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Influence of selective caries excavation on marginal penetration of class II composite restorations in vitro

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Selective caries excavation may support pulp preservation. This in vitro study investigated the influence of selective removal of demineralized dentin on marginal integrity of composite restorations as determined by dye penetration. Dentinal caries-like lesions were produced in the approximal surfaces of 40 extracted human molars (ethylenediaminetetraacetate, 0.5 M, 96 h). The following test procedures were established: complete excavation, selective excavation, and caries-free control. Two class II cavities with enamel at the cervical margins were prepared per tooth and demineralization volume was determined by micro-computed tomography for the purpose of a stratified distribution to receive complete excavation or selective excavation. After complete or selective excavation (30 cavities each), adhesive composite restorations were placed. Cavities without demineralized dentin (20 cavities) served as control. The marginal integrity of restorations was evaluated by dye penetration with and without thermocycling or mechanical loading. Results were analyzed by non-parametrical statistical tests (Mann-Whitney *U* Test) with an $\alpha = 0.05$ level of significance. Dye penetration did not differ significantly among completely excavated, selectively excavated, or undemineralized teeth, but was increased by thermocycling and mechanical loading in all experimental groups. Selective caries removal did not increase marginal penetration in class II restorations. The presence of remaining demineralized dentin surrounded by sound dentin did not impair marginal integrity of restorations with margins placed in sound enamel.

Konstantin J. Scholz and Michael Hinderberger share first authorship.

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Complete excavation of caries, as it was traditionally performed in the course of restorative therapy, has been widely superseded by less invasive techniques, such as infiltration technique, atraumatic restorative treatment, or selective caries excavation (1). While atraumatic restorative treatment might have its limitations (2), clinical studies show promising success rates for selective excavation (3,4).

Based on a meta-analysis, Isolan *et al.* (5) concluded that caries-affected dentin in general leads to a lower bond strength than sound dentin, irrespective of the adhesive approach used (5). Dissolution of minerals, degradation of collagen, increasing porosities, and changes in elemental composition of carious dentin might be factors leading to a decrease in bond strength (6-8). Concerns about the use of selective caries excavation have been raised with respect to the possible progression of carious lesions and compromised mechanical properties due to the softness of the carious dentin serving as the foundation for the restoration (9). A soft base underneath a restoration may trigger marginal gap formation, which itself may lead to recurrent or secondary caries (10,11). Besides the influence on mechanical performance, residual caries in dentin might pose a risk for the dental pulp. In a randomized clinical trial, cavities showed a higher bacterial load after selective excavation but before restoration compared to cavities that were completely excavated (12). In the same study, a decrease of total viable microorganisms in general, as well as streptococcus species and lactobacillus species specifically, was observed after three months for both excavation strategies and no significant differences between selective and complete excavation remained. In another in vivo study, 3–4 months after selective excavation harder and darker dentin was found compared to the date of restoration, irrespective of the restorative material (13). Furthermore, selective caries excavation can be biologically advantageous because pulp exposure

is avoided. Indirect pulp capping using bioactive materials may reduce the concentrations of bacterial lipopolysaccharides and lipoteichoic acid and it can provide a barrier against the potentially cytotoxic effects of adhesive system monomers (14-16). Apart from the biological advantages of selective excavation, little is known about the influence of residual carious dentin on the mechanical properties of restorations (4,17). The marginal seal of a restoration is crucial when selective excavation is performed in order to ensure long term arrest of microbial activity in the remaining carious dentin through a tight seal (17). Avoiding marginal leakage is a critical factor for restoration success, especially the exclusion of carbohydrates from reaching residual carious dentin. In vitro, dye penetration has been frequently applied as a method for measuring marginal leakage of dental restorations (18-20).

The aim of this in vitro study was to investigate the influence of complete or selective excavation of artificial caries lesions in human molars on the marginal integrity of subsequently placed class II composite restorations. Since it is difficult to control lesion size and interindividual factors in clinical studies, this in vitro study used micro-computed tomography (micro-CT) to ensure teeth with similar demineralization volumes were stratified into two groups. A controlled in vitro design was chosen to examine the microleakage as determined by dye penetration on similar sized restorations with the complete margins in enamel. All groups were investigated without and with thermocycling and mechanical loading on multiple sections. The null hypothesis was that leaving demineralized dentin in a cavity before placing class II composite restorations in human molars leads to a similar amount of marginal dye penetration compared to complete excavation in vitro.

Material and methods

Sample preparation

Forty caries-free human third molars were collected and placed in 0.5% chloramine solution directly after extraction and stored for a maximum of 6 months. The University of Regensburg Ethics Committee (Reference: 19-1327-101) approved the use of extracted teeth on the condition that informed consent was obtained from each patient.

After removal of soft tissue remnants, the roots of the sample teeth were embedded in acrylic resin (Paladur, Kulzer, Hanau, Germany) 2 mm apical the cemento-enamel junction. During the entire experimental period, the teeth were stored in demineralized water.

Caries model

In order to allow standardized dentin demineralization in proximity to the pulp chamber, an approximal cylindrical pit (1.3 mm diameter, 2 mm depth) was prepared perpendicularly to the tooth axis with a diamond bur (Meisinger, Neuss, Germany) at 200,000 r.p.m. and with extensive water-cooling. This demineralization pit was located 3.5 mm above the cemento-enamel junction and directed towards the pulp chamber, all the way through the enamel into dentin. The pit was closed with a customized guttapercha place holder and the whole crown was encased in polyether impression material, leaving the ends of the guttapercha place holder protruding from the impression material (Impregum, 3M Espe, St. Paul, USA). After the polyether had set, the guttapercha was removed, providing direct access to dentin.

