Original Article

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Effect of 30-day toothbrushing on permeability of desensitizer occluded root dentin: *In vitro* study

Yossaya Laemlak, **Pisol Senawongse,** Pipop Saikaew

Department of Operative Dentistry and Endodontics, Faculty of Dentistry, Mahidol University

Objective: This study aims to evaluate dentin permeability after treating with desensitizers under toothbrushing for 30 days and to analyze which method would give the most sustainable plugged dentinal tubules.

Materials and Methods: Root dentin disc with approximate thickness of 0.4-0.6 mm prepared from upper premolars were immersed with 17% EDTA solution to gain maximum dentin permeability. The specimens were randomly divided into 4 groups; positive control (Er,Cr:YSGG laser), negative control (no treatment), brushing with Colgate® Sensitive ProRelief™ toothpaste and dab-on with Colgate® Sensitive ProRelief™ toothpaste. The second dentin permeability was assessed and then all the specimens were subjected for toothbrushing with total 1200 strokes to simulate daily toothbrushing for 30 days with brushing force 200 g and frequency 120 strokes/min. Then, dentin permeability was re-evaluated after brushing procedure. The specimens were randomly selected from each group for dentinal surface observation under SEM. Data were analyzed with 2-way ANOVA and Dunnett T3 multiple comparison.

Results: Significant reduction of dentin permeability was found after dentin were treated with Er,Cr:YSGG laser, brushing with *Colgate® Sensitive ProRelief™* toothpaste or dab-on with *Colgate® Sensitive ProRelief™* toothpaste ($p<0.05$). No significant difference of dentin permeability among three treated groups was found ($p\geq0.05$). The brushing after treatment had no effect to the permeability of treated specimens.

Conclusion: All three desensitizers were able to reduce dentin permeability and had ability to maintain the effectiveness up to 30 days.

Keywords: dentin permeability, dentin sensitivity, desensitizers, lasers, toothbrushing

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Introduction

Hypersensitivity is one of the most common complaints that bring patients to dental clinics. It can affect people in age between 20-50 years but a peak of 30-40 years is found [1]. Hypersensitivity associates with exposed dentin and the short sharp pain condition is its characteristic [2]. Non-noxious stimuli such as thermal, osmotic, tactile or evaporative can cause this exaggerate response [3]. The lost enamel and cementum on either crown or root is a significant factor that cause hypersensitivity of the teeth. The most common type of teeth presenting hypersensitivity are canines and premolars. The buccal area at cervical is the most common site [1]. Brännström and colleagues who proposed the hydrodynamic theory stated that the external stimuli can cause the movement of dentinal fluid and it activates mechanoreceptor in pulp, thus the pain begins [4]. In addition, dentinal tubules in hypersensitive dentin are widely open than in normal dentin. This presence leads substances in oral cavity directly connect to pulp [5].

Two main mechanisms to relieve the pain from hypersensitivity are nerve desensitization and tubule occlusion. Nerve desensitization aims

Correspondence author: Pisol Senawongse
Department of Operative Dentistry and Endodontics, Faculty of Dentistry, Mahidol University
6 Yothi Road, Ratchathewi, Bangkok 10400, Thailand
Tel.: +66 2200 7825 ext. 25 E-mail: pisol.sen@mahidol.ac.th
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to depolarize nerve ending. Tubule occlusion aims to occlude dentinal tubules to prevent the dentinal fluid movement. The latter accords with the hydrodynamic theory and it is found that occluding tubules has much more benefit in pain relief [6]. There are many desensitizing therapy in reducing the pain from hypersensitivity such as desensitizing toothpastes, adhesive resins, vanishes or lasers. These methods provide an immediate effect to relieve the pain [7, 8].

Laser is one of the methods that is effective in reducing pain from hypersensitivity. It produces a chromatic radiation and emits light at particular wavelengths. There are several studies showed the effect of laser in depolarizing nerve endings or occluding tubules [9-12]. The ability to seal dentinal tubules has been reported that it gives more durable effect than other desensitizers [11]. The type of laser which is used in treating hypersensitivity is high-power lasers such as Nd:YAG, Er:YAG, Er,Cr:YSGG. They lead the recrystallization process from increasing the surface temperature and the closure of dentinal tubules occurs [10-12]. Therefore, the application of laser is professional apply in clinic.

