

Margin Integrity and Secondary Caries of Lined or Non-lined Composite and Glass Hybrid Restorations After Selective Excavation *In Vitro*

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Clinical Relevance

Using glass hybrid or lining a cavity before restoring it with composite resins could decrease margin integrity. This might not be relevant for risk of secondary caries.

SUMMARY

Objectives: For deep carious lesions, selective carious tissue removal (leaving soft dentin close to the pulp) is suggested. Afterward, different restoration materials, such as resin composites or glass hybrids (GHs), can be placed. Many dentists also apply setting or non-setting calcium hydroxide liners before restoration. We compared margin integrity

and susceptibility for secondary caries in differently restored premolars *in vitro*.

Methods: In 48 extracted human premolars, artificial residual lesions were induced on pulpo-axial walls of standardized cavities. Teeth were restored using a GH (Equia Forte) or adhesively placed resin composite restoration (OptiBond FL and Tetric EvoCeram) without any liner (RC), resin composite restoration with a non-setting calcium hydroxide liner (RC_NCH), or resin composite restoration with a setting calcium hydroxide liner (RC_SCH). After thermomechanical cycling, groups (n=12) were compared regarding their gingivocervical margin integrity (proportion of irregularities, microgaps, gaps >5 µm, overhangs). Teeth were then submitted to a continuous culture *Lactobacillus rhamnosus* biofilm model. After 14 days, bacterial numbers in biofilms, along tooth-restoration margins and mineral loss (ΔZ) of secondary lesions, were determined.

Results: GH and RC_NCH showed significantly higher proportions of irregularities than RC

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and RC_SCH ($p < 0.05$ /Mann-Whitney). GH also showed significantly more gaps than alternative restorations ($p < 0.05$). Bacterial numbers and ΔZ did not differ significantly between groups ($p > 0.05$).

Conclusions: GH and composites lined with non-setting calcium hydroxide showed reduced margin integrity compared with non-lined composites or composites lined with setting calcium hydroxide. This did not increase susceptibility for secondary caries.

INTRODUCTION

For deep carious lesions in teeth with vital pulps, selective (incomplete) carious tissue removal is recommended, leaving soft dentin in proximity to the pulp to avoid pulp exposure and postoperative pulp complications. Then, an adhesive restoration is placed, which seals, and thus inactivates, residual bacteria. The resulting residual carious dentin is soft, is less elastic, and allows only very limited bonding of dental adhesives in the lesion area,¹ all of which are feared to compromise the integrity and stability of the placed restoration. This, in turn, could limit the longevity of the restoration, leading to fracture or reduced margin integrity, which would presumably increase the susceptibility for secondary caries.^{2,3}

Consequently, a variety of strategies have been developed to bond to this carious dentin and to restore the resulting extended cavity.^{1,4,5} Besides these restorative challenges, dentists also aim to reduce pulpal risks, for example via placing a cavity liner, which supposedly protects the pulp and reduces postoperative pulp complications. Moreover, such liners should also exert antibacterial or remineralization effects.

The most commonly placed lining material is calcium hydroxide (CH), which has been shown to provide remineralization *in vitro*⁶ and clinically.^{7,8} CH is also antibacterial and induces reactionary dentin development.⁹⁻¹¹ Two different application forms of CH are available, setting and non-setting CH. Setting CH sets after mixing with salicylate esters via a chelating reaction, resulting in a moderately stable lining layer with low compression strength; it releases fewer calcium and hydroxyl ions than non-setting CH,^{12,13} which potentially exerts higher antibacterial effects. Both materials are susceptible for degradation via acid-etching¹⁴ and dissolve over time, leaving voids and tunnels prone to bacterial recolonization.^{15,16} They are conventionally covered with a self-conditioning material (like

resin-modified glass ionomers) before placing an adhesive restoration (eg, acid-etching or applying self-etch adhesives). In summary, CH are widely used, though their application might mechanically compromise the subsequent restoration (eg, its margin integrity or fracture resistance), the clinical impact of which remains unclear at present.

