Available online at http://www.journalijdr.com

IJDR

ISSN: 2230-9926

International Journal of Development Research
Vol. 09, Issue, 04, pp.26934-26938, April, 2019

ORIGINAL RESEARCH ARTICLE

OPEN ACCESS

# IN VITRO EVALUATION OF DENTINAL TUBULE OCCLUSION BY NOVAMIN (BIOACTIVE GLASS)

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**ARTICLE INFO**

***Article History:***
Received 08th January, 2019
Received in revised form
17th February, 2019
Accepted 26th March, 2019
Published online 29th April, 2019

***Key Words:***

Bioactive glass,
Dentine Hypersensitivity, Novamin,
Tubule occlusion.

**ABSTRACT**

**Aim**–The aim of this study was to evaluate the dentinal tubule occluding ability of Novamin (bioactive glass) containing desensitizing dentifrices using scanning electron microscope. **Materials and Methods –** Forty-five 1mm thick dentine discs were obtained from orthodontically extracted human premolar teeth. Each disc was then split into two halves producing a total of 90 dentine specimens. One half of each disc was allotted to Group -1(Control group) and the other half was allotted to group-2 (Test group). GROUP 1: brushed without dentifrices. GROUP 2: brushed with dentifrices containing Novamin. After brushing for 2 minutes twice a day, the specimens were stored in artificial saliva. Specimens were analyzed after 7 days under SEM. **Results –** The novamin containing desensitizing agent significantly resulted in effective dentinal tubule occlusion. 91.5% of the dentinal tubules showed complete dentinal tubule occlusion, 6.61% showed partial occlusion and 1.89% remained unoccluded. **Conclusion -** The inclusion of novamin into a toothpaste formulation may be an effective approach to treat dentinal hypersensitivity as Novamin showed high percentage of dentinal tubule occlusion by precipitating hydroxycarbonate apatite over the entire dentine surface.



**Citation: Dr. Prabhati Gupta, Dr. Suhail Majid Jan and Dr. Roobal Behal. 2019.** "In vitro evaluation of dentinal tubule occlusion by novamin (bioactive glass)", *International Journal of Development Research*, 09, (04), 26934-26938.

## INTRODUCTION

Dentine hypersensitivity is a relatively common problem in periodontal practice. It may occur spontaneously when the root becomes exposed as a result of gingival recession or pocket formation, or it may occur after scaling and root planing and surgical procedures. Dentine hypersensitivity is a transient pain arising from exposed dentine, typically in response to chemical, thermal, tactile or osmotic stimuli, which cannot be explained by any other dental defect or pathology (Rees JS *et al*., 2002). It is a common problem found in many adult populations with prevalence figures ranging from 4 to 74% (Rees JS *et al*., 2002). This wide variation in prevalence may be due to various factors like chronic trauma from tooth brushing, gingival recession, erosion of enamel, anatomical factors, etc. (West N *et al*.,1998). Effective and long-lasting treatment of dentine hypersensitivity is thus of paramount interest to both patient and clinician. The hydrodynamic theory, first proposed by Gysi in 1900 and proven by Brannstrom in 1963, implicates a change in fluid flow through the patent dentinal tubules as a result of external stimulation, most notably evaporative cold application.

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This induces a discharge of pulpal afferents of the intradental nerves (Matthews B *et al*., 2000), and consequently nociceptor activation in the pulp/dentine border area (Markowitz K *et al*., 1991) emanating centrally as pain. There have been two basic approaches to the treatment and prevention of dentine hypersensitivity. The first approach is to treat the tooth with a chemical agent such as potassium nitrate or potassium chloride that penetrates into the dentinal tubules and raises the potassium ion level. This sustained neural depolarization reduces sensitivity by inactivating voltage-gated sodium channels, thereby blocking active potential generation (Orchardson R *et al*., 2000). A second approach is to treat the tooth with a chemical or physical agent that creates a deposition layer and mechanically occludes dentinal tubules, which reduces sensitivity by prevention of pulpal fluid flow e.g. potassium oxalate, ferric oxalate, strontium chloride (Dragolich WE *et al*,1993). Novamin (calcium sodium phosphosilicate) is a bioactive glass in the class of highly biocompatible materials that were originally developed as bone regenerative materials (Hench LL *et al*.,1993) .These materials are reactive when exposed to body fluids and deposit hydroxycarbonate apatite (HCA), a mineral that is chemically similar to the mineral in enamel and dentine. When incorporated into a dentifrice, particles are deposited onto the dentine surface to mechanically occlude dentinal tubules (Du