Self-applied desensitizing toothpaste may be the first recommendation for treatment of hypersensitivity. The occluding mechanism from oxalate, arginine, stannous fluoride or calcium sodium phosphosilicate, had been reported to achieve in pain relief [8]. Arginine has the sufficient evidences of efficacy for hypersensitivity treatment [8, 13, 14]. Lavender and co-workers showed that ProArgin desensitizing toothpaste which contains 8% arginine is highly effective in occluding tubules [13]. Moreover, a single topical application or dab-on technique of ProArgin was also found to be useful in exposed sensitive dentin [15].

The durability of the occluded tubules is still suspected for therapy in hypersensitivity. Some evidences shown that desensitizing methods may give long-term effect once apply to exposed dentin. Toothbrushing in daily life can cause abrasion to dentin and play an important role in dentinal tubule opened [16]. Hence, it may affect durability of desensitizing therapy.

The overall aims of this study were to investigate dentin permeability after treating with desensitizers under toothbrushing for 30 days and to analyze which method would give the most sustainable plugged dentinal tubules. The null hypotheses were first, there would be no difference in dentin permeability and tubule patency after their applications. Second, there would be no difference in dentin permeability before and after toothbrushing for 30 days.

Materials and methods

The study protocol was approved by the Faculty of Dentistry/Faculty of Pharmacy, Mahidol University Institutional Review Board. Seventy-two upper premolars were stored in 0.1% Thymol solution at 4 °C for up to six months after extraction according to the approved study protocol were used in this study.

Dentin sample preparation

All upper premolars were sectioned at CEJ perpendicular to the long axis of teeth to obtain the roots with low speed diamond saw (Isomet™, Buehler, Lake Bluff, IL, USA). The root was sectioned twice parallel to long axis of root to obtain a dentin disc with the approximately 0.7 mm thickness. The surface was polished with 600-grit silicon carbide paper to get the approximate thickness of 0.4-0.6 mm. Pulp tissue was evaluated to be absent. The dentin discs were cleaned in ultrasonic water bath for 10 minutes to remove debris. Then, they were randomly divided into 4 groups (n=18) for receiving different desensitizing therapy.

Maximum dentin permeability

Before treating with desensitizer, the specimens were immersed in 17% EDTA solution pH7.4 for 5 minutes to mimic hypersensitive dentin. The EDTA solution removed remaining intratubular inorganic substance and dentinal tubules were opened resulting in gaining maximum dentin permeability [17]. The specimens were then rinsed with distilled water and stored in 100% humid environment at room temperature until baseline of dentin permeability was evaluated. Two specimens per group were randomly selected for observation micro-morphology of dentin after 17% EDTA immersion.

Application of treatment for desensitization

After the maximum dentin permeability had been assessed, the different treatment was applied to each group. Group 1 Positive control: surface of each dentin disc was irradiated with Er,Cr:YSGG laser (BIOLASE, San Clemente, CA, USA) with 0.25 W, 30 Hz, 0% water and 0% air condition. The laser was radiated perpendicular to dentin surface and moved along surface for 30 seconds [9].

Group 2 Negative control: specimens were immersed in tap water without any additional treatment. Group 3 *Colgate® Sensitive ProRelief™* toothpaste (brushing): dentin surface was brushed with Colgate® Sensitive ProRelief™ toothpaste (Colgate-Palmolive, Chonburi, Thailand) using a toothbrush (Systema super spiral original toothbrush, Lion, Bangkok, Thailand) for 2 minutes. Then it was rinsed with tap water. Group 4 *Colgate® Sensitive ProRelief™* toothpaste (single application): surface of specimen was massaged with Colgate® Sensitive ProRelief™ toothpaste for 1 minute with a prophy brush. Then, it was rinsed with tap water [15].