Ethylenediaminetetraacetate (EDTA Disodium Salt 2-hydrate; AppliChem, Darmstadt, Germany) was used as demineralization solution (0.5 M, pH 7). Initially, the demineralization agent was injected into the pits using needles designed to prevent vapor lock (BD Microlance 20G; Becton Dickinson, Franklin Lakes, NJ, USA). Subsequently, each tooth was placed in 400 ml EDTA, which was stirred continuously (300 r.p.m.) for 96 h at room temperature. Immediately afterwards, the teeth were removed from EDTA, the polyether impression material was taken off, and the teeth were extensively rinsed with demineralized water.

A mesial and a distal approximal adhesive class II cavity was prepared (200,000 rpm, water-cooling) with cylindrical diamond burs (Reference number 806 314 111 524, diameter 1.4 mm; Meisinger). Cavities had an oro-vestibular dimension of 4 mm with a cervical margin above the cemento-enamel junction and were prepared in a standardized way to create occlusal and approximal boxes, leaving a demineralized area at the pulpal dentin (Fig. 1). Standard dental radiographs were taken to identify irregularities (e.g., subclinical carious lesions, insufficient demineralization) during the experimental procedure. Occlusally, the two cavities in each tooth were separated by at least 1 mm of sound enamel supported by underlying dentin. Cavities had a minimum extension of 2 mm in mesio-distal direction. Sharp edges on approximal cavity margins were minimally beveled 45° to remove loose enamel prisms and slightly enlarge the adhesive surface in enamel.

The volume of demineralized dentin was determined by micro-CT. Therefore, each tooth was kept wet wrapped in a parafilm (Parafilm M; Merck, Darmstadt, Germany) and scanned (phoenix v|tome|xs 240/180 research edition; General Electric Measurement & Control, Wunstorf, Germany) with the following parameters: duration = 170 min, Images = 3,000, Averaging = 15, Skip = 2, tube voltage = 80 kV, beam current = 500 μA, voxel size = 35 μm. Demineralized dentin was defined as tissue showing an absorption coefficient between 0.028 and 0.063 (Fig. 1).

Sample allocation

As Fig. 2 illustrates, 20 teeth (Category 1) were demineralized on one approximal surface, whereas the opposite approximal surface was not demineralized. Furthermore, 20 teeth (Category 2) were artificially demineralized on both approximal surfaces. For Category 1, teeth were sorted in ascending order of demineralization volume as determined by micro-CT. Teeth with similar demineralization volumes were distributed stratified into two groups: one cavity with complete excavation or one cavity with selective excavation. The endpoint of caries removal was defined by hardness using polymer burs for excavation (PolyBur, Gebr. Brasseler, Lemgo, Germany). In the selectively excavated cavities, demineralized dental hard tissue was removed from the outermost 0.5 mm next to all outer

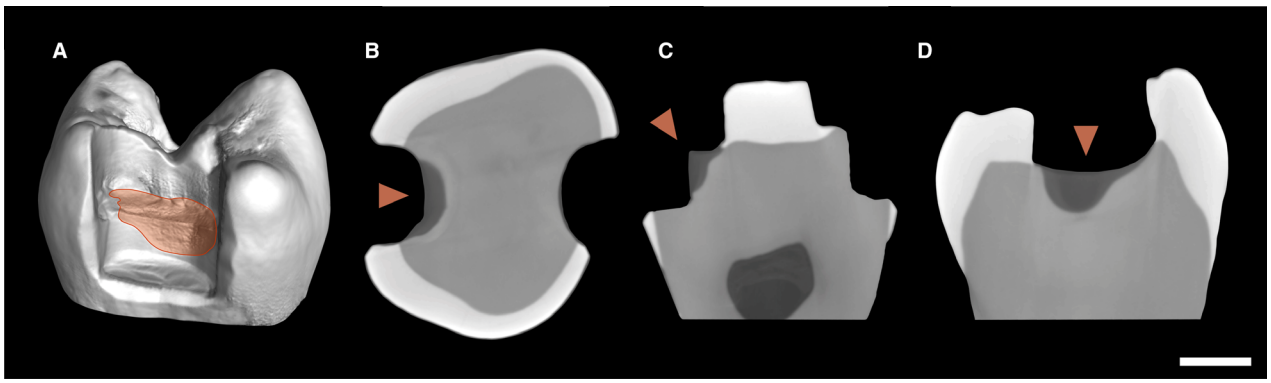

Fig. 1. Example micro-CT images. Micro-CT images of a tooth with demineralized dentin on one side only (Category 1) before excavation. (A) 3-D reconstruction of a tooth with demineralized dentin (absorption coefficient: 0.028–0.063), which is highlighted in orange. (B) Horizontal sectional view with demineralized dentin on left side (arrow). (C) Mesio-distal vertical section with demineralized dentin on left side (arrow). (D) Oro-vestibular vertical section with centrally located demineralized dentin (arrow). Scale bar: 2 mm. [Colour figure can be viewed at wileyonlinelibrary.com]

cavity margins. The demineralized dentin left centrally remained untouched. The central demineralized dentin showed stable but tactile softened consistency, as described in the literature (17). Opposing approximal surfaces, which were not demineralized, were also prepared for adhesive class II restorations of equal size as the control. For Category 2, one demineralized surface of each tooth was randomly allocated by coin toss to complete excavation and the other demineralized surface to selective excavation (Fig. 2).

