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Research Article

Scanning Electron Microscopic Evaluation of The Effectiveness of Desensitizing Agents on Dentinal Tubule Occlusion and Durability – An *In vitro* Study.

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Abstract

Aim: The purpose of this study was to evaluate the effectiveness of four desensitizing agents on dentinal tubule occlusion soon after their application and after brushing for one week.

Materials and Methods: Eighty specimens were obtained from 40 extracted sound human maxillary premolars. Each tooth was mesiodistally sectioned to obtain 40 buccal and 40 lingual surfaces, and enamel was removed in order to simulate hypersensitive dentin. Specimens were divided into four groups with 10 specimens each. Group 1 samples were treated with Colgate pro- relief toothpaste, Group 2 samples were treated with Aclaim Toothpaste, Group 3 samples were treated with Superseal and Group 4 samples were treated with Diode laser. These specimens were examined under scanning electron micro scope (SEM) with and without brushing for 1 week to find out the occluding ability and durability of the respective products.

Statistical Analysis: The results were statistically analyzed by paired T test and Oneway ANOVA with post hoc tukey test for inter-group comparision.

Results: There is statistically difference in the mean values in Colgate Pro-relief & Aclaim($p<0.001$) in before and after brushing which indicates both groups unable to maintain dentinal tubular occlusion and no Statistically difference in mean values of Laser and Super Seal($p=0.992$).which indicates both groups maintained tubular occlusion.

Conclusion: Diode laser and Super seal showed better durability followed by Aclaim and Colgate pro-relief even after brushing for 7 days twice daily.

Keywords: Desensitizing Agents; Dentinal Tubule Occlusion; Colgate Pro-Relief Toothpaste; Aclaim Toothpaste; Super Seal; Diode Laser

Introduction

Dentine hypersensitivity (DH) is characterized by short sharp pain arising from exposed dentine in response to stimuli typically thermal, evaporative, tactile, osmotic, or chemical and which cannot be ascribed to any other form of dental defect or pathology [1]. Dentine hypersensitivity (DH) is one of the commonest causes of pain encountered in regular dental practice. The prevalence of DH in the population is found to be at a peak in the age of 20 to 40 years [2], more so in buccocervical region of teeth due to branching of dentinal tubules at the dentinoenamel junction (DEJ) [3]. The success of desensitizing agents is directly proportional to its ability to seal or occlude the dentinal tubules and reduce the diameter of the opened tubules thereby decreasing the hydrodynamic pain transmission mechanism [4]. The oral environment being dynamic, the desensitizing agent has to withstand the challenges of salivary dissolution, acid attack from microbes and food components as well as chemical, mechanical and thermal trauma to provide long-lasting pain relief for the patient [5].

The incorporation of arginine in the dentifrices was reported in the late 1990s. It adsorbs on the surface of the insoluble calcium carbonate particles, forming positively charged agglomerates that readily bind with the negatively charged dentine of the exposed tubule walls to form an occluding adhesive plug [6].In investigating the mechanism of action of arginine and calcium carbonate paste (Colgate sensitive Pro-Relief) using scanning electron microscopy, confocal laser scanning microscopy and atomic force microscopy petrou., *et al*. found that the technology totally occluded the dentinal tubules rapidly [7].

Recently, Aclaim toothpaste has been advocated for treatment of DH which offers nanocrystals of hydroxyapatite. These nanocrystals mimic natural hydroxyapatite in composition, structure, nano-dimenisions as well as functionally. Acting as filler, nano particles easily penetrate into the exposed dentinal tubules and strongly adsorb to dentine apatite, thus sealing exposed dentinal tubules. The tooth paste releases calcium and phosphate ions, which precipitate and recrystallize to form a biomimetic apatite layer over exposed dentinal tubules [8].

Super Seal is a potassium oxalate based, acid resistant desensitizer with a unique formula that demineralizes the organic and mineral debris of the smear layer and outer most ring of peritubular dentin and restructures the demineralized material as a calcium oxalate precipitate that block the dentinal tubules [9].

