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The evaluation of the desensitization effect of a desensitizing agent and desensitizing toothpastes *in vitro*

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This study was evaluating how three desensitizing toothpastes used at home influence the effect associated with desensitizing agents after application in the clinic. Fifty dentine disks measure it permeability and 32 dentine disks with similar permeability levels were selected. Following Dental desensitizer treatment, dentine disks were randomly divided into three subgroups (n=10) that received applications of three toothpastes, respectively. The permeability (Lp) of each specimen was measured after each treatment. One specimen was selected from each group for scanning electron microscopy (SEM) observation. After each treatment, the Lp values decreased significantly for each group (p<0.05) and either completely or partially blocked the dentine tubules upon SEM observation. However, no significant differences in Lp values were observed amongst subgroups (p>0.05). After using the Dental desensitizer, Sensodyne, Crest and Colgate desensitizing toothpastes both can continued to reduce the permeability of the dentine disk, and no significant differences were found amongst them.

Keywords: Dentine hypersensitivity, Dentine permeability, Desensitizing toothpastes, Desensitizing agent, Scanning electron microscopy

INTRODUCTION

Dentine hypersensitivity (DH) refers to dentine exposure to an external stimulus that produces pain, which cannot be attributed to a defect or any other specific tooth lesion cause. The typical stimuli such as those related to temperature, blowing, mechanics or chemicals. Moreover, DH is characterized by its rapid onset, sharp pain and brief duration[1)], which is a common and multiple clinical symptom encountered by adults. Its incidence amongst adult is more than 50 percent, and the symptom degrees vary[2)]. This disease is common among people aged 20–49 years, especially 30-to-39-year-olds. It most often occurs in the area of the incisors and premolar cervical, multiple occurs on the lip or buccal of the teeth, and is rare on the tongue, and it is prone to recurrence after treatment[3)]. This disease is often caused by a comprehensive set of factors including the wear of teeth, severe abrasion, teeth cervical exposure caused by gingival recession, periodontal cleaning and scraping, the external bleaching of live teeth pulp, the preparation of dental crowns and so on, all of which can lead to exposed dentine tubules[4)].

DH negatively affects the quality of life of individuals because of the associated sharp pain, which can affect dietary choices and changes in oral hygiene[5)]. Therefore, DH is becoming an increasingly prominent problem in dentistry[6)]. In 1900, Gysi first described DH and the possible causes of the sharp pain[7)]. The mechanism of the occurrence of DH has not been determined, although most people accept hydrodynamic theory because it is in accordance with clinical practice. This theory states that dentine exposure (due to many reasons) causes the loss of enamel and dentine tubules in open state, which increases permeability so that the flow of liquid into the small dentine tubules causes pain when exposed to an external stimulus[8)]. Several studies have shown that the morphological changes of DH include not only the number of dentine tubules that are open but also the diameter of the dentine tubules in the teeth, whose DH is more than that of teeth without DH[9)]. According to this theory, any treatment that can seal the dentine tubules and reduce the flow of liquid into the dentine tubules can reduce the dentine sensitive effect. An array of different types of tooth-desensitizing agents and desensitizing toothpaste have been created based on this principle to reduce the permeability of dentine[10)]. In addition, laser desensitization, dentine adhesive desensitization, sodium fluoride desensitization, *in-situ* precipitation desensitization, electrocoagulation desensitization and desensitization *via* traditional Chinese medicine have been used in the clinic[11)]. All of these methods can be used to treat DH. However, the evaluation of the clinical curative effect of these treatments primarily depends on the subjective feelings of the patient. Therefore, it is not easy to assess or collect statistical data[12)].

