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Results: Colgate Sensitive Pro-Relief and Gluma desensitizer produced a greater number of partially occluded tubules NovaMin[R] (SHY-NM[TM]) and Laser Diode therapy produced a greater number of completely occluded tubules. The differences among all the groups were recorded as statistically significant ($P \leq 0.05$).

Conclusion: All materials were significantly effective in occluding dentinal tubules but, Laser diode therapy and Novamin[R] (SHY-NM[TM]) containing dentifrice showed the best results for total occlusion of dentinal tubules.

Keywords: dentinal hypersensitivity; novamin; gluma; diode laser, pro-arginine; scanning electron microscope.

I. Introduction

Dentinal Hypersensitivity (DH) is defined as one of the most painful and least predictably treated chronic conditions in dentistry.[1]

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The nature of the exposed dentin is of relevance, as not all patients exhibiting dentin exposure will develop sensitivity. The number and diameter of dentinal tubules at the tooth surface is shown to be significantly increased in hypersensitive dentin.[2,3] While the exact mechanism of dentinal hypersensitivity is still controversial, hydrodynamic theory is one of the most accepted hypothesis.[4] The dentinal tubules, which are open and wide, contain a fluid. According to this theory, this fluid expands when exposed to heat and contracts when exposed to cold or touch. The contraction and expansion change the pressure in the fluid phase, which in turn activates mechanoreceptor nerves close to the pulp. When nerve receptors are activated, sodium ions enter the dentin and potassium exits. This ion exchange polarizes the nerves and causes pain.[3] The concept of tubule occlusion as a method of dentin desensitization is a logical conclusion based on the hydrodynamic hypothesis.[5]

The two principal treatment options available are, preventing fluid flow, plugging the dentinal tubules, or desensitizing the nerve, making it less responsive to stimulation.[6]

In the last fifteen years, with the introduction of Lasers which gave urther possibilities to DH therapy.[7, 8-11]

It is now possible to show that the role of laser in DH therapy is twofold. The low-level power lasers also called "soft lasers," act directly on nerve transmission. The other, high-power lasers such as diode 980 nm and 808 nm, Nd: YAG 1064 nm, CO_2 10600 nm, Er, Cr: YSGG 2780 nm, and Er: YAG 2940 nm act on DH by provoking a melting effect along with the crystallization of dentine inorganic component and also the coagulation of fluids into the dentinal tubules.[12] Among the "high power" devices, the diode lasers which are the most studied and also the ones that gave the best results in several clinical protocols even in very high-grade DH cases.

Gluma desensitizer, (Heraeus Kulzer GmbH, Wehrheim, Germany), is a combination product consisting of an aqueous solution of 35% hydroxyethyl methacrylate and 5% glutaraldehyde, that has been reported to have been an effective desensitizing agent. The glutaraldehyde intrinsically blocks dentinal tubules,

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counteracting the hydrodynamic mechanism that leads to dentin hypersensitivity.[13]

A new product consisting of calcium sodium phosphosilicate NovaMin^{R}, (SHY-NM^{TM}), has now been introduced. NovaMin is the trade name that has been given to bioactive glass (e.g., Bioglass), that has been ground into a fine particulate with a median size of less than 20 microns. It reduces sensitivity by blocking open tubules and by supplying calcium (Ca^{2+}) and phosphate (PO^{43-}) ions to form hydroxycarbonate apatite (HCA). It is composed of elements that are naturally occurring in the body and it reacts to form a mineral layer that is chemically and structurally very similar to natural tooth material.[14]

In 2009, Colgate-Palmolive company acquired the rights to the novel technology, now known as Pro-Argin technology, First introduced by Kleinberg et al in 2002, The pro- arginine therapy uses arginine, an amino acid, calcium carbonate a source of calcium and Bicarbonatea pH buffer.[15]

The main objective of this study was to evaluate and compare the occlusion of dentinal tubules for treatment of DH by four novel Desensitizing agents:

In-Office Treatment: Diode Laser (Zolar) and Gluma Desensitizer.

Patient Applied Products for Home Use: Novamin^{R} containing Toothpaste (SHY-NM^{TM}) and Colgate Sensitive Pro-Relief Toothpaste.