MQ *et al*., 2008). Saliva can solubilise materials adhering to teeth and contains calcium and phosphate ions that can interact with tooth surfaces (Gandolfi MG *et al*., 2008 and Arrais CA *et al*., 2003). Therefore, it is essential to evaluate whether desensitizing agents could occlude dentinal tubules effectively under the circumstances similar to oral environment (Wang Z *et al*., 2010). The evidence for the dentine occlusion effect of Novamin, containing desensitizing dentifrices under stimulated oral environment is limited. For these reasons, selection of artificial saliva as post-treatment immersion medium to evaluate the efficacy of novamin containing desensitizing product appears to be necessary. Thus the aim of this study was to evaluate the dentinal tubule occluding ability of Novamin containing desensitizing dentifrices using scanning electron microscope.

## MATERIALS AND METHODS

This study was conducted at the Department of Periodontics, Government Dental College Srinagar. Scanning electron microscopic evaluation was done at University of Kashmir.
Forty-five premolar teeth extracted for orthodontic reasons were collected from the Department of Oral and Maxillofacial Surgery, Government Dental College, Srinagar. All the teeth were cleaned thoroughly and stored in 10% formalin for no longer than a month prior to their use. The exclusion criteria involved presence of fluorosis or hypocalcification, caries, periodontal disease, wasting diseases in premolars and teeth of patients receiving or undergoing treatment for dentinal hypersensitivity. Forty-five dentine discs, each with a thickness of 1mm approximately, were cut perpendicular to the long axis of the tooth just above the cementoenamel junction from the region between apical limit of dentinoenamel junction and coronal limit of pulp chamber by means of the double sided diamond disc (Summa Disk 0692, 3/4″ Regular) attached to water-cooled air motor (SDE-M40E) and straight handpiece (ND, ES-30A, JAPAN). Each disc was carefully prepared and inspected to ensure that they were free of coronal enamel or pulpal exposures. Each disc was then split into two halves using a dental chisel. A groove was prepared on the pulpal surface of each half of the disc for the purpose of orientation. One half of each disc was allotted to Group-1 (Control group) and the other half was allotted to Group-2 (Test group).Thus each group had 45 specimens. These dentine specimens were then mounted on 2mm thick polyvinyl plates using Cyanoacrylate adhesive (Fewi kwik). After preparation of the specimens, the occlusal surface of each dentine disc half was sanded with silicon carbide paper for 30 seconds to create a standard smear layer. The smear layer was subsequently removed by dipping the specimens into 0.5M EDTA solution (pH 7.4) for 2 minutes to open dentinal tubules to simulate the hypersensitive dentine. The etched specimens were rinsed with distilled water and kept wet.

| GROUP | Treatment done |
|---|---|
| 1 (Control Group) | EDTA-etched specimens were brushed for 2 minutes twice a day for 7 days without dentifrices |
| 2 (Test group) | EDTA-etched specimens were brushed for 2 minutes twice a day for 7 days with dentifrices containing Bioactive glass or novamin (Vantej™) |

