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# EVALUATION OF THE EFFECTIVENESS OF THREE DIFFERENT DESENSITIZING AGENTS IN DENTINAL TUBULE OCCLUSION USING SCANNING ELECTRON MICROSCOPE

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# ABSTRACT

**Objective:** This study was conducted to evaluate and compare the laboratory effect of three different treatment modalities in dentinal tubules occlusion. **Materials and methods:** Total of (27) teeth were enrolled in the study. Dentin discs prepared from teeth to apply desensitizing agents evaluated through occluding dentinal tubules by scanning electron microscope (SEM) examination. Discs were used in the study which were randomly divided into three groups (n=9) according to desensitizing agent (D) used while D1: Gluma adhesive, D2: Citrene varnish and D3: Charm varinsh. Each group was divided into three sub groups(n=3) according to storage time (T) where T1: 1 week, T2: 1 months and T3: 3 months. **Results**: The results of this in-vitro study revealed that there was high significant difference ( $p \le 0.05$ ) in percentage of dentinal tubules occlusion before and after treatment for all tested groups. Totally there was statistically significant difference ( $p \le 0.05$ ) between the three groups as proven by Chi square test where Citrine varnish group was the highest followed by charm sense varnish group and the lowest was Gluma group in management of dentin hypersensitivity. **Conclusions:** Citrine varnish , Charm varnish and Gluma were effective in occluding dentinal tubules, with Citrine varnish being the most effective up to 3 months.

KEYWORDS: Dentinal tubule occlusion, desensitizing agents, SEM

## INTRODUCTION

Dentin hypersensitivity (DH) is as an acute, nonspontaneous, short-duration pain resulting from exposure of the dentin to chemical, mechanical, osmotic, or thermal stimuli unlikely to be ascribed to any other form of dental pathology<sup>(1,2)</sup>.

DH develops when dentinal tubules are exposed to oral cavity. The exposure of dentin and its resulting sensitivity is likely to be caused by one or two mechanisms: either with the removal of enamel or the exposure of the root surface with the loss of the overlying cementum <sup>(3-5)</sup>. The commonly accepted theory to explain the pain related to DH is the hydrodynamic theory <sup>(6)</sup>. In the perspective of this theory, when dentinal tubules are exposed, the pressure differences in the surrounding tissue affect the flowing direction of the dentinal fluid inward and outward. This flowing may stimulate mechanoreceptors in intratubular nerves or in the superficial pulp that is recognized by the patient in the form of a rapid and sharp pain<sup>(7)</sup>.

There are several methods used for management of DH. These methods include instructions for proper brushing, dietary advice, use of desensitizing products, the use of adhesive systems, and adhesive restorations<sup>(3,8)</sup>.

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Fluoride varnishes were introduced on the market to increase the efficiency and permanence of fluoride when in contact with the tooth surface, in order to allow a slow and continuous release of fluoride <sup>(7)</sup>. Varnishes consist of natural resin-based vehicles for fluoride, and are highly adhesive to the tooth structure. They are easy to apply and are low-cost materials <sup>(8)</sup>. The fluoride is dissolved in an organic solvent, which evaporates when applied, leaving a thin layer of the material covering the exposed tooth surfaces. The mechanism of action is the deposition of calcium fluoride on the tooth surface, with the formation of fluorapatite <sup>(9)</sup>.

A product containing the combination of an aqueous solution of 35% hydroxyethyl methacrylate and 5% glutaraldehyde is considered to be an efficient desensitizing agent. Dentinal tubules are inherently blocked by the glutaraldehyde, and this counteracts the hydrodynamic mechanism that gives rise to DH <sup>(10)</sup>.

Dentin hypersensitivity is related to the number of the exposed tubules on the tooth surface. The main aim of successful hypersensitivity treatment is the partial or complete occlusion of dentinal tubules<sup>(11)</sup>. Occluding dentinal tubules is accepted as an effective method in lowering permeability of dentin, reducing the movement of the dentinal fluid and alleviating pain <sup>(12)</sup>.

Therefore, the present study aimed at evaluating and comparing the laboratory effect of three different desensitizing agents in dentinal tubules occlusion.

