Abstract

The aim of this randomized in vitro study is to clarify whether resin based restorative materials (RBRM) behave in a way comparable to non-resin based restorative materials (non-RBRM) in dentine-limited cavities with regard to their marginal fit. For this purpose, cylindrical standardized cavities (diameter: 3.0 +/- 0.1 mm, depth: 1.5 mm) were placed on buccal surfaces of sixty human molars and restored with three non-RBRM (glass ionomer cement, amalgam, phosphate cement) and three adhesive (composite, compomer, ormocer) restoratives. Aging of the samples was achieved by thermal cycling (500 cycles). The marginal gaps could be made visible with a dye penetration test (methylene blue 2%). Using a diamond internal hole saw, 5 cuts were made through the cavity and measured under an incident light microscope at 10x magnification. The result of the statistical evaluation of the additionally determined percentage marginal gap depths was compared with the numerical evaluation of the penetration depths of 0, 1 and 2 required by the ISO test setup. The ISO classification reveals statistical differences in the penetration behaviour of phosphate cement to the other materials, whereas the statistical evaluation of the percentage measurement distances revealed significant differences between the nonadhesive and the adhesive restorative materials, with significantly smaller marginal gaps for the cavities of the RBRM group.

Introduction

There has always been a great interest in an optimal adaptation of dental restorative materials to the cavity walls to minimize the ingress of oral fluids and microorganisms [1,2]. Most adhesive (RBRM) and non-adhesive (non-RBRM) restorative materials show varying degrees of marginal leakage because of dimensional changes and a lack of adaptability to cavity walls [1,3-8]. Although no current product satisfies all the requirements of an ideal restorative material, adhesive techniques enable some procedures that cannot be provided with non–RBRM restorations, for example with amalgam or conventional cements. RBRM procedures, however, are technique sensitive compared to amalgam restorations.

In the absence of definitive clinical data, laboratory microleakage studies are internationally described methods of screening restorative materials for marginal seal [2,9]. Standardization of such methods is necessary in order to obtain comparable results from different laboratories. In this respect, it seems important to standardize quality of teeth, type of cavity, and the quantification of leakage. The type of tracer substance does not seem to be of major importance [9]. A standardized microleakage methodology can be a valuable setting to compare materials on a relative scale and to make some controlled interstudy comparisons [10].

In vitro evaluations might also act as a predictable indicator of in vivo leakage [3,11,12]. However, this is discussed controversially by the dental research community. The exact adherence to the ISO specifications for microleakage testing could possibly be a solution towards more predictability [3].

Microleakage investigations of various adhesive and non-adhesive restorative materials exist only in a limited number of products and to our knowledge never in direct comparison including modern RBRMs in dentine cavities and exactly in accordance with the internationally recognized ISO specifications [9,13].

The purpose of this in vitro study, therefore, was to compare the sealing ability of non–RBRM (glass ionomer cement, phosphate cement and amalgam) and RBRM restorative materials (composite, compomer and ormocer) in
standardized dentine cavities using 2 % methylene blue dye. We were interested in the following research questions:

Is there an average difference between the RBRM and non-RRM group?

Are there differences between the individual material variations used?

