



over-the counter (OTC) dentifrices propose tubule occlusion as their mode of action. Strontium chloride is the active ingredient in the original Sensodyne® dentifrice (GlaxoSmithKline, London, UK) and was the first tubule-occluding agent incorporated into a dentifrice; later products also contain strontium acetate.<sup>12</sup> Fluoride was first proposed as a desensitizing agent in 1941<sup>13</sup> and has subsequently been used in dentifrices, gels, mouth rinses and varnishes.

Recently, a bioactive glass (NovaMin®, developed by NovaMin Technology Inc, Alachua, FL, USA) based on the original 45S5 Bioglass® (US Biomaterials Corp., Jacksonville, FL, USA) composition has been incorporated as a remineralising ingredient in dentifrice formulations for treating Dentinal Hypersensitivity. It relieves the symptoms by precipitating hydroxycarbonate apatite (HCA) onto the tooth surface and subsequently occluding the dentinal tubules. Bioactive glass is a calcium sodium phosphosilicate material (24.5% CaO, 24.5% Na<sub>2</sub>O, 6.0% P<sub>2</sub>O<sub>5</sub>, and 45% SiO<sub>2</sub>) that was originally developed as an implant material to regenerate bone and is recently adapted for use in oral care products. NovaMin reacts rapidly with saliva to release sodium, which increases the salivary pH, as well as calcium and phosphate, creating the ideal conditions for tooth remineralization. NovaMin has been shown to occlude dentinal tubules and remineralize dentin; therefore, it could be used in the treatment of dentinal hypersensitivity.

Another combination product consisting of an aqueous solution of 5% glutaraldehyde and 35% hydroxyethyl methacrylate (Gluma desensitizer, Heraeus Kulzer GmbH, Wehreim, Germany) has been reported to be an effective desensitizing agent. The glutaraldehyde intrinsically blocks dentinal tubules, counteracting the hydrodynamic mechanism that leads to dentin hypersensitivity.<sup>14</sup>

The major objective of this study was to evaluate and compare the effect of Gluma desensitizer and bioactive glass (novamin) on dentinal tubule occlusion under scanning electron microscope after their initial application as desensitizing agents on dentin in the treatment of dentinal hypersensitivity.

## MATERIALS AND METHODS

In this study, extracted sound human premolars were included, all of which had been extracted for orthodontic reasons and had no history of scaling, root planing, or prophylaxis in the previous six months. The teeth were

cleaned of gross debris and the crown and the apical third of each tooth were removed, and the remaining teeth were sectioned of thickness 2 mm to provide one to two dentin specimens each. Out of all the sections, 60 specimens were taken. Teeth were sectioned with a carborundum disc attached to a cutting machine. The dentin specimens were then placed in an ultrasonic cleaner in distilled water for 30 seconds, etched with 6% citric acid for 2 minutes to remove the smear layer and rinsed in distilled water. The specimens were then divided into three groups with 20 specimens in each group, which were Group 1- control, Group 2- Gluma Desensitizer group and Group 3- Novamin group. The specimens were then dried and the test specimens were treated with the desensitizing agents as per the manufacturer's instructions. The control group was surface treated with distilled water, the second group, with Gluma desensitizer, and the third group with NovaMin.

In the Gluma group, a small amount of Gluma desensitizer was applied onto the dentin discs using small cotton pellets as per the manufacturer's instructions and left for 30–40 seconds. The surface was then dried by applying a stream of compressed air until the fluid film had disappeared and the surface was no longer shiny, and then rinsed thoroughly with water. In the NovaMin group, bioactive dentifrice paste was applied to the dentin specimens with the help of a swab. It was left for 2 minutes and then lightly rinsed away. Each sample was air-dried at a constant room temperature (21–23°C) in a desiccator and sputter coated with gold. A scanning electron microscope (JEOL Model 5400, JEOL Co., Tokyo, Japan) was used to examine the specimens.

