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# Impact of access cavity cleaning on the seal of postendodontic composite restorations *in vitro*

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## Abstract

**Aim:** The aim of the study was to investigate the influence of cavity cleaning and conditioning on marginal integrity of directly placed post-endodontic composite class-I-restorations *in vitro*.

**Methodology:** A total of 168 fully intact teeth without caries or fillings received pre-endodontic composite restorations (class-II) after their extraction. Occlusal endodontic access-cavities were prepared, and root canals were instrumented and filled with gutta-percha and an epoxy resin-based sealer. Prior to post-endodontic class-I-restoration, access cavities were completely contaminated with sealer, cleaned with alcohol and pre-treated as follows: cleaner only (alcohol), glycine-polishing, $Al_2O_3$ sandblasting, carbide bur (immediate as well as delayed restoration). A positive control (not contaminated with sealer and adhesive used) and negative control (cleaner used but no adhesive) were established. Half of the teeth from each group were subjected to thermocycling and mechanical loading (TCML). Marginal integrity of post-endodontic restoration was evaluated in oro-vestibular or mesio-distal sections after $AgNO_3$ dye penetration (DP) by standardized photomacroscopic imaging and expressed in per cent of margin length along all segments and separately for enamel, dentine and composite, respectively. Results were analysed non-parametrically ($\alpha = .05$).

**Results:** No restorations or teeth fractured or debonded completely. Without TCML, the median DP of all segments was significantly higher for the negative control compared with all other groups in oro-vestibular cutting direction (53%; $p = .002$) and in mesio-distal cutting direction (51%; $p \leq .041$). The other groups without TCML revealed 16%–24% DP (oro-vestibular) and 12%–24% DP (mesio-distal). With TCML, the median DP in oro-vestibular cutting direction for all segments ranged between 48% and 62% for all groups, a significant difference was only observed between glycine-polishing and carbide bur ($p = .041$). In mesio-distal cutting direction, the median DP in negative control was 69% with TCML and significantly higher compared with all other groups ($p = .002$). For all other groups, the median DP of all segments ranged between 28% and 40% with TCML without significant differences. Error rates method (k = 7) revealed a significant influence of TCML in general on penetration of all segments in both oro-vestibular and mesio-distal cutting directions.

**Conclusion:** Additional access cavity pre-treatment after alcohol cleaning did not improve the marginal integrity of post-endodontic composite restorations. Thorough cleaning of the access cavity with alcohol seems to assure an acceptable marginal integrity to the tooth and restorative composite.

**KEYWORDS**

aluminium oxide, dentine bonding agents, glycine, postendodontic restoration, resin cements, root canal therapy

## INTRODUCTION

Nonsurgical root canal treatment is an essential part of conservative dentistry. As reported by a systematic review summarizing 33 epidemiological studies, the prevalence of endodontically treated teeth is high, with an average of two per patient (Pak et al., 2012). Fortunately, the success rate of primary endodontic treatment is still over 80% even after up to 10 years (de Chevigny et al., 2008; Friedman et al., 2003).

Several clinical studies indicate that primarily the quality of the coronal restoration determines the long-term success of endodontically treated teeth (Craveiro et al., 2015; Ray & Trope, 1995; Siqueira Jr et al., 2005; Thampibul et al., 2019) besides presence or absence of preoperative apical periodontitis (de Chevigny et al., 2008; Gillen et al., 2011) or the perfection of the root canal filling. In this context, marginal leakage allows bacteria to penetrate towards the disinfected and obturated endodontic system, potentially leading to apical periodontitis (de Chevigny et al., 2008; Gillen et al., 2011; Siqueira Jr et al., 2014). Reinfections as well as tooth-fractures are among the most common complications after endodontic treatment (Ng et al., 2010). Preceding tooth decay including necessary restorations as well as the preparation of an access cavity during the course of endodontic treatment reduce the fracture resistance of teeth (Corsentino et al., 2018; Lang et al., 2016). Thus, the postendodontic restoration is of great importance for the treatment success, both for mechanical reasons and for prevention of bacterial reinfection.

The adhesion of composite restorations can be affected by endodontic irrigants such as sodium hypochlorite, which degrades organic structures (Abuhaimed & Neel, 2017; Dikmen et al., 2015). Furthermore, the adhesion of self-etch as well as etch-and-rinse systems can be impaired by the inevitable contamination of the cavity walls with endodontic sealer during canal obturation (Devroey et al., 2020; Wattanawongpitak et al., 2009). Ethanol cleaning reduces the residual amount of sealer at the cavity walls, but a complete removal of sealer remnants may not be achieved (Devroey et al., 2020), which may make additional cleaning necessary. *In vitro* studies are indicative that a surface treatment using bur preparation or blasting with $Al_2O_3$ or, more recently, bioglass can enhance microtensile bond strength to dentine or performance in bi-material curve test (Sinjari et al., 2020; Spagnuolo et al., 2021; Zimmerli et al., 2012). Thus, improvement of the adhesive bond might also be achieved by bur preparation or airborne particle abrasion (glycine, $Al_2O_3$) of the access cavity walls following root canal obturation (Flury et al., 2015; Frankenberger et al., 2007; Lima et al., 2020; Mujdeci & Gokay, 2004; Oztas et al., 2003; Shimizu et al., 2014).

Clinically apparent defects, such as infractures, fractures or discoloured restoration margins, are often preceded by a gradual and latent degradation of the adhesive bond between restorative material and tooth, which is accompanied by penetration of microorganisms. The reinfection associated with loss of adhesion usually precedes the loss of the restoration and thus represents the earliest indication of restorative deficiencies. In this context, a well-established *in vitro* approach to evaluate the marginal seal and leakage and thus reveal the weak points of dental restorations is dye penetration (Durham et al., 2017; Gamarra et al., 2017; Scholz et al., 2020).

This *in vitro* study aimed to investigate the impact of additional endodontic cavity pretreatment after ethanol cleaning on the marginal integrity between directly placed postendodontic composite restorations and dental hard tissues or preendodontic composite restorations by dye penetration. The null-hypothesis was that additional mechanical or micro-abrasive protocols prior to filling of the endodontic cavity do not influence the marginal integrity of postendodontic restorations without or with thermocycling and mechanical loading (TCML).

# MATERIALS AND METHODS

## Specimen selection

One hundred and sixty eight human caries-free upper and lower molars were collected and stored in 0.5% chloramine solution (4°C) directly after extraction for a maximum of 6 months. The University of Regensburg Ethics Committee (Reference: 19-1327-101) approved the use of extracted teeth. The study was planned and performed in accordance with the PRILE 2021 guidelines for laboratory studies in Endodontology (Nagendrababu et al., 2021). The teeth were cleaned from all soft tissue remnants on outer surfaces and stored in deionized water ($1.82 \times 10^7$ µSv, TKA GenPure, TKA xCAD; TKA Wasseraufbereitungssysteme) during the entire experimental period. Visual-tactile inspection and standard dental radiographs were performed to exclude teeth with visible infractures, carious lesions, or other irregularities. All steps of the specimen preparation are shown in Figure 1a–f.