Materials and methods

Specimen preparation based on the ISO specification

Sixty extracted human molars were distributed among six experimental groups and stored in distilled water. After cleaning the molars, they were decapitated using a separating disc (Gebr. Brasseler, Lemgo, Germany) 1-2 mm below the enamel–cement junction. The cut surface was apically sealed with composite (Herculite XRV, Kerr GmbH, Biberach, Germany) and the corresponding dentine adhesive (Syntac Classic, Ivoclar Vivadent Corporate, Schaan, Liechtenstein) after prior conditioning of the cut surface with 15 sec. phosphoric acid etching (Dentsply Sirona, Constance, Germany). In accordance with ISO/TS 11405:2015 [4], the rest of the tooth was embedded with ISO/Ts 11405:2015 [4], the rest of the tooth was embedded in cold polymerisate (Technovit, Heraeus-Kulzer GmbH, Hanau, Germany) with the exception of the buccal surface. The treatment of the buccal tooth surface (alignment parallel to the underside) was also carried out with 180 grained sandpaper until a 4 mm diameter dentine surface was levelled in the clamping device by means of a polishing unit (Jean Wirtz KG, Düsseldorf, Germany). The treatment of the buccal tooth surface (alignment parallel to the underside) was also carried out with 180 grained sandpaper until a 4 mm diameter dentine surface was exposed. This surface was polished with 600 grit sandpaper (3M AG, Rüschlikon, Germany). The grinding process was carried out under water cooling with low contact pressure and an average speed of the polishing unit (Jean Wirtz, Düsseldorf, Germany) of 1400 rpm. A visual control of the samples for the completeness of the enamel ablation was achieved with the aid of magnifying glasses (Carl Zeiss AG, Oberkochen, Germany). Depth extraction was initially carried out by drilling a pilot hole using a diamond grinding wheel (diameter 1.8 mm, ISO No.: 806 314 108 524 018, Hager & Meisinger GmbH, Neuss, Germany; grinding wheel change after 10 samples). The carbide grinding wheel with the largest diameter of 2.3 mm (ISO no. 500 104 107 006 023, Busch & Co, Engelskirchen, Germany; grinding wheel change after 10 samples) was used to expand the cavity. As the use of the same would have led to large variations in sample geometry until the required cavity design was achieved, the sucking extension to the required extent of the cavity was achieved by using two additional drills with increasing diameters. Two longitudinally toothed steel drills with diameters of 2.7 mm (ISO No. 310 204 107 071 027, Busch, Engelskirchen; change of grinding wheels after 5 samples) and finally 2.9 mm (ISO No. 310 104 107 002 029 (Hager & Meisinger GmbH, Neuss, Germany; change of grinding wheels after 2 samples) were used.

Restoration materials

The materials used are shown in table 1. The cavities to be filled non-adhesively (Ketac Fil Aplicap, 3M GmbH, Seefeld, Germany; Vivacap Non-Gamma–2, Ivoclar Vivadent Corporate, Schaan, Liechtenstein, Germany; Harvard Phosphatzeement, Hofmann Dental Manufaktur GmbH, Berlin, Germany) underwent H2O2 cavity cleaning with subsequent drying. The samples to be adhesively filled (Esthet-X, Dyract Xtra, Ceram-X, Dentsply Sirona, Konstanz, Germany) were additionally subjected to the dentine adhesive conditioning with Prime & Bond NT (rinsing of the 15 sec. H2O2-cleaning

| Number | Group / Material | Manufacturer | Title | Composition |
|--------|-----------------|--------------|-------|-------------|
| 1      | Glass ionomer cement | Espe, Seefeld, G | Ketac-Fil Aplicap | Calcium Sodium Fluorophosphorous Aluminum Silicate, Polyacrylic Acid, Malic Acid, Tartaric Acid |
| 2      | Amalgam | Ivoclar Vivadent Dental GmbH, Ellwangen, G | Vivacap | Silver, tin, copper, mercury |
| 3      | Phosphate cement RBRM | Richter & Hoffmann-HARVARD Dental GmbH, Berlin, G | Harvard Cementnormalsetting | Zinc Oxide, Magnesium Oxide, Phosphoric Acid |
| 4      | Composit (+ Adhesive) | Dentsply De Trey, Konstanz, G | Esthet-X | Urethane-modified Bis GMA, TEGDMA, BA01 (Ba-Al-fluoroborosilicate glass), EDAB, nano-silicone dioxide, camphorquinone |
| 5      | Compomer (+ Adhesive) | Dentsply De Trey, Konstanz, G | Dyract Xtra | ethoxylated bisphenol A dimethacrylate, UDMA, TEGDMA, TMPTMA, TCB resin, dimethylaminobenzoic acid ethyl ester, strontium fluoride glass, camphorquinone |
| 6      | Ormocer (+ Adhesive) | Dentsply De Trey, Konstanz, G | Ceram-X | Methacrylate-modified polyoxalanes, dimethacrylate resin, ethyl-4 (dimethylamino) benzoxates, barium-aluminum borosilicate glass, methacrylate-functionalized silicodioxide nanofillers, camphorquinone, iron oxide, titanium oxide and Aluminium sulphone silicate, pigments |
|         | Adhesive | Dentsply De Trey, Konstanz, G | Prime & Bond NT | Urethane dimethacrylate, di- / trimethacrylate resins, PENTA, acetone, functionalized amorpossilica, cetylamine hydrofluoride, butyldydroxylouene, ethyl-dimethylaminobenzoate, camphorquinone |