Many scholars who study DH have also conducted clinical research and used the appropriate *in vitro* experiment method to evaluate the efficacy of desensitization agents or desensitizing toothpastes[13)]. The *in vitro* evaluation method of studying the effect of desensitizing agents and desensitizing toothpastes is generally performed by observing the closed rate of the dentine tubules *via* scanning electron microscopy (SEM) and the change in the permeability of the dentine disk through hydraulic conductance[14)]. SEM observation

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is advantageous because it is convenient and concise, brief and intuitive. However, SEM can only be used to observe the surface of the dentine disk, and it cannot directly observe the treatment effect. The observe area is small of the sample, which prone to bias of the results. In particular, no quantitative index exists. No such problem exists for hydraulic perfusion, which can be used to quantitatively measure the changes in dentine permeability and then evaluate the effect of desensitizing agents and desensitizing toothpastes[15]. Besides, this method has a large area of the sample of detection, which the results are more representative with the effect of desensitization, and it is also reduce the bias of the results. Therefore, it is better to adopt the combination of hydraulic perfusion and SEM to evaluate the efficacies of desensitization agents and desensitizing toothpastes.

At present, specialized desensitizing agents exist in the clinic, and numerous household desensitizing toothpastes are available on the market, both of which relieve dentine sensitive symptoms[16]. However, it is not clear that the effect of desensitization can be influences when different commercial desensitizing toothpastes use at home after using desensitizing agent in the clinic.

The purpose of this study was to use SEM and hydraulic infusion to evaluate how three commercial desensitizing toothpastes used at home influences the desensitization effect of using desensitizing agents in the clinical. In addition, to compare whether the results of both evaluation methods are consistent. This study has the potential to provide experimental evidence regarding the evaluation and treatment of DH by using both desensitizing agents and desensitization toothpastes.

MATERIALS AND METHODS

Preparation of human dentine disks

A total of 50 third molars were collected from people who seeking extractions. All teeth were collected after patients provided informed consent. This study was approved by the ethics committee of the School and Hospital of Stomatology at Peking University. All molars had complete crowns and were not subject to root canal therapy or obvious caries lesions. The teeth were cleaned by eliminating the soft tissue and debris and stored in a 0.5% chloride T solution with deionized water at 4ºC. All molars were used within 2 months after extraction, and 75% ethanol was used to soak and sterilize the teeth for 15 min before the dentine disks were prepared.

Dentine disks Φ>6 mm were obtained from these teeth by cutting perpendicular to the long axis of the teeth with a low-speed water-cooled diamond saw (Isomet-Buehler, Lake Bluff, IL, USA). The dentine disk that was the closest to the pulp cavity was selected, which was carefully prepared and inspected to ensure that it was free of coronal enamel and pulpal exposure. Therefore, only one dentine disk sample was obtained from each tooth. Ultimately, 50 dentine disks were collected with thicknesses of 0.5±0.05 mm. All dentine disks were stored in a 24-well cell culture plate with 0.9% sodium chloride solution.

The measurement of dentine disk permeability

The protocol for the permeability experiment was established according to the hydraulic conductance model of Outhwaite *et al.*[17]. The schematic diagram of the experimental device is shown in Fig. 1. To simulate normal pulp pressure, the glass water tank contained deionized water at a pressure of 32 cm H_2O (3.14 kPa) on the pulp side of the dentine disks. The measurement area of the dental disk was 0.283 cm^2, which was calibrated using two 6-mm diameter rubber rings. Only the center of the dentine disk was used to measure permeability. The measurement process of dentine permeability was the same as that applied by Pashley *et al.*[18]. This study recorded the time taken for the air bubble to move up to 10 μL after it showed stable motion for 1 min. The permeability of the dentine disks was calculated using the following equation: Lp=Jv/(A×t×P). In this study, Jv=10 μL, A=0.283 cm^2, and P=32 cm H_2O. Each dentine disk was measured three times, and the average was obtained. All experiments were performed at room temperature.