Adhesive restoration

Teeth were cleaned with demineralized water in an ultrasonic bath (Bransonic 221; Branson, Danbury, CT, USA) for 5 min. A metal matrix band was placed over the cavities in each tooth (Hawe Tofflemire Matrices; Kerr, Bioggio, Switzerland). Enamel was selectively etched for 20 s using 37% H_3PO_4 (Total Etch; Ivoclar Vivadent, Schaan, Liechtenstein), rinsed with water for 20 s and gently air-dried. A two-bottle self-etch adhesive system (Clearfil SE; Kuraray, Chiyoda, Japan) was used on all surfaces with a saturated applicator tip (Flocked Applicator Tips; Dentsply I, York, PA, USA). First, the primer was rubbed into the dentin and then applied to the enamel for 20 s and dried by mild air-flow. Subsequently, the bonding agent was applied for 10 s across dentin and enamel, distributed evenly with mild air-flow and light-cured for 10 s (Satelec Mini LED; Acteon Group, Mérignac Cedex, France; 1,250 mW cm^{-2}). Cavities were restored with three increments of a nano-hybrid resin composite material (Tetric Evo Ceram XL Bleach; Ivoclar Vivadent) creating a flat occlusal surface. The first and second increments were

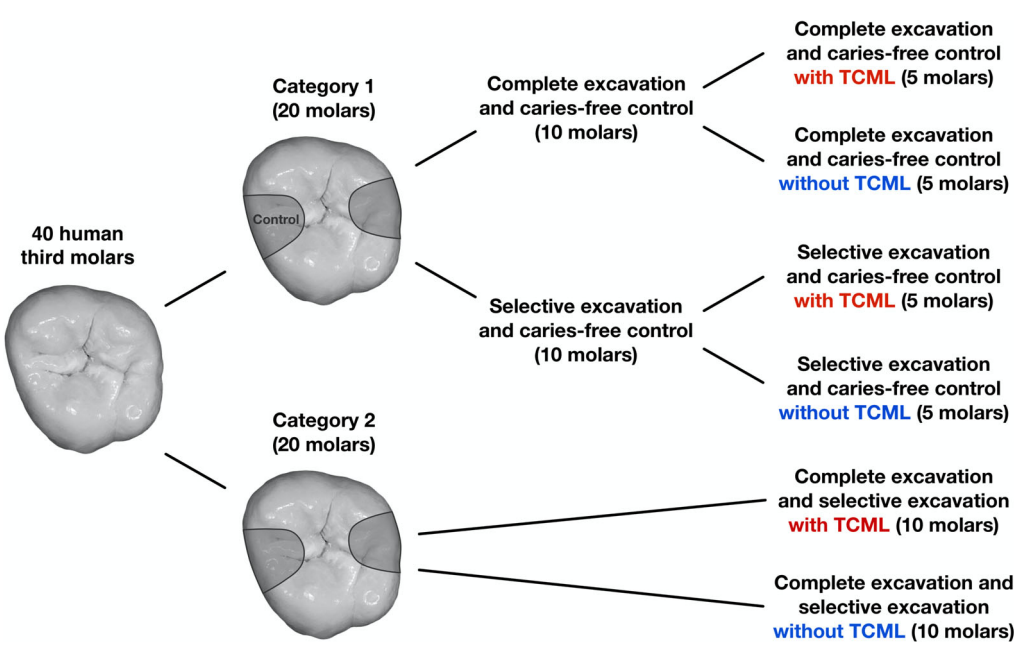

Fig. 2. Group allocation. Allocation to Categories 1 and 2, treatment groups complete excavation, selective excavation and caries-free control and application of thermocycling and mechanical loading. In total, 80 restorations in 40 human molars were investigated. Positions of restorations on occlusal and proximal surfaces are symbolized by grey fields. [Colour figure can be viewed at wileyonlinelibrary.com]

light-cured for 20 s each; the third increment was light-cured for 40 s. After removing the matrix band, the entire restoration was light-cured for 20 s and polished (Busch diamond polisher #9588 and #9578; Busch, Engelskirchen, Germany). The teeth were then stored in demineralized water for five days at room temperature.

Thermocycling and mechanical loading

Tooth pairs with similar demineralization volumes were selected from all combinations of treatment groups (complete excavation, selective excavation, and caries-free control). As shown in Fig. 2, one tooth per pair was randomly assigned by coin toss to thermocycling and mechanical loading (TCML: 5,000 cycles of 30 s at 5–55°C and 500,000 cycles at 72.5 N, 1.6 Hz), and the other tooth was stored (4°C) in demineralized water for the same time (19,21). Thus, the same numbers of cavities with similar demineralization volumes were evaluated without and with thermocycling and mechanical loading. Central mechanical loading was performed upon a metal stop representing the opposing cusp. This metal stop was placed in the middle of the occlusal surface not in contact with the adhesive restorations.

