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The influence of a selected desensitizer on marginal microleakage of a class V composite restoration subjected to thermocycles: an in vitro study

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Abstract: Introduction: Dentin hypersensitivity is a painful clinical condition. The frequency of its occurrence varies from 8 to 57%, depending on tested group and different methods of investigations. Recommended desensitizing agents have different mechanism of action and effectiveness. We are still looking for solutions that will improve their effectiveness and simultaneously allow for wider use of e.g. as a base material, counteracting postoperative hypersensitivity, reducing marginal microleakage.

The aim of the study was to assess the effect of a selected desensitizing agent occluding dentin tubules with calcium hydroxyapatite on marginal microleakage formation of a class V composite restorations subjected to thermocycles.

Materials and Methods: In study it was used 40 molars and premolars, which were alternately assigned into two groups. In both groups standardized cavities were prepared. In the study group (study group — SG) before application of bonding agent Teethmate Desensitizer (f. Kuraray, Noritake Dental Inc., Okayama, Japan) was used. In the control group (control group — CG) OptiBond All-in-one (f. Kerr, Bioggio, Switzerland) bonding agent was used and cavities were filled using composite material Gradia Direct (f. GC Europe N.V., Leuven, Belgium). After storage in saline, teeth were subjected to 600 thermocycles, passive dye penetration test was done, teeth were cut in the area of filling, according to its long axis. Under light microscope magnification value of microleakage was measured and marginal microleakage rate (M) was counted. The results of the tests were statistically analyzed using the package STATISTICA 12.0 (StatSoft, USA).

Results: The average value of M for the SG group was 0.46 (min 0.05, max 0.76, SD 0.226) and for CG was 0.22 (min 0, max 0.74, SD 0.235). The differences between M values were statistically significant ($p = 0.0094$).

Conclusion: A reduction in the number of retention sites for the bonding system, facilitates the formation of microleakage in the experimental conditions and reduces the degree of adhesion of the composite material to the hard tissues of the tooth.

Keywords: dentin hypersensitivity, desensitizer, margin microleakage, bonding systems, *in vitro* study.

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Introduction

Dentin hypersensitivity, which exerts a negative influence on the quality of life, occurs relatively commonly in the European population. The incidence of dentin hypersensitivity ranges from 8 to 57% of the population and may reach 60–98% in periodontal patients. Variance in these results are caused by the diversity of the population and research methods (questionnaires, clinical trials). Symptoms most often occur between 30 and 40 years of age, affecting mainly canines and premolars, and are reported more often by women than by men [1–4].

Patients with hypersensitivity experience short-term acute pain in response to thermal, mechanical, osmotic, or chemical stimuli or dehydration of dentin [5], lasting until the moment the stimulus is discontinued [6]. The most common factor causing pain is cold (about 75% of respondents) [3, 5]. The main cause of this type of pain, which cannot be attributed to any other dental pathology, is open dentinal tubules [3, 6–8].

There are several theories explaining dentin hypersensitivity. The mechanism of its formation is best explained by the hydrodynamic theory. A stimulus acting on open dentinal tubules causes an increase in the flow of dentinal tubular fluid, leading to stimulation of pain receptors in the nerves inside the tubules or in superficial layers of pulp. As a result, the patient feels pain [9].

We offer a growing number of preparations relieving hypersensitivity, characterized by various levels of effectiveness and mechanisms of action. Most methods of treatment used in dentists' offices and at home by the patient function by closing the dentinal tubules and reducing the flow of dentinal tubular fluid [6, 10]. Available chemical and physical agents induce a smear layer or block the dentinal tubules. Materials to close the tubules may include resins, strontium compounds, aluminum, potassium, and iron oxalates, or materials containing calcium and precipitating proteins. Another mechanism involves the blockage of nerve activation and pain transmission with potassium salts. The two methods can be combined [10].

