Stomatologija. Baltic Dental and Maxillofacial Journal, 21: 113-8, 2019

An *in vitro* evaluation of microleakage of class V composite restorations using universal adhesive under different level of cavity moisture conditions

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SUMMARY

Objective of this study was to evaluate microleakage using self-etching universal adhesive on direct class V composite resin restorations under different cavity moisture conditions.

Materials and Methods. 30 extracted human molars were divided into three groups (n=10) to compare the adhesive "Prime&BondActive"under optimal, overly dry, and overly wet cavity moisture conditions. Class V cavities were filled with nanocomposite and polished. All teeth were thermocycled for 3000 cycles, sealed and immersed in 1% methylene blue solution for 24 hours. Consequently, teeth were sectioned and analyzed under a microscope. Dye penetration lengths were scored in millimetres and permeation degrees.

Results. The results showed that self-etching universal adhesive was least permeable (lower dye penetration depths) under optimal working field conditions. During overly wet cavity moisture conditions performans decreased insignificantly.

Conclusions. According to the present study there is no significant difference of microleakage using universal adhesive in class V composite restorations with different moisture level.

However, too wet working field conditions tend to increase microleakage formation. In clinical performance, it could be suggested to keep an appropriate cavity moisture level.

Key words: microleakage, universal adhesive, class V composite restoration.

INTRODUCTION

The basic requirements for dental adhesive is to provide an effective bond to hard tissues and to achieve hermetically sealing of cavity. Adhesives are often regarded as techinique-sensitive, because error in the clinical application procedure may lead to debonding, microleakage of restoration or insufficient cavity sealing (1, 2). The microleakage is considered to be a major factor influencing the longevity of dental restorations (1). Consequently, the evaluation of microleakage of composite restoration may reflect the sealing ability of adhesive system (2).

Therefore, it is important to use a proper adhesive, which minimizes the extent of microleakage at the tooth-restoration interface (3). The current trend is to develop simplified self-etching materials such as one-step self-etch systems (all-in-one) (2). They incorporate all the components of an adhesive system into a single solution and combine all three bonding steps into a single application. Generally, self-etching adhesives are preferred materials because they save chair time and reduce procedural errors (4).

The structural attributes of dental hard tissues like enamel, dentin, and cementum as well as a prerequisite of field moisture control with regard to the adhesive technique demand high requirements on the treatment of cervical lesions (1). Therefore, microleakage studies of class V restorations are still very relevant.

Clinically, self-etching adhesive technique leads to a question concerning the wettability of the dentin. It is not uncommon to have overly wet regions and overly dry surfaces in the same preparation, which causes a non-uniform resin bonding (5). This increases the risk of gap formation at the tooth-restoration interface and subsequently increases the risk of microleakage (5, 6). Therefore there is a

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need for the studies investigating universal adhesive system on moisture sensitivity.

In the present study we evaluate *in vitro* microleakage of class V direct resin composite restorations using universal self-etching adhesive with respect to cavity moisture level.

MATERIALS AND METHODS

The study was set as randomized parallel group in vitro investigation to evaluate the microleakage on extracted human teeth using universal self-etching "Prime&Bond Active TM" (Dentsply DeTrey GmbH, Germany). The study took place at the Clinic of Dental and Oral Pathology of Lithuanian University of Health Sciences (LUHS). Thirty caries free human molar teeth were collected from LUHS Maxillofacial Surgery Clinic. Right after the extraction, the teeth were kept in NaCl 0.9% solution. The remaining soft tissues, dental plaque and calculus have been removed.

Cavity design

Class V cavities in molar teeth were made on lingual (palatal) or vestibular tooth surfaces on the cemento-enamel junction using green diamond rose burrs (G801.023 D04.001FG) and cavity shape was formed using green diamond cylindrical burrs (G836.012 99507FG). New identical burrs were used for each group. The burrs were used in a high-speed handpiece (400,000 rpm) with water cooling. The preparations were done using the same measurements: 3 mm × 3 mm × 15 mm (depth). In order to prepare equal size cavities, Williams periodontal probe was used. After the preparation, each cavity was thoroughly cleaned and dried.All cavities were prepared by the same operator.

Groups

The teeth were randomly divided into three groups (n=10 each) to analyse the adhesive's performance in different cavity moisture levels: group A – ideal/optimal cavity moisture level**,** group B – overly dry cavity surfaces**,** group C – overly wet cavity surfaces.