Dye penetration

After thermocycling and mechanical loading or storage, nail varnish was applied to the surface of the teeth. The restoration, the cemento-enamel junction, and 1 mm of tooth surface around the restoration margin remained uncovered. The teeth were immersed in 50 wt% $AgNO_3$ solution (S-6506; Sigma-Aldrich Chemie, Steinheim, Germany) for 120 min in the dark. Afterwards, the teeth were rinsed with demineralized water, immersed in a photographic developing solution (Tetenal Ultrafin Plus; Tetenal, Norderstedt, Germany), and exposed to fluorescent light (Philips Master PL-S 840/2P, 11 W = 900 Lumen, WD 100 mm) for 6 h. After copious rinsing with demineralized water, samples were stored in a humidity chamber prior to sectioning.

Teeth were cut longitudinally in mesio-distal direction using a water-cooled rotating diamond saw with blade thickness of 300 μm (Leitz 1600; Leica Microsystems, Wetzlar, Germany) to obtain as many sections of 300 μm thickness as possible (depending on tooth-size). Standardized images were taken from both sides of the sections using a photomacroscope (Makroskop M420; Wild, Heerbrugg, Germany, magnification x3.15; and Axiocam 105 color; Carl Zeiss, Oberkochen, Germany; 2,560 × 1,920 pixels). Images were analyzed using Optimas 6.51 software (Bioscan, Washington DC, USA) and a standardized scheme adapted to the cavities of complete excavation group, selective excavation group, and caries-free control (Fig. 3). The entire interface between tooth and restoration was subdivided into enamel segments, dentin segments, and demineralized segments. Penetration between demineralized dentin and sound dentin was recorded in selectively excavated cavities as well. For further analysis, the occlusal margin was defined as segment and the approximal margin was defined as the sum of the approximal enamel segment and the horizontal dentin segment next to it.

Data treatment and statistical analysis

Dye penetration depth was calculated by measuring penetration depth per segment or segments combined as percent of total length of the respective segment(s). Length was measured using the Optimas software's line morphometry tool. Dye penetration in all segments (sum of all segments of a tooth), in enamel segments, in dentin segments, for approximal margins, and occlusal margins was calculated separately (Fig. 3).

Additionally, the maximum dye penetration (%) from all sections of every tooth was used as the representative value for that tooth. Maxima, minima, medians, 25%- and 75%- percentiles were calculated for each group.

Data were analyzed nonparametrically and Mann-Whitney *U*-Test was used to test for statistically significant differences between groups (SPSS version 25.0, IBM, Armonk, NY, USA). The level of significance was set to $\alpha = 0.05$. To evaluate the impact of thermocycling and mechanical loading, the level of significance α was adjusted to $\alpha^*(k) = 1 - (1 - \alpha)^{1/k}$ by the Error Rates Method (k = number of paired tests performed). Regression analysis was performed for dye penetration and volume of demineralized dentin.

Results

The median volume (25–75% percentile) of the demineralized dentin was 1.8 mm^3 (1.4–2.3 mm^3). Linear regression coefficients ranging from 0.004 to 0.11 for all segments indicate that there is no correlation between demineralization volume and dye penetration for all segments (Fig. 4). The measured median (25–75% percentile) extension of prepared cavities in mesio-distal direction was for complete excavation 2.8 (2.7–3.1) mm, for selective excavation 2.6 (2.2–3.0) mm, and for caries-free control 2.7 (2.5–3.0) mm.

Two-hundred and sixty-six images were evaluated for complete excavation: 285 for selective excavation and 176 for the caries-free control cavities. The median number of images from sections per tooth was nine in every group. The number of images per tooth ranged from 6 to 14.

The median dye penetration for all segments was ≤8.9% in all groups with no thermocycling or mechanical loading, and it ranged between 43.9% and 52.5% in all groups with thermocycling and mechanical loading (Table 1, Fig. 5). There was no significant difference between complete excavation, selective excavation, or caries-free control for all segments.

Within enamel, dye penetration in all groups was ≤17.9% when no thermocycling and mechanical loading had taken place, and it ranged between 60.7% and 73.9% after thermocycling and mechanical loading (Table 1, Fig. 6). There was no significant difference between complete excavation, selective excavation, or caries-free control for enamel. Dye penetration within dentin was ≤ 1% with no thermocycling or mechanical loading and 26.9–49.7% after thermocycling and mechanical loading for all groups. With no thermocycling or mechanical loading, there was significantly more dye penetration in dentin following selective caries excavation compared to the caries-free control ($P = 0.026$).

Dye penetration for all groups from the approximal margins ranged between 4.3% and 11.3% without