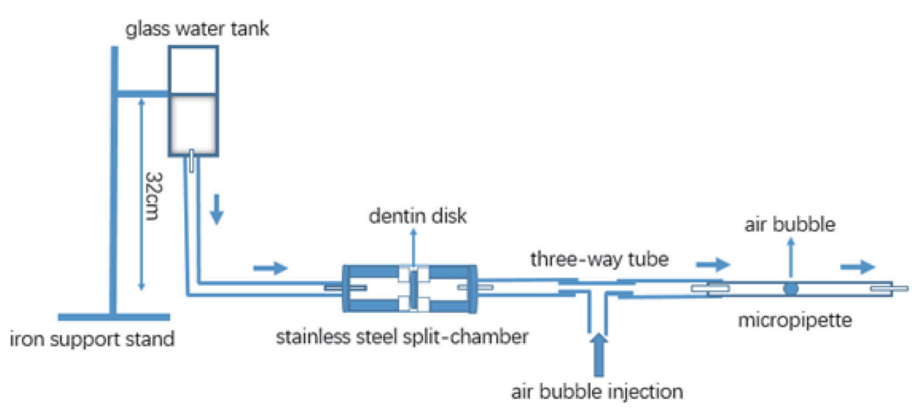

Fig. 1 Diagram of the equipment used to measure dentine disk permeability.

Experimental design

A total of 50 dentine disks were prepared with thicknesses of 0.5±0.05 mm, and the smear layer was removed *via* acid-etching on both sides of the dentine disk with 35% phosphoric acid for 30 s. Then, the etched dentine disks were rinsed with deionized water for 1 min, and their permeability was measured. After measured, 32 pieces of the dentine disks were selected with similar permeability values and treated as A group. The mean of the permeability of A group (LpA) was recorded, which was assigned a value of 100% permeability. Besides, a dentin disk from A group was selected for SEM observation. The Dental desensitizer was applied on the rest 31 dentine disks for 2 min and treated as B group. Its permeability was also measured after rinsed with deionized water for 30 s and the mean of the permeability of B group (LpB) was recorded. Furthermore, a dentin disk from B group was selected for SEM observation. In the next step, the remain 30 dentine disks was randomly divided into three subgroups (C, D, and E) that rubbed for 30 s with Sensodyne, Crest, and Colgate desensitizing toothpastes in one side for 30 s, respectively. Then, the dentine disks were rinsed with distilled water for 30 s to remove visible desensitization toothpastes. The permeability of dentine disks were measured and the mean of the permeability of each subgroup (LpC, LpD, and LpE) was recorded after the use of the different desensitizing toothpastes. All the above operations are done by the same person. Furthermore, a dentine disk from each subgroup was selected for SEM observation, respectively. The above process was summarized in Fig. 2. The compositions of the desensitizer and desensitizing toothpastes used in this study and treatment protocols for the groups and subgroups are listed in Table 1.

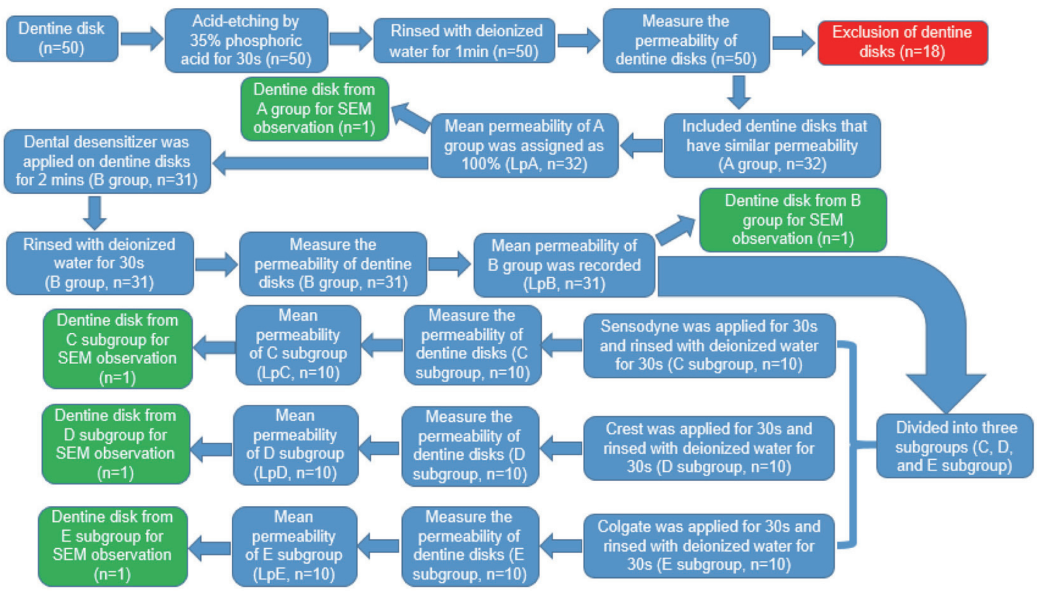

Fig. 2 Experimental design of the study.