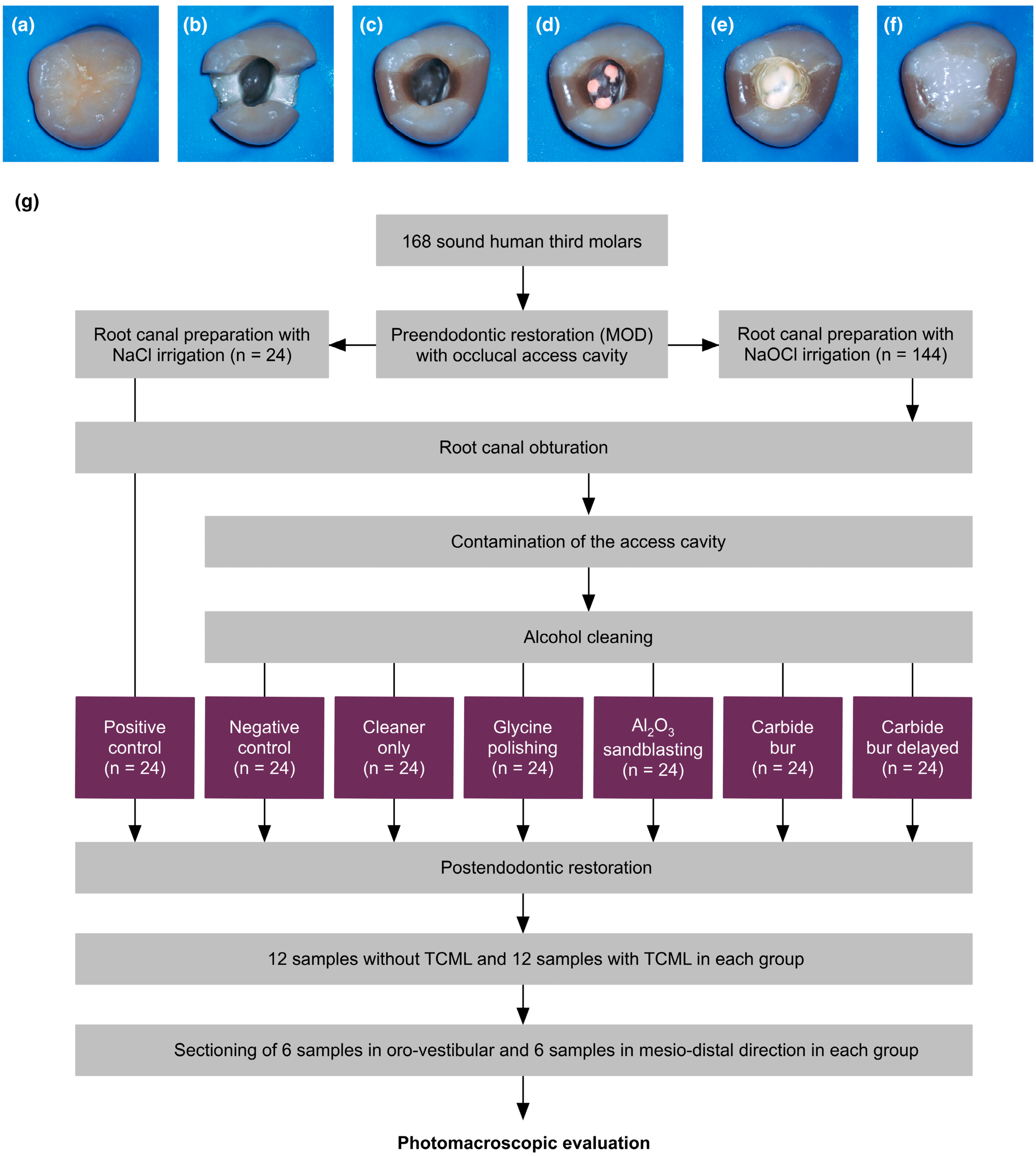


**FIGURE 1** Single steps of specimen preparation: Caries-free molar (a), access and MOD-cavity preparation (b), preendodontic restoration (c), root canal filling (d), sealer contamination (e), and postendodontic restoration (f). Flowchart of the individual steps and sample allocation (g).

## Endodontic access and preendodontic restoration

Occlusal endodontic access cavities were prepared with a high-speed contra-angle handpiece at 200 000 rpm with extensive air-water-cooling (Diamond Access Bur, Dentsply Sirona Endodontics; 851012 FG safe end bur; Busch) to expose root canal orifices and allow a straight-line access of instruments. Furthermore, mesial and distal boxes with a width of 4 mm in oro-vestibular dimension and cervical margin 0.5 mm below the cemento-enamel junction were prepared using cylindrical diamond burs (Reference number 806314111524, diameter 1.4 mm; Hager & Meisinger). The mesial and distal boxes had a minimum extension of 2 mm in mesio-distal direction. Sharp edges on approximal cavity margins in enamel were minimally bevelled at 45° to remove loose enamel prisms and maximize the adhesive surface in enamel. A metal matrix band was circularly placed over the tooth (Hawe Tofflemire Matrices; Kerr), and the canal orifices were covered with a foam pellet (Pele Tim; VOCO). Enamel margins were etched selectively for 20 s using 37% $H_3PO_4$ (Total Etch; Ivoclar Vivadent), rinsed with water for 20 s, and gently air-dried. A two-bottle self-etch adhesive system (Clearfil SE; Kuraray) was applied (Flocked Applicator Tips; Dentsply International) on the mesial and distal proximal boxes according to the manufacturer's instructions. First, the primer was rubbed into dentine and then applied to enamel without agitation for 20 s and dried by mild air-flow. Subsequently, the bonding agent was applied for 10 s across dentine and enamel, distributed evenly with mild air-flow, and light-cured for 10 s (working distance 5 mm; Satelec Mini LED, Acteon Group; light intensity ≥1000 mW/cm$^2$ according to a Cure Rite Visible Curing Light Meter; Dentsply Caulk). Cavities were restored with up to three 2-mm-thick increments of a nano-hybrid resin composite material (Filtek Supreme XTE, colour C4D; 3 M) each light-cured for 40 s. The foam pellet was removed, and the inner walls of the endodontic cavity were finished using a diamond bur (851012 FG safe end bur, Busch) at 200 000 rpm and with extensive air-water-cooling. After removing the matrix band, the proximal preendodontic composite class-II-restorations was polished (diamond polisher #9588 and #9578, Busch) on the proximal and occlusal aspects.

Before proceeding with endodontic treatment, 12 upper and 12 lower molars were randomly allocated to each of the seven experimental groups (positive control, negative control, cleaner only, glycine-polishing, $Al_2O_3$-sandblasting, carbide bur, and carbide bur delayed), which will be described in detail below (Figure 1g).