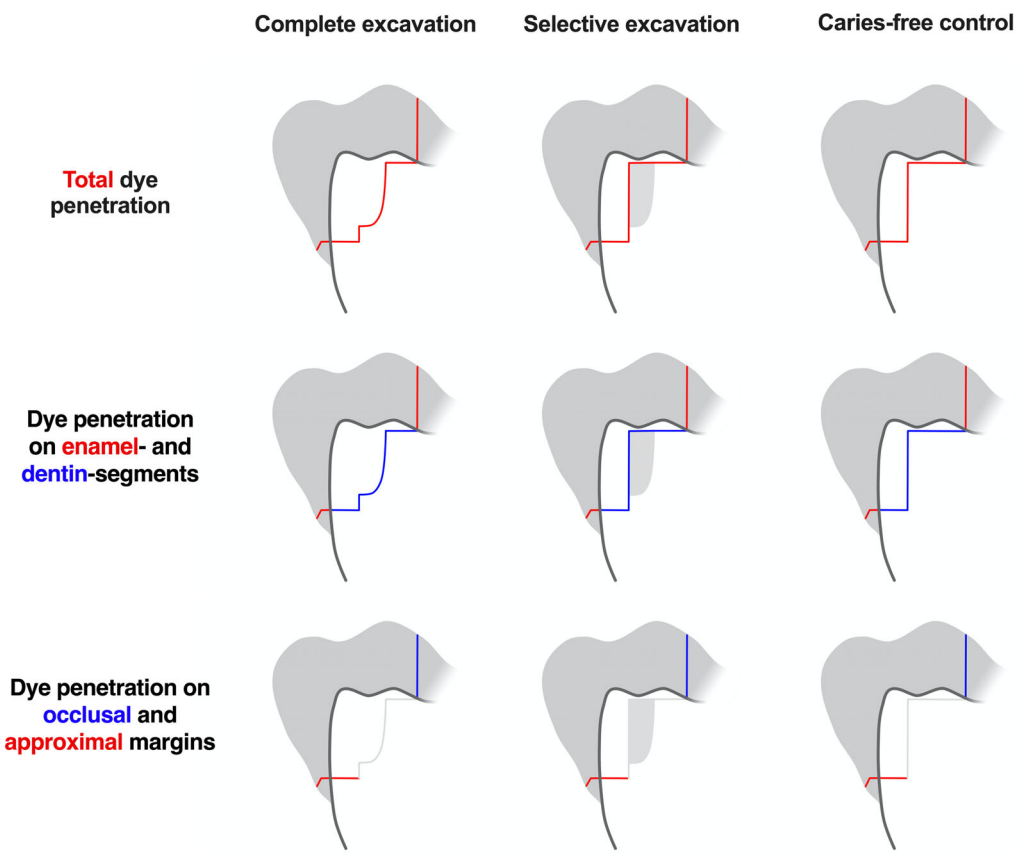

Fig. 3. Evaluation-schemes for marginal dye penetration. Complete excavation, selective excavation and caries-free (undemineralized) control. Marginal dye penetration was evaluated for all segments (upper row, results shown in Fig. 5), by tooth substance (middle row, red = enamel, blue = dentin, results shown in Fig. 6) and margins (lower row, blue = occlusal, red = approximal, results shown in Fig. 7). The border between demineralized and sound dentin on the pulpo-axial wall was also evaluated in selectively excavated teeth. Every identifiable segment was evaluated in each section. [Colour figure can be viewed at wileyonlinelibrary.com]

thermocycling or mechanical loading and between 83.7% and 100.0% in the presence of thermocycling and mechanical loading (Table 1, Fig. 7). Dye penetration at the occlusal margin ranged from 15.1% to 21.9% without thermocycling or mechanical loading and 70.4%–92.3% in the presence of thermocycling and mechanical loading for all groups. When occlusal and approximal dye penetration were compared, the only statistically significant difference was for the caries-free control without TCML, which showed more dye penetration from the occlusal surface ($P = 0.007$) than from the approximal..

Dye penetration was significantly ($P < 0.011$) higher when thermocycling and mechanical loading had been performed than without thermocycling or mechanical loading for all locations and excavation procedures (Fig. 8). No restorations with fractures or loss of retention were observed.

Discussion

Caries-affected dentin is a challenging substrate for restorations for several reasons (5). The in vitro bond strength of composite materials to caries-affected dentin, in general, is reported to be lower than that to sound dentin (5,22). However, the clinical consequences remain unclear, as in vitro studies on the influence of the remaining carious dentin on the mechanical properties and fracture resistance have disagreed (10,23,24). The clinically derived hypothesis of this study was that remaining demineralized dentin does not affect the marginal integrity and, thus, the success of composite restorations.

To investigate the influence of restoration geometry in our model, the dimensions of the cavities in the caries-free control teeth matched those of the selective excavation group but without any artificial caries formation. This kind of control is not always considered in the literature (25,26). Two cavities of equal extension were prepared in each tooth and specimens were paired according to the volume of the artificial demineralization, as measured by using a micro-CT.

Despite the general limitations of dye penetration tests to predict clinical success (27), this test allows a reliable, fast, and commonly accepted preclinical comparison of marginal integrity (18,20,28). Silver ions with a particle size of 0.059 nm are much smaller than