Lasers, on the other hand, are a promising and upcoming treatment modality in management of DH. The action of lasers in DH depends on the laser wavelengths and parameters used [10]. The effect of laser as desensitizing agent is exemplified only when etiological factors are removed [11]. While low output lasers (He-Ne, diode laser, etc) cause photo-biomodulation in the dentin and bring about analgesia in the neural complex [12]. Studies reported that Nd; YAG, Er: YAG, CO_2, and diode lasers produce an efficient desensitizing effect [13].

There are many in-vivo studies shown decrease in hypersensitivity after using desensitizing agents, and in-vitro studies conducted capacity of desensitizing agents for occluding dentinal tubules, but durability is an important factor that helps us to know how long a desensitizing agent works on occlusion of dentinal tubules, hence present study was undertaken to evaluate the tubule occluding ability and durability of 1.Arginine containing tooth paste-Colgate pro-relief 2.Nanohydroxyapatite containing tooth paste- Aclaim 3.In office desensitizing agent – Super Seal 4. Diode laser.

Material and Methods

Sample size:

Eighty specimens obtained from 40 extracted sound human maxillary premolars were included in the study according to inclusion and exclusion criteria given below.

Sample size:

1. Teeth free of Restoration,
2. Teeth free of Dental caries
3. Teeth free of Enamel defects

Exclusion criteria

1. Teeth with Dental caries
2. Teeth with Restorations
3. Teeth with Malformations
4. Teeth with Fractures

Randomization

All specimens were randomly assigned to eight groups as follows:

- **Group1 (P)**- 10 buccal surfaces were treated with Colgate Pro-Relief tooth paste without brushing.
- **Group 2 (A)**- 10 lingual surfaces were treated with Aclaim tooth paste without brushing
- **Group 3 (S)** - 10 buccal surfaces were treated with Super Seal without brushing.
- **Group 4(D)**- 10 lingual surfaces were treated with Diode laser without brushing
- **Group 5 (P+B)** - 10 buccal surfaces were treated with Colgate pro-relief tooth paste with brushing
- **Group 6 (A+B)** - 10 lingual surface were treated with Aclaim tooth paste with brushing
- **Group 7 (S+B)**-10 buccal surfaces were treated with Super Seal with brushing
- **Group 8 (D+B)** -10 lingual surfaces were treated with Diode laser with brushing

Experimental procedure

- After cleaning gross debris each tooth was sectioned mesiodistally using minitome (low speed diamond saw, Struers, Denmark to obtain 40 buccal &40 lingual

surfaces. Cavities of 3mm length x3mm widthx2mm depth was prepared using diamond wheel and straight fissure bur (PIVO) under coolant on cervical areas of buccal and lingual surfaces.

- Exposed dentin surface was etched using 17% EDTA (MAARC) for 40 minutes and was sonicated in distilled water for 12 minutes in order to open dentinal tubules completely

Treatment of samples:

- **Group 1 (P):** Specimens were treated with Colgate pro-relief for 7 days twice daily for 2 minutes using tweezer and cotton swab.
- **Group 2 (A):** Specimens were treated with Aclaim for 7 days twice daily for 2 minutes using tweezer and cotton swab.
- **Group 3 (S):** Specimens were treated with Super Seal according to manufactures instructions.
- **Group 4 (D):** Specimens were treated with Diode laser according to manufactures instructions.
- **Group 5 (P+B):** Specimens were treated similar to group 1 and brushed twice daily for 7 days for 2 minutes with the help of power brush.
- **Group 6 (A+B):** Specimens were treated similar to group 2 and brushed twice daily for 7 days for 2 minutes with the help of power brush.
- **Group 7 (S+B):** Specimens were treated similar to group 3 and brushed twice daily for 7 days for 2 minutes with the help of power brush.
- **Group 8 (D+B):** Specimens were treated similar to group 4 and brushed twice daily for 7 days for 2 minutes with the help of power brush.

The laser device used was 980 nm diode laser (Zolar photon plus Diode laser, Zolar technology and MFG Canada), at the laser parameters of 1 W, continuous wave at 190 J for 15 seconds.

Artificial saliva (AS) was used as a substitute for saliva in the present study. All specimens from each group were kept in 10 ml of AS at pH 7.4 for 7 days which was replaced every 24 h.

