, dispense liquid and apply/rub with applicator for 30 s, air-blast for 5–10 s, rinse
Nanoseal (NAN)	Reacts with tooth structure and forms fluoroaluminocalciumsilicate precipitate that blocks dentin tubules	Clean, rinse, mix A & B, apply to dentin for 20 s, rinse with water
Toothmate desensitizer (TMD)	Powder-liquid mix reacts to form hydroxyapatite precipitate Sealing of dentin	Clean, rinse, dispense and mix powder and liquid (15 s), apply with applicator, rub for 30 s, rinse

The partial occlusion was greatest for Gluma and least for Toothmate. Analysis of variance revealed significant differences among partial occlusion for the desensitizing agents between Gluma and Nanoseal; Gluma and Toothmate (Tables 3 to 10 and Graphs 1 to 3).

The complete occlusion was greatest for Gluma followed by MSC and NAN, and was found to be least for Toothmate. Moreover, ANOVA followed by Tukey's *post hoc* analysis revealed significant differences among complete occlusion for the desensitizing agents between Gluma and Toothmate.

## DISCUSSION

Gluma desensitizer has the mechanism that purportedly depends on aggregate or incomplete occlusion of the

**Table 3:** Mean depth of penetration of four agents into dentinal tubules

Mean			
Group I	Group II	Group III	Group IV
0.24	0.26	0.18	0.18
0.64	0.22	0.22	0.24
0.26	0.18	0.34	0.26
0.44	0.34	0.16	0.2
0.34	0.46	0.34	0.08
0.42	0.2	0.42	0.24
0.38	0.16	0.16	0.18
0.34	0.34	0.08	0.14
0.28	0.42	0.24	0.36
0.32	0.12	0.16	0.24

**Table 4:** Comparison of mean depth of penetration between four study groups using one-way ANOVA test

Groups	n	Mean	SD	Standard			f-value	p-value
				error	Min	Max		
I	10	1.07	0.38	0.12	0.47	1.56	0.666	0.58
II	10	1.35	0.61	0.19	0.73	2.85		
III	10	1.25	0.47	0.15	0.68	2.28		
IV	10	1.29	0.34	0.11	0.80	1.86		

SD: Standard deviation

**Table 5:** Mean partial obliteration of tubules

Group I	Group II	Group III	Group IV
0.58	0.36	0.46	0.32
0.32	0.4	0.44	0.2
0.44	0.36	0.3	0.32
0.5	0.28	0.24	0.36
0.5	0.34	0.22	0.3
0.36	0.44	0.32	0.3
0.34	0.32	0.36	0.48
0.48	0.42	0.44	0.44
0.38	0.3	0.46	0.32
0.48	0.38	0.32	0.38

**Table 6:** Comparison of mean partial occlusion between four study groups using one-way ANOVA test followed by Tukey's *post hoc* analysis

Groups	n	Mean	SD	Standard			f-value	p-value
				error	Min	Max		
I	10	0.37	0.12	0.04	0.24	0.64	4.343	0.01*
II	10	0.27	0.11	0.04	0.12	0.46		
III	10	0.23	0.11	0.03	0.08	0.42		
IV	10	0.21	0.08	0.02	0.08	0.36		

SD: Standard deviation; \*p-value ≤0.05 is significant

**Table 7:** Multiple comparison using Tukey's *post hoc* analysis for partial occlusion

Group	GI vs GII	GI vs GIII	GI vs GIV	GII vs GIII	GII vs GIV	GIII vs GIV
p-value	0.19	0.03*	0.01*	0.83	0.60	0.98

\*p-value ≤0.05 is significant

**Table 8:** Mean complete obliteration of dentinal tubules

Group I	Group II	Group III	Group IV
0.24	0.26	0.18	0.18
0.64	0.22	0.22	0.24
0.26	0.18	0.34	0.26
0.44	0.34	0.16	0.2
0.34	0.46	0.34	0.08
0.42	0.2	0.42	0.24
0.38	0.16	0.16	0.18
0.34	0.34	0.08	0.14
0.28	0.42	0.24	0.36
0.32	0.12	0.16	0.24