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for 15 sec., 15 sec. exposure to phosphoric acid (35%), application (20 sec. exposure time) of Prime & Bond NT to the moist, non-wet surface, five second blowing, 10 second light curing). The restorations were placed strictly according to the manufacturer’s instructions. The finishing was carried out using Sof-Lex discs (grain size SF, F and M; 3M GmbH, Seefeld, Germany) of three different grain sizes under water cooling with the blue contra-angle handpiece (KaVo Dental, Biberach, Germany). In preparation for thermocycling, the test specimens were heated to body temperature by being stored for 24 hours in distilled water in a drying cabinet (Memmert GmbH, Schwabach, Germany) at 37 °C. The simulated ageing of the fillings, analogous to the conditions in the oral cavity, took place by means of a temperature change load (W26; Haake, Karlsruhe, Germany) of 500 cycles in 5 °C and 55 °C warm water baths (distilled water). The dwell time per water bath was 20 seconds, the transport time, which consisted of 7 seconds drip off time and 3 seconds transfer time, was a total of 10 seconds.

Methodology of penetration determination

The detection of microleakage of the 6 different materials (glass ionomer cement, amalgam, phosphate cement, composite, compomer, ormocer) was carried out by visualizing the marginal gaps using the dye penetration test. In preparation, the occlusal, mesial, distal and cervical tooth surfaces of the test specimens that protruded from the embedding material were protected against penetration of the dye solution over these surfaces by allowing the embedding material to flow over them again. Applying nail varnish twice (Nivea, Beiersdorf AG, Hamburg, Germany) at a distance of 1.5 mm from the edge of the filling prevented the dye solution from penetrating the buccal surface. The test specimens were then stored in a petri dish filled with methylene blue solution in a drying cabinet (Memmert GmbH, Schwabach, Germany) at 37 °C with the flattened surface facing downwards for a period of 10 minutes.

In order to be able to produce saw cuts, the test specimens had to be socketed (Technovit, Heraeus–Kulzer GmbH, Hanau, Germany). Five 220 μm thick consecutive cuts were made with a diamond saw (Leica, Bensheim, Germany) in the longitudinal axis of the tooth (in vestibulo–oral direction).

The evaluation of the dye penetration depth was carried out under an incident light microscope (Leica, Bensheim, Germany) with a magnification factor of 10 at 10 volts, 3200 °K and with the aid of a measuring cross (Kappa GmbH, Gleichen, Germany), a video camera (Kappa GmbH, Gleichen, Germany) and a monitor (Sony, Cologne, Germany). Three sections could be measured and documented per segment or cavity; an occlusal (x1), a cervical (x2) and a pulpal section (y), whereby the penetration depths were measured starting from the edge of the cavity and thus a percentage penetration depth was recorded. In addition, an evaluation of the penetration depths (Table 2) based on ISO/TS 11405:2015 (E) [4] was carried out.