In order to obtain better results in the suppression of dentin hypersensitivity, ever-newer solutions are being sought, including some which enable the use of material that eliminates dentin hypersensitivity by serving as base material as well as reducing marginal microleakage and post-operative hypersensitivity and sealing enamel microcracks. One example is Teethmate Desensitizer (TD) (manufactured by Kuraray Noritake Dental Inc., Okayama, Japan), available in Japan, Europe, and North America [11]. Its ingredients include a powder and a liquid. The powder consists of tetracalcium phosphate (TTCP: $Ca_4(PO_4)_2O$) and anhydrous dicalcium phosphate (DCPA: $CaHPO_4$), which, when mixed with the liquid contained in the kit (the main component of which is water), are easily converted to calcium hydroxyapatite (HA: $Ca_{10}(PO_4)_6(OH)_2$) [11–13]. The crystals thus produced are capable of blocking the dentinal tubules [14].

Numerous studies prove that TD is an effective preparation in the suppression of dentin hypersensitivity. In their clinical study, Mehta et al. showed that TD resulted in a statistically significant reduction in hypersensitivity immediately as well as 6 months after application. An average reduction of 3 to 4 points on the Visual Analog Scale (VAS), used for subjective assessment of pain by patients (0 = no pain, 10 = unbearable pain) [15], was also obtained following use of the preparation.

Marginal microleakage is defined as the diffusion of bacteria, molecules of chemical compounds, and/or ions contained in saliva in a liquid-filled crack or structural irregularity which is naturally present or occurs between restorative material and the prepared tooth surface [16]. Its existence may cause post-procedural hypersensitivity and discoloration at the border between the filling and tooth tissues, and may give rise to secondary caries leading to the failure of the reconstruction [17]. It is recommended to apply different prevention methods of microleakage of composite restorations. One of these might be proper preparation and conditioning of tissues and application of appropriate bonding system.

The aim of the study was to assess the effect of a selected desensitizing agent occluding dentin tubules with calcium hydroxyapatite on marginal microleakage formation of a class V composite restorations subjected to thermocycles.

Materials and Method

Forty molars and premolars, which were alternately assigned to two groups (I: study group, or SG, and II: control group, or CG; Fig. 1), were used in the study. Standardized V-class defects were prepared in the teeth, in accordance with Black. The enamel was then etched for 30 seconds, the dentin for 15 seconds, using 36% orthophosphoric acid (Arkona, Bydgoszcz, Poland). In group SG, the desensitizing agent Teethmate Desensitizer (Kuraray Noritake Dental Inc., Okayama, Japan) was used, according to recommendation, prior to application of the bonding system. The recommended volume of powder and liquid was measured out with the measuring cup provided and mixed for more than 15 seconds. The material was rubbed into the dentin surface for about 35 seconds, using an applicator. The defect was then rinsed with water spray and cleaned of excess material with a microbrush. The bonding system OptiBond All-

Fig. l. Molars and premolars used in the study and randomly assigned to two groups.

In-One (Kerr, Bioggio, Switzerland) was applied to the surface prepared in this manner, followed by Gradia direct composite material (GC Europe N.V., Leuven, Belgium). In the CG group, the OptiBond All-In-One bonding system was used and the defect was filled with Gradia direct composite material.

The examined teeth were stored in saline solution for a period of 6 months, then subjected to 600 cycles of variable temperatures using a thermocycler. One thermocycle consisted of 60 seconds at 5°C and 60 seconds at 50°C. These cyclic temperature changes were supposed to imitate thermal conditions in the mouth which lead to fatigue in composite material. The teeth were then left for 6 hours in methyl blue, enabling the dye to passively penetrate the space that had been created between the filling and tooth tissues, thus illustrating the extent of microleakage.

Next, the teeth were cut symmetrically along the long axis in the area of fillings made with the use of a disc with a fine diamond coating. In this way, two cross sections were obtained from each tooth and examined using a light microscope with a micrometer attachment at a magnification of 12× (Fig. 2).

Teeth were randomly assigned to students. In each group, measurements were carried out by two individuals, which made it possible to monitor the activities being conducted.