To avoid such lining material and the required additional treatment steps, but nevertheless to remineralize the residual lesion, a variety of restoration materials have been proposed, for example fluoride-doped and fluoride-releasing composites, or glass ionomer cements (GICs). The latter have been shown to remineralize dentin *in vitro* and *in vivo*.¹⁷⁻²³ However, GICs have mainly been placed temporarily over residual lesions, as their limited flexural strength does not allow for their use to permanently restore extended cavities. Recent advances in this material class, however, have widened the indication spectrum of GICs, and one very recent development was the introduction of glass hybrids (GHs). These are reinforced GICs, with a second, smaller, and more reactive silicate particle and higher-molecular-weight acrylic acid molecules, which supposedly increase matrix cross-linking. This, in turn, is thought to improve the material's flexural strength. Covering these restorations with a resin layer is supposed to further improve wear resistance and esthetic appearance. It remains unclear if GH may truly be used to restore extended cavities, as the margin integrity and fracture resistance of teeth restored with GH are unknown. Moreover, the secondary caries susceptibility of teeth restored with GH is not clear, which could be linked to restoration margin characteristics of these materials and their fluoride release.

Therefore, the present study aimed to assess the margin integrity and secondary caries susceptibility of lined and non-lined composite resin and GH restorations. We hypothesized that lining materials significantly decrease margin integrity of composite restorations after selective excavation, and we also assumed that GH would have significantly lower margin integrity than composite. In line with this, we hypothesized that mineral loss of biofilm-induced secondary caries lesions is significantly larger adjacent to lined compared with non-lined composites and with GH compared with composite.

METHODS AND MATERIALS

Experimental Setup

We compared four groups of restoration strategies: (1) Resin composite without liner, (2) resin composite

(RC) on top of a setting CH liner placed at pulpal cavity walls, (3) RC placed on top of a non-setting CH, and (4) GH. We assessed three parameters. First, margin integrity after thermomechanical cycling was determined using replica analysis via scanning-electron microscopy. Second, restored teeth were then submitted to a continuous-culture single-species biofilm model, and bacterial numbers in biofilms developed on margin areas were enumerated. Last, secondary lesions were assessed using microradiography.

Specimen Preparation

A sample size of 12 teeth per group had been determined a priori based on a previous study using a similar design.²⁴ Forty-eight extracted human upper second premolars obtained with informed consent under an ethics-approved protocol (EA4 102/14) were selected according to their mesial-distal (mean [range] = 6.5 [6.3/6.7] mm) and buccal-lingual width (8.7 [8.5/8.9] mm), with a maximum deviation of 0.2 mm from the means in each dimension set as the limit. Teeth were cleaned, examined for cracks, and embedded (Technovit 4071, Heraeus Kulzer, Hanau, Germany) in chromed brass tubes (15 mm length, ϕ 15 mm; Bauhaus, Belp, Switzerland) 2 mm below the cemento-enamel junction using a gauge. Standardized cavities were prepared using water-cooled copy-milling (Celay, Mikrona, Spreitenbach, Switzerland). Minor adjustments were performed with rotating instruments. Cavity surfaces were controlled for cracks or abnormalities using a stereomicroscope (Stemi Zoom, Zeiss, Oberkochen, Germany). Teeth were then covered with nail varnish (43K, Manhattan, Mainz, Germany), with two rectangular windows (1.5 \times 2 mm) left unprotected on the mesial and distal pulpal-axial walls (Figure 1).