Then, two specimens in each group were randomly selected for observation of occluding pattern of dentinal tubule after treatment under a scanning electron microscope (JSM-6610LV; JOEL, Tokyo, Japan) at the magnification of 2000x

Toothbrushing procedure

In order to evaluate the sustainability of occluded tubules, all specimens were subjected for toothbrushing procedure which was calculated for simulation of 30 days brushing. Each specimen was brushed with a slurry toothpaste (Colgate® Anticavity Toothpaste, Colgate-Palmolive, Chonburi, Thailand). The slurry toothpaste was prepared by mixing water and toothpaste with a ratio of 3:1 by weight according to EN ISO 11609:2010 standard (Dentistry-toothpaste: Requirements, test methods, and marking). The procedure was performed by a toothbrush (Systema super spiral original toothbrush, Lion, Bangkok, Thailand) and toothbrushing machine (TBS-V8, KMITL, Bangkok, Thailand) with loading of 200g and 1200 strokes (120 strokes/min) [18, 19]. Afterwards, four specimen per group were randomly selected to confirm occluding pattern under SEM. The remaining specimens from each group were subjected for evaluation of the dentin permeability.

Dentin permeability analysis

Dentin permeability (Lp) was evaluated from hydraulic conductance model. The flow rate of infusion pump and a constant pressure that applied to this device were set to 0.36 μL/min and 20 cmH_2O. The specimen was connected to a custom-made artificial pulp chamber. It was attached to a polymethyl methacrylate (PMMA) plate by a pair of rubber rings with a 2 mm^2 central hole for distilled water diffusion. The system permitted the water passing through specimens from inner to outer root surface. The infusion pump allowed the linear movement of air bubble which was induced into capillary tube. The movement of bubble was captured by a digital camera and measured by ImageJ software program (NIH Image, Bethesda, Maryland, USA).

The specimen was measured for three times. First measurement (Lp1) evaluated after immersion of EDTA was the maximum dentin permeability. The second (Lp2) was the measurement after application desensitizer. The third (Lp3), it was measured after the specimen went through toothbrushing process.

Dentin permeability was calculated from fluid flow (μLmin^{-1}), which was assessed from the linear movement of the air bubble. This value was divided by surface area of exposed dentin (cm^2) and hydrostatic pressure (cmH$_2$O/psi). The formula of dentin permeability was shown as formula below.

$$\textit{dentin permeability (Lp)} = \frac{\textit{fluid flow}\ (\mu\text{Lmin} - 1)}{\textit{surface area}\ (\text{cm2})\ \chi\ \textit{hydrostatic pressure}\ (\text{cmH2O})}$$

Scanning electron microscope (SEM) analysis

To verify pattern of occlusion, the specimens in each group were randomly selected to observe after coating with palladium (K500X Sputter coater, SPI Supplies, West Chester, PA, USA) under the scanning electron microscope with 20 kV and 2000x magnification. The observation was performed at center of specimen at the same area that dentin permeability was analyzed [17]. The flowchart of the experimental method in this study Is shown in Figure 1.

Statistical analysis

For analysis the difference of dentin permeability, the collected data was analyzed using SPSS version 26 (IBM Corp, NY, USA). The normal distribution and homogeneity of variance of the dentin permeability were verified with a Kolmogorov - Smirnov test and Levene's test. The influences of the type of desensitizers and toothbrushing condition were analyzed with 2-way ANOVA. The differences among groups were further analyzed with Dunnett T3 multiple comparison. The level of statistical significance was set up at p=0.05.