Brushing was performed at a load of 200 g with oscillations of 7800 strokes per minute with help of customized toothbrush machine. In between, the samples were stored in artificial saliva.

Scanning electron microscope analysis

Specimens which were stored in Artificial Saliva were washed with distilled water and air-dried then kept in hot air oven for 2 hours at 37ºc and stored in vacuum desiccator for another 2 hours; the samples were sputter coated to aid conductivity. Photomicrographs were taken from each specimen surface examined at 2000x magnifications under SEM to check the obliteration of dentinal tubules and durability.

Steps involved in SEM analysis are as follows

1. Gold sputtering
2. Teeth mounted in SEM machine
3. Vaccumization
4. Image processing.

Sem observation

In the present study the scanning electron microscope evaluation was done in Department of physics, S.V university, Tirupati using SEM EVO MA 15.All specimens were observed under 2000x magnification, with EHT – 20.00 kv. The photographs were saved and analyzed for dentinal tubule occlusion using Image J software (Version 1.47, National Institute of Health, USA).

The percentage of partially and/or fully occluded tubules was calculated for each representative micro graphs using following simple formula:

Percentage of partially or fully occluded tubules =

$$\frac{\text{Number of partially or fuly occluded tubules}}{\text{Total number of tubules}} \times 100$$

Results and Discussion

From the SEM analysis mean dentinal tubular scores are highest for Group 3-Superseal (95.94 ± 1.12) followed by Group 4 -Diode Laser (95.60 ± 1.43), Group 2 - Aclaim (92.27± 3.73) and Group 1-Colgate Pro-relief (70.72 ± 3.52).There is no significant difference present between Super Seal and Laser (p=0.992)which indicates the both have similar dentinal tubular occlusion.

Order of Efficacy of Various Materials (Without Brushing)

Group 3 ≥ Group 4 > Group 2 > Group 1

After brushing mean dentinal tubular scores are highest for Group 4 - Diode Laser (95.40±1.71) followed by Group 3 – Super seal (95.12±1.53), Group 2 - Aclaim (63.18±3.65) and Group 1-Colgate Pro-relief (58.78±2.31).There is no significant difference present between Super Seal and Laser (p=0.992) which indicates the both have maintained dentinal tubular occlusion.

Order of Efficacy of various materials (with brushing):

Group8 ≥ Group 7 >Group 6>Group 5

On comparison of groups without and with brushing there is statistically difference in the mean values in Colgate Pro-relief & Aclaim ($p<0.001$) in before and after brushing which indicates both groups unable to maintain dentinal tubular occlusion and no Statistically difference in mean values of Laser and Super Seal ($p=0.992$).which indicates both groups maintained tubular occlusion.

Thus, it can be concluded that Diode laser and SuperSeal showed better durability followed by Aclaim and Colgate Sensitive Pro relief even after brushing for 7 days twice daily.

Discussion

Dentin may experience short and severe pain known as hypersensitivity, often caused by acid corrosion, wear, or abrasion. Interstitial fluid movement within the dentinal tubules is the basis for the transmission of sensations [14]. A possible approach to reducing or eliminating the painful symptoms of dentin hypersensitivity is the interruption of stimuli transmission to the nerve endings of odontoblastic processes by reducing the fluid movement inside the dentinal tubules through the narrowing or occlusion of tubule openings. Dentinal tubules can be sealed on the dentin surface, occluded within their orifices or in the subsurface dentin within their tubules. It can be assumed that intradentinal closure or seal is the most promising approach with regard to long-term success [15].

The teeth most commonly affected by DH are canines, premolars, incisors and molars in the descending order [16,17], and hence, in the present study forty maxillary sound premolars were chosen for the study and each tooth was mesiodistally sectioned to obtain buccal and lingual surfaces [18,19]. Cavity preparation was done at the cervical region as the number of dentinal tubules is more numerous in that region3 with measurement of 3mm x 3mm x 2mm for standardization. The prepared cavity of all of the samples were etched with 17% EDTA (MAARC) for 40 minutes and ultrasonicated in distilled water for 12 min prior to treatment. This step was done to ensure that the prepared dentin surface was free of any smear layer or smear plugs simulating the open tubules of the sensitive dentin. SEM investigation was selected because it is a non-destructive approach for surface analysis. It also provides high-resolution, three dimensional images and topographical information. In this study, a magnification 2000x was used for calculating mean tubular occlusion this was in accordance with previous studies [18,20,21].