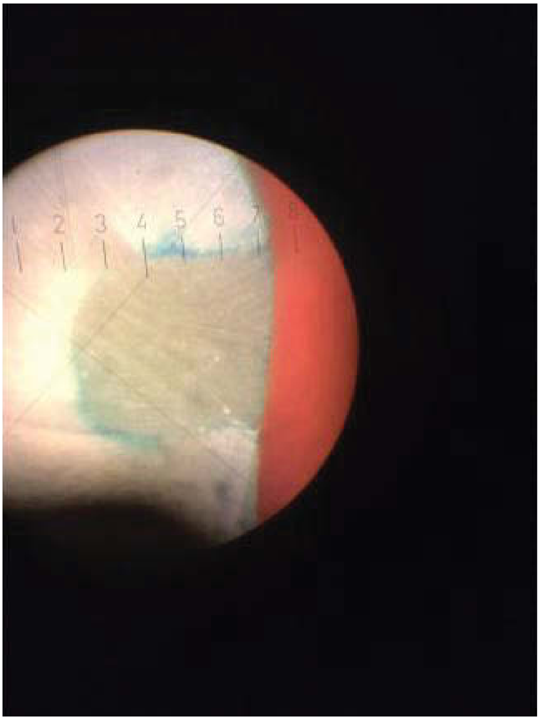

Fig. 2. Image from a light microscope with a visible micrometer scale and passive dye penetration along the filling walls (magnification of 12×).

In each tooth, the lengths of the walls of the defect were measured (marked with capital letters from A to F), along with the lengths of sections stained with methyl blue penetrating between the material and hard tissues of the tooth (marked with lowercase letters from a to f) (Fig. 3). The marginal microleakage index M, calculated using the formula $M = (a + b + c + d + e + f)/(A + B + C + D + E + F)$ was assessed (Fig. 3).

$$M=\frac{\text{sum of all measurements of the depth of dye penetration in both halves [mm]}}{\text{sum of the lenght of all walls and of the bottom of the defect in both halves [mm]}}$$

Fig. 3. Formula for calculation of the index of marginal microleakage M.

The results of the tests were statistically analyzed using the package STATISTICA 12.0 (StatSoft, USA). The calculations included: arithmetic mean (sm), median (m), minimum (min), maximum (max), and standard deviation (SD). In both groups the Shapiro-Wilk test was used to confirm that the distribution of the variable M was consistent with normal distribution. The distribution of the M variable in the CG group differed significantly from normal distribution (p = 0.00685); therefore, a rank sum test (the Mann-Whitney U test) was used for subsequent analysis (Student's T-test could not be used, as the assumption had not been fulfilled).

Results

The average value of the microleakage indicator was M = 0.46 (min. 0.05, max. 0.76, SD 0.226) for the SG group and M = 0.22 (min. 0, max. 0.74, SD 0.235) for the CG group. Detailed values are presented in Table 1. Based on statistical analysis, the average values of the microleakage index M were found to differ statistically significantly between the groups (p = 0.0094 <0.05), as shown in Fig. 4.

Table 1. Detailed values of analysis for the SG and CG groups.

	SG	CG
arithmetical mean (sM)	0.460811358182667	0.216539424483477
median (m)	0.453446241995281	0.142276422764228
minimum (min.)	0.0508826583592939	0
maximum (max.)	0.762278978388998	0.746073298429319
standard deviation (SD)	0.226618125044925	0.235549714549446

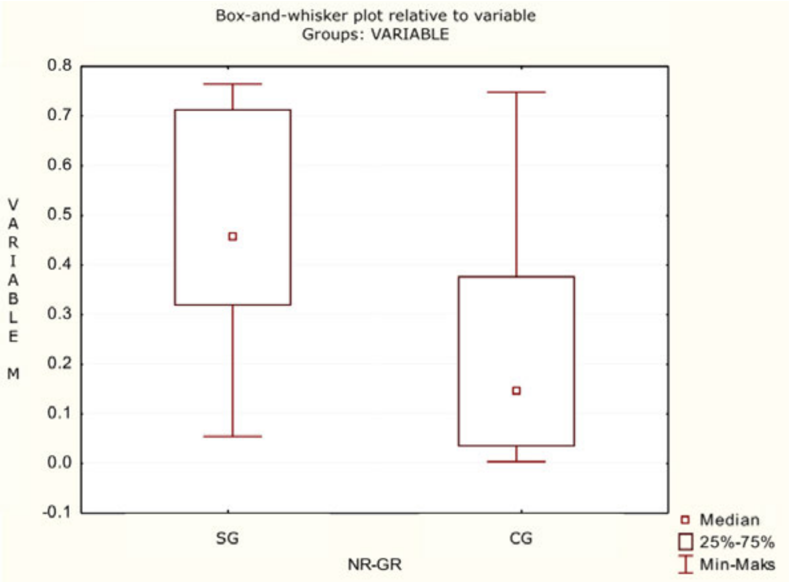

Fig. 4. Distribution of microleakage M indicator values with the median indicated.