Table 1 The ingredients, manufacturers and treatment protocols of the desensitizer and desensitizing toothpastes used in this study

Materials	Ingredients	Manufacturer	Treatment protocols
Dental desensitizer	Polymer polyethylene glycol, glycerine, silica, Bioactive mineral powders	Datsing, Beijing, China, LOT:16070701	The Dental desensitizer was applied to the dentine disks that belong to A group for 30 s, and then rinsed with distilled water for 15 s.
Sensodyne	Sodium fluoride, Hydrated silica, Potassium nitrate, Sodium hydroxide, Zinc citrate	GSK, Suzhou, Jiangsu, China, LOT:16122804	Sensodyne was applied to the dentine disks that belong to C subgroup for 30 s, and then rinsed with distilled water for 15 s.
Crest 7 effect toothpaste	Hydrated silica, Stannous chloride, Sodium fluoride, Gluconic acid sodium	Procter & Gamble, Guangzhou, China, LOT:63391864BB	Crest was applied to the dentine disks that belong to D subgroup for 30 s, and then rinsed with distilled water for 15 s.
Colgate sensitive Pro. Relief™	Calcium carbonate, Arginine bicarbonate, monofluorophosphate, Sodium bicarbonate	Colgate, Guangzhou, China, LOT:6287™112J	Colgate was applied to the dentine disks that belong to E subgroup for 30 s, and then rinsed with distilled water for 15 s.

SEM analysis
The dentine disks created for SEM observation are listed in Table 2. All of the selected dentine disks were dried in a desiccator for 3 days, and these dentine disk sputter coating with Au/Pd was applied for examination under an SEM (CARL ZEISS, EVO18, Oberkochen, Germany) at 10 kV acceleration voltage to visualize the transverse section. Representative images of the dentine disks were acquired at a magnification of 500×.

Statistical analyses
The permeability of each dentine disk was expressed as a percentage (Lp%) of the dentine disk that was etched with 35% phosphoric acid, which was the original maximum permeability value of each dentine disk. Means and standard deviations of the Lp% value were calculated for each group or subgroup. The data were analysed by one-way ANOVA to determine whether significant differences were present between groups/subgroups. The significance levels were set at α=5%. All statistical analyses were performed using SPSS 20.0 (SPSS, Chicago, IL, USA).

RESULTS

Permeability test of dentine disk
Table 3 and Fig. 3 show the results of the permeability test on the dentine disks before and after desensitizer treatment or combined with different desensitizing toothpastes. The LpA that measuring the dentine disks etched with 35% phosphoric acid was considered the baseline permeability values. Following the Dental desensitizer treatment, the LpB decreased significantly compared with the LpA (p<0.05). After treatment with the different commercial desensitizing toothpastes, the LpC, LpD and LpE all decreased significantly compared with the LpB (p<0.05). However, no significant difference was observed amongst LpC, LpD and LpE (p>0.05).