## Endodontic treatment

During endodontic treatment in groups negative control, cleaner only, glycine-polishing, $Al_2O_3$-sandblasting, carbide bur, and carbide bur delayed, root canals were continuously irrigated with a total of 9 ml sodium hypochlorite (D Microlance 20G, Becton Dickinson; 2%, Speiko; 21°C, 1 ml between files) for a total of 30 min per tooth. All rotary instruments were used according to the manufacturer's instructions (X-Smart Plus, Dentsply Sirona; 300 rpm, 2.0 Ncm). Root canals were enlarged coronally (ProTaper Next SX, Dentsply Sirona), and a glide path was established to size 20.02 (K-files, VDW). Subsequently, canals were shaped with rotary NiTi-files up to size 40.06 (ProTaper Next X1-X4, Dentsply Sirona).

Each root canal was irrigated with 5 ml 17% ethylenediaminetetraacetate (EDTA Disodium Salt 2-hydrate, AppliChem; 21°C), 4 ml 2% sodium hypochlorite with sonic activation by a polyamid tip (EDDY, VDW, 30 s, 195 000 rpm), and subsequently 1 ml 2% sodium hypochlorite without activation. Canals were dried (40/06 paper points, Dentsply Sirona) and filled with guttapercha and epoxy-resin based sealer (AH Plus, Dentsply Sirona) by thermoplastic obturation (Sybron Endo Elements free guttapercha, Kerr). Afterwards, standardized contamination of the access cavity was performed using a foam pellet and 0.05 ml root canal sealer, which was left for 60 s and cleaned subsequently using a foam pellet saturated with a sealer removal solution containing ethanol and tertiary butanol (AH-Plus Sealer Cleaner, Dentsply Sirona). The access cavity was rinsed with water for 20 s and gently air-dried.

The positive control served as the control group without any contamination. For this, the walls of the endodontic access cavity were never in contact with sodium hypochlorite, EDTA, epoxy-resin based root canal sealer, or sealer removal solution. Canals were enlarged coronally, irrigated with 5 ml 0.9% NaCl per canal, and obturated with thermoplasticised guttapercha only.

## Access cavity pretreatment

The endodontic access cavities were pretreated according to different protocols. Group cleaner only underwent no further pretreatment steps in addition to alcohol-based sealer cleaner. The microabrasive pretreatment protocols in group glycine-polishing (25 µm glycine powder; Clinpro, 3 M; PROPHYflex 3, KaVo Dental) and $Al_2O_3$-sandblasting (27 µm $Al_2O_3$; RONDOflex plus 360, KaVo Dental) were performed with water-cooling at 1 bar pressure for 10 s in a working distance of 20 mm from the cavity floor, using a tilting motion to reach all adjacent surfaces of the access cavity. In group carbide bur and carbide bur delayed, mechanical pretreatment was conducted on all inner access cavity walls without water-cooling using a slow-speed contra-angle handpiece with tungsten carbide burs at 4000 rpm (1SXM 018 WST-LG RUND SXM-VERZ HM, Busch). In the carbide bur delayed group, the procedure was performed after 24 h with an intermediate provisional occlusal restoration (foam pellet, Fuji II LC, 20 s light-curing) that was removed using a high-speed contra-angle handpiece at 200 000 rpm with extensive air-water-cooling (Diamond Access Bur, Dentsply Sirona Endodontics).

## Postendodontic restoration

After cavity pretreatment, occlusal enamel margins of all teeth were selectively etched for 20 s (37% $H_3PO_4$; Total Etch, Ivoclar Vivadent), rinsed with water for 20 s, and gently air-dried. A two-bottle self-etch adhesive system (Clearfil SE, Kuraray) was used as described above for the endodontic access cavity in groups positive control, cleaner only, glycine-polishing, $Al_2O_3$-sandblasting, carbide bur, and carbide bur delayed. For the negative control, no adhesive was used. In all groups, a 1 mm layer of opaque flowable composite (Venus Baseliner, Kulzer Mitsui Chemicals Group) was placed on top of the gutta-percha onto the cavity floor and light-cured for 40 s. The endodontic access cavities were filled with at least two increments of nano-hybrid composite material, each no thicker than 2 mm (Filtek Supreme XTE, shade WD; 3 M), creating a flat occlusal surface. As for the preendodontic restoration, each increment of the postendodontic restoration was light-cured for 40 s. All curing steps during the postendodontic restoration procedure were performed with the shortest possible working distance in contact with the cusps of each tooth. The postendodontic class-I-restorations were polished (661 030 FG Arkansas, diamond polisher #9588 and #9578, Busch), and standard dental radiographs were taken to ensure the quality of all foregoing steps. The teeth were then stored in deionized water for 24 h at 21°C.

## Thermocycling and mechanical loading

Teeth were embedded in acrylic resin (Paladur, Kulzer) up to 2 mm apically of the cemento-enamel junction. Half of the samples from each group (six upper and six lower molars) were randomly assigned to thermocycling and mechanical loading (5000 thermo-cycles of 30 s at 5 and 55°C and 500 000 mechanical cycles at 72.5 N load and 1.6 Hz) established in other studies (Krifka et al., 2009, 2011; Schenke et al., 2008; Scholz et al., 2020), whereas the other samples were stored in deionized water (21°C). Teeth were loaded centrally with a hemispherical metal stop simulating the opposing cusp (Naumann et al., 2009; Scholz et al., 2020). This stop was placed in the occlusal surface of the postendodontic composite restoration without contact to the restoration margins.

## Dye penetration

After TCML or storage, the surfaces of the teeth were covered with nail varnish. However, the composite restoration, the cemento-enamel junction, and 1 mm around the restoration margins remained uncovered. All teeth were immersed in 50 wt% $AgNO_3$ solution (S-6506: Sigma-Aldrich Chemie, pH-value 4.3) for 120 min in the dark. Subsequently, teeth were rinsed with demineralized water, immersed in a photographic developing solution (Tetenal Ultrafin Plus, Tetenal AG), 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, the samples were stored at 100% humidity prior to sectioning.

Without and with TCML, 6 (three upper and three lower molars) out of 12 teeth per group were randomly allocated to sectioning in an oro-vestibular or mesio-distal orientation using a water-cooled rotating diamond saw with a blade thickness of 300 μm (Leitz 1600, Leica Microsystems) to obtain as many sections of 300 μm thickness as possible. Standardized images were taken from both sides of the sections using a photomicroscope (Makroskop M420, Wild, magnification 3.15×; Axiocam 105 colour, Carl Zeiss; 2560 × 1920 pixels) as reported previously (Schmalz et al., 1995; Scholz et al., 2020).