II. Materials and Methods

a) *Sample preparation:* Thirty freshly extracted mandibular molars were selected and stored in normal saline until use(n=30)

The teeth were cleaned of any gross debris. Tooth cuts were made with a carborundum disc attached to a cutting machine. The crown and the apical third of each tooth were removed, and the remaining teeth were sectioned to provide one to two dentin specimens each. Sectioned samples of 2-mm thickness were made. The dentin specimens were then placed for 30 seconds in an ultrasonic cleaner in distilled water, etched with 17% aq EDTA (Ethylene Diamine Tetra Acetic Acid) for 2 minutes to remove the smear layer and rinsed in distilled water. The control specimens were then dried, and the test specimens were treated as per the manufacturer's instructions with the desensitizing agents.

b) *Desensitising Agents:* 30 extracted mandibular molars were used. (n=30) They were divided into 5 groups including the control group in which no treatment was done. The Diode Laser and three commercially available desensitizers were investigated in this study.

Group 1: Diode laser (ZOLAR) at 980nm therapy was used on dentin discs, in a non-contact mode for 30 seconds.

Group 2: Novamin^{R} containing toothpaste SHY-NM^{TM} Is applied on the dentin discs for 30- 40 seconds using an applicator tip and air-dried.

Group 3: Gluma desensitizer (Heraeus Kulzer GmbH, Wehrheim, Germany) is applied on the dentin discs for 30-40 seconds using applicator tip and air-dried

Group 4: Pro-arginine group of toothpaste Colgate sensitive pro-relief is applied on the dentin discs and left for 30-40 seconds using applicator tip and air-dried.

c) *SEM Evaluation:* All specimens were dehydrated in graded acetone, sputter-coated with gold-palladium and critical point dried. The specimens were then examined under a scanning electron microscope (Zeiss Sigma VP, Zeiss, Oberkochen, Germany) at 20 kV acceleration voltage. Standardized images of the dentine discs were acquired at a specific magnification of 3000×. Twenty images were acquired per disc. and surface scans were made to study the covering of the discs.[Fig 1(a)(b)(c)(d)(e)]

d) Quantitative Analysis of dentinal tubule occlusion: The standardized SEM images in the form of microphotographs were imported into ImageJ software (NIH, USA) and converted into binary images. The black (open dentine tubules) and white (occluded dentine tubules and dentine) pixels were counted, and the numbers were transferred into a worksheet using Microsoft Excel.

III. 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 were calculated. Nonparametric tests were done as the data obtained did not show a normal distribution. 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 an intergroup comparison was performed, respectively, and statistical significance was calculated (Table 1). The mean of the ratio of completely occluded tubules to total tubules and partially occluded to total tubules for each group were plotted (Figs.2 and 3). All of the statistical analyses were performed using IBM SPSS ver. 21 (IBM Co., Armonk, NY, USA).

After combining the mean values of occluded tubules/total number (partial and total) the more occluded tubules were seen in this order

Group 1>Group 2>Group 3> Group 4

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IV. Discussion

Dentin Hypersensitivity is defined as a pain arising from exposed dentin, typically in response to chemical, thermal, or osmotic stimuli, that can't be explained as arising from any other form of dental defect or pathology. [16]

SEM studies of hypersensitive dentin surfaces reveal that they need more patent tubules per unit area than nonsensitive dentin. Absi et al.[17] and Yoshiyama et al.18reported that most of the tubules were occluded in naturally desensitized dentin. Based on transmission electron microscopic studies, Yoshiyama et al.18reported that tubular occlusions could be due to an extension of the intratubular dentin layer or deposition of substances in the tubules. It has been shown by Brannstrom in human studies that the patency of the dentinal tubules is a major characteristic of sensitive dentin. A significant direct correlation between the density of open dentinal tubules the intensity of pain responses induced from exposed cervical dentin surfaces has also been reported. The condition of dentin with either open or blocked tubules is decisive regarding the hydraulic conductance of dentin and thus stimulus-induced fluid flow in the dentinal tubules. Hence, blocking off the tubules should effectively abolish dentinal pain symptoms.[19] Dentine tubule occlusion is achieved in two different ways, either by the deposition of an occluding layer on top of the dentin or by the introduction of occluding material into dentine tubules.

In the Morris et al. [20] study they highlighted a 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. [20] 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. Thus, 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.[21]

Conventional therapies for the treatment of DH comprehend the topical use of desensitizing agents, either professionally or by using protein precipitants,[22] tubule-occluding agents, [23, 24] tubule sealants,[25] and, recently, lasers.[26]

Several studies27-29 describe a synergistic action of lasers using desensitizing agents. The laser system can favor the permanence of the desensitizer for a longer time than when they are used alone. For this reason, if a laser device is employed additionally to a standard desensitizing agent, the latter remains above the tooth surface for 60 seconds before the irradiation.