Teeth were submitted to an established protocol to induce artificial residual caries lesions resembling those remaining after selective excavation using the criterion of leathery dentin remaining in proximity to the pulp.²⁵ Briefly, teeth were exposed to an acetic acid solution containing 50 mM acetic acid, 3 mM $\text{CaCl}_2 \times 2 \text{ H}_2\text{O}$, 3 mM KH_2PO_4 , and 6 μM methylhydroxy-diphosphonate (pH 5.30, 37°C) for 12 weeks. Depths and mineral loss of induced lesions were determined on 10 random samples via microradiography (see the sections that follow) after the conclusion of the experiment. Artificial residual lesions had a mean (standard deviation) depth of 76 (27) μm and a mean ΔZ of 1575 (736) vol% $\times\mu\text{m}$.

After demineralization, the nail varnish was mechanically removed, and surfaces were checked again. A Tofflemire matrix was placed, and cavities were restored according to one of four protocols:

1. Tetric EvoCeram RC (Ivoclar Vivadent, Schaan, Liechtenstein), placed incrementally in laminate technique, after conditioning the cavity using phosphoric acid 37% (Orbis, Münster, Germany) and chlorhexidine 2% (Charité Hausapotheke, Berlin, Germany) for rewetting, and placement of OptiBond FL (Kerr Italia, Salerno, Italia). Adhesive treatment was performed as follows: primer application for 30 seconds, evaporation of the solvent, and application of bond. After each increment, light-curing was performed for 20 seconds using a light-emitting diode curing light (Valo, Ultradent, Salt Lake City, UT, USA) with an intensity of 1400 mW/cm².
2. Tetric Evo Ceram, as described, with non-setting CH (UltraCal XS, Ultradent, Cologne, Germany) placed prior to cavity conditioning as a liner (RC_NCH). CH was only placed on the artificial lesion and was covered with a thin layer of Vitrebond Plus (3M ESPE, St Paul, MN, USA) which was light-cured for 20 seconds.
3. Tetric EvoCeram, as described, with setting CH (Kerr Life, Scafati Salerno, Italy) being used as liner (RC-SCH), which was also covered with Vitrebond Plus before restoring the cavity.
4. GH (Equia Forte, GC, Tokyo, Japan), placed in a bulk, after conditioning with polyacrylic acid (Equia Cavity Conditioner, GC), according to manufacturer instructions. Afterward, GH was covered with resin coating (Equia Coat, GC) on the occlusal surface, followed by light-curing for 20 seconds. Note that gingivocervical margins were not covered, as this would not be clinically possible in most circumstances.

The restorations were polished (SoFlex and Greenie/Brownie, Henry Schein, Melville, NY, USA) after placement and checked under loupes and a stereomicroscope (Stemi Zoom).

Thermomechanical Cycling

Teeth were submitted to thermal cycling of 10,000 cycles between 5°C–55°C, with 12 s/30 s dwell/equilibration time, respectively, in distilled water (liquid cycler, Haake, Karlsruhe, Germany). Afterwards, 1.2×10^6 mechanical cycles were performed,²⁶ with a ceramic ball (ϕ = 5 mm; Steatite, Hoechst, Wunsiedel, Germany) being loaded onto the occlusal surface 1.5 mm with a load of 5 kg²⁷ in a