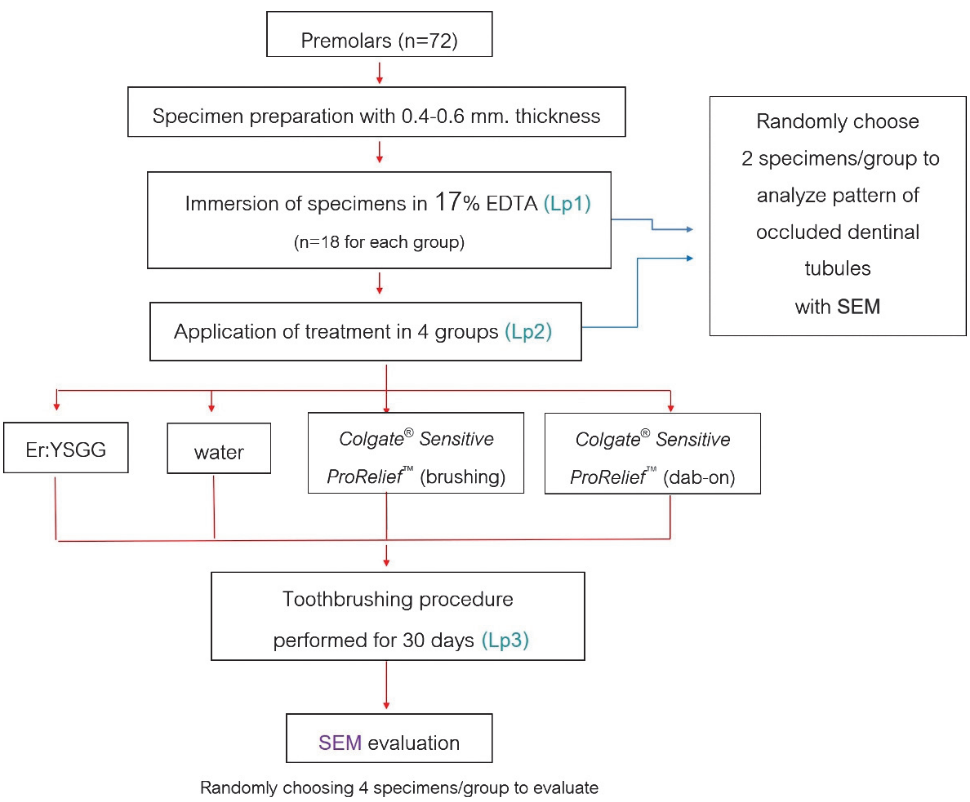

Figure 1 Flowchart of the experimental method in this study

Result

According to 2-way ANOVA, significant effect of the type of desensitizers ($p<0.01$) and toothbrushing condition ($p<0.01$) were found with the interaction between two factors ($p<0.01$). In addition, the power of test was investigated, which was 1.0. The effects of sample size (η^2) were calculated that were 0.91 for desensitizer factor, 0.93 for toothbrushing condition factor and 0.85 for the interaction between two factors. The strong effect of sample size was found in this study.

Means ± standard deviations of dentin permeability of experimental groups are demonstrated in Table 1. There were no statistically significant differences among dentin permeability of all experimental groups before treatment. Significant reduction of dentin permeability was found after dentin were treated with Er,Cr:YSGG laser, brushing with *Colgate® Sensitive ProRelief™* toothpaste and dab-on with *Colgate® Sensitive ProRelief™* toothpaste ($p<0.05$). No significant difference of dentin permeability among dentin treated with Er,Cr:YSGG laser, brushing with *Colgate® Sensitive ProRelief™* toothpaste and dab-on with *Colgate® Sensitive ProRelief™* toothpaste was found ($p\geq0.05$). The further brushing with Colgate® Anticavity toothpaste after treatment had no effect to neither increase nor decrease the permeability of treated specimens. The final of experimental times, the significance between surface treated groups did not present either.

The percentages reduction of dentin permeability was further calculated. The use of Er,Cr:YSGG laser could reduce the dentin permeability for 94.80±2.50%. While using *Colgate® Sensitive ProRelief™* toothpaste in brushing and dab-on techniques could reduce the permeability of dentin for 91.18±5.87% and 89.61±7.77%, respectively. Therefore, no statistically significant differences were found among these percentages ($p=0.14$).

The SEM images of dentin specimens before and after irradiation with Er,Cr:YSGG laser, brushing or dab-on with *Colgate® Sensitive ProRelief™* toothpaste are demonstrated in Figure 2.

From the SEM images, specimens after treating with 17% EDTA solution before application with desensitizers showed clearly opened dentinal tubules. After treatment, a high number of patent-opened tubules were observed in the control group which received no treatment. The surface of specimens irradiated with Er,Cr:YSGG showed a significant change. It was relatively smooth and rarely found widely opened tubules. For specimens after brushing with *Colgate® Sensitive ProRelief™* toothpaste, most of dentin surface were covered with irregular particles. These particles also plugged into dentinal tubules and only few opened tubules were observed. In dab-on technique with *Colgate® Sensitive ProRelief™* toothpaste, the layers of crystalline particles were covered on the surface of dentin and the patent tubules were hardly seen. This group showed more dense deposit from toothpaste than the regular brushing technique.