The surface of specimens was visualized under scanning electron microscope at a magnification of ×2000 and the photographs of the representative areas were obtained (Figure 1-3). The total number of tubules, number of open tubules, number of completely occluded tubules and number of partially occluded tubules were counted in each photograph of all of the specimens. The following criteria was used for determining the type of occlusion when counting the tubules. 1) The tubules that showed complete obliteration of the canals with the reaction products were considered completely occluded. 2) The tubules that showed reduction of the diameter of the tubule by more than fifty percent or circumferential closure of the tubule with the presence of a central opening in the canal were considered partially occluded.

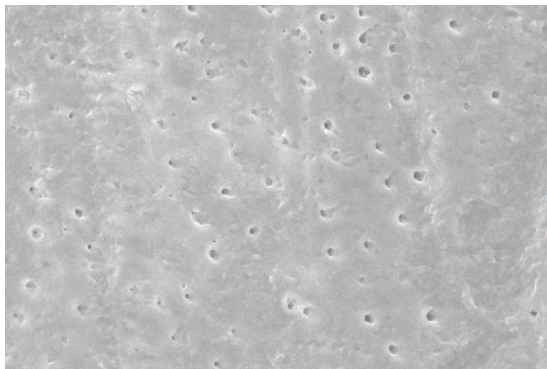


Figure 1: Group 1 (control)

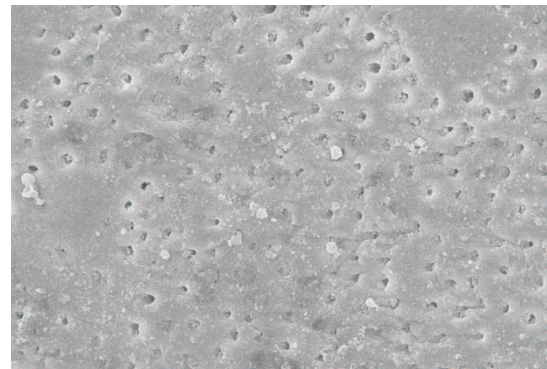


Figure 2: Group 2 (Gluma desensitizer)

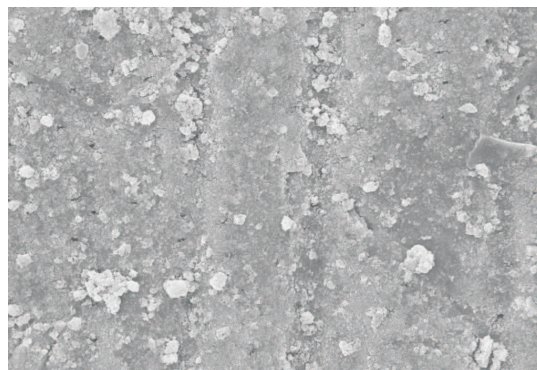


Figure 3: Group 3 (Bioactive Glass)

**RESULTS**

The total number of tubules was counted from the various images captured by the SEM. Out of the total tubules; those that were completely occluded, partially occluded, and open tubules were counted. The ratio of completely occluded tubules to the total tubules as well as the ratio of partially occluded tubules to the total tubules was calculated. The data obtained was statistically analyzed using the Kruskal-Wallis test and

Wilcoxon rank sum test, through which comparison among the groups as well as intergroup comparison was performed, respectively, and statistical significance was calculated (Table 1). The mean of the ratio of completely occluded tubules to total tubules as well as partially occluded to total tubules for each group were plotted (Figures. 4,5). All of the statistical analyses were performed by using IBM SPSS ver.<sup>21</sup> (IBM Co., Armonk, NY, USA).

Group	No of specimens	Mean ±SD	Mean rank	Kruskal Wallis Test	Wilcoxon rank - sum Test
1-Control	20	0.132±0.066 <sup>a)</sup> 0.050±0.020 <sup>b)</sup>	10.50 <sup>a)</sup> 10.50 <sup>b)</sup>	Group 1, 2 and 3	Group 1 & 2 <sup>c)</sup>
2-Gluma desensitizer	20	0.400±0.065 <sup>a)</sup> 0.533±0.070 <sup>b)</sup>	31.50 <sup>a)</sup> 50.35 <sup>b)</sup>		Group 1 & 3 <sup>c)</sup>
3-Novamin	20	0.540±0.052 <sup>a)</sup> 0.361±0.046 <sup>b)</sup>	49.50 <sup>a)</sup> 30.65 <sup>b)</sup>		Group 2 & 3 <sup>c)</sup>