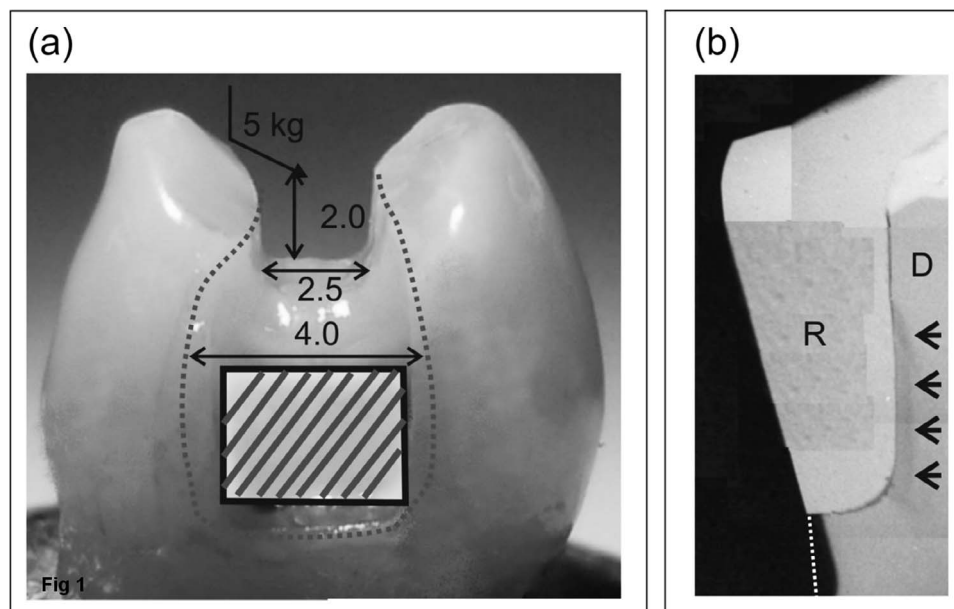


Figure 1. Specimens. (a): Standardized cavities were prepared using copy-milling. Since teeth were chosen according to their buccal-lingual and mesial-distal dimensions, the remaining cuspal-supporting hard tissue was standardized as well. Cervical margins were located 1.0 mm above (mesial) or below (distal) the cemento-enamel junction. Artificial rectangular lesions (1.5×2 mm) were created at pulpo-axial walls (hatched grey area). After placement of a composite restoration (dotted line), teeth were submitted to mechanical cycling using a ceramic ball loaded 1.5 mm below the cuspal aspect on the inner incline of the palatal cusp with a force of 5 kg (black arrow). (b): Microradiograph of a restored sample. The restoration (R), dentin (D), and the artificial residual lesion (arrow heads) are shown. On the outer dentin surface, a secondary caries "outer" lesion induced during biofilm challenge is detected (the dotted line indicates the original outline of dentin). Note that the microradiograph is composed of several single images, leading to (quadrangular) artifacts (as can be noted mainly in the restoration area).

dual-axis chewing simulator (Kausimulator CS-4.8, Willytech, Feldkirchen-Westerham, Germany) in distilled water.

Quantitative Margin Analysis

Gingivocervical margins were assessed using scanning electron microscopy as described.²⁸ Briefly, margins were replicated using polyvinyl-siloxane impression material (Honigum, DMG, Hamburg, Germany) and epoxy resin (Stycast 1266, Henkel, Westerlo-Oevel, Belgium), and assessed both directly after finalization of the restoration and after thermomechanical cycling. Margin adaptation was evaluated at $200\times$ magnification (AMRAY 1810, Amray, Bedford, MA, USA) using defined criteria²⁹: Perfect margin, irregularities or submarginations, microgaps (hairline cracks), distinct gaps >5 μm , and overhangs/positive ledges.

Secondary Caries Model

To assess the susceptibility for caries adjacent to the restoration, teeth were covered using nail varnish, leaving a window (2×2 mm) on the mesial and distal gingivocervical margins uncovered. A computer-controlled continuous-culture biofilm model³⁰ was used for bacterial demineralization. Overnight cultures of *Lactobacillus rhamnosus* (DSM 20021) were

prepared in deMan-Rogosa-Sharpe medium at 37°C . Teeth and bacterial suspension were daily incubated within the biofilm chamber at 100% humidity at 37°C for 30 minutes. After a 1-hour pause, the cycle started: specimens were provided with a flow of 100 mL sterile MRS supplemented with 2% sucrose and amphotericin B for 45 minutes, followed by 200 mL sterile defined mucine medium³¹ for 30 minutes using peristaltic single-canal pumps (Type PR1, Seko, Mainz, Germany) with a resting period of approximately 6 hours. Each cycle was performed five times a day. Once a day, 250 ppm sodium fluoride rinses were provided for 10 minutes. After 14 days, cultivation was concluded, and biofilms on the defined windows were removed, transferred to 0.9% sodium chloride, serially diluted from 10^{-1} to 10^{-7} , and plated onto deMan-Rogosa-Sharpe agar for enumeration of colony-forming units after 48 hours. Induced lesions adjacent to restorations were assessed using transversal microradiography.