Discussion

Teethmate Desensitizer is gaining greater and greater popularity due to its biocompatibility and its ability to block dentinal tubules and favorably reduce dentin permeability in the oral environment [11, 14, 18]. Its biocompatibility results from the precipitation of calcium hydroxyapatite (HA), which is the main inorganic component of teeth and the hardest mineral in the human body. Formation of HA occurs as a result of mixing tetracalcium phosphate (TTCP) and anhydrous dicalcium phosphate (DCPA) with water or an aqueous solution [11, 12, 19]. The resulting crystals block the dentinal tubules, reducing the permeability of dentin [20] (Fig. 5, 6). The potential for HA formation in the oral environment, as the result of supersaturation of human saliva with calcium phosphates [21, 22], is also essential and, clinically speaking, may be an important long-term process maintaining the action of TD [13, 23].

Due to its mechanism of action, the preparation is also recommended for sealing enamel microcracks as well as dentin exposed in abrasions, erosions, gingival reces-

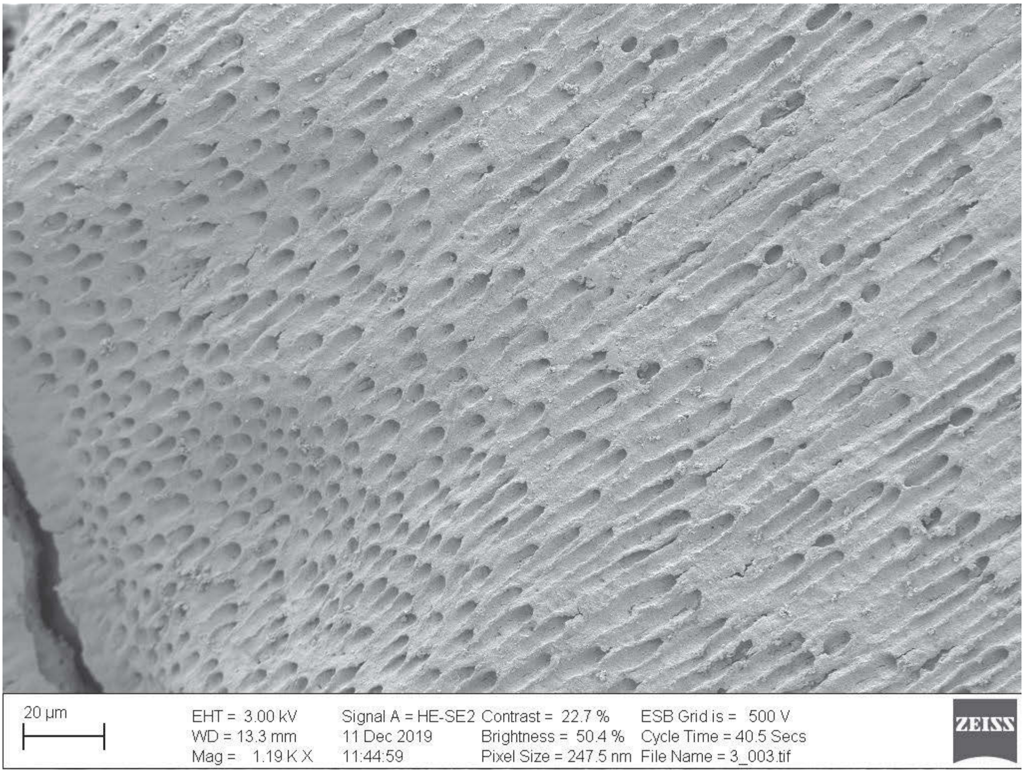

Fig. 5. Open dentinal tubules after conditioning with 40% citric acid solution, 2 minutes ultra sound activation and 20 minutes 5% sodium hypochloride solution (magnification of 1200×), SEM, Merlin Gemini II (f. Zeiss, Germany). Proprietary material.

sions, in periodontal disease, and traumatic mechanical cleaning of teeth. TD reducing the roughness of the tissues prevents the formation of extrinsic stains as well [24].