## Image evaluation

Images were analysed using Optimas 6.51 software (Bioscan) according to standardized schemes as depicted in Figure 2. Specifically, the entire interface between tooth and restoration (all segments) was evaluated and additionally subdivided into enamel segments, coronal (vertical) and cervical (horizontal) dentine segments, gutta-percha segments, and segments between preendodontic composite class-II-restorations and postendodontic composite class-I-restorations. Dye penetration (%) was calculated by penetration depth per segment in relation to total length of the respective segment (Figure 2). Length was measured using the Optimas-software line morphometry tool (mm, 4 decimal places) and median, 25% and

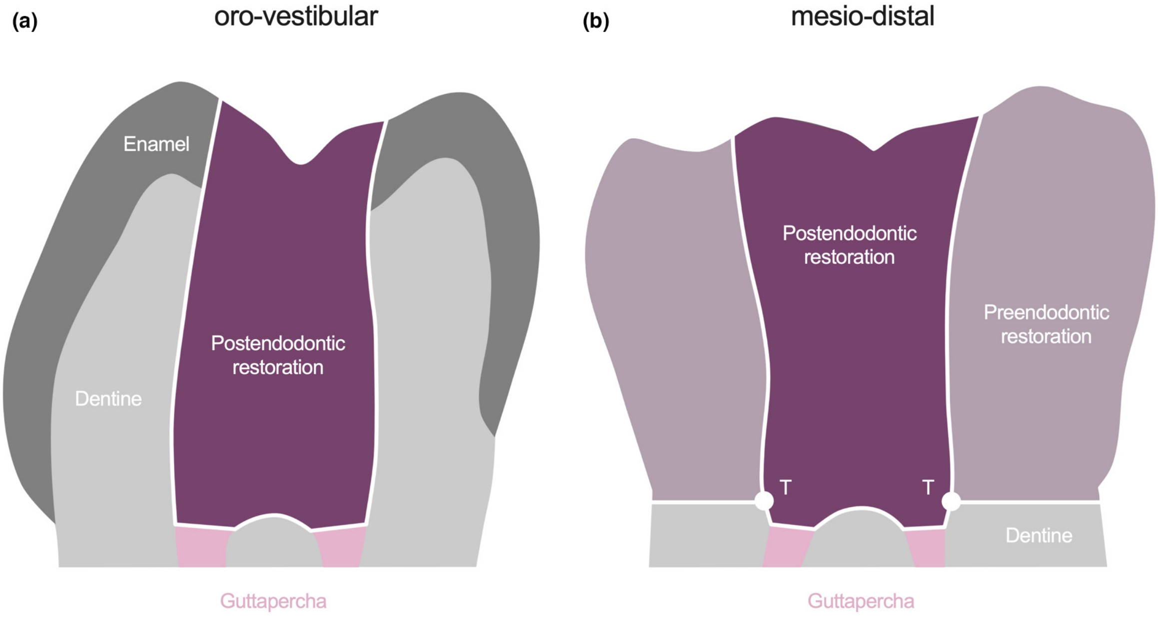


**FIGURE 2** Evaluation-schemes for marginal dye penetration in oro-vestibular (a) and mesio-distal cutting direction (b). The T-point is marked by "T".

75% percentiles of dye penetration (%) were calculated for each group ($n=6$).

In all mesio-distal sections, the points of intersection between cervical dentine, preendodontic, and postendodontic composite restoration were defined as "T-point". The penetration patterns of both T-points per image were recorded as "no penetration", "cervical dentine penetration", "occlusal composite penetration", or "mixed penetration". Teeth without visible penetration at the T-point in any image were also counted. For the teeth with visible penetration at the T-point in any image, the proportion of modes to reach the T-point was calculated as median, 25% and 75% percentiles.

## Statistical analysis

Data were analysed nonparametrically, and the Mann–Whitney *U*-Test was used to test for statistically significant differences between groups at $\alpha=.05$ level of significance (SPSS version 27.0, SPSS). To evaluate the impact of TCML, the level of significance α was adjusted to $\alpha \times (k) = 1-(1-\alpha)^{1/k}$ by the Error Rates Method (k = number of paired tests performed).

## Low vacuum scanning electron microscopy

Two additional teeth per group were prepared as described above according to the respective pretreatment protocols (positive control, negative control, cleaner only, glycine-polishing, $Al_2O_3$-sandblasting, carbide bur, and carbide bur delayed). After storage in deionized water (4°C) for 24 h, all teeth underwent TCML. For scanning electron microscopy, samples were embedded in resin (Paladur clear, Heraeus) and a central section of 1.5 mm thickness in oro-vestibular and mesio-distal direction was obtained using a water-cooled rotating diamond saw (Leitz 1600, Leica Microsystems). The sections were polished with SiC-Sandpaper CarbiMet P4000 and Mastertex Polish cloth (both: Buehler, ITW Test & Measurement) under copious rinsing with deionized water for 60 s and mounted onto aluminium stubs using Leit-Tabs (both: Baltic Präparation). Micrographs of the adhesive interfaces were taken using low-vacuum scanning electron microscopy (LV-SEM; FEI Quanta 400 FEG, Thermo Fisher Scientific, FEI Deutschland) with a large field detector using secondary electron mode, X-ray Pressue Limiting Aperture of 500 μm, 1.5 Torr, 4 kV accelerating voltage, spot size 3, approximately 10 mm working distance, 30 μm end aperture, and image resolution of 2048 × 1768 pixels.

# RESULTS

The median (25% and 75% percentile) number of images that could be acquired and evaluated per tooth was 8 (7.25–10) in oro-vestibular cutting direction and 8 (6–8) in mesio-distal cutting direction. No fractures of restorations or teeth or complete loss of retention were observed. The dye penetration values for all groups and segments are provided in Table 1. In general, besides the negative

**TABLE 1** Dye penetration (%) for all pretreatment protocols with and without TCML (thermocycling and mechanical loading)