Each specimen from Group 2 was brushed with undiluted dentifrice (approximately 1 gram). A powered toothbrush (Oral – B Cross Action Power™, China) with bristles of medium hardness was applied to the dentine surface at an inclination of about 90º under a constant loading (150grams) for 2 minutes. The brushing load was measured with a top loading balance during brushing. At a time only one specimen could be brushed. After brushing the specimens for 2 minutes, they were rinsed with distilled water and stored in artificial saliva (pH 7.2). The composition of artificial saliva was (mMoles/L): distilled water 700ml, Ca $(OH)_2$ 1.56 mMol/L, KCl 150.00 mMol/L, HCl 36.00 mMol/L, $H_3PO_4$ 0.088 mMol/L, buffer 99.7 mMol/L. This procedure was followed twice a day for 7 days. Dentine specimens obtained after treatment were dried in a desiccator and sputter coated with gold in a vacuum evaporator (J.F.C. 100 JEOL). Photomicrographs of the dentine surfaces were obtained using a scanning electron microscope (JSM-S10-A, Jeol, Japan) at 20 kV. Each SEM photograph was assessed for the percentage of completely occluded, partially occluded and unoccluded tubules. SEM photomicrograph from central area of each sample was obtained at 20 kV at ×1000 magnification. The SEM photomicrographs were evaluated quantitatively. Quantitative analysis was performed by counting the number of dentinal tubules in an area of 50µ. Percentage of occluded tubules was obtained by dividing the total number of occluded tubules by total number of tubules in each photomicrography. This result was then multiplied by 100 to obtain the percentage of occluded tubules for each photomicrography. Similarly the percentages of partially occluded and unoccluded tubules were calculated in each photomicrograph.

## RESULTS

Figures 1and 2 show SEM photomicrographs (at 1000x) of the two Group specimens. SPSS (version 20.0) and Microsoft Excel software were used to carry out the statistical analysis of the data. Data was analyzed with the help of descriptive statistics viz., means and standard deviations. Comparison of data between the groups in terms of complete occlusion, partial occlusion and unoccluded dentinal tubules was analyzed using one-way analysis of variance (ANOVA). P-value less than 0.05 was considered statistically significant.

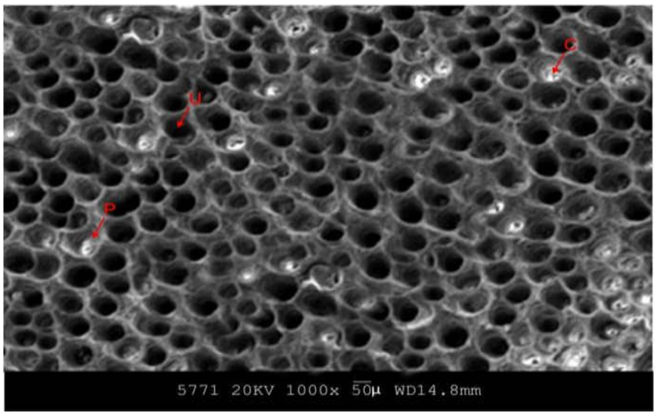


**Figure 1. SEM Photomicrograph of Group – 1 dentine specimen brushed with distilled water without dentifrice. (*U=Unoccluded Tubule, P=Partially Occluded Tubule, C=Completely Occluded Tubule)**

Tables 1-3 demonstrate the mean, standard deviation and other descriptive values of the two groups in terms of complete occlusion, partial occlusion and unoccluded dentinal tubules respectively. On applying ANOVA to compare the mean percentage of completely occluded dentinal tubules of all the four groups, p value of <0.001 was obtained which was statistically highly significant (TABLE-1).

**Table 1. Complete dentinal tubule occlusion in the 2 groups**

| | | No. | Mean (in %) | Std. Deviation | Std. Error | 95% Confidence Interval for Mean | | Range | p-value (ANOVA) |
|---|---|---|---|---|---|---|---|---|---|
| | | | | | | Lower Bound | Upper Bound | | |
| C | Group 1 | 45 | 4.58 | 1.079 | 0.279 | 3.981 | 5.176 | 2.76-6.52 | <0.001 (SSD) |
| | Group 2 | 45 | 91.50 | 0.999 | 0.258 | 90.944 | 92.050 | 90.16-94.45 | |

*C : Complete occlusion, SSD : Statistically significant difference.