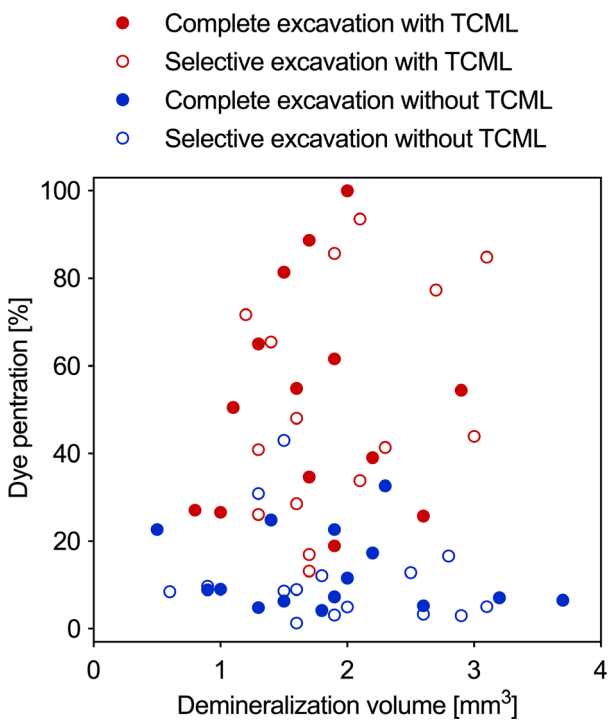

Fig. 4. Dye penetration depending on demineralization volume. Dye penetration (%) of restorations after complete or selective excavation, without and with TCML of all segments plotted against volume of demineralized dentin before caries excavation. Every circle represents one cavity. TCML: thermocycling and mechanical loading. [Colour figure can be viewed at wileyonlinelibrary.com]

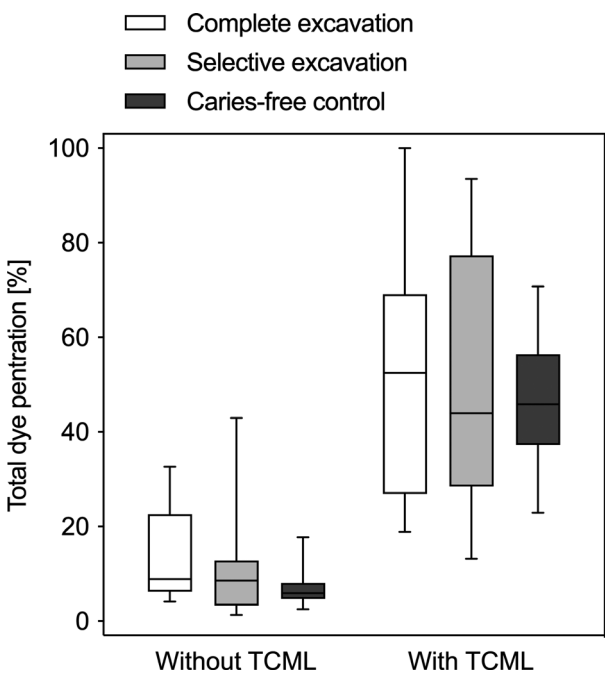

Fig. 5. Total dye penetration. Results of dye penetration for all segments within each tooth under the influence of TCML (Maximum, minimum, median, 25%–75% percentile). TCML: thermocycling and mechanical loading.

cariogenic bacteria (0.5–1 μm), so $AgNO_3$-penetration is a strict in vitro test parameter (29). However, because nutritional substrates, such as sugars, may penetrate marginal gaps and feed bacteria in the residual caries lesion, dye penetration using $AgNO_3$ may be a reasonably good model in order to determine whether sucrose is able to penetrate the margins of restorations. In our study, as many sections as possible were evaluated to accurately identify the weakest spot in every restoration. Optical coherence tomography (OCT) is a promising and clinically applicable alternative analytical method to the dye penetration test used in this study (30,31), as it allows repeated non-invasive measurements on the same specimen. With the OCT-method, restorations with a maximum thickness of 2.5 mm may be analyzed (32). As our restorations mostly had a thickness above 2.5 mm, OCT would be limited only to the outer margins. Moreover, all OCT-systems available today for use with extracted teeth still suffer from low resolution. Both aspects favor dye penetration for this in vitro investigation. High resolution scanning electron microscopy (SEM) studies analyzing marginal integrity using replicas and SEM-images of

Table 1

Dye penetration (%) for all three treatment protocols treated with or without thermocycling or mechanical loading

	Without TCML [% dye penetration]			With TCML [% dye penetration]		
Segment groups	Complete excavation (n = 15)	Selective excavation (n = 15)	Caries-free control (n = 10)	Complete excavation (n = 14)*	Selective excavation (n = 15)	Caries-free control (n = 10)
All segments	8.9 (6.3–22.6)	8.6 (3.3–12.8)	6.0 (4.7–8.1)	52.5 (27.0–69.2)	43.9 (28.5–77.3)	45.9 (37.3–56.4)
Enamel	17.1 (14.0–23.5)	17.9 (10.6–26.3)	12.0 (10.3–26.3)	72.6 (49.2–85.2)	60.7 (42.6–84.3)	73.9 (63.5–84.9)
Dentin	0.0 (0.0–15.7)	1.0 (0.0–10.5)	0.0 (0.0–0.0)	49.7 (18.4–82.9)	26.9 (16.9–87.0)	37.8 (19.5–49.1)
Approximal	7.5 (0.0–66.2)	11.3 (1.7–53.9)	4.3 (2.9–10.4)	86.9 (47.0–100.0)	83.7 (45.3–100.0)	100.0 (73.8–100.0)
Occlusal	21.9 (13.5–25.5)	15.2 (10.8–30.3)	15.1 (12.0–21.9)	92.3 (52.8–100.0)	70.4 (49.2–89.4)	83.3 (58.4–100.0)

Penetration data in the form of median (25%–75% percentile) is given for all segments, and limited to margins in enamel or dentin, or to penetration into approximal and occlusal margins (See Fig. 3). TCML: thermocycling and mechanical loading.
*One restoration was excluded due to remaining demineralized dentin detectable by microscopy.