Focusing on the effectiveness of only the diode laser, this was investigated by several authors. Matsumoto et al. [30] showed an 85% improvement in teeth treated with a laser; Aun et al.[31] reported success in laser-irradiated teeth in 98% of their cases; Yamaguchi et al.[32] noticed an effective improvement index of 60% within the group treated with laser compared to the 22.2% of the control non-lased group; Another study administered by Brugnera et al. showed the immediate analgesic effect using a diode laser.[33]

Gluma desensitizer is a 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 a 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.[34]

NovaMin^{R} (SHY-NM^{TM}) is a bioactive glass-ceramic material that falls into a class of newer materials that provide calcium and phosphate upon reaction. In the case of products with NovaMin, the active ingredient, calcium sodium phosphosilicate that reacts when exposed to aqueous media and provides calcium and phosphate ions that form a HydroxyCarbonate Apatite (HCA) with time.[35] The combination of the residual NovaMin particles and the HCA layer results in the physical occlusion of dentinal tubules, which will relieve hypersensitivity.[36]

The results of the present study revealed that NovaMin-treated dentin specimens showed more complete tubule occlusion. This is in accordance with the findings of Litkowski 14 and Du Min et al. [37] who found NovaMin to be a more effective desensitizer.

Pro-arginine, the effective components of this new technology are Arginine, an amino acid positively charged at physiologic pH (6.5-7.5), Bicarbonate, a pH buffer, and insoluble Calcium carbonate, a source of calcium. The arginine present in the products is obtained from vegetable sources. In 2009, Colgate-Palmolive launched Colgate Sensitive Pro-Relief in-office desensitizing paste.[39]

When the desensitizing paste is applied to exposed dentin, Arginine (positively charged) and calcium carbonate, found in saliva naturally, work together to accelerate the natural mechanisms of occlusion by binding to the negatively charged dentine surface to deposit a dentin-like mineral, as a plug within the dentin tubules and a protective layer on the dentin surface. This consists of arginine, calcium carbonate, phosphate, and salivary glycoproteins. Freeze fracture images have shown that this plug reaches a depth of 2 μm into the tubule. It is effective in reducing dentin fluid flow thereby relieving hypersensitivity.[39]

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In the present study, we have shown that professionally applied dental (in-office) products containing Diode Laser (Zolar)and Gluma desensitizer are both capable of occluding the dentin tubules to varying degrees and may have the clinical potential to reduce dentin hypersensitivity.

In house application of dental products like Colgate Sensitive Pro-relief and Novamin (SHY-NM), also occluded dentinal tubules and reduced dentin hypersensitivity.

All the novel desensitizers occluded the tubules but Diode laser and NovaMin have shown superior results with respect to 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 dentin specimen utilized, etching process, time and mode of application of the desensitizing agent, or a combination of these variables. Further future clinical trials with larger sample size, comparison with positive controls and negative controls, different concentration should be taken under consideration to validate the result of this new product as an efficacious desensitizing agent. However, the results were consistent in demonstrating a significant effect in reducing the sensitivity with the desensitizing agents used.

V. Conclusion

In conclusion, NovaMin^{R} (SHY NM^{TM}) and laser diode therapy showed a greater number of completely occluded tubules and Gluma desensitizer and Colgate sensitive Pro-relief produced a greater number of partially occluded tubules.

There was a statistically significant difference between the five groups when the ratio of complete and partial occlusion was calculated against the total number of tubules.

Hence, the Diode Laser application and NovaMin^{R} (SHY NM^{TM}) application could be more effective in providing relief from dentinal hypersensitivity.