**Table 2. Partial dentinal tubule occlusion in the 2 groups**

| | | No. | Mean (in %) | Std. Deviation | Std. Error | 95% Confidence Interval for Mean | | Range | p-value (ANOVA) |
|---|---|---|---|---|---|---|---|---|---|
| | | | | | | Lower Bound | Upper Bound | | |
| P | Group 1 | 45 | 7.64 | 1.110 | 0.287 | 7.027 | 8.256 | 6.34-10.12 | <0.001 |
| | Group 2 | 45 | 6.61 | 0.842 | 0.217 | 6.145 | 7.077 | 4.46-7.89 | <0.001 |

*P : Partial occlusion.

**Table 3. Unoccluded dentinal tubules in the 2 groups**

| | | No. | Mean (in %) | Std. Deviation | Std. Error | 95% Confidence Interval for Mean | | Range | p-value (ANOVA) |
|---|---|---|---|---|---|---|---|---|---|
| | | | | | | Lower Bound | Upper Bound | | |
| U | Group 1 | 45 | 87.78 | 0.949 | 0.245 | 87.254 | 88.306 | 86.26-89.92 | <0.001 |
| | Group 2 | 45 | 1.89 | 0.716 | 0.185 | 1.495 | 2.289 | 1.09-3.72 | <0.001 |

*U :Unoccluded tubules.

**Table 4. Inter group comparison in terms of complete dentinal tubule occlusion**

| Group Comparison | Mean Difference | t-value | p-value |
|---|---|---|---|
| Between Group 1 and Group 2 | -86.92 | 228.93 | <0.001(SSD) |

*SSD : Statistically significant difference.

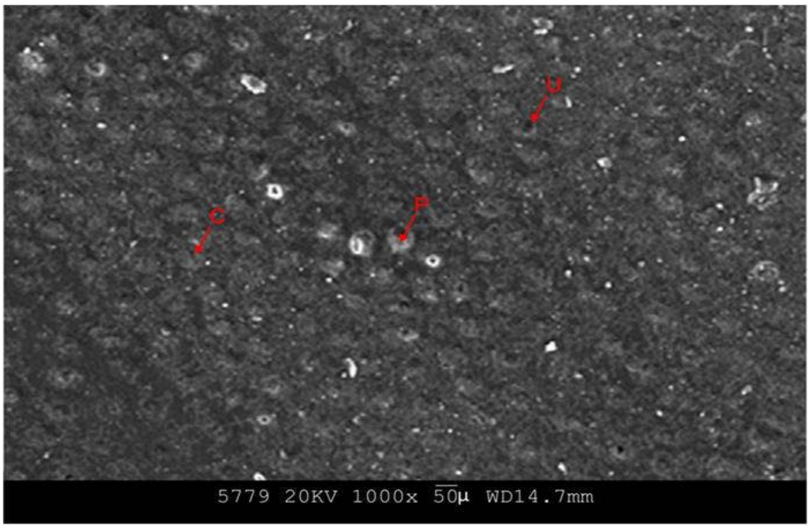


**Figure 2. SEM Photomicrograph of Group – 2 dentine specimen brushed with dentifrices containing Novamin. (*U=Unoccluded Tubule, P=Partially Occluded Tubule, C=Completely Occluded Tubule)**

Table 4 demonstrates the comparison between the two groups in terms of complete dentinal tubule occlusion. On comparing the mean percentage of completely occluded dentinal tubules between two groups, p value of <0.001 was obtained which was statistically highly significant (TABLE-4).

## DISCUSSION

Life expectancy is increasing and the patients are retaining their natural teeth for a longer time due to effective treatment strategies for caries and periodontal disease. Consequently, there is a higher risk of cervical dentine hypersensitivity as a result of physiological gingival recession with aging (Rees JS *et al*., 2002).