| Marginal dye penetration | | TCML | Positive control | Negative control | Cleaner only | Glycine-polishing | $Al_2O_3$-sandblasting | Carbide bur | Carbide bur delayed |
|---|---|---|---|---|---|---|---|---|---|
| Oro-vestibular | All segments | Without | 16 (11–26) | 53 (51–61) | 12 (6–42) | 22 (9–30) | 16 (10–26) | 19 (13–40) | 16 (10–29) |
| | | With | 51 (44–72) | 56 (49–78) | 54 (43–65) | 62 (50–69) | 55 (46–72) | 48 (29–54) | 49 (32–65) |
| | Enamel | Without | 66 (45–70) | 100 (91–100) | 52 (35–74) | 59 (47–84) | 62 (46–73) | 63 (53–72) | 51 (43–64) |
| | | With | 94 (76–100) | 100 (100–100) | 100 (94–100) | 100 (93–100) | 100 (100–100) | 100 (100–100) | 92 (87–100) |
| | Coronal and pulpal dentine | Without | 4 (0–25) | 46 (39–62) | 4 (0–42) | 10 (0–25) | 6 (2–16) | 7 (0–43) | 11 (0–32) |
| | | With | 45 (36–68) | 45 (38–72) | 47 (35–56) | 52 (44–67) | 48 (35–63) | 29 (18–41) | 38 (24–59) |
| Mesio-distal | All segments | Without | 22 (12–45) | 51 (39–56) | 24 (8–26) | 16 (10–31) | 22 (12–34) | 17 (7–30) | 23 (16–29) |
| | | With | 39 (31–46) | 69 (62–75) | 28 (18–40) | 38 (31–44) | 40 (28–46) | 36 (29–42) | 35 (19–48) |
| | Cervical and pulpal dentin | Without | 33 (22–51) | 42 (28–56) | 47 (20–64) | 29 (18–56) | 44 (28–62) | 40 (10–47) | 47 (39–55) |
| | | With | 61 (48–96) | 73 (50–84) | 56 (37–73) | 61 (56–88) | 87 (61–92) | 70 (78–94) | 59 (36–96) |
| | Postendodontic and preendodontic composite | Without | 22 (7–49) | 72 (68–84) | 12 (8–24) | 14 (3–23) | 8 (1–14) | 10 (1–28) | 10 (6–17) |
| | | With | 20 (7–46) | 83 (70–100) | 9 (4–30) | 25 (14–36) | 6 (2–18) | 16 (8–21) | 22 (8–34) |

*Note*: Penetration data in the form of median (25% and 75% percentile) are given for all segments, limited to margins in dentine, enamel, and composite.

Abbreviation: TCML, Thermocycling and mechanical loading.

control, none of the cleaning and conditioning procedures tested revealed significantly more dye penetration than the positive control in both cutting-directions and without or with TCML, respectively.

## Dye penetration without TCML

Without TCML (Figure 3a), the median dye penetration for all segments in oro-vestibular cutting direction was significantly higher for negative control, showing 53% dye penetration compared to all other groups ($p = .002$). In mesio-distal cutting direction negative control with 51% median, dye penetration also was significantly higher than all other groups ($p \leq .041$). Median dye penetration of all segments and both cutting directions for all other groups without TCML ranged between 16% and 24% without any significant differences among groups.

## Dye penetration with TCML

With TCML (Figure 3b), the median dye penetration for all segments in oro-vestibular cutting direction ranged between 48% and 62% for all groups with a significant difference only between glycine-polishing and carbide bur ($p = .041$).

In mesio-distal cutting direction, the median dye penetration of all segments in the negative control was 69%, which was significantly higher than all other groups ($p = .002$). For all other groups, the median dye penetration of all segments ranged between 28% and 40% without any differences among groups.

## Dentine or composite boundaries and T-point analysis in mesio-distal cutting direction

Among all groups, only the negative control showed higher percental penetration between preendodontic and postendodontic restoration (composite boundaries) than penetration between preendodontic restoration and dentine (dentine boundaries) without (Figure 4a) and with TCML (Figure 4b). Composite boundaries for all groups except that of the negative control revealed a median dye penetration between 8% and 22% without TCML and between 6% and 25% with TCML (negative control: without TCML 72%, with TCML 83%). Dye penetration into dentine boundaries for all groups ranged between 29% and 47% without TCML and between 56% and 87% with TCML.

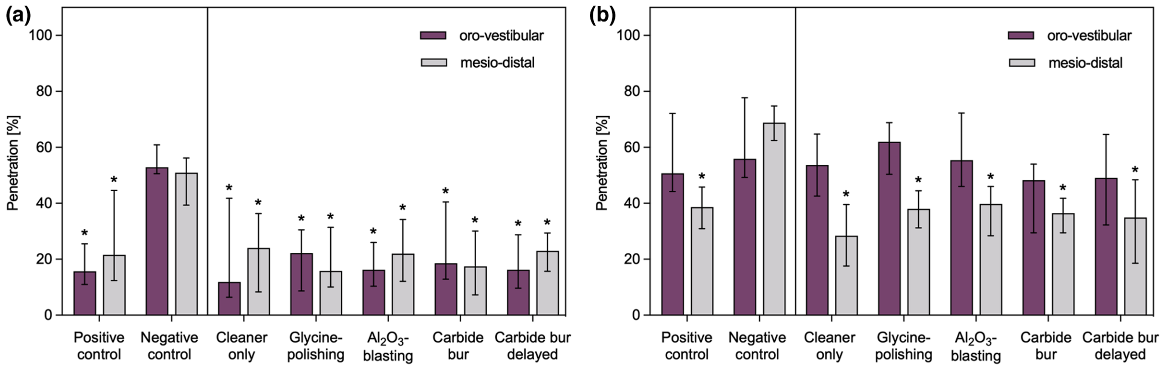


**FIGURE 3** Results of dye penetration for all segments of the respective pretreatment protocols ($n = 6$ per bar, median, 25% and 75% percentile). The asterisk marks a statistically significant difference between the group and the negative control in mesio-distal and oro-vestibular cutting direction, respectively. Without TCML (a) and with TCML (b). Among the other groups, a significant difference could only be observed with TCML between glycine-polishing and carbide bur ($p = .041$). TCML, Thermocycling and mechanical loading.

Regarding to the composite and dentine boundaries, the negative control showed significantly more colour penetration at the boundaries between preendodontic and postendodontic restorations (composite boundaries) without ($p = .002$) and with TCML ($p \leq .004$). Among the other groups, a significant difference was only observed between cleaner only and $Al_2O_3$-sandblasting for dentine boundaries with TCML ($p = .026$). Qualitative details and the proportion of modes to reach the T-point for teeth in mesio-distal cutting direction, that is, from cervical and from occlusal or from both directions is shown in Table 2. Penetration modes to reach the T-point revealed penetration between preendodontic and postendodontic restoration only for the positive control with TCML (mixed penetration) and mainly for the negative control without and with TCML (mixed penetration and isolated occlusal composite penetration, respectively). In all other groups, penetration to the T-point occurred exclusively between cervical dentine and preendodontic restoration (Figure 5).