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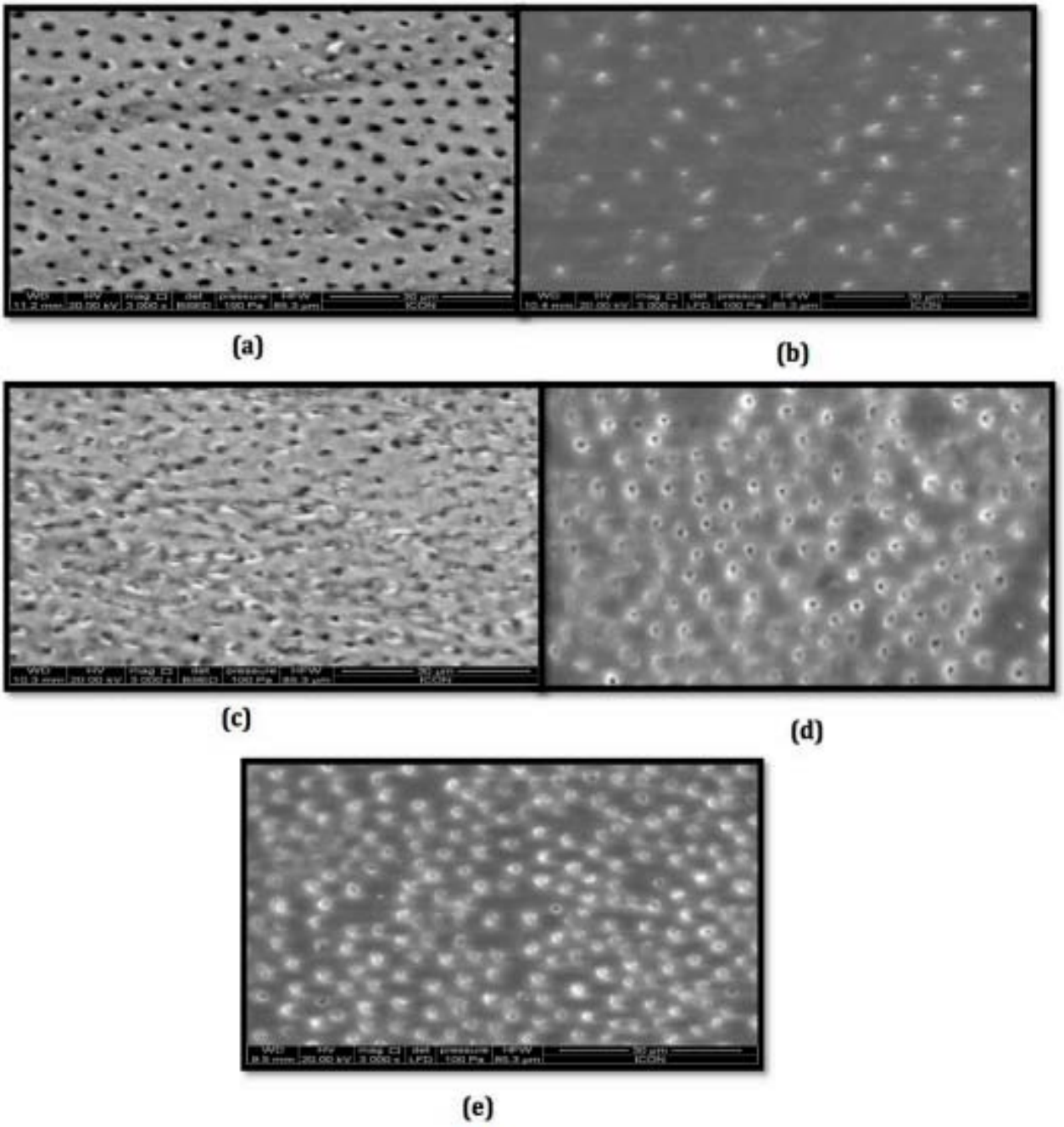


Figure 1(a) to (e): Representative image of dentinal tubule occlusion following the use of four desensitizing agents. (a) Control group (b) Laser group (c) Novamin group (d) Gluma desensitizer (e) Colgate Sensitive Pro-relief

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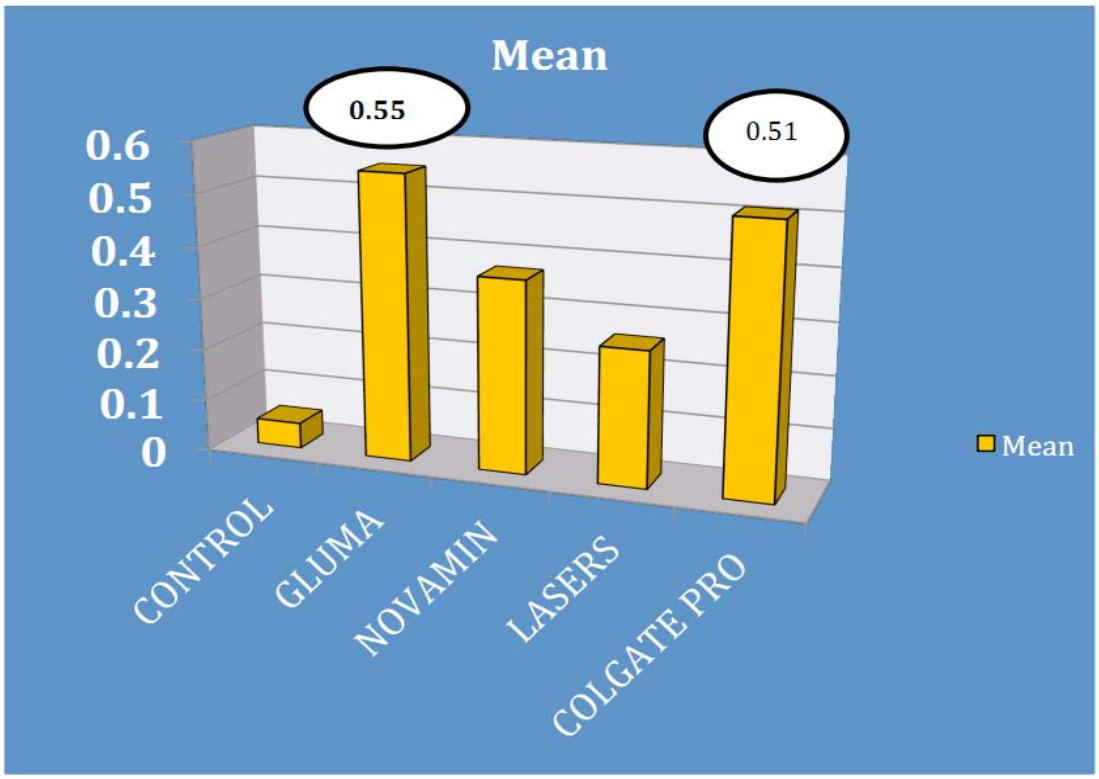