Transversal Microradiography

Teeth were embedded in acrylic resin (Technovit) and divided midsagittally through the induced demineralized lesion. Two thin sections (200 μm) per tooth were prepared (band saw 300cl; Mikroschleifsystem 400 CS, abrasive Paper 800 and 1200). A nickel-filtered copper X-ray source (PW3830,

Pananalytical, Kassel, Germany) operating at 20 kV and 20 mA was used to obtain radiographs. Films (35 mm B/W positive, Fujifilm, Tokyo, Japan) were exposed for 10 seconds and developed under standardized conditions according to the manufacturer's recommendations. Microradiographs were analyzed using a digital-image-analyzing system (CFW 1312M, Scion, Frederick, MD, USA) interfaced with a universal microscope (Axioplan 60318, Zeiss, Oberkochen, Germany) and a personal computer (Transversal Microradiography for Windows 5.25, UMCg, Groningen, The Netherlands), with integrated mineral loss (ΔZ) being assessed. We controlled for the presence of both outer and wall lesions,³² but no wall lesions could be found. In case the lesion was heterogeneous, for example, deeper in proximity to the restoration, the deepest section of the lesion was analyzed. Of the two assessed lesions per margin, means were calculated for further analysis.

Statistical Analysis

Data were analyzed using SPSS 20 (IBM, Armonk, NY, USA). Normal distribution was checked using the Shapiro-Wilk test. Group-wise comparisons were performed using the Mann-Whitney U test. Level of significance was set at $\alpha=0.05$.

RESULTS

Regarding margin integrity (Figure 2), GH and RC_NCH showed significantly higher proportions of irregularities along enamel margins than RC and RC_SCH ($p<0.05$; Mann-Whitney U test). GH also showed significantly more gaps along dentin and enamel margins than alternatives ($p<0.05$).

Bacterial numbers (Table 1) did not differ significantly between groups ($p>0.05$).

Mineral loss of secondary caries lesions was significantly higher in dentin than enamel ($p<0.05$) but did not differ significantly between groups (Figure 3). No wall lesions were detected in any of the groups, that is, all lesions were outer lesions.

DISCUSSION

When treating deep lesions, dentists are challenged to manage pulpal vitality (which is why many place cavity liners for pulp protection) and to restore the resulting extended cavity.¹¹ The latter is especially relevant when performing selective carious tissue removal, leaving soft residual carious dentin in proximity to the pulp, which might compromise the

margin integrity of restorations and therefore affect treatment success or tooth/restoration survival. The present study investigated whether cavity liners affect the integrity of composite restorations and assessed the suitability of a new material class, GHs, for restoring cavities after selective excavation. We found GHs to have relatively poor gingivocervical margin integrity, with a large proportion of irregularities or distinct gaps. Moreover, we found non-setting CH lining to detrimentally affect margin integrity. We thus accept our first hypothesis. However, and clinically probably more relevant, materials did not differ significantly in their susceptibility for secondary caries or biofilm accumulation in cervical areas. We therefore refute our second hypothesis.