## Influence of TCML and tooth aspect

In oro-vestibular cutting direction, TCML led to significant deterioration of marginal integrity for all segments and all groups ($p \leq .041$) except the negative control. In mesio-distal cutting direction, TCML increased marginal dye penetration in all groups by tendency with a significant influence for the negative control ($p = .002$), $Al_2O_3$-sandblasting ($p = .026$) and carbide bur ($p = .016$). TCML thus had a greater influence on the interfaces between enamel or dentine and the postendodontic restoration as investigated in the orovestibular direction (Figures 3 and 5). In the mesiodistal direction, TCML led to higher dye penetration, especially regarding the dentine boundaries (Figures 4 and 5). Error rates method (k = 7) revealed a significant influence of TCML in general on dye penetration for all segments in both oro-vestibular and mesio-distal cutting direction. No significant differences between oral and vestibular penetration were detected in either group. Only for the positive control with TCML, a significant

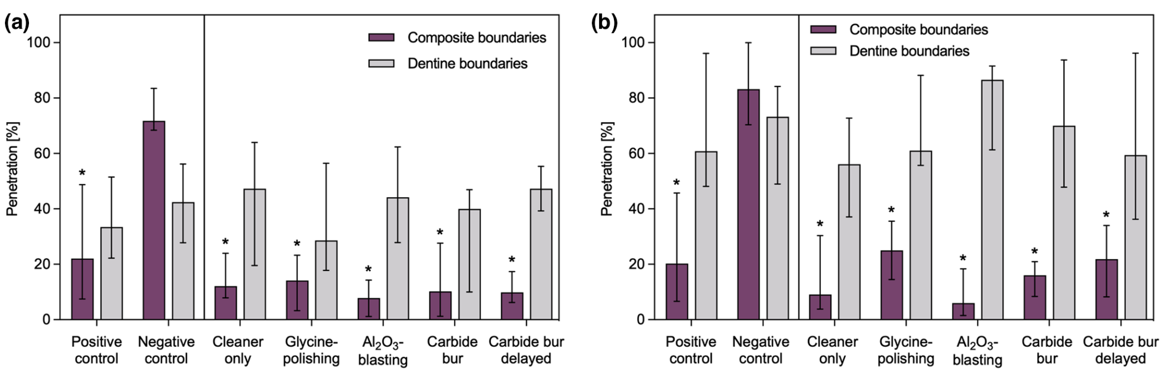


**FIGURE 4** Results of dye penetration for composite boundaries (between preendodontic and postendodontic restoration) and dentine boundaries (between cervical dentine and preendodontic restoration) of the respective pretreatment protocols (median, 25% and 75% percentile) in mesio-distal cutting direction. Without TCML (a) and with TCML (b). * = significant difference compared to negative control. Among the other groups, a significant difference was only observed between cleaner only and $Al_2O_3$-sandblasting for dentine boundaries with TCML ($p = .026$). TCML, Thermocycling and mechanical loading.

**TABLE 2** Proportion (%) of modes to reach the T-point for teeth in mesio-distal cutting direction with visible penetration from cervical or occlusal direction at the T-point in any image (median, 25% and 75% percentiles)

| Modes to reach the T-point | TCML | Positive control | Negative control | Cleaner only | Glycine-polishing | $Al_2O_3$-sandblasting | Carbide bur | Carbide bur delayed |
|---|---|---|---|---|---|---|---|---|
| *N* (out of 6) | Without | 2 | 6 | 4 | 3 | 4 | 3 | 5 |
| | With | 5 | 3 | 6 | 6 | 3 | 4 | 6 |
| No penetration | Without | 53 (17–53) | 50 (39–52) | 50 (27–56) | 72 (50–72) | 44 (22–69) | 69 (50–69) | 57 (50–65) |
| | With | 50 (28–66) | 63 (8–62) | 56 (21–82) | 39 (29–58) | 38 (6–38) | 15 (13–70) | 51 (10–72) |
| Isolated cervical dentine penetration | Without | 26 (11–26) | 9 (0–23) | 50 (44–73) | 28 (19–28) | 56 (31–78) | 31 (8–31) | 43 (35–50) |
| | With | 50 (13–60) | 0 (0–0) | 44 (18–76) | 61 (42–69) | 63 (50–63) | 82 (30–86) | 48 (28–70) |
| Isolated occlusal composite penetration | Without | 0 (0–0) | 14 (0–33) | 0 (0–0) | 0 (0–0) | 0 (0–0) | 0 (0–0) | 0 (0–0) |
| | With | 0 (0–16) | 25 (0–25) | 0 (0–0) | 0 (0–0) | 0 (0–0) | 0 (0–0) | 0 (0–0) |
| Mixed penetration | Without | 21 (0–21) | 25 (5–44) | 0 (0–0) | 0 (0–0) | 0 (0–0) | 0 (0–0) | 0 (0–0) |
| | With | 0 (0–19) | 0 (0–0) | 0 (0–2) | 0 (0–1) | 0 (0–0) | 0 (0–5) | 0 (0–7) |

Abbreviation: TCML, Thermocycling and mechanical loading.