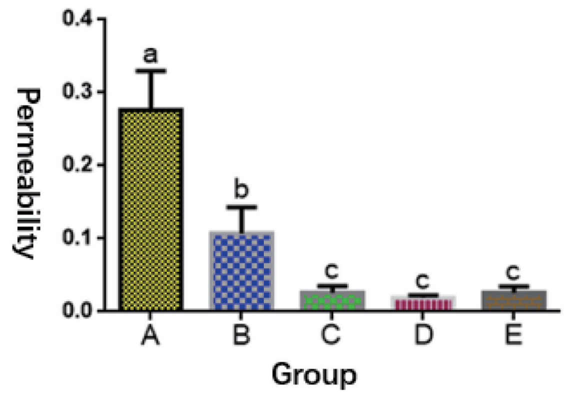

Fig. 3 The results of the dentine disk permeability test that before and after desensitizer treatment or combined with different desensitizing toothpastes. The permeability of dentine disks that etched with 35% phosphoric acid are expressed as percentages of the baseline permeability, which was considered equal to 100%; the error bars indicate the standard deviations of each group or subgroup. Different letters in the error bars that indicates a statistically significant difference between group or subgroups (p<0.05).

Table 2 The dentine disks created for SEM evaluation

Coding	Thickness (mm)	Treatment
A	0.5±0.05	35% phosphoric acid for 30 s
B	0.5±0.05	Dental desensitizer for 2 min
C	0.5±0.05	Dental desensitizer for 2 min+Sensodyne for 30 s
D	0.5±0.05	Dental desensitizer for 2 min+Crest for 30 s
E	0.5±0.05	Dental desensitizer for 2 min+Colgate for 30 s

Table 3 The results of the dentine disk permeability test before and after desensitizer treatment or combined with different desensitizing toothpastes

Group	Number	Treatments	Permeability (means±SD) ($\mu l \cdot min^{-1} \cdot cm^{-2} \cdot cmH_2O^{-1}$)	Relative permeability (%)
A	32	35% phosphoric acid for 30 s and rinsed with deionized water	LpA=0.276±0.0537	100
B	31	Dental desensitizer	LpB=0.107±0.0361	37.91±9.58
C	10	Dental desensitizer+Sensodyne	LpC=0.026±0.0092	8.71±2.40
D	10	Dental desensitizer+Crest	LpD=0.019±0.0039	6.87±0.89
E	10	Dental desensitizer+Colgate	LpE=0.026±0.0084	9.73±2.39

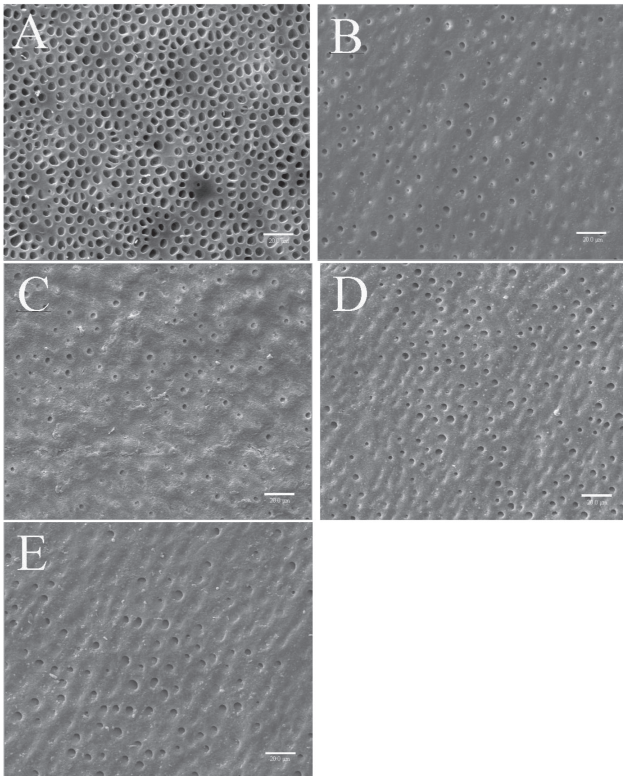

Fig. 4 Representative SEM images of dentine disks, whose occlusal surface received the application of the following treatments: (A) Dentine disks that etched with 35% phosphoric acid; (B) Dental desensitizer only; (C) Dental desensitizer+Sensodyne; (D) Dental desensitizer+Crest and (E) Dental desensitizer+Colgate.