Nevertheless, the cementum in the cervical region and along the root is very thin which can be easily removed during non-surgical periodontal therapy increasing the risk for dentinal hypersensitivity (Sauro S *et al*., 2010). The most acceptable hydrodynamic theory postulates that the most pain provoking stimuli increase the outward flow of the fluid in the tubules. This increased outward flow of the fluid in the tubules in turn causes pressure change across the dentine which activates the A-delta intradental nerves at the pulp dentine border or within the dentinal tubule resulting in pain (Pashley DH, 1986 and Addy M, 2002). The differences in tubule diameter and the number of tubules are important. According to Poiseuille's law, the fluid flow is proportional to the fourth power of the radius of the tubule or dentine permeability is proportional to the product of tubule number and diameter (Kolker JL *et al*., 2002). This information has important implication for treatment strategies, reducing the number of open tubules or decreasing the diameter is mode of reducing the hypersensitivity by many chemical compounds (Kolker JL *et al*., 2002 and Kerns DG *et al*., 1991). The present study evaluated the effects of Novamin (Bioactive glass or Calcium sodium phosphosilicate) on dentinal tubule occlusion using Scanning electron microscope (SEM). GROUP 1 specimens were brushed with distilled water without dentifrices and GROUP 2 with dentifrices containing Novamin (Vantej™). Each SEM photograph was assessed for percentage of completely occluded tubules, partially occluded tubules and unoccluded tubules. The Group 1 specimens showed the mean percentage of completely occluded dentinal tubules as 4.58±1.079 % and partially occluded dentinal tubules as 7.64±1.110 %. The results of this study are in agreement with the studies conducted by Wang Z *et al*. in 2010 and Wang Z *et al*. in 2011 which demonstrated that the toothbrushing with distilled water reduced the dentine permeability by leaving some smear debris in the dentinal tubules which results in occlusion of some dentinal tubules. In the present study, the small percentage of dentinal tubule occlusion was seen in Group 1 specimens. This may be because of the smear layer formed by the brushing process (Yoshiyama M *et al*., 1990, Trowbridge HO *et al*.,1990 and Goldstein GR *et al*., 1991). The Group 2 specimens showed the mean percentage of completely occluded dentinal tubules as 91.50±0.999 % and partially occluded dentinal tubules as 6.61±0.842 %. The results of this study are in agreement with the studies conducted by Wang Z *et al*. in 2010, Sauro S *et al*. in 2010, Burwell A *et al*. in 2010, Joshi S *et al*. in 2013, West NX *et al*. in 2011. In the present study, the Group 2 dentine specimens showed a higher number of tubule occlusion with the characteristic granular deposition all over the dentine surface. Novamin is known to induce osteogenesis in physiological systems and would appear to offer suitable surface reactivity. Since there are obvious similarities between bone and dentine, there is a possibility of novamin being useful as a means of more permanent occlusion of the dentinal tubules (Gillam DG *et al*., 2002). Bioglass particles in the toothpaste apparently deposited onto the dentine surface and reacted with calcium and phosphorus in the artificial saliva that mechanically occluded dentinal tubules. Such a reaction of bioglass when exposed to body fluids such as saliva, tended to deposit hydroxycarbonate apatite, a mineral that is supersaturated with respect to artificial saliva (Suge T *et al*., 2008). This could probably explain why Novamin or bioactive glass treated specimens showed the maximum percentage of dentinal tubule occlusion after immersion in artificial saliva. Thus it was concluded that Novamin (Group 2) showed the higher percentage of dentinal tubule occlusion by precipitating hydroxycarbonate apatite over the entire dentine surface. Thus this study would suggest that the inclusion of novamin into a toothpaste formulation may be an effective approach to treat dentinal hypersensitivity The future scope of this study is to determine the depth of dentinal tubule occlusion.

## Conclusion

Novamin containing desensitizing dentifrice demonstrated surface coverage after brushing with the formulated toothpaste. The formation of a hydroxyapatite layer occluding the dentine tubules following artificial saliva immersion may be considered an important stepping stone for further evaluation of these bioactive glass compositions. In conclusion, the results from the present in vitro study would appear to support the growing evidence in the published literature that toothpaste formulations containing bioactive glasses or novamin occlude dentine tubules and therefore may be an effective approach treating dentinal hypersensitivity.

**Acknowledgements** – Nil.

**Conflicts of Interest** – Nil.

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