**Table 9:** Comparison of mean complete occlusion between four study groups using one-way ANOVA test followed by Tukey's *post hoc* analysis

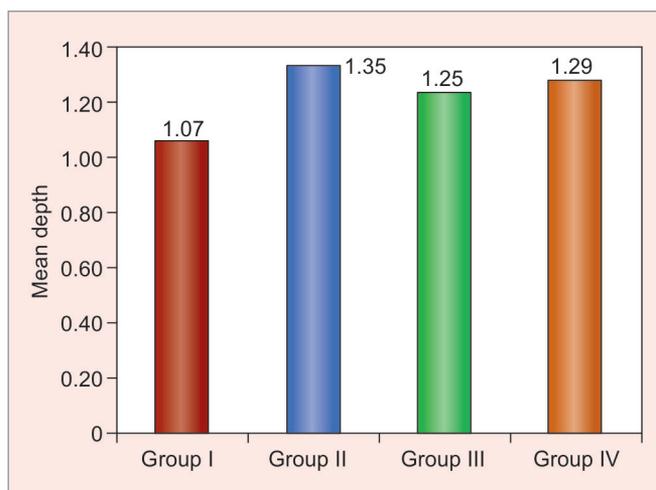
Groups	n	Mean	SD	Standard		f-value	p-value	
				error	Min			
I	10	0.44	0.08	0.03	0.32	0.58	3.114	0.04*
II	10	0.36	0.05	0.02	0.28	0.44		
III	10	0.36	0.09	0.03	0.22	0.46		
IV	10	0.34	0.08	0.02	0.2	0.48		

SD: Standard deviation; \*p-value ≤0.05 is significant

**Table 10:** Multiple comparison using Tukey's *post hoc* analysis for complete occlusion

Group	GI vs GII	GI vs GIII	GI vs GIV	GII vs GIII	GII vs GIV	GIII vs GIV
p-value	0.13	0.10	0.04*	1.00	0.95	0.98

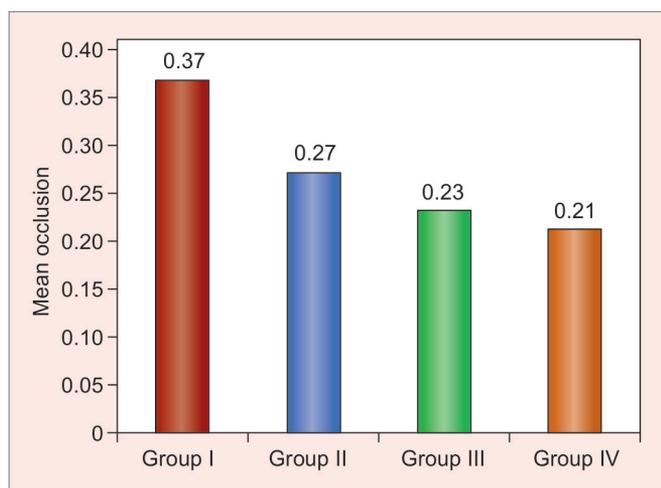
\*p-value ≤0.05 is significant



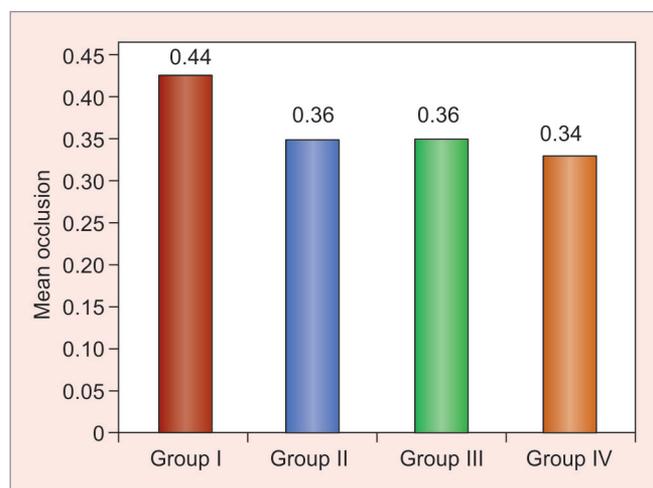
**Graph 1:** Comparison of mean depth of penetration between four study groups