Although, according to recommendations, Teethmate Desensitizer can also be applied to prepared dentin prior to application of direct reconstruction material or indirect prosthetic reconstruction, an approximate twofold increase in microleakage observed in the group with this preparation gives such an indication into question.

Good adhesion of a filling or prosthetic reconstruction is one of the important conditions for the durability of reconstruction. In the case of reconstruction using the direct method, not only the properties of the material used for this purpose but also the methods of application and of polymerization, the technique for working on tooth tissues, and their conditioning prior to filling are essential. Bonding systems enable

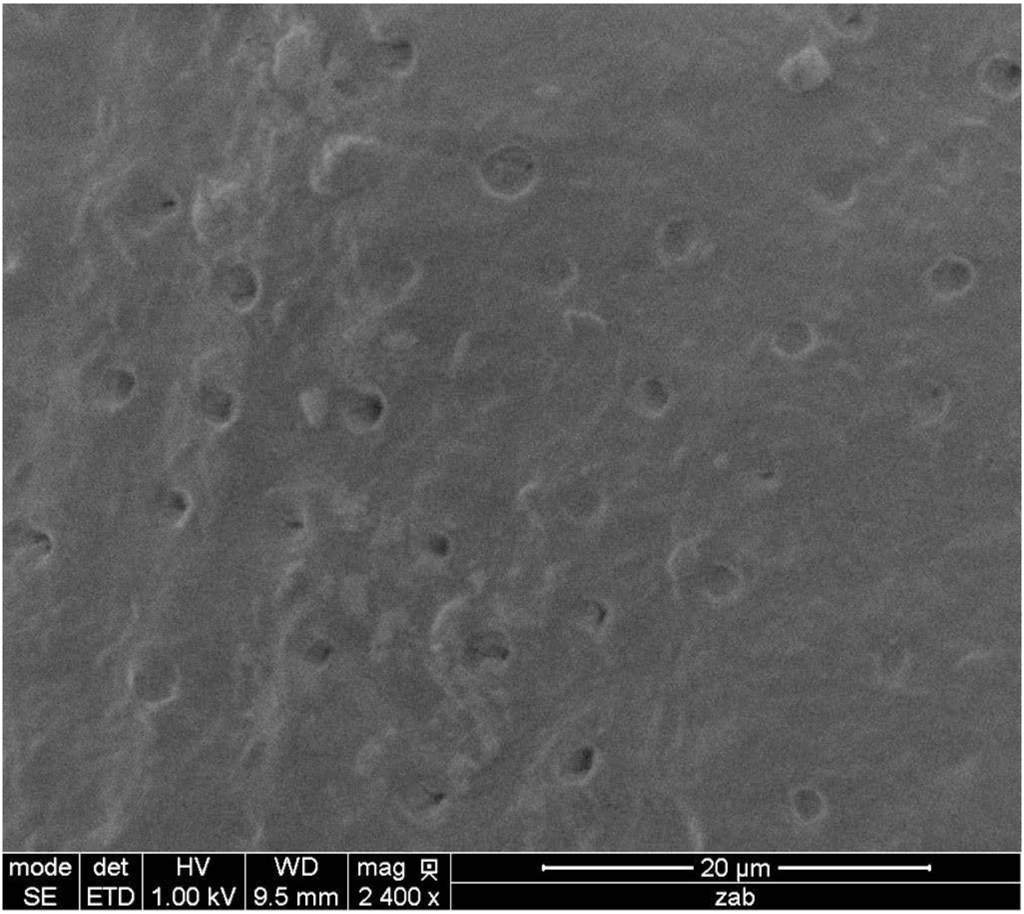

Fig. 6. Dentin surface after conditioning with 36% orthophosphoric acid and one-time application of TD. Most of dentinal tubules are filled with material (magnification of 2400×), SEM, Inspect S50 (f.FEI, USA). Proprietary material.