Application of adhesive

After the preparation, the adhesive was applied on the cavity surfaces following the manufacturer's instructions and in the study group's at different levels of cavity moisture (Table).

Cavity filling

Selective enamel etching was performed on enamel margins only. In all groups, the same 38% phosphoric acid was used. The acid was washed and dried specifically according to the study group's working field conditions or the manufacturer's instructions. Afterwards, the adhesive was applied (Table 1). To polymerize the adhesive and composite, the same dental curing light was used, the light intensity reached a minimum of 1200 mW/cm^2 and a maximum of 1500 mW/cm^2. Each group's adhesive was cured for the period of time as instructed by the manufacturer.

Thirty class V cavities were filled with universal nano-ceramic composite "Ceram X" (Dentsply). The depth of all cavities was less than 2 mm. All restorations were thoroughly polished in a low-speed handpiece (20,000 rpm) using "Ceram X gloss"blue and white polishers. New identical polishers were used for each group. After this step, the fillings were carefully explored with a dental probe in order to check the quality of the restorations.

Thermocycling

After the restorations were finished, the thermocycling process (at the laboratory of Kaunas University of Technology) of 3000 cycles was performed. A total length of a full cycle was 136 seconds. The submersion process was done in a clean room with a person providing 24/7 on-site supervision for a total uninterrupted period of 4 days, 17 hours and 13 minutes**.**

Preparation for permeability testing

After the thermocycling, the teeth apices were sealed with dental wax. All 30 teeth were covered in two layers of clear gel nail lacquer leaving 1mm space to the restoration margins. After that, all teeth specimens were immersed in due (1% methylene blue solution) for 24 hours.

Teeth sectioning

For tooth sectioning Buehler "Isomet Low-Speed Diamond Saw" (Model:11-1280-250) was used with a ±0.0001 inches (±5 µm) precision via a manual micrometer. Series 15LC blade disk was used (No.: 11-4254). Each tooth block was transversally sectioned in the middle of the restoration (Figure 1).

Evaluation of microleakage

Each cut of the tooth was analyzed and photographed using Olympus BX43 microscope with white LED lamp (U-LHLEDC). Section's dye penetration depths were scored in permeation degrees and millimeters (Figure 2).

Statistical analysis

Results were statistically analyzed using SPSS statistical software (version 22.0, SPSS Inc., Chicago, Illinois).The means and standard deviations were calculated for the parameters. N represented the number of samples from which the quantitative data were obtained. The paired differences were assessed using Student's t test. The multi-group comparison of all the groups was tested for significance using one-way analysis of variance (ANOVA) and non-parametric Kruskal-Wallis tests. Differences were considered significant when $p<0.05$.

RESULTS

The results of the study showed that mean permeability depth (dye penetration depths) was 0.209 mm (SD 0.131) with a range from 0.00 to 1.35 mm in group A. In specimens with overly dry cavity conditions (group B) mean permeability depth was 0.116 mm (SD 0.061) with a range from 0.00 to 0.55 mm and in group C (overly wet cavity) – mean permeability was 0.668 mm (SD 0.436), with a range from 0.0 to 4.47 mm (Figure 3). ANOVA and non-parametric Kruskal-Wallis tests showed no significant differences of permeability depth between study groups ($p>0.05$) (Figure 3).

There was no statistically significant difference of microleakage (degrees of permeation) in all study groups with different level of moisture. No significant difference was found in a