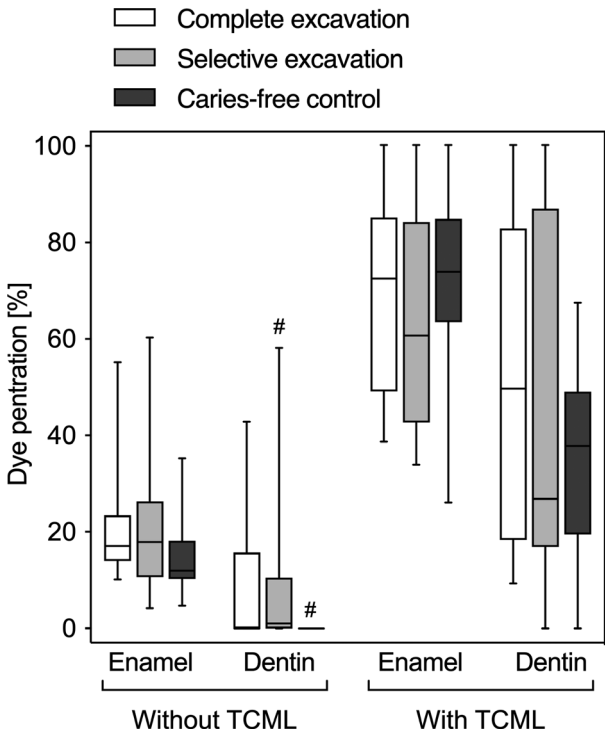

Fig. 6. Dye penetration on enamel and dentin segments. Results of dye penetration for enamel segments and dentin segments for treatment groups complete excavation, selective excavation and caries-free control under the influence of TCML (Maximum, minimum, median, 25%–75% percentile; # = statistically significant difference, $P = 0.026$). TCML: thermocycling and mechanical loading.

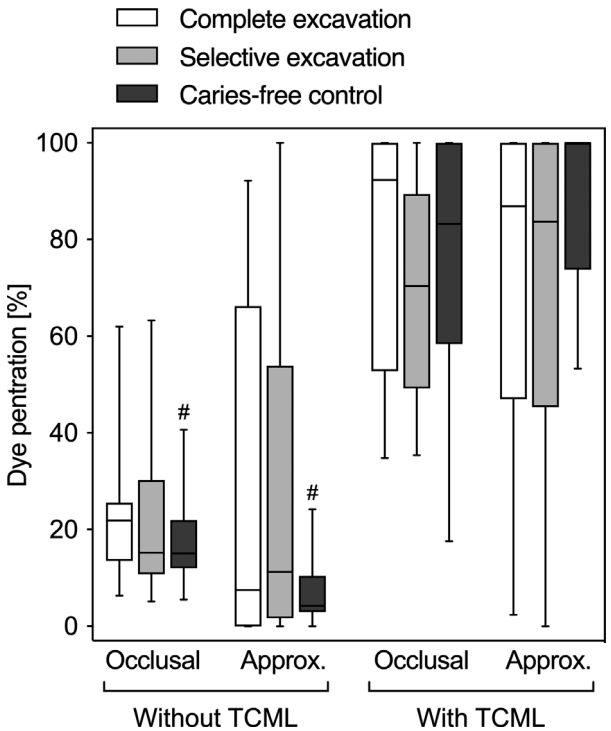

Fig. 7. Dye penetration on occlusal and approximal margins. Dye penetration for all groups subdivided in penetration from occlusal and approximal direction under the influence of TCML. (Maximum, minimum, median, 25%–75% percentile; # = statistically significant difference, $P = 0.007$). TCML: thermocycling and mechanical loading.

the adhesive interface allow marginal gap analysis at the outermost part of the tooth-restoration interface, but do not provide information on the depth of defects without cutting of the specimens (33). In the past, we observed that the marginal quality became so good with modern adhesive techniques that direct restoration margins were either no longer identifiable or interpretation of the marginal quality became difficult. Direct SEM-visualization without replicas allows marginal analysis at high resolution (30), but it is compromised by artifacts at the adhesive interface due to vacuum exposure, which is not the case using dye penetration.

The in vitro demineralization models commonly use organic acids, chelators, or bacterial biofilms to mimic carious lesions (34-36). Although in vitro caries models never reproduce all aspects of the clinical situation, sample acquisition is simple and specimens can be treated in a standardized and reproducible way (37). For example, EDTA is claimed to lead to nearly complete mineral loss within the lesion body (38). Micro-CT measurements in our study, however, revealed a demineralized volume in every cavity before complete excavation and selective excavation, which was depictable in micro-CT and, thus, not completely depleted from calcium and phosphate (Fig. 4). This stable demineralization might be due to the complex geometry of our model with long diffusion paths, which is more similar to the way demineralization takes place clinically than the complete demineralization often used in in vitro studies.

It is widely accepted that color and stainability of dentin are not reliable parameters in the determination of the endpoint of caries excavation (39,40). Furthermore, bacteria-based detection techniques, such as fluorescence aided caries excavation (FACE), could not be used in our study, since we did not use a bacterial demineralization protocol (41-44). Therefore, polymer burs were chosen to provide an objective endpoint for complete excavation in the present study (45,46).