An outstanding desensitizing agent should exert continuous good occlusion effects of the dentinal tubules against all adverse external environments, thus achieving durable anti-sensitivity effects. Our study thus designed a procedure of brushing with distilled water alone for 7 days with a load of 200mg and oscillations of 7800 strokes per minute with the help of Customized Oral B Cross Action Battery Power Toothbrush this was in accordance with previous studies [18,22].

There are many in-vitro studies conducted using desensitizing dentifrices, anti-inflammatory substances, iontophoresis, neodymium-doped yttrium aluminum garnet (Nd: YAG) and erbium YAG (Er: YAG) lasers, Diode (GaAlAs) lasers and other conventional treatments using composite resins and dentin adhesive to evaluate the dentinal tubule occlusion and permeability. However, only few studies were conducted to find out their durability. Durability is an important factor that helps to know how long desensitizing agents work on obliteration of dentinal tubules, hence the present study was undertaken to evaluate the tubule occluding ability and durability of four desensitizing agents, namely, Colgate Pro-relief dentifrice (Colgate-Palmolive India Ltd.), Aclaim dentifrice (Group Pharmaceuticals Ltd. Malur, India), Superseal desensitizer(Phoenix Dental, Inc. USA), Zolar photon plus diode laser(Zolar technology and MFG Canada).

The present study is the first *in vitro* study comparing the dentinal tubule occlusion and durability of Desensitizing dentifrices – Colgate Sensitive Pro-Relief dentifrice (Colgate-Palmolive India Ltd.), Aclaim dentifrice (Group Pharmaceuticals Ltd. Malur, India), In office desensitizing agent - Super Seal(Phoenix Dental, Inc. USA), and Zolar photon plus Diode laser (Zolar technology & MFG Canada).

In this study, all the four desensitizing agents relatively showed dentinal tubule occlusion, but Diode laser and Super Seal demonstrated higher degree of occlusion. Diode Laser and Super Seal showed the best durability when compared to Aclaim and Colgate pro-relief when brushed twice daily for 2 min with oscillation of 7800 strokes per minute for the 7 days.

Durability of Aclaim over Colgate Pro relief may be attributed due to predominant dentinal tubule occlusion with apatite mineral

not only on the dentin surface but also deep inside the dentinal tubules to a depth of 10 to 15 µm from the dentin surface [23]. Whereas in Colgate Pro relief depth of penetration was 2µm into the tubule [24]. Chemically, these agents are composed of calcium and phosphate, and the saliva in the oral cavity is supersaturated with respect to HAP, thus the chances of dissolution of these compounds by saliva is limited.

Durability of SuperSeal over Colgate Pro-relief and Aclaim may be attributed due to demineralization of the tubules by attacking peritubular dentin (the very hard mineralized dentin of each tubule complex) most likely due to the low pH of the solution and restructures the demineralized material as a insoluble calcium oxalate crystals that block the dentinal tubules [25]. Granular precipitate was formed within dentinal tubules at depths of 7-12µm.

Durability of Diode Laser over Colgate Pro-relief and Aclaim may be attributed to photo thermal effects, heating and melting the surface hard tissue. When the dentin cools, it recrystallizes, thereby obliterating the dentinal tubules [26].

Diode Laser and Super Seal treatment seems to be more effective and durable than using desensitizing dentifrices, because they induce morphological changes to the dentin surface.

Conclusion

The following conclusions were drawn within the limits of the present study

1. All four desensitizing agents (Colgate Sensitive Pro-relief dentifrice, Aclaim dentifrice, Super Seal Desensitizer, Diode laser) relatively showed dentinal tubule occlusion, despite their chemical compositions and application procedures. Diode Laser and Super Seal demonstrated higher degree of occlusion.
2. Diode Laser and Super Seal were able to maintain the occlusion effect even after brushing twice daily for 2 min with oscillation of 7800 strokes per minute for 7 days with a load of 200g.However, their long-term effectiveness of action must be determined through future clinical studies.

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Volume 3 Issue 7 July 2019