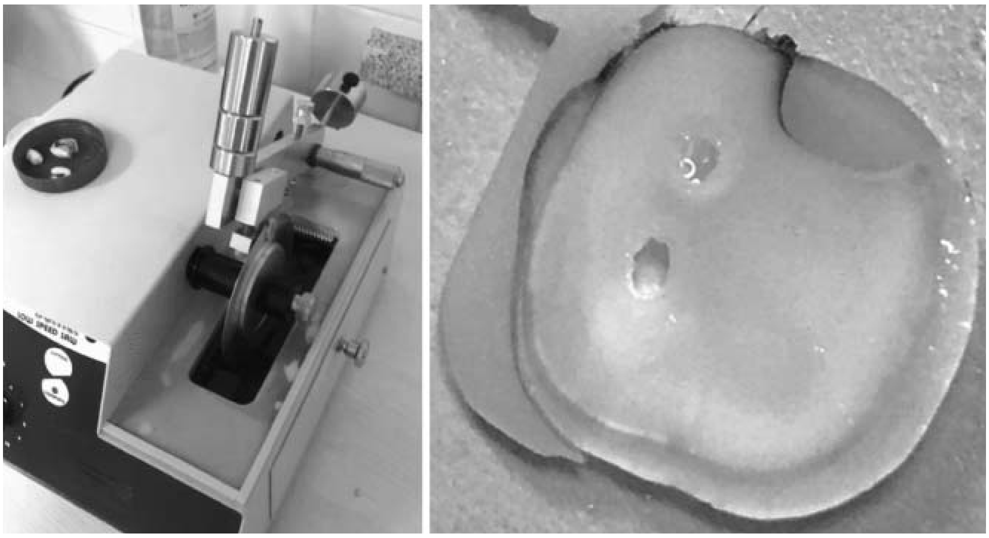

Fig 1. Teeth sectioning process with device Buehler "IsoMet Low Speed Saw" (on the left); a tooth section on the right

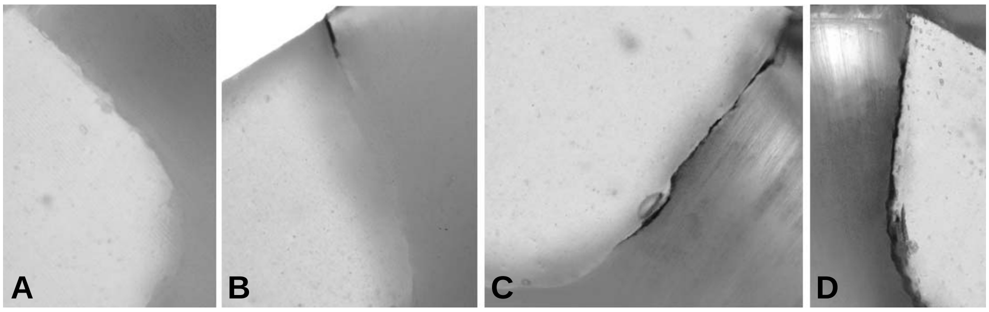

Fig 2. Images of microscope Olympus BX43 (100) illustrating microleakage (permeability depth) of teeth specimens: A – 0 degrees (no dye penetration); B – 1 degrees (dye penetration to ½ or <½ of the wall); C – 2 degrees (dye penetration >½ of the wall, without involving axial wall); D – 3 degrees (dye penetration to the axial wall, involving axial wall).

Table. Application protocol for universal adhesive

Study groups	Adhesive application's procedure, in respect to different levels of cavity moisture
Group A (ideal/optimal cavity moisture level, according manufacturer's instructions)	Selective enamel etching with 38% phosphoric acid for 15 seconds. Acid washed with water flow for 15 seconds. Cavity gently dried for 5 seconds until glossy surface. Adhesive applied with a single-use applicator and rubbed in for 20 seconds. Adhesive distributed with medium air flow for 5 seconds. Instructions point out to avoid too strong air flow. Polymerization for 10 seconds.
Group B (overly dry cavity surfaces)	Selective enamel etching with 38% phosphoric acid for 15 seconds. Acid washed with water flow for 15 seconds. Cavity dried for 20 seconds until matte surface. Adhesive applied with a single-use applicator and rubbed in for 20 seconds. Adhesive distributed with medium air flow for 5 seconds. Instructions point out to avoid too strong air flow. Polymerization for 10 seconds.
Group C (overly wet cavity surfaces)	Selective enamel etching with 38% phosphoric acid for 15 seconds. Acid washed with water flow for 15 seconds. Cavity dried for 2 seconds until surface seemed more wet than glossy. Adhesive applied with a single-use applicator and rubbed in for 20 seconds. Adhesive distributed with medium air flow for 5 seconds. Instructions point out to avoid too strong air flow. Polymerization for 10 seconds.