SEM evaluation

Typical SEM images of the transverse sections of the dentine disks that belong to A, B, C, D and E groups or subgroups are shown in Fig. 4 at 500× magnification. Compared with the treatment of 35% phosphoric acid, all desensitization treatments were morphologically modified for the dentine disk surface. Dentine tubules are clearly completely or partially blocked; however, some dentine tubules remain open in some images. Brushing dentine with the Dental desensitizer created a more obvious blockage of the dentine tubules (Fig. 4B). Brushing dentine with the Dental desensitizer and Sensodyne desensitizing toothpaste blocked most of the dentine tubules (Fig. 4C). Brushing dentine with the Dental desensitizer and Crest desensitizing toothpaste did not produce obvious changes compared with the application of Dental desensitizer only (Fig. 4D). Brushing dentine with the Dental desensitizer and Colgate desensitizing toothpaste created a layer of deposits (Fig. 4E).

DISCUSSION

Hydrodynamic theory is widely regarded as the major mechanism of DH. Therefore, two methods have been developed to treat DH[19]. The first method is to obstruct the conduction of nerve impulses of the A-beta and A-delta nerve fibres in the pulp and affect the transmission of pain signals caused by external stimuli by increasing the potassium ions concentration. Several different potassium-containing desensitizing toothpastes are available on the market. The second method is to reduce the flow of fluid through the small dentinal tubules by blocking the hydrodynamic mechanism in these dentine tubules. Therefore, many desensitizers and desensitizing toothpastes are used for the clinical or home treatment of DH. Desensitization can be accomplished by effectively blocking the dentine tubules.

The purpose of this study was to use SEM and hydraulic infusion to evaluate the desensitization effects of three commercial desensitizing toothpastes at home after application a desensitizing agent in the clinic. In addition, this study examined whether the results of the two evaluation methods are consistent.

We choose one desensitizer for the clinic and three commercial desensitizing toothpastes for the home for use in this study. The Dental desensitizer contained bioactive mineral powders that might have reacted with the phosphorous of the dentine to mechanically occlude the dentine tubules[20]. Sensodyne and Crest both contain sodium fluoride and hydrated silica, which react with hydroxyapatite or merely physically block open dentine tubules. Colgate contains not only calcium carbonate and sodium bicarbonate but also arginine bicarbonate, which has been shown to successfully occlude open dentine tubules in several clinical studies[21]. In addition, Sensodyne contains potassium ions because it is designed to reduce nerve excitability in people with hypersensitive teeth; however, the effect of these potassium ions cannot found in this study[22].

In vitro hydraulic perfusion, a reportedly suitable method was used to determine the permeability of dentine disks before and after the use of desensitization materials in this study. This experimental method can control the factors that influence the results and improve their reliability and clinical relevance. Previous studies used SEM to evaluate the effect of the desensitizing agent and observe the blockage of the dentine tubules after using desensitization materials[23]. However, SEM is a qualitative research method, whereas *in vitro* hydraulic perfusion method is quantitative and can be used to accurately understand the effect of desensitization. In addition, SEM can only be used to select a few small areas of the dentine disk to evaluate the curative effect; therefore, the experimental results can be biased. Using *in vitro* hydraulic perfusion, the test area of the dentine disk is larger, the average of the whole test region can be obtained, and the experimental results are more objective and stable.

The dentine disk model of sensitive teeth is an important factor when evaluating the *in vitro* desensitization effect of desensitizing agents on people with DH syndrome[24]. This experiment adopted a dentine disk etched by 35% phosphoric acid for 30 s to remove the smear layer and simulate the opening condition of the dentine tubules, thereby establishing a repeatable and unified research model for the objective and accurate evaluation of the effect of desensitizing agent for sealing sensitive teeth. Although previous studies that the test area of the dentine disk used was 0.071 cm^2 or even smaller, we chose a 0.283 cm^2 test area for this experiment[25]. We believe that larger test areas produce more average and representative results. Furthermore, previous studies were performed in presence of 20 cm,70 cm H_2O or even higher levels of pressure; We selected 32 cm H_2O to simulate the normal pulp pressure in this study[26].