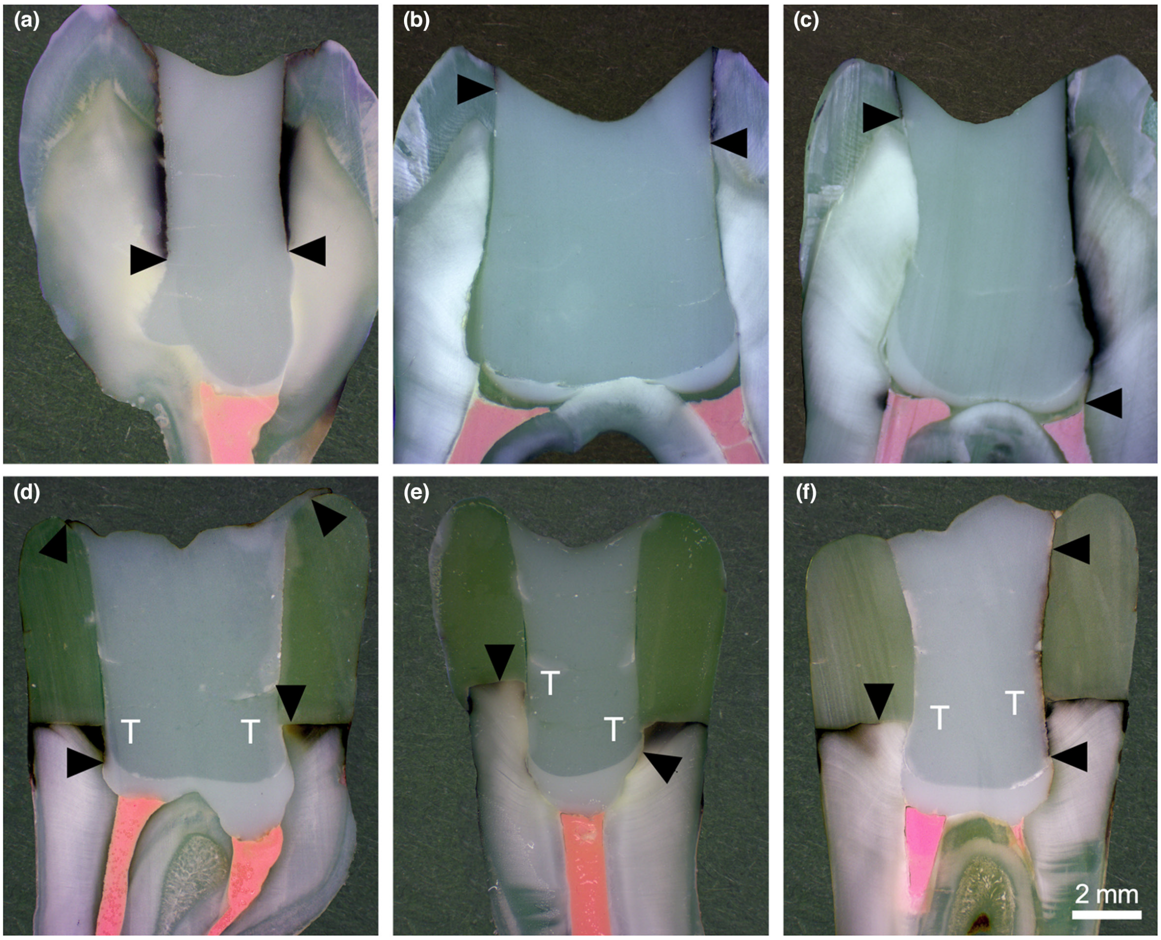


**FIGURE 5** Exemplary tooth-sections for evaluation. Black arrow heads: Maximum dye penetration of the corresponding image and aspect. Upper row: Oro-vestibular cutting direction. (a): Negative control without TCML; dye penetration extends into dentine on both sides. (b): $Al_2O_3$-sandblasting without TCML; dye penetration limited to enamel. (c): Positive control with TCML; dye penetration extends into dentine. Bottom row: Mesio-distal cutting direction. (d): Positive control with TCML: Dye penetration limited to cervical dentine; Only left T-point shows penetration (dentine boundaries). (e): Carbide bur with TCML: Dye penetration limited to cervical dentine; Only right T-point shows penetration (dentine boundaries). (f): Negative control without TCML; Dye penetration on dentine and composite boundaries; Right T-point shows penetration into both dentine and composite boundaries. TCML, Thermocycling and mechanical loading.

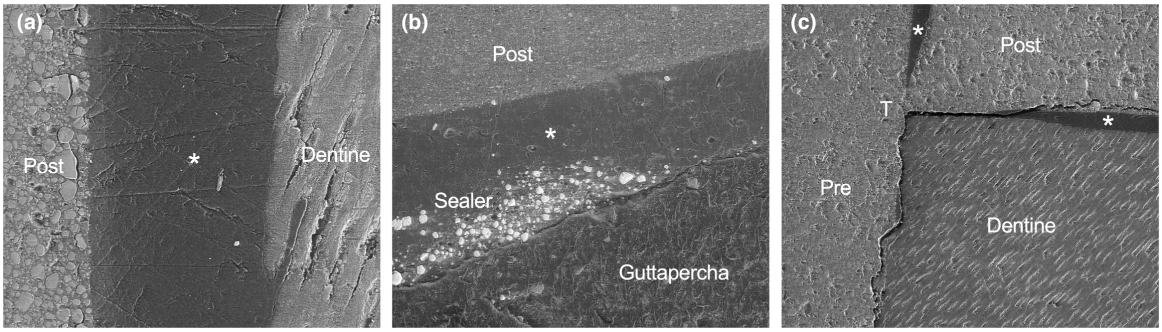


**FIGURE 6** Exemplary low-vacuum SEM images (* = adhesive, post = postendodontic restoration, pre = preendodontic restoration; T = "T-point"). (a): Group glycine-polishing with TCML, oro-vestibular cutting direction (horizontal field width 90.13 µm); mid-coronal interface between postendodontic restoration and dentine shows micromorphological adhesive interaction without visible sealer contamination. (b): Group glycine-polishing with TCML, oro-vestibular cutting direction (horizontal field width 340 µm); apical interface between postendodontic restoration and guttapercha shows different amounts of sealer contamination. (c): Group cleaner only with TCML, mesio-distal cutting direction (horizontal field width 340 µm); visible microgap-formation between dentine and pre- or postendodontic restoration, but not between preendodontic and postendodontic restoration. TCML, Thermocycling and mechanical loading.

difference between mesial and distal dye penetration was shown with higher mesial penetration ($p = .041$). Areas of sufficient adhesive adaption between dentine and postendodontic composite, sealer remnants, and microgap initiation particularly in cervical dentine could be detected using LV-SEM and are shown exemplarily in Figure 6.

# DISCUSSION

The present *in vitro* study aimed to evaluate the marginal integrity of directly placed preendodontic composite class-II-restorations in combination with postendodontic composite class-I-restorations in endodontically treated teeth after different pretreatment protocols. Neither of the investigated pretreatment protocols showed advantages over alcohol cleaning alone. TCML led to significant deterioration of marginal integrity in general where the boundaries to dentine and enamel were affected the most but without fractures or complete loss of retention in any specimen.

A general problem with all root canal treated teeth is the mechanical weakening of the tooth crown, which can lead to infractures, fractures, and adhesion loss at the boundaries to restorations. This makes stable postendodontic restorations even more important for the long-term survival of endodontically treated teeth. The clinical study by Safavi et al. indicated a tendency of higher clinical success rates after root canal treatment when definitive cast crowns that cover the load-bearing occlusal surface are placed (Safavi et al., 1987), whereas Mergulhao et al. found no significant differences in fracture strength in their study investigating endodontically treated teeth restored with different restorations without cusp reduction (Mergulhão et al., 2019), and information on the influence of different restoration types on the microleakage of endodontically treated teeth is generally sparse.

Following a root canal treatment, direct restoration of the access cavity is common practice. An advantage of direct adhesive restorations is the immediate completion of treatment and preservation of tooth structure.

This study is based on an experimental setup with extracted teeth resembling the clinical situation of endodontically treated posterior teeth. As an endodontic database study including 7372 patients identified deep caries and former extended restorative procedures as causative factor for non-surgical root canal treatment in 68.1% of the cases (Iqbal et al., 2008), we prepared mesial and distal cavities with cervical dentine margins and placed preendodontic composite class-II-restorations.

Generally, the marginal integrity between postendodontic composite restorations and enamel, dentine, or preendodontic build-up may be impaired by endodontic procedures. In particular, materials or chemicals used during root canal disinfection or filling can affect the adhesive strength. Wattanawongpitak et al. performed an *in vitro* study investigating coronal root canal dentine showed a significantly inferior microtensile bond strength after etch-and-rinse adhesive or self-etch adhesive application in specimens treated with EDTA followed by NaOCl (Wattanawongpitak et al., 2009). A study of de Rose et al. assessing the internal adaptation of composite restorations placed in endodontic cavities by scanning electron microscopy indicated a decline of the adhesive bond to dentine and enamel due to contact with NaOCl (Rose et al., 2015). Since NaOCl is the most effective antibacterial root canal irrigant and essential in endodontic therapy, a sufficiently long NaOCl contact time was selected to allow NaOCl to penetrate the dentinal tubules, and EDTA was applied to remove the smear layer according to best clinical practice

(Ayhan et al., 1999; Rossi-Fedele & Rödig, 2022; Ruksakiet et al., 2020; Violich & Chandler, 2010).