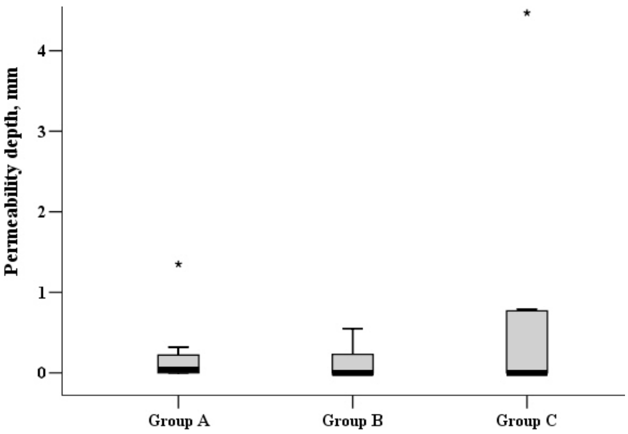

Fig 3. Comparison of permeability depth (microleakage) between the study groups

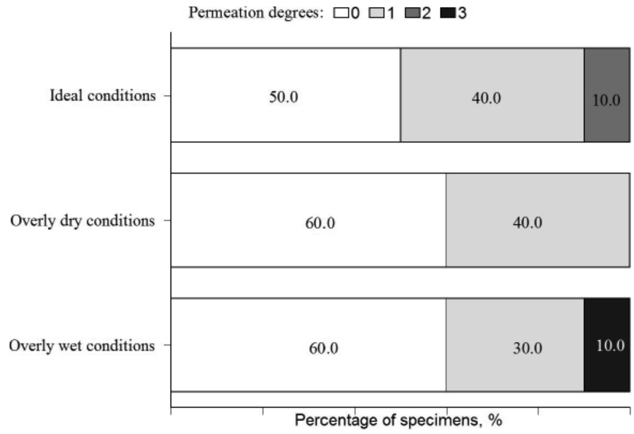

Fig 4. The distribution of study specimens (%) according to the degrees of permeation (microleakage)

degree of permeation between study groups (Figure 4). About half of the specimens of all the study groups had dye penetration, and only in specimen of group C the highest microleakage was detected.

DISCUSSION

It is important to understand adhesive system's interaction with hard dental tissues in order to obtain an efficient adhesion, to reduce risk of microleakage formation and to increase the lifetime of resin composite restoration. The important factor that contributes to bonding efficiency is the humidity of dentin (7). Clinically, the self etching adhesive technique leads to a question concerning the wettability of the dentin and the appropriate bonding to dentin (1). It is often hard to maintain ideal dampness of class V cavity while performing the adhesion step. Therefore, it is important to use a proper adhesive, which minimizes the extent of microleakage at the tooth/restoration interface in overly dry, ideal and overly wet cavity surface.

Microleakage in the cervical area might appear because of the low number of dentinal tubules and organic substrate in the dentin. Studies show that there is no adhesive system, which would prevent cervical microleakage in Class V restorations (4). The enamel on the dental cervical margins is thin, aprysmatic and bonds to resin poorly. After polymerization, resin composite moves towards stronger link in the occlusal margin and pushes itself away from the weaker bond in the gingival margin (3). Sealing ability of adhesives in class V composite restorations depends on chemical composition of adhesive, localization of cavity margins and the method of cavity preparation (4). All microleakage studies are *in vitro* investigations, because accurate in vivo evaluation of the interface between the restoration and dental tissue is not possible. The clinical examination is mostly limited to the evaluation of marginal staining and adaptation (8). Some researchers state that microleakage of clinical restoration is lower compared to the microleakage performed in the laboratory. The present study also identified that all restorations showed some kind of permeability during the dye penetration method (6).

The limitation of our study was the dye penetration method. Dye penetration methods are commonly used in order to investigate microleakage in *in vitro* studies. However, it is difficult to compare the results of different laboratory investigations, because various dye penetration methods, concentration and types are used (3, 9). Although the comparison of quantitative analysis data with penetration depth of three commonly used tracers (basic fuchsin, methylene blue and silver nitrate solutions) showed no significant difference in tracer penetration. However, higher penetration occurred at the dentin than at the enamel margin for all three tracers (10). In the present study microleakage was evaluated using 1% methylen blue solution penetration test. Even the small surface area of methylen blue particles may lead to an overestimation of mikroleakage at tooth-restoration interface (11). Furthermore, this method is not very objective because of tooth sectioning step. It requires destructive sectioning and depends on the quality and randomized place of the restoration. New diagnostic technologies like mikro-CT 3D technique, which allows to test microleakage without tooth sectioning could be used for more accurate result (6).