In the present study, enamel was slightly beveled and etched selectively before applying the self-etch adhesive system, as these procedures facilitate reduced marginal discoloration and increased retention (47). The self-etch adhesive system used allowed a chemical interaction with dentin by 10-methacryloyloxy-decyl-dihydrogenphosphate (10-MDP) monomers without exposure of vulnerable collagen (48). Because our study aimed to evaluate the influence of complete or selective excavation, and not to test other restorative procedure parameters, all cavities had complete margins in enamel and were restored according to one clinically accepted protocol (25,47,49).

Leakage at the margin of restorations increases over time due to thermal and mechanical stress (28,50). Corroborating previous in vitro studies (28,51), we found

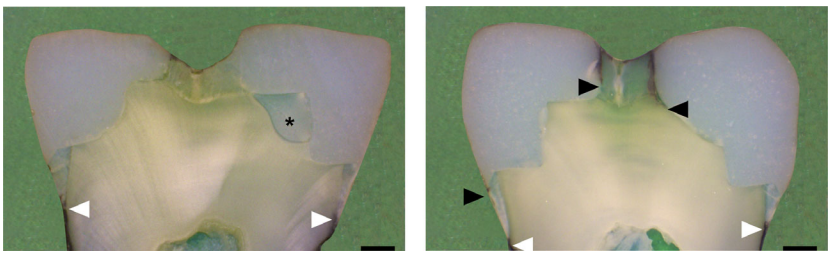

Fig. 8. Example tooth-sections for evaluation. Left image: Section of a tooth (Category 2 – carious lesions produced on both, mesial and distal, surfaces of the tooth) without TCML. Composite restoration after complete excavation on left side, composite restoration after selective excavation with residual demineralized dentin (*) on right side. Restoration margins are located above the cemento-enamel-junction (white arrow heads). No dye penetration is visible at the restoration-tooth interfaces. Scale bar: 1 mm. Right image: Section of a tooth (Category 1 – carious lesion produced on one, mesial or distal, surface of the tooth) with TCML. The composite restoration of caries-free control is located on the left side, the composite restoration after complete excavation on the right side. Restoration margins are located above the cemento-enamel-junction (white arrow heads). Occlusal dye penetration (black arrow heads) appeared on both occlusal margins reaching dentin at the restoration on the right side. Little approximal dye penetration (black arrowhead) could be detected for the left restoration. Scale bar: 1 mm. TCML: thermocycling and mechanical loading. [Colour figure can be viewed at wileyonlinelibrary.com]

marginal dye penetration to be significantly higher after thermocycling and mechanical loading than without thermocycling and mechanical loading, where dye penetration was seen mostly in enamel segments. Unlike other authors, we investigated dye penetration both initially *and* under the influence of thermocycling and mechanical loading (25,52). To stay in line with previous studies, we aimed not to exceed physiological forces in our in vitro model by using a mechanical load of 72.5 N (10,28,53). A significantly decreased marginal integrity after thermocycling and mechanical loading was observed in all experimental groups, highlighting the validity of the chosen model of artificial aging (i.e. thermocycling and mechanical loading). Furthermore, the marginal integrity of the caries-free controls with less extension in pulpal direction did not differ from the marginal integrity observed in the other groups. The greater extension of restorations after complete excavation or selective excavation did not reduce the marginal integrity of the restorations having enamel margins compared to the restorations made in caries-free control teeth. Some authors have speculated that the volume of the caries lesion may have an effect on the marginal quality of restorations (25). Our findings do not support this speculation, because in our study demineralization volume did not correlate with dye penetration, irrespective of whether the samples were exposed to thermocycling and mechanical loading or not.

As is typical clinically, the length of approximal enamel segments after preparation was much shorter than at the occlusal margin. Composites have been shown to provide better bond strength to enamel than to dentin in several in vitro studies (25,28,54). Even though the approximal margin consists of a higher proportion of dentin, dye penetration between the occlusal and approximal margin was not significantly different in our experiments. This might indicate that even a short enamel segment at approximal margins yields a tight seal and protects the dentin from penetration. The main finding of this study is that there is no effect on the marginal seal of adhesive composite restorations, irrespective of whether there were no caries at all, or whether caries were removed completely or selectively prior to placing the restoration. A conservative approach, using selective caries excavation in case of well-established dentin caries lesions, can therefore be recommended.

However, the second finding of this study is that marginal seals deteriorate with wear (tested here using thermocycling and mechanical loading). This can be interpreted to mean that every adhesive restoration, despite the presence or absence of residual caries, loses its tight seal with time. Hence, with time, every restoration margin will become permeable for sucrose and other nutritive molecules. Whether this might enable residual bacteria to reestablish metabolic activity, or even proliferate, needs to be further evaluated (55). Likewise, the impact thereof on pulp hemostasis and biology and its reaction patterns is highly interesting and warrants further investigation.

Within the limitations of our in vitro study, we could not find any significant differences in the marginal integrity of restorations made in teeth exposed to complete excavation, selective excavation, or caries-free control lesions. Thus, the null hypothesis could not be rejected for each situation with and without thermocycling and mechanical loading. However, aging, as simulated by thermocycling and mechanical loading, significantly increased the dye penetration of composite restorations. The presence of demineralized dentin close to the pulp following selective excavation compared to complete excavation does not compromise marginal integrity in class II cavities with enamel margins.

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