Statistics

For the statistical analysis of the results, the Kruskal–Wallis Multiple–Comparison Z–Value Test with a significance level of p ≤ 0.05 was applied. The test was performed using the statistical analysis package NCSS (Version 6.0.2.1. Kaysville, Utah).

Results

The individual results are presented in tables 3,4. The statistical evaluation revealed significant differences between the adhesive filling materials with lower penetration depths and the non–adhesive ones. The highest penetration values determined were obtained by evaluating the samples filled with phosphate cement (42.56%). Lower microleakage values were shown by the non–adhesive filling material amalgam (average 27.13%), followed by glass ionomer cement (average 28.57%), but without statistical significance.

However, the results evaluated on the basis of the ISO classification reveal only statistical differences between phosphate cement as a material and the other groups.

| Table 2: Measurement of microleakage. |
|----------------------------------------|
| Parameter according to ISO/TS 11405:2015(E) | Scoring |
|----------------------------------------|--------|
| No penetration                          | 0      |
| Penetration into the dentine/material interface, but not including the pulpal floor of the cavity | 1      |
| Penetration including the pulpal floor of the cavity | 2      |

| Table 3: Penetration values (%) of all the respective material groups with their corresponding values (SD = standard deviation, Min = minimum, Max = maximum and significances). |
|---------------------------------------------------------------|
| Group                                                                 |
| Non-RBRM              | Mean  | Median | SD    | Min | Max | Significant to |
|-----------------------|-------|--------|-------|-----|-----|---------------|
| Nr. 1 Glass ionomer cement | 28.57 | 23.68  | 15.79 | 16.00 | 100.00 | 4, 5, 6       |
| Nr. 2 Amalgam          | 27.13 | 21.45  | 15.98 | 3.74 | 69.83 | 4, 5, 6       |
| Nr. 3 Phosphate cement | 42.56 | 34.32  | 25.88 | 6.26 | 100.00 | 4, 5, 6       |

| Table 4: Penetration values (ISO specification) of all lines of the respective material groups with their corresponding values (SD = standard deviation, Min = minimum, Max = maximum and significances). |
|---------------------------------------------------------------|
| Group                                                                 |
| Non-RBRM              | Mean  | Median | SD    | Min | Max | Significant to |
|-----------------------|-------|--------|-------|-----|-----|---------------|
| Nr. 1 Glass ionomer cement | 1.02  | 1.00   | 0.14  | 1.00 | 2.00 | 3             |
| Nr. 2 Amalgam          | 1.14  | 1.00   | 0.35  | 1.00 | 2.00 | 3             |
| Nr. 3 Phosphate cement | 1.30  | 1.00   | 0.46  | 1.00 | 2.00 | 1, 2, 4–6     |

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**Discussion**

The results of this study show that the property of micromechanical bonding of the adhesively bonded materials (composite, compomer, ormocer) to the dentine results in significantly reduced marginal gap formation. The ranking of the results of the marginal adaptation within the non-adhesive group (without statistical significance) resulted in the following order: amalgam < glass ionomer cement < phosphate cement and is based on the material-specific properties. The high microleakage values of the phosphate cement presented in this study can be explained on the one hand by the lack of plastic deformation of the cement after frequent intermittent load changes, with the subsequent loss of adhesion of this material to the dentin [14,15]. On the other hand, the property of high solubility of phosphate cement in combination with a missing mechanism of bonding the cement to the tooth structure, instead of adaptation to it, contributes to marginal gap formation. Amalgam, through the application technique of condensation with subsequent burnishing alone, all processes that lead to a close adaptation of this material to the tooth structure, shows a lower marginal gap formation (27.13% on average) than phosphate cement. Thus, although amalgam does not have the property of bonding with the tooth structure, it can show slightly better penetration results than glass ionomer cement, which chemically adheres to the tooth structure [16]. The lower ability of the glass ionomer cements to avoid marginal gap formation compared to the materials of the adhesive group is due to the lower adhesive strength of the bonding agent to dentin (2–5 MPa [17,18]) to the tooth structure dentin compared to the required adhesive strength of at least 20 MPa [19] by micromechanical bonding of the adhesively bonded materials.