## MATERIAL AND METHODS

#### Study setting

The ethical approval of this in-vitro study was by the ethics committee of faculty of dental medicine, al-Azhar university, Cairo, Egypt (EC Ref No:114/136).

### **Teeth selection:**

Thirty six intact human premolars extracted for orthodontic reasons were assigned for this study. Teeth were examined to be free from decay, defect, or restorations. They were cleaned to remove any plaque, calculus then and stored in distilled water at room temperature till testing.

#### **Dentin disc preparation**

Dentin discs were prepared from crown segment using grinding machine under copious coolant. Crowns of the selected teeth were separated from their root segment with a diamond disc.

The samples were sectioned longitudinally (bucco-lingual direction)using diamond disc in cutting machine ( DEMCO, Dental maintenance CO, Bonsall, Calf. U.S.A, Model E96. Sectioned discs of 5mm height,4mm width ,and 1mm thickness were obtained from each tooth.

The outer surfaces of the dentin discs were polished using a polishing machine (NANO 2000, Pace Technologies, Tucson, AZ, USA) with 400, 800, 1000, and 1200 grit paper, 1 and 0.5  $\mu$ m diamond polishing compound. The dentin discs were then cleaned in digital ultrasonic cleaner containing deionized water for 30 min.

#### Grouping of the specimens

A total 36 discs were used in the study which were randomly divided into four groups (n=9) according to desensitizing agent (D) used into D0: a control group and received no treatment.

D1: Gluma adhesive (glutraldehyde based adhesive), D2: Citrine varnish (sodium fluoride based varnish) and D3: Charm varnish(sodium fluoride based varnish). Each group was divided into three sub groups(n=3) according to storage time (T) where T1: 1 week, T2: 1 months and T3: 3 months.

#### **Treatment protocol:**

To simulate hypersensitive dentin, all specimens immersed in 17% ethylene diamine tetra acetic acid (EDTA) for 2 min to remove the smear layer and open the dentin tubules. Group1: (n= 9) dentin discs was etched and not received treatment.

Group2: (n=9) dentin discs treated with Gluma adhesive (Heraeus Kulzer GmbH, Hanau, Germany) as followed

A thin layer of Gluma was then applied by agitating with an applicator tip for 20 s, gently airdried for 5 s and light cured for 20 s.

Group 3: (n=9) dentin discs treated with Citrine varnish (Dhrama, USA) by\_application of a disposable brush, left to dry for 3 min.

Group 4: (n=9) dentin discs treated with Charm varnish (Dentkist- Korea)by\_application of a disposable brush, left to dry for 3 min.

After application of the three tested materials, each sample was placed in test tube filled with 10ml of artificial saliva solution prepared in faculty of science Al- Azhar University (9g/1 NaCl, 0.24/1CaC12, 0.43g/1KCl and 0.2g/1NaHCO3, PH:7.8). The immersion process was done after warming of the solution till it reaches 37°C. The samples were then incubated in an laboratory incubator at 37°C.

# Scanning Electron Microscopy(SEM) examination

The treated surface of each group were examined using scanning electron microscope (SEM) (Model Qunta 200, FEL, Netherland) for evaluating the closure of the dentinal tubules. The analysed sample was placed at analysing chamber in the scanning electron microscope (SEM), and then by using the SEM a certain area was specified that was captured at a certain magnification. Examination was performed at 25K V of accelerating voltage, at standardized working distance of 11.3 mm at 2000X magnification to provide quantitative assessment. The images were provided with good contrast and brightness setting which remained constant for all dentin specimens after treatment All specimens were evaluated before and after treatments and storage. After treatment, quantitative analysis of occluded dentinal tubules was done by SEM analysis. The total number of tubules, number of open tubules, and number of occluded tubules were counted in each photograph of all the specimens.

#### **Image analysis**

After capturing the image, an order is measurements software (XT document) to begin to make its measurements up on specific area. For quantitative evaluation, the patency and closure of dentinal tubules of (SEM) photographs for each specimen after treatment were assessed using image analysis software (XT document). Counting, measuring the dimensions' of the dentinal tubules was within the middle portion of each photograph. The program was used to calculate the count in micron of the dentinal tubules for standardized portion of each photograph.