The observed margin integrity behavior of GHs is closely related to the composition of the material, which is largely unpolishable. In conventional GICs, this leads to unacceptable wear, which is why these materials are not recommended for permanent restorations in most countries. For GHs, the load-bearing surface is covered with a methacrylate resin, which has been found to significantly decrease wear.³³ However, this resin is not applied on proximal surfaces, which is why certain margin irregularities are most likely unavoidable in gingivocervical proximal margins. However, we also found significant proportions of distinct gaps after thermocycling, which might indicate local debonding. In contrast, non-lined RC showed a perfect margin integrity, with only few teeth showing any imperfections along the margin in both enamel and dentin. This once more confirms that excellent bond and restoration integrity can be achieved after selective excavation with current adhesive systems. Placing a non-setting CH liner seems to negatively affect margin integrity, with a large proportion of enamel and dentin margins showing irregularities. It seems that the permanently soft lining material decreases the mechanical support of the composite restoration, leading to a possibly different bending behavior under load and eventually decreased margin integrity. It should be noted that this was found even while liners were placed only on the lesion area, that is, very locally, which might not always be clinically possible. This possible detrimental effect of CH is especially remarkable, as the positive attributes of CH lining, such as remineralization or antibacterial effects, are also increasingly questioned.^{8,34-37} Thus, CH lining could have only very limited benefit for the pulp but negative effects on the restoration,³⁸⁻⁴⁰ while requiring an additional