Also, contamination of dentine with sealer may affect the marginal integrity between postendodontic restorations and preendodontic restorations or dental hard tissues. Analogous to clinical procedure, in the present study, we used alcohol (ethanol and tertiary butanol) in a commercially available sealer removing solution to clean the cavity walls from epoxy-resin based root canal sealer following root canal obturation. In an *in vitro* study examining pre- and postoperative photographs, cleaning with an ethanol-saturated microbrush alone or with additional calcium carbonate air polishing led to the most efficient removal of epoxy-resin-based root canal sealer compared to other pretreatment methods, for example, round burs or air-water spray (Devroey et al., 2020). However, none of the pretreatment methods was able to completely remove the sealer from dentine (Figure 6), which is in accordance with a study of Kriznar et al., where despite meticulous alcohol cleaning, approximately 0.1 mm residues of epoxy-resin based root canal sealer or $Ca(OH)_2$ could be detected in endodontic cavities using phase contrast–enhanced μCT (Devroey et al., 2020; Križnar et al., 2019). Therefore, we investigated if additional microabrasive or mechanical treatment can improve the marginal integrity of preendodontic composite class-II-restorations and postendodontic composite class-I-restorations.

Despite its general limitations to predict clinical success or bond strength of coronal restorations (Heintze, 2007; Scholz et al., 2020), dye penetration on as many sections as possible to accurately identify the weakest spot in every restoration allows for a reliable preclinical comparison of microleakage of the coronal restoration towards the root canals, which is considered to be the most important risk factor responsible for apical periodontitis in endodontically treated teeth (Jafari & Jafari, 2017). As there might be a positive correlation between dye penetration and cusp fracture resistance in endodontically treated teeth with deep MOD-restorations (Ausiello et al., 1999), dye penetration might, beneath the investigation of the marginal seal preventing apical reinfection, even be a relevant surrogate for the stability of restored teeth after different postendodontic restorative strategies. To allow the most accurate evaluation of penetration in the sections, even between pre- and post-endodontic restorations, we used two different shades (C4D and WD) of the same nano-hybrid resin composite material, which did not differ in application or light-curing time according to the manufacturer.

Similar to previous *in vitro* studies, marginal dye penetration was significantly higher for restorations exposed to a physiological level of thermal and mechanical stress than without TCML (Krifka et al., 2011; Rocca et al., 2018; Scholz et al., 2020). According to the results of this study, another study investigating endodontically treated teeth reported thermomechanical loading to significantly increase dye penetration on adhesively sealed pulp chambers underneath a coronally placed temporary glass-ionomer cement restoration without a significant influence of bur pretreatment or the adhesive strategy (Ebert et al., 2009). Another *in vitro* study found a positive correlation between dye penetration in composite restorations and cusp fracture strength in endodontically treated teeth with deep MOD-restorations (Ausiello et al., 1999).

The presented results on dye penetration and particularly the analysis of the T-points comply with a previous *in vitro* study investigating the marginal integrity between endodontic temporary restorative materials and composite build-ups or bovine dentine (Kameyama et al., 2020). In the present study, the T-point analysis revealed penetration mainly between cervical dentine and preendodontic composite restorations and not between preendodontic composite class-II-restorations and postendodontic composite class-I-restorations when a self-etch adhesive was used. This is in accordance with several *in vitro* studies presenting better bond strength and marginal integrity of adhesively luted restorations to enamel compared with dentine (Barkmeier et al., 1999; Krifka et al., 2011) and improved bond-strength and reduced nanoleakage when dentine is surrounded by peripheral enamel margins (Kasaz et al., 2012). In our study only for the negative control, where no self-etch adhesive was applied before postendodontic restoration, penetration between preendodontic and postendodontic composite was observed. Exemplary low-vacuum scanning electron microscopic images under low-voltage conditions (Scholz et al., 2021) were also indicative for predominant disintegration of dentine margins. The marginal leakage at the cervical dentine of the preendodontic restoration has developed, although TCML was only performed after complete postendodontic restoration of the teeth. Clinically, the preendodontic restoration is in some situations already stressed thermically and mechanically before a potential stabilization by placing the postendodontic restoration can occur. This may also be the reason why the percental penetration of all segments without TCML tends to be lower in the oro-vestibular cutting direction but after TCML tends to be lower in the mesio-distal cutting direction. Consequently, the analysis of the T-points showed a lower proportion of cervical penetration than isolated occlusal composite penetration or mixed penetration only in the negative control, where no adhesive was applied between preendodontic and postendodontic restorations.

The main finding of this study is that highly visible penetration was observed in all groups and also occurred in the positive control. The marginal integrity between the preendodontic composite and cervical dentine is less stable than between the preendodontic and postendodontic composite and further deteriorates with thermocycling and mechanical loading irrespective of additional pretreatment steps. Overall, the null hypothesis could not be rejected as different pretreatment-protocols of the endodontic access cavity did not significantly improve the marginal integrity of the postendodontic composite class-I-restorations.

A future improvement, as recently investigated, could be the application of bioactive components for blasting or as restorative materials as they may have the potential to reduce bacterial penetration (Khvostenko et al., 2016; Spagnuolo et al., 2021). Furthermore, based on the presented results, additional studies should investigate the option of complete direct restoration or indirect, cusp-covering restorations after root canal treatment to prevent the disintegration of the adhesive bond and resultant apical reinfection aiming for a long-term survival of endodontically treated posterior teeth.

## CONCLUSIONS

Different microabrasive or mechanical pretreatment protocols in addition to alcohol cleaning the sealer-contaminated endodontic cavity and using a self-etch adhesive combined with selective enamel etching did not lead to improved marginal integrity between postendodontic composite class-I-restorations and enamel, dentine, or beforehand placed preendodontic composite class-II-restorations. Cervical restoration margins of preendodontic composite restorations placed in dentine appear to be a particularly critical weak spot of directly restored endodontically treated teeth.

### AUTHOR CONTRIBUTIONS

Conception and design: Konstantin J. Scholz, Matthias Widbiller. Data acquisition, analysis and interpretation: Konstantin J. Scholz, Woocheol Sim, Silvio Bopp, Karl-Anton Hiller, Matthias Widbiller. Drafted the manuscript: Konstantin J. Scholz, Matthias Widbiller. Revised the manuscript: Konstantin J. Scholz, Karl-Anton Hiller, Kerstin M. Galler, Wolfgang Buchalla, Matthias Widbiller.

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### CONFLICT OF INTEREST

The authors declare no conflict of interest.

### ETHICAL APPROVAL

The use of extracted teeth was approved by the University of Regensburg Ethics Committee (Reference: 19-1327-101).

### DATA AVAILABILITY STATEMENT

Data available on request from the authors.

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