#### Statical analysis

Data analysis was performed in several steps. Initially, descriptive statistics for each subgroup results. Two-factor analysis of variance ANOVA test of significance was done for comparing variables affecting mean values (treatment groups and time). One way ANOVA followed by pair-wise Tukey's post-hoc tests were performed to detect significance interaction between subgroups. Statistical analysis was performed using Asistat 7.6 statistics software for Windows. P values  $\leq 0.05$  were considered to be statistically significant in all tests.

#### RESULT

Percentage of the total number of occluded tubules was obtained by dividing the total number of occluded tubules by the total number of tubules in each photomicrography. The mean data was used to compare the different samples and number of applications tables (1,2,3) figures (1,2,3).

# Percentage of occluded dentinal tubules after one week

There was high statistically significant difference between the three groups. There was high statistically significant difference between **control** group, which showed the highest value of mean value  $(383\pm73)$ , followed by **Gluma** group  $(131\pm34)$ , then **Cham varnish** group  $(50\pm7)$  with the lowest mean value for **Citrine varnish** group  $(25\pm4)$ . Table (1) represented mean values and standard deviation of percentage of occluded dentinal tubules after application of 3 desensitizing agents (Gluma, Citrine varnish and Charm varnish) **after one week**. Tukey's post-hoc test for comparison showed no significance between **Cirine varnish** and **Charm varnish** groups

**TABLE (1)** Mean values and standard deviation of percentage of dentinal tubules occluded after application of 3 desensitizing agents (Gluma, Citrine varnish and Charm varnish) **after one week:** 

Group	Mean ± SD	p-value
Gluma	131 <sup>b</sup> ± 34	
Citrine varnish	25°±4	0.0001*
Charm varnish	50°±7	<0.0001*
Control	383ª±73	

\*: Significant at  $p \le 0.05$ ; different letter indicates significant difference at  $\alpha = 0.05$  by Tukey's multiple comparison test.



FIG (1) Column chart showing mean values of percentage of occluded dentinal tubules after application of 3 desensitizing agents (Gluma, Citrine varnish and Charm varnish) after one week.

# Percentage of occluded dentinal tubules after 4 weeks:

There was high statistically significant difference between the three groups. There was high statistically significant difference between *control* group, which showed the highest value of mean value (277±34), followed by **Gluma** group (104± 7), then **Charm varnish** group (47±4) with the lowest mean value for **Citrine varnish** group (31±10). Table (2) Mean values and standard deviation of percentage of occlusion of occluded dentinal tubules after application of 3 desensitizing agents (Gluma, Citrine varnish and Charm varnish) **after 4 weeks**. Tukey's post-hoc test for comparison showed no significance between **Citrine varnish** and **Charm varnish** groups

**TABLE (2)** Mean values and standard deviation of percentage of dentinal tubules occlusion after application of 3 desensitizing agents (Gluma, Citrine varnish and Charm varnish) **after 4 weeks** 

Group	Mean ± SD	p-value
GLUTA	104 <sup>b</sup> ± 7	<0.0001*
Citrine varnish	31°±10	
Charm varnish	47°±4	
Control	277ª±34	

\*: Significant at  $p \le 0.05$ ; different letter indicates significant difference at  $\alpha = 0.05$  by Tukey's multiple comparison test.



FIG (2) Column chart showing mean values of percentage of dentinal tubules occluded after application of 3 desensitizing agents (Gluma, citrine varnish and charm varnish) **after 4 weeks** 

# Percentage of dentinal tubules occlusion after 3 months:

There was high statistically significant difference between the three groups. There was high statistically significant difference between *control* group, which showed the highest value of mean value ( $250\pm7$ ), followed by **Gluma** group ( $117\pm7$ ), then **Charm varnish** group ( $49\pm2$ ) with the lowest mean value for **Citrine varnish** group ( $29\pm12$ ). Table (3) represented Mean values and standard deviation of percentage of occlusion of occluded dentinal tubules after application of 3 desensitizing agents (Gluma, Citrine varnish and Charm varnish) **after 3 months**.