The total penetration distances (%) of the materials in the adhesive group are all on average approximately 15.00% (composite: 15.52%; compomer: 15.48%; ormocer: 13.15%) and therefore do not differ significantly from each other. This can mainly be explained by the fact that the cavity conditioning is identical using a uniform dentine adhesive system (15 second phosphoric acid etching; Prime & Bond NT application) and the matching bonding mechanism of all three materials with the tooth structure (micromechanical bonding). Furthermore, the three materials have only slightly different material properties. These have an influence on the marginal gap formation, which results in only minimal variations with regard to the microleakage behaviour. Studies for the direct comparison of RBRM and non-RBRM exist only in a limited number. For example, one group of authors found significantly lower microleakage values in composite materials when comparing amalgam to composite restorations [20].

The statistical evaluation of the results of the entire segment based on ISO 2015 [9] shows different significance compared to the percentage evaluation. Thus the behaviour of the marginal sealing of all materials used only differs from the high penetration values of the phosphate cement. The articles available in relevant literature [21,22], however, rather support the result of the statistical evaluation of the percentage representation of the penetration distance. Since in literature the microleakage depth is frequently expressed numerically by 0 – 2, 3, 4 or even 5 and these reflect the identical tendencies as in the study at hand by means of percentage evaluation achieved results, the result of the present ISO evaluation, which differs from the relevant literature, seems to be based on the too rough depth division of the marginal gaps into 0, 1 and 2. An extension of the degrees of depth scaling is therefore to be regarded as sensible. Only in this way could a possibly modified ISO evaluation (e.g. "leakage levels" of 0–5) represent a basis for discussion, confirmed by the data to be found in relevant literature.

The test setup presented here was designed on the basis of the ISO standards [4,13], since standardized procedures can be used to quantitatively compare the results of future investigations that are now carried out identically in the test setup. A comparison of the present study with investigations of the available literature is difficult if not impossible alone due to the test specimen used. The deeper dentin layers exposed by the levelling process have a higher proportion of tubular openings than superficial layers, which means that the substrate is much less solid. This shows that a comparison with the results of conventional investigations makes only limited sense. As expected, the results of experiments with Class II (enamel-dentine-limited cavities) and Class V cavities whose cavity boundaries lie on the surface of the tooth differ from the results of the present study due to the different filling margin boundaries with different influences on the restorative materials. An explanation for the predominantly higher microleakage behaviour of the hydrophilic (= adhesive) filling materials of the present study on the experiments in technical literature [23,24] may be found in the "differing substrate" with a higher water and mineral content.

One limitation of our study is the in vitro setting [25,26]. Although such microleakage assessment is in principle even easier than in vivo measurements (and therefore done very frequently), the reliability of conventional microleakage protocols remains controversial [27]. Clinically, restorations can potentially fail because of restoration/tooth fracture, loss of anatomy and function (chemical and mechanical wear), or interface degradation, leading to marginal leakage, pulpal pathology and recurrent decay. Therefore, the best approach to evaluate the ability of a restorative system to resist such strains and degradation patterns is a clinical trial. After bond strength tests, the second evaluation level would be provided by adaptation studies (score of 3) and, in particular, those studies that submit samples to cyclic loading (scores of 4 to 5). Such protocols appear more discriminative in terms of predicting clinical behaviour since they mimic a global interaction of the restorative system with the tooth in a simulated oral environment. They also provide meaningful information about the quality of interfaces following fatigue [25].

**Conclusion**

The results of the present study show that RBRM (composite, compomer, ormocer) is to be preferred with regard to the "microleakage" behaviour in comparison to non-RBRM (glass ionomer cement, amalgam, phosphate cement).
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