Table 1 Means ± standard deviations of dentin permeability

	Before treatment	After treatment	After brushing
No treatment	2.61±0.33[a]	2.69±0.31[a]	2.93±0.23[a]
Er-Cr:YSGG	2.76±0.18[a]	0.15±0.54[b]	0.20±0.09[b]
Brushing	2.55±0.10[a]	0.22±0.15[b]	0.31±0.19[b]
Dab-on	2.87±0.42[a]	0.29±0.22[b]	0.59±0.33[b]

The data with the same superscript demonstrates no statistically significant differences ($p\geq0.05$)

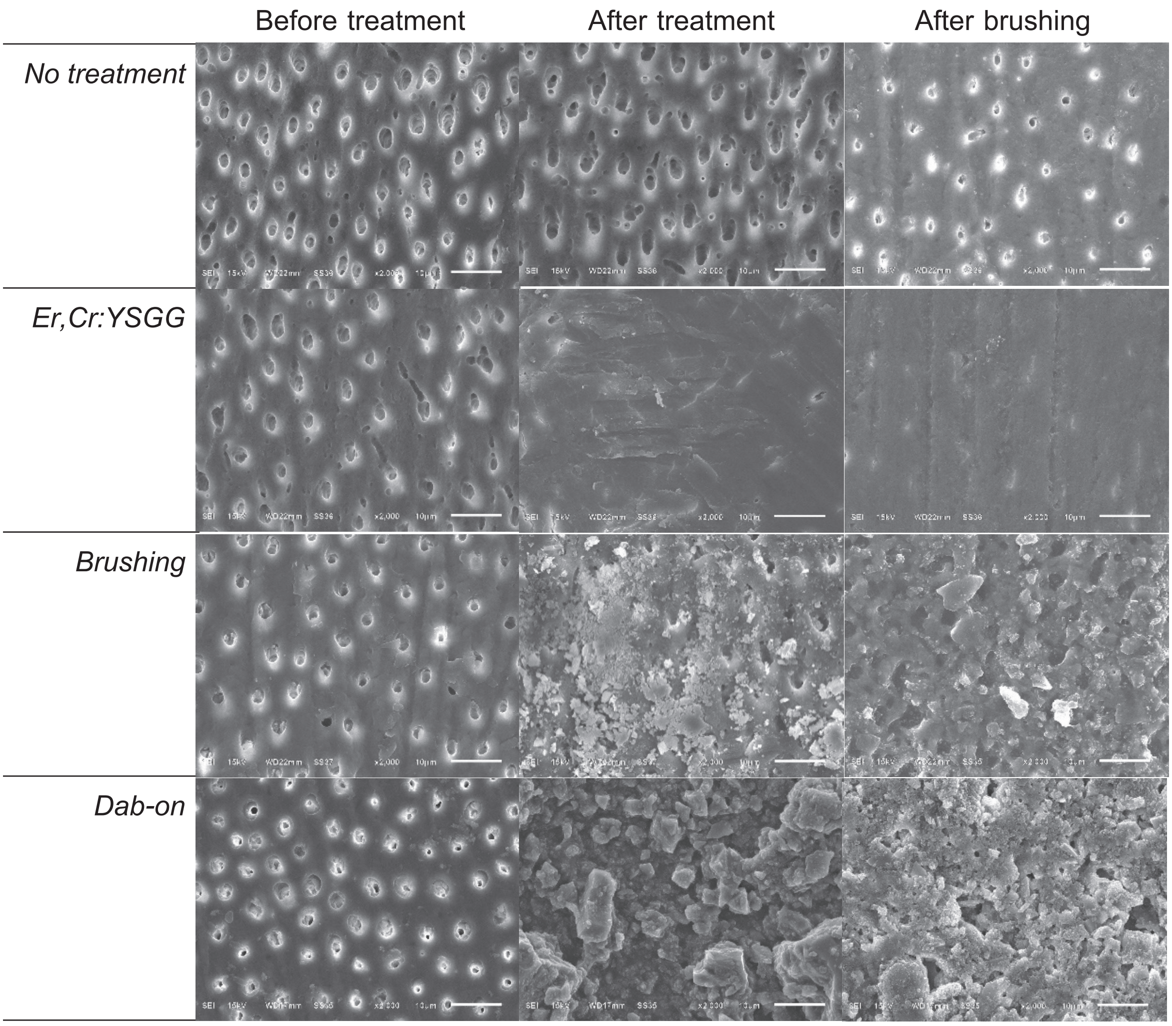

Figure 2 The SEM images of dentin specimens after surface treatment

After toothbrushing process, the control group showed a high number of opened tubules similar to after treatment. The group of Er,Cr:YSGG laser after brushing also did not show any change of dentin surface comparing with the after treatment. This group showed the slightly scratch lines on the dentin surface with no opened dentinal tubules in the path where the laser irradiated. The patent tubules were more visible in the *Colgate® Sensitive ProRelief™* toothpaste groups both brushing and dab-on technique. Therefore, in brushing group, the entrance of dentinal tubules still occluded with the particles after brushing. For *Colgate® Sensitive ProRelief™* toothpaste by using dab-on technique, most of the area was still covered with the layer of irregular particles which was denser than before brushing. The entrance of patent dentinal tubules was scarcely observed. Thus, in both experimental time intervals, these two groups continued to show a layer of deposits with more tubules exposed when the brushing process was done.

Discussion

Dentin hypersensitivity is a common disease with a high incidence. The exposed radicular dentin was found mostly involving with the etiology of this condition in adults [20]. The hydrodynamic theory is mostly used to describe the mechanism of hypersensitivity. Base on this theory, there are many desensitizing therapies which have been developed for reducing the pain. Occluding dentinal tubules is the main point of the treatment to reduce the diameter of dentinal tubules [4]. Many previous researches had studied the effect of those treatments in reducing dentin permeability by their occluding tubules action [14]. Only few studies had used the root surface to evaluate the permeability of dentin [21].

This study aimed to evaluate the reductions in root dentin permeability with tubule occlusion mechanism by using Er,Cr:YSGG laser, brushing and dabbing with *Colgate® Sensitive ProRelief™* toothpaste. A modified custom-made artificial pulp chamber model was used for this in vitro study. The flow rate 0.36 μL/min and hydrostatic pressure 20 cmH_2O were used for this device to simulate human pulpal pressure [22]. Furthermore, root dentin specimens were selected for use in this study due to mostly involving in etiological reason.