Figure 2: Graphical Representation of Partially Occluded Tubules of all study groups.

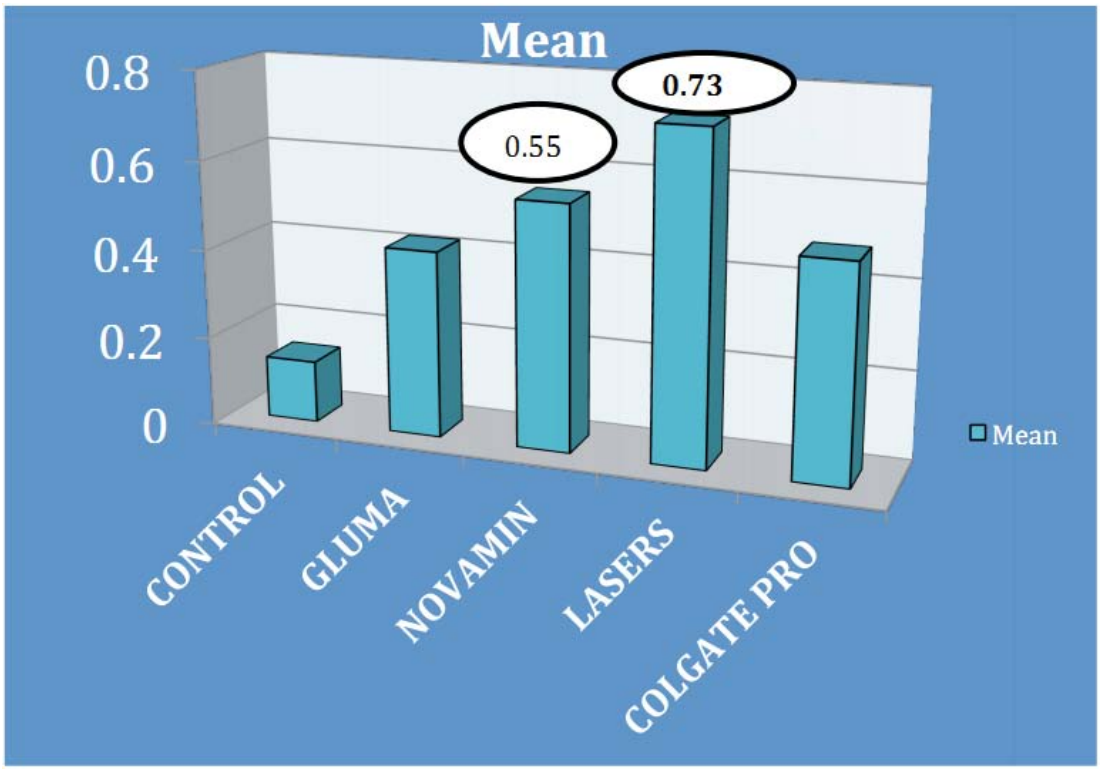


Figure 3: Graphical Representation of Totally Occluded Tubules of all study groups.

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Table 1: Descriptive Statistical data of Partially and Totally Occluded scores of the study Groups.

		PARTIALLY OCCLUDED		TOTALLY OCCLUDED	
		Mean	SD	Mean	SD
Groups	CONTROL	.05	.01	.14	.01
	GLUMA	.55	.03	.42	.01
	NOVAMIN	.37	.01	.55	.02
	LASERS	.26	.02	.73	.02
	COLGATE PRO SENSITIVE	.51	.01	.48	.01

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