On the other hand, one of the strong points of our study was permeation measurement using microscope. In other studies the dye penetration was measured using micrometers as well. This method seemed to be more accurate, because of full restoration measurements, without involving only one wall of the cavity. In the present study, it was decided to use both models: micrometers and permeation degrees to gain more accuracy.

Thermocycling is usually used in *in vitro* studies to age the restoration in order to predict the longevity of the restoration (2, 3, 6, 9). It was evaluated that 2500 thermal cycles make a significant difference for bond strength (9). We used 3000 cycles, which was an optimal amount in order to examine the microleakage. On the other hand, in the study by Yuan *et al.* 2017 it was stated that 6000 thermal cycles imitate 5 years, 12,000 thermal cycles – 10 years and 18,000 cycles 15 years of clinical wear (12). According to this study, it could be assumed that with 3000 thermal cycles the restorations were aged by 2.5 years. By contrast, thermocycling may reduce sealing in class V restoration. After evaluation of the sealing ability of adhesives in class V cavities before and after thermocycling, the author concluded that thermocycling did not affect the occlusal bonding, but reduced the gingival sealing in class V composite restorations (2). It can be estimated that thermocycling may influence the results of the study.

Selective phosphoric-acid etching of the enamel cavity margins is today highly recommended, followed by applying a self-etch procedur**e** (1). Szesz et al 2016 ascertained that selective enamel etching prior to the application of adhesive can improve retention, avoid marginal color changes and ameliorate longevity in class V composite restorations (13). Sampaio et al 2016 stated that selective enamel etching technique can stop the formation of marginal gaps and make the restoration more lasting (14). Therefore, selective enamel etching step was included in our research as well despite the use of self-etching universal adhesive.

However, the filling technique might have had negative impact on our results. Some researchers conclude that polymerization shrinkage is doubled when the depth of the cavity increases from 1 mm to 2 mm (9). In our study the width of the restoration was 2 mm, therefore the polymerization shrinkage might have had an impact on the appearance of microleakage in the final result. By contrast, some studies suggested that the composite filling technique did not have any impact on polymerization shrinkage and it is more important to choose the right adhesive strategy than filling technique. Incremental layering technique has its flaws too. This method increases the risk of cavity surface contamination and formation of air bubbles. Therefore we decided to restore the teeth using only one increment, because the depths of the cavities were less than 2 mm.

According to the manufacturer's instructions: the self-etching universal adhesive "Prime&Bond Active" should demonstrate minimal microleakage in various dampness conditions. However, self-etching adhesives are more technique sensitive (1). The chemical composition of adhesive may influence the bonding strenght as well as other properties of the agent (15). This could be explained by well-balanced hydrophilic and hydrophobic monomers in its composition. The combination of such materials allows universal adhesives to create a bridge between hydrophilic tooth substrate and hydrophobic resin restoration (1). Munoz et al 2015 concluded that universal adhesives, which contain MDP (10 Methacryloyloxydecyl dihydrogen phosphate), demonstrated more stable results in bond strength and had less nanoleakage (16). However, the data showed that self-etching universal adhesive was sensitive to overly wet working field conditions, though it did not reach statistically significant level. Tested adhesive in the present study was least permeable under optimal and overly dry working field conditions. Overly wet working field conditions influenced the permeability of the adhesive most negatively. That can be explained by the fact, that in overly wet working field conditions the adhesive bonds worse and the bond strength reduces. It may happen due to the dilution of the adhesive (17). Over-drying the surface of dentin can demineralize it and lower resin monomer penetration to the etched surface (5). In result, the resin marks and hybrid layer cannot fully form and due to this the bond strength reduces as well. This prompts gap formation between the junction of the tooth and restoration, which increases the risk of microleakage (5, 6). More accurate methods like micro-CT 3D technique may be helpful in more precise evaluation of different bonding systems.

CONCLUSION

Despite the limitation of this *in vitro* study there is no significant difference of microleakage using universal adhesive in class V composite restorations with different moisture level. However, too wet working field conditions tend to induce microleakage formation.

In clinical performance, it could be suggested to keep an appropriate cavity moisture level.

STATEMENT OF CONFLICT OF INTEREST

The authors state no conflict of interest.

ACKNOWLEDGEMENT

Authors would like to thank Irena Nedzelskiene for statistical analysis.

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Received: 06 06 2019
Accepted for publishing: 24 12 2019