According to the results, all experimental groups except control group were able to reduce dentin permeability after treatments when comparing with the maximum dentin permeability. In the point of view, the first null hypothesis was rejected. There was a significant difference in dentin permeability between no treatment group and the others. Laser application presented the lowest dentin permeability value followed by brushing with *Colgate® Sensitive ProRelief™* toothpaste and dab-on technique with *Colgate® Sensitive ProRelief™* toothpaste, respectively. However, among three treatment groups, the statistically significant differences were not found.

Er,Cr:YSGG laser showed a significant difference in dentin permeability reduction both after treatment and brushing process. It could reduce dentin permeability for 94.80±2.50%. This result could be described by the melting effect from Er,Cr:YSGG laser. It is a high-power laser and its system supposes for hypersensitivity treatment [23]. This laser increases the surface temperature which is followed by recrystallization and melting process on dentinal surface. Then, the closure of dentinal tubules is occurred [11]. This was a reason why the SEM images of this application showed the quite smooth surface of specimen with no patent tubules observed. The result from this application of Er,Cr:YSGG laser was similar to previous studies [9, 24]. They reported that the Er,Cr:YSGG laser could reduce the tubular diameter by melting peritubular and occluding tubules in irregular pattern. The study of Al-Omari, which supported this experiment, was also described that this laser could reduce dentinal fluid flow significantly after irradiation on dentin surface [25].

To get melting process and avoid ablation effect, the laser condition was set at 0.25 W with 0% air and 0% water [9, 10]. The melting surface will be happened if this laser is used without water [10]. However, contrary to this research, there was a study showed that Er,Cr:YSGG laser could not reduce dentin permeability significantly compared to control group. This finding was affected by different laser condition setting [17].

For *Colgate® Sensitive ProRelief™* toothpaste with brushing and dab-on technique, these applications could reduce dentin permeability significantly like the laser group. The reduction rates were 91.18±5.87% and 89.61±7.77%, respectively and the difference between two techniques were not found. The SEM images in both groups also presented a large amount of crystal-like deposits on the surface covering the tubular entrances and the opened tubules were rarely seen.