The mean (SD) permeability values of the dentine disk with thicknesses of 0.5±0.05 mm etched with 35% phosphoric acid were 0.276±0.0537 $\mu L \cdot min^{-1} \cdot cm^{-2} \cdot cmH_2O^{-1}$, respectively. The results of this study revealed different hydraulic conductivity values from other studies using the same thicknesses[27]. The most likely causes of this difference are the age of the donor's teeth, the position of the section from the coronal teeth essence, the test area changes, and the small dentine tubules' densities and diameters. The mean (SD) permeability of the dentine disks with thicknesses of 0.5±0.05 mm that was applied with Dental desensitizer was 0.107±0.0361 $\mu L \cdot min^{-1} \cdot cm^{-2} \cdot cmH_2O^{-1}$. After desensitization, a significant reduction was observed in dentine permeability ($p<0.05$). After treatment with different commercial desensitizing toothpastes (Sensodyne, Crest and Colgate), the mean (SD) permeability of the dentine decreased significantly for all subgroups compared with the values associated with teeth that only had Dental desensitizer applied ($p<0.05$). However, the mean (SD) permeability values of the dentine that received different desensitizing toothpastes after Dental desensitizer application were similar and no significant differences were found amongst them ($p>0.05$). The null result might be because the effect of the Dental desensitizer masks the desensitization effect of the three desensitizing toothpastes.

Typical SEM images were taken of the transverse section of the dentine disks on which each desensitizer was applied alone or in combination with different desensitizing toothpastes. Dentine tubules were clearly blocked; however, some dentine tubules remained open. In addition, these SEM results do not correspond well with the reduction in permeability of the dentine disks. For example, SEM images of dentin disks that using Dental desensitizer+Crest, which showed that many of the dentin tubules still open. However, the permeability of it is small. The reason for this phenomenon may be that when desensitizing agent and desensitizing toothpaste are applied to dentin disks, their components are squeezed into deep dentine tubules. Although many dentine tubules on the outer surface are remaining open according to the image of SEM, they are blocked inside that resulting in low permeability. Another possible reason for this result is that Crest desensitization toothpaste has a good blocking effect but with a poor adhesion, which lead to exposure of dentine tubules.

The permeability of teeth differs, even across different areas of the same teeth. During the process of human tooth growth, permeability is affected by various factors such as wear, damage, the essence of the teeth and the essence of the dentine. These factors can lead to differences in the test results of dental disk permeability[28]. Therefore, this experiment used dentine disk models before and after self-control and selected a sensitive dentine disk model with similar permeability after etching with 35% phosphoric acid to alleviate the problem of the dentine permeability showing great individual differences.

Some desensitization therapies use lasers treat DH[29]. Therefore, the therapeutic effect of laser desensitization can be also evaluated using in vitro hydraulic perfusion method.

The limitation of the experimental protocol is that the effect of potassium ions, which block or inhibit nerve endings, could not be detected. Therefore, the clinical application of potassium ions requires further exploration. In addition, teeth are affected by various mechanical forces (*e.g.*, pressure, tension and torque) during chewing, as well as by temperature changes, microorganisms and salivary enzymes. All of these elements affect the efficacy of desensitization; however, we did not control these effects in this experiment. This study detected the effect of the immediate closure of the dentine tubules due to desensitization materials; however, the long-term closed effect was not evaluated. It is necessary to conduct a longitudinal experiment when evaluating the long-term sealing effect of desensitization materials[30]. Cold/heat circulation stimulation, saliva immersion and toothbrush-wear experiments should also be examined in a longitudinal experiment. As the next step, we will assess the long-term effects of desensitization on the combined use of desensitizers and different desensitizing toothpastes.

CONCLUSION

In summary, after applying Dental desensitizer, the permeability of the dentine disk decreased substantially. After using the Dental desensitizer, different desensitizing toothpastes continue to reduce the permeability of dentine disk; however, no significant difference was found amongst them. Hydraulic perfusion is a more objective and stable method than SEM observation when evaluating the effect of desensitization materials.

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