permanent and tight adhesion of the composite material to tooth tissues. In the search for the optimal system, research is being conducted on acquisition of a preparation with a high degree of binding strength which is simple to use. The systems currently in use belong to the fourth, fifth, sixth, and seventh generations. They differ in manner of application and strength of binding. In order to simplify the procedure of applying fillings, self-adhesive composite materials (compobonds) which do not require prior tissue etching or application of a bonding system are also being developed [25–27].

In obtaining a tight filling, an important role is played by the proper microstructural development of enamel and dentin. Proper etching significantly develops the adhesion surface of the reconstruction, opening dentinal tubules and exposing collagen fibers and apatites. Loss of any of these retention sites can result in reduced adhesion of restorative materials.

In our study, the increase in microleakage was most likely caused by the closure of a portion of dentinal tubules and the loss of a significant number of retention sites for the bonding system, thus facilitating the loss of tightness in thermocycle conditions.

The extent of the loss of dentinal tubules as retention sites may depend on the number of applications of the preparation. In studies conducted in a number of centers, assessments were made with the use of a scanning electron microscope. After one application of Teethmate Desensitizer, single open dentinal tubules remained visible, which was rarely observed after the product had been used three times. In fact, repeated application of the agent significantly reduces the permeability of dentin, which in the case of hypersensitivity is of key importance; however, when planning subsequent restorations, the potential for reducing retention surfaces for the bonding system should be taken into account, as well as potentially reduced tightness of the reconstruction to be performed [28, 29].

In vitro tests showed an immediate reduction in dentin permeability of up to 92% regardless of the surface structure (with or without exposure of the collagen fiber network) following TD application, which may have an impact on the clinical reduction of hypersensitivity but it can significantly reduce retention sites as well [13, 20].

In comparative studies, Teethmate Desensitizer exhibited properties similar to those of Gluma Desensitizer PowerGel (GLU), whose main components are glutaraldehyde and HEMA, and which works through the precipitation of proteins in dentinal tubules [15, 30]. *In vitro* studies conducted by Ishihata et al. showed a reduction in dentin permeability of 30 to 50%, both at the outset of application of TD and GLU and after one month. Both products can be assessed as effective desensitizing agents [11].

Alike in research on dentin permeability and tubule occlusion made by Machado et al. In their study 1mm thick demineralized (17% EDTA solution) dentin specimens were conditioned with selected dental agents: Clinpro White Varnish, Clinpro XT Varnish, Teethmate Desensitizer, Desensitizer Nano, Nupro prophylaxis paste. Most of them were efficient in reducing number of open dentinal tubules and dentin permeability [20].

Despite the cited results of research on the effectiveness of TD in suppressing dentin hypersensitivity, in the case of its use prior to application of a bonding system and composite material, the potential for reduced adhesion should be taken into consideration. In our experiment, the influence of Teethmate Desensitizer on the value of the microleakage indicator M was observed, increasing it nearly twofold compared to the group in which the agent reducing hypersensitivity was not used. It is likely that its capacity to block dentinal tubules reduced the strength of the bonding system and reduced the adhesion of the composite material. Further research, such as assessment of the value of the M index with two and three applications of Teethmate Desensitizer, is required to explain this phenomenon.

Limitations

In this study, it has been examined the impact of temperature change on the microleakage development of composite restoration with or without dentin desensitizer. *In vivo* there are many more factors which might affect microleakage development. Among these one may identify: pH changes in oral cavity environment, occlusion forces, in-restoration and in-tooth tissue stress, saliva composition. Further research might be conducted to examine other correlations. Furthermore, it can be assumed, that a double or triple TD application affects M variable.

Conclusions

A reduction in the number of retention sites for the bonding system facilitates the formation of microleakage in the experimental conditions and reduces the degree of adhesion of the composite material to the hard tissues of the tooth.

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Conflict of interest

None declared.

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