This promising in vitro results in dentin occlusion was supported by many previous studies. Yang and colleagues reported that Colgate® Sensitive ProRelief™ toothpaste produced successive and significant permeability reduction after every

1 minute after brushing [14]. The study of Lavender also showed the images presenting dentin surface and tubular orifices were covered with particles [13]. This observation was also confirmed by Li's experiment which used the same toothpaste as this experiment [26]. Although this paste had shown the effect in tubular occlusion, contrary result, which demonstrated that it was unable to reduce the permeability significantly from the control, had also been reported [17].

Moreover, a single topical application with this toothpaste, the method using a prophy brush to massage the dentin surface was applied to mimic burnishing with a prophy cup. The present study displayed a satisfied result that dab-on technique could reduce hydraulic conductance of dentin like the previous studied [15].

The effectiveness of the toothpaste used in this study might be related to its composition of arginine. There are evidences in systematic reviews supported that arginine-containing toothpaste could relieve the symptoms of dentin hypersensitivity [8]. Arginine is the desensitizing agent that is contained in *Colgate® Sensitive ProRelief™* toothpaste in 8% ratio. The company claimed that it impersonates the natural process of precipitation in tubules. It is biomolecule and its occluding effect is presented when conjugating with calcium, carbonate and phosphate. The positive charge of arginine is attracted by negative charge of dentin and the alkaline pH activates precipitation of calcium, phosphate, carbonate and arginine. The tubular diameter will decrease with these insoluble substances, thereby, inhibiting the hydraulic conductance of dentin [14, 26, 27].

The above desensitizers exhibited the effectiveness in dentin permeability reduction. For treating hypersensitivity, these treatments should provide long term effect in occluding tubules after their application. As daily toothbrushing is an abrasive procedure. It has been postulated as one of the important factors in dentinal tubules exposure [28, 29]. To standardize the brushing procedure, the brushing force and brushing frequency were set at 2 N and 120 strokes/min. The brushing stroke per day was set at 40 strokes, this simulated the clinical situation of daily toothbrushing [19].

Conforming to the results, the second null hypothesis was accepted due to all three experimental groups showed no significant difference in the dentin permeability after brushing with Colgate® Anticavity toothpaste. The SEM images of desensitized dentin after brushing process, especially in Er,Cr:YSGG group still displayed the same dentinal surface as before brushing. The quite smooth surface with no opened tubules was observed and the brushing process produced a slightly scratches on the surfaces when compared to the before brushing image. The SEM images of other desensitized two groups demonstrated more than half of dentin surface covering with the crystalline layer after brushing although relatively more opened tubules were observed. Therefore, no significant difference of dentin permeability among irradiating Er,Cr:YSGG and application of arginine containing dentifrice were observed. This was in agreement with the result of Joao-Souza *et al* [17]. The previous work was also shown that toothbrushing with 200g (2N) had no significant effect in increasing patency when comparing to control group [18]. From the data of this study, it could presume that the brushing force 200 g with total 1200 strokes did not reach the threshold in opening tubules. These could imply that all three desensitizing techniques had an effectiveness and resistance to maintain occluded dentinal tubules for 30 days.

This study was performed using only toothbrush with spiral bristle, meanwhile, difference of either design of bristles or stiffness of bristles of toothbrush might not be considered. These differences might affect dentin permeability and tubular patency after application of desensitizer. The further study might be necessary to investigate in this matter.

The limitations of this study were that it was demonstrated in vitro to simulate in vivo as much as it could. However, this experiment did not completely mimic the clinical condition due to the oral environment had many variable factors which might affect the dentin permeability. There were saliva, the variable dentin surfaces and the brushing condition. Toothbrushing was a dynamic action that brushing force depended on individual person. In addition, the used of root dentin was only small area to determine dentin permeability. It was not the whole surface of root dentin. Thus, it might affect the interpretation of the permeability.

Conclusion

Under the conditions and limitations of this experiment, all treatments which were irradiated with Er,Cr,YSGG laser and application of *Colgate® Sensitive ProRelief™* toothpaste by either brushing or dab-on technique provided the significant reduction in dentin permeability after the application. The significant increasing in dentin permeability of three treatments was not found after toothbrushing condition either. Thus, it was considered that these desensitizers could potentially maintain the reduction in dentin permeability for up to 30 days.

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