tubules by protein coagulation and precipitation upon response with glutaraldehyde and hydroxyethyl methacrylate.<sup>21</sup> In a spectroscopic examination, the response mechanism among glutaraldehyde and 2-HEMA was portrayed as a two-step reaction.

Glutaraldehyde reacts with serum albumin inciting precipitation that causes a second step polymerization



**Graph 2:** Comparison of mean partial occlusion between four study groups



**Graph 3:** Comparison of mean complete occlusion between four study groups

of HEMA.<sup>22</sup> Specimens treated with Gluma desensitizer demonstrated a resinous layer of thickness 1 to 2  $\mu\text{m}$  blocking the surface of the tubules. This is as per the findings of Joshi and Gowda<sup>23</sup> and Arrais et al.<sup>24</sup> For its water solvency, HEMA is outstanding, and the HEMA substance of Gluma may hence, advance profound infiltration of the GA component into the tubules.<sup>25</sup> This would explain the greater presence of precipitates in the tubules. MSC contains oxalic acid and a fluoride containing acid polymer.

As indicated by the manufacturer, calcium oxalate upon application to dentin is precipitated and the acid polymer is claimed to give a surface sealing film. NanoSeal is a desensitizing compound introduced in the Japanese market recently. Regarding the composition, this product seems to be a spin-off from silicate cement.

It is hypothesized that, upon application of the acidic mix to the dentin surface  $\text{CaF}_2$ ,  $\text{Ca}_3\text{PO}_4$  and phosphosilicate are precipitated into dentinal tubule entrances and on intertubular dentin. Immediately after application and throughout the entire assessment time, visual analog scale rating was reduced by almost three scores. The slight regain in sensitivity recorded at 6 months recall might indicate that the precipitate is gradually removed by mechanical action and/or erosion in dietary acids. Teethmate Desensitizer is a calcium phosphate-based material.

Amid over two decades, there has been extensive enthusiasm to create calcium phosphate mixes for treatment of DH. Calcium phosphate compounds are transferred to hydroxyapatite, the main mineral phase in teeth. This means that such products can be characterized as true biocompatible and biomimetic materials.

Teethmate Desensitizer is the first calcium phosphate-containing desensitizer biomimetic materials to be marketed. In order to remove smear layer, control group specimen were polished and later etched showed patent

tubules and similar tubule density as described for sensitive areas.

In our investigation, the vast majority of the tubules in the control group was observed to be open, with some of them blocked with a smear layer. On the contrary, a large portion of the tubules in the segments treated with desensitizing agents—GLU, MSC, NAN, TMD—were partially or completely occluded.

In the present investigation, we have demonstrated that professionally applied in-office products containing Gluma desensitizer, MSC, NAN, and TMD are equipped for blocking the dentin tubules to varying degrees and may have clinical potential to decrease DH. All desensitizers impeded the tubules, yet Gluma has demonstrated prevalent outcomes regarding tubule impediment.

The outcome of the present study is constrained to physical findings of the change in the dentinal tubules and do not present *in vivo* differences that may be produced because of the physiological impact of these desensitizing operators. Contrasts between our outcomes and those of other studies may be related to the dentin specimen used, etching process, time and application mode of desensitizing agents, or a combination of these variables.

## CONCLUSION

Overall, Gluma desensitizer was found to be most effective. The newer materials MSC, NAN, and TMD appear to be promising in-office desensitizing agents. However, further research is required to access the dissolution resistance or solubility level of precipitates that occlude the dentinal tubules and also their ability to reduce fluid flow through dentin.

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