SD- Standard Deviation

a) Ratio of completely occluded and total tubules.

b) Ratio of partially occluded and total tubules.

c) Significant difference where  $p \leq 0.05$

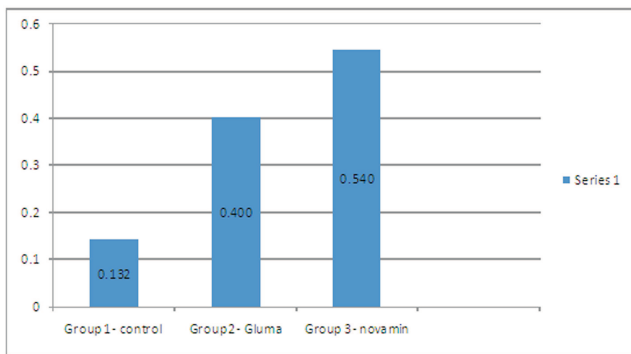


Figure 4: Ratio of completely occluded tubules to total tubules. The bar graph depicts the mean ratio of the number of completely occluded tubules to the total number of tubules. The mean value is highest for the NovaMin group, which indicates more completely occluded tubules than the other groups.

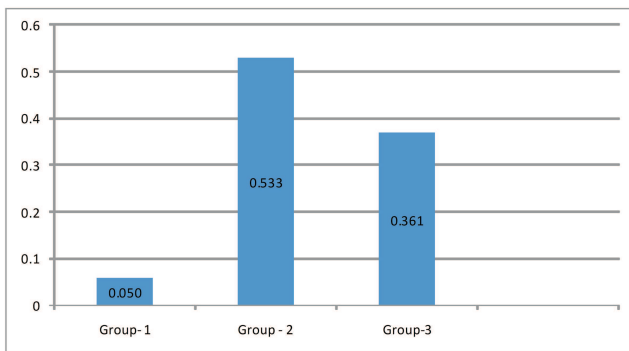


Figure 5: Ratio of partially occluded tubules to total tubules. The bar graph depicts the mean ratio of the number of partially occluded tubules to the total number of tubules. The mean value is highest for the Gluma desensitizer group, which indicates more partially occluded tubules than the other groups.

## DISCUSSION

SEM studies of hypersensitive dentin surfaces show that they have tubules that are more patent per unit area as compared to normal nonsensitive dentin. Furthermore, tubules in superficial parts of hypersensitive dentin are on average twice as wide as tubules in nonsensitive dentin. Absi et al<sup>15</sup> and Yoshiyama et al<sup>16</sup> reported that in naturally desensitized dentin, most of the tubules were occluded. Based on transmission electron microscopic studies, Yoshiyama et al<sup>16</sup> reported that tubular occlusions could be due to extension of the intratubular dentin layer or deposition of substances in the tubules. Some of the occlusions in their study were crystals of inorganic salts, but some may be organic in origin. However, the nature of the occluding layer is important. Some surfaces where the tubules were observed to be occluded with a “dense pellicle” were found to be very sensitive. Pashley and Carvalho<sup>17</sup> noted that tubules apparently occluded with

a smear plug are permeable to both solvent and solute. Thus, the surface appearance alone may not correlate with sensitivity or permeability of dentin.

Dentin will only be sensitive if the tubules are patent from the pulp to the oral environment, and this patency will change with production and removal of the smear, hence resulting in a hypersensitive condition.<sup>18</sup> Most studies on tubule occlusion have focused on coronal dentin, where important variables such as the dentin surface area, thickness, and surface characteristics can be controlled. The hydraulic conductance of radicular dentin has been observed to be much lower than that of coronal dentin; there is a good correlation between tubule density and diameter and the measured hydraulic conductance.<sup>19</sup>

Morris et al<sup>20</sup> showed in their study the very powerful placebo effect inherent in clinical dentin sensitivity studies, particularly when dealing with small numbers of subjects and eligible teeth. Furthermore, the large standard deviations reported by Morris et al<sup>20</sup>, because of the highly subjective nature of pain and/or the variability of the individual pain response reported in dentin sensitivity studies, makes it extremely difficult to detect significant differences between groups without utilizing a large number of subjects. With this in mind, the in vitro examination of products using a reproducible model such as the dentin disc, can aid the understanding of the potential occluding, and thus desensitizing properties of possible desensitizing agents.<sup>21</sup>