**Table (3):** Mean values and standard deviation of percentage of occluded dentinal tubules after application of 3 desensitizing agents (Gluma, Citrine varnish and Charm varnish) **after 3 months** 

Group	Mean ± SD	p-value
GLUTA	117 <sup>b</sup> ± 7	<0.0001*
<b>Citrine varnish</b>	29 <sup>d</sup> ±12	
Charm sense varnish	49°±2	
Control	250ª±7	

\*: Significant at  $p \le 0.05$ ; different letter indicates significant difference at  $\alpha = 0.05$  by Tukey's multiple comparison test.



FIG (3) Column chart showing mean values of percentage of dentinal tubules occlusion after application of 3 desensitizing agents (Gluma , Citrine varnish and Charm varnish)after 3 months.



FIG (4) SEM image of dentin sample with smear layer removing (Control group).



FIG (5) SEM image of dentin sample treated with Gluma adhesive



FIG (6): SEM image of human dentin surface treated with Citrine varnish



FIG (7): SEM image of dentin sample treated with Charm varnish.

## DISCUSSION

In-vitro studies on dentin hypersensitivity encompass the use of dentin discs in study dentinal morphology and its interaction with different dental materials. To study dentin hypersensitivity, various methods have been employed, utilizing SEM analysis to estimate dentin reactivity with desensitizing agents <sup>(13)</sup>.

The removal of smear layer was necessary to stimulate the sensitive dentin with dentinal tubules patent to the oral cavity, as it was reported that the smear layer was responsible for 86% of total resistance to the movement of fluids<sup>(14)</sup>. Thus dentin discs were etched to remove the smear layer formed during the preparation to exclude any alteration in the permeability. Gluma (Heraeus Kulzer GmbH, Hanau, Germany) is a commercially available desensitising agent consists of glutaraldehyde and hydroxyethyl methacrylate (HEMA). Glutaraldehyde occludes dentinal tubules by coagulation of amino acids and proteins present in the dentin, whereas HEMA can work by occluding the dentinal tubules (15).

Sodium fluoride (NaF) has also been indicated for treating dentine hypersensitivity and it is available in a variety of forms. The use of fluoridating varnishes with sodium fluoride (in high concentrations) as the active ingredient has been advocated to increase time of action of NaF in contact with exposed dentin, thus aiming to enhance its effectiveness in decreasing dentine sensitivity<sup>(16)</sup>.

In this study image analysis was used to measure the number of open and occluded on SEM micrographs of specimens surfaces, the software polygon tool was used to draw the outline of dentinal tubules based on gray pixel intensity differences between dentinal tubules and the outer area, these intensity differences changes according to patent dentinal tubules become occluded by the application of the desensitizing agent, if total occlusion happen no difference in the intensity would be detected, and finally the total open and occluded areas were calculated <sup>(17)</sup>.

As a result, the citrine fluoride varnish treated group showed the highest degree of dentinal tubule occlusion effects during the first, fourth weeks and three months while aging, and the charm fluoride varnish treated group showed the second highest degree. The lowest group of the dentinal tubule occlusion effects was the Gluma treated group.

After three months, the Gluma treated group showed the lowest degrees of dentinal tubule occlusion effects. Jang et al. (18) also reported that the SE-Bond treated group showed a higher occlusion level than the Gluma treated group. In this study, the fluoride varnish treated group showed a higher dentinal tubule occlusion level than the Gluma group. This corresponded to the study done by Lee <sup>(19)</sup> who found that fluoride varnish treated group showed a higher occlusion level than Gluma group. This might be due to the fluoride varnish that slowly permeated into the dentinal tubules. As a result, its effects were lasted for a longer period than in the Gluma group. In the Gluma treated group, most dentinal tubules were open because the deposits were thin enough to be abraded through storage time. According to this study, the citrine and charm fluoride varnish treated groups showed higher levels of occlusion and active ingredient efficiency than the Gluma treated group. Therefore, the use of fluoride varnish may be effective as hypersensitivity treatments.

#### CONCLUSIONS

Citrine varnish, Charm varnish and Gluma were effective in occluding dentinal tubules, with Citrine varnish being the most effective within 1 to 2 weeks and sustained up to 3 months when compared with Gluma.

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