Gluma desensitizer is an aqueous solution containing 5% glutaraldehyde and 35% hydroxyethyl methacrylate. Because glutaraldehyde is a biological fixative, it has been suggested that the dentinal tubules are occluded as an effect of reaction with plasma proteins from dentinal fluid. Hydroxyethyl methacrylate is a hydrophilic monomer compound of dentin bonding agents with the ability to infiltrate into acid-etched and moist dental hard tissue.<sup>22</sup>

NovaMin is a material that has been shown to reduce sensitivity by blocking open tubules in both in vitro and in vivo studies (United States patents 5,735,942 and 6,086,374).<sup>23,24</sup> NovaMin is a bioactive glass-ceramic material that falls into a class of newer agents that provide calcium and phosphate upon reaction. In the case of products with NovaMin, the active ingredient is a calcium sodium phosphosilicate that reacts when exposed to aqueous media and provides calcium and

phosphate ions that form a HCA with time.<sup>25</sup> The combination of the residual NovaMin particles and the HCA layer results in the physical occlusion of dentinal tubules, which will relieve hypersensitivity.<sup>26</sup>

In our study, most of the tubules in the control group were found to be open (Figure 1), with some of them occluded with a smear layer; on the other hand, most of the tubules in the sections treated with Gluma desensitizer (Figure 2) and NovaMin (Figure 3), which work on the principle of tubule occlusion by infiltration of precipitation products, were partially or completely occluded. In our study, after initial application, Gluma desensitizer produced a greater number of partially occluded tubules and fewer completely occluded tubules, while in the case of specimens treated with NovaMin, a greater number of completely occluded tubules and fewer partially occluded tubules were observed. In both the cases, the difference was statistically significant. This might be due to the mechanism of Gluma desensitizer, which reportedly is based on total or partial closure of the tubules by protein coagulation and precipitation upon reaction with glutaraldehyde and hydroxyethyl methacrylate. An *in vitro* study reported that NovaMin occluded a significantly greater number of dentinal tubules relative to untreated controls and it also occluded significantly more tubules than another test material Gluma desensitizer.<sup>27</sup> The results of the current study revealed that NovaMin-treated dentin specimens showed more complete tubule occlusion. This is in accordance with the findings Du Min et al.<sup>28</sup>, who found NovaMin to be a more effective desensitizer. An *in vivo* study reported that Gluma desensitizer was not effective in relieving dentinal hypersensitivity after 4 weeks; this can be attributed to a relatively large number of open and partially occluded tubules remaining after treatment.<sup>29</sup>

In the present study, it was shown that professionally applied dental (in-office) products containing NovaMin and Gluma desensitizer are both capable of occluding the dentin tubules to varying degrees and may have the clinical potential to reduce dentin hypersensitivity. Both desensitizers occluded the tubules but NovaMin has shown superior results in terms of complete tubule occlusion on initial application. The results of the present study are limited to physical findings of the change in the dentinal tubules, and do not present *in vivo* differences that may result from the physiological effect of these desensitizing agents. Differences between our results and

those of other studies may be related to the differences in dentin specimen utilized, etching process, time and mode of application of the desensitizing agent, or a combination of these variables. Significant differences in results can be produced on multiple applications and testing the materials under the vigorous conditions. In this study, it has been shown that NovaMin and Gluma desensitizer are materials with different modes of action and produce varying degrees of obliteration of tubules at initial application and hence could have differences in reduction in sensitivity based on the type and amount of blockage of tubules. Both materials produced varying degrees of tubule occlusion in the form of complete and partial occlusion.

### CONCLUSION

In the present study bioactive glass was found to produce more completely occluded tubules while Gluma desensitizer caused more partial occlusion on initial application. There was a statistically significant difference between the two groups when the ratio of complete and partial occlusion was calculated against the total number of tubules. Hence, NovaMin application could be more effective in providing relief from dentinal hypersensitivity when compared with Gluma Desensitizer.

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