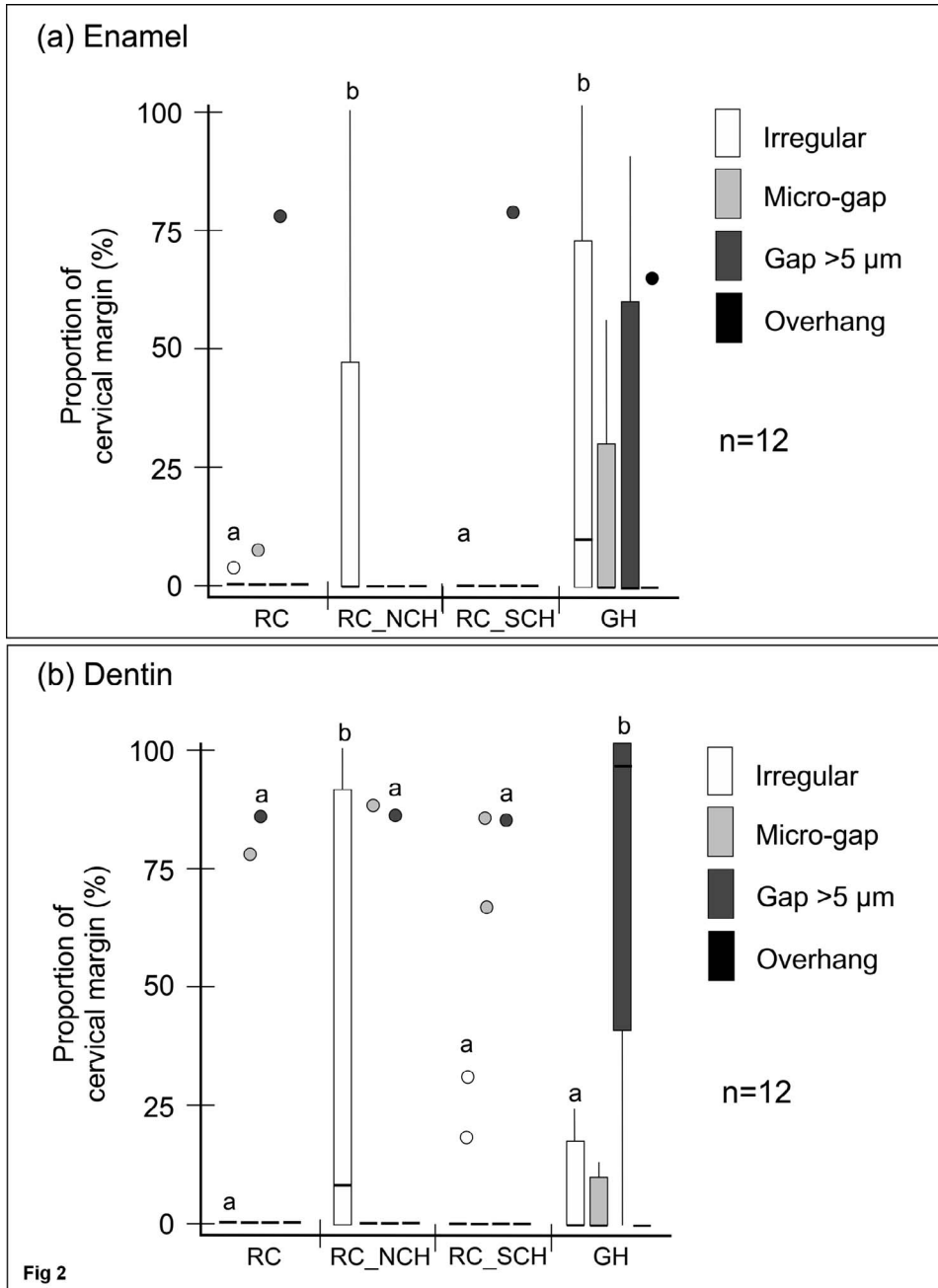


Figure 2. Margin integrity of different restorations after thermomechanical cycling. Gingivo-cervical margins in (a): enamel and (b): dentin were assessed for their integrity, with the proportion of irregular margins (white), margins with microgaps (light grey), or margins with distinct gaps (dark grey) as well as overhangs/positive ledges (black) being quantified. Note that the percentage of perfect margins is not given, as it results from these four margin categories. Different superscript letters indicate significant differences between groups ($p < 0.05$, Mann-Whitney U test). Letters are only provided for categories with significant differences between groups. n =number of specimens per group. Box and line: Interquartile range and median; whiskers: range; circles: outliers. GH, glass hybrid; RC, resin composite restoration without any liner; RC_NCH, resin composite restoration with a non-setting calcium hydroxide liner; RC_SCH, resin composite restoration with a setting calcium hydroxide liner.

treatment step, that is, increased efforts. GH, in contrast, is easy to apply even under moderately adverse conditions, and was found to provide significant remineralization via fluoride and strontium release.⁴¹

Such release could also exert antibacterial effects and reduce bacterial colonization along margins and prevent or slow down the development of secondary lesions. We did not find such secondary caries preventive effect of GH. In our study, margin behavior generally did not correlate with mineral

loss of lesions adjacent to restoration. It seems that while microscopic margin integrity assessment is a sensitive technique to compare materials, it has limited external validity. As shown in a previous study, the observed irregularities or interfacial gaps do not translate into the development of true “secondary” lesions (constituting an outer and a wall lesion). Instead, all induced lesions were outer lesions adjacent to a restoration, possibly without any causal association with the restorative material or integrity.^{32,42} This, however, might be specific to

Table 1: Bacterial Numbers in Biofilms at Restoration Margins ^a		
Group	Enamel	Dentin
RC	8 (3/43)	26 (8/79)
RC_NCH	7 (4/26)	13 (9/19)
RC_SCH	11 (5/21)	11 (9/46)
GH	18 (13/49)	28 (20/43)

Abbreviations: GH, glass hybrid; RC, resin composite restoration without any liner; RC_NCH, resin composite restoration with a non-setting calcium hydroxide liner; RC_SCH, resin composite restoration with a setting calcium hydroxide liner.

^a Biofilms were assessed at gingivocervical enamel and dentin margins in 12 samples per group. Median and 25th/75th percentiles of $10^5 \times$ colony-forming units are given. Colony-forming units did not differ significantly between groups ($p > 0.05$, Mann-Whitney U test).

the present study or *in vitro* investigations, where margin integrities are overall relatively high, that is, even distinct gaps measure only few micrometers. It might be that larger gaps are required for the formation of a proper “wall lesion”^{2,3,43} via leakage of bacteria or acids along the restorative interface.^{32,44} Recent studies also highlighted that masticatory loading might influence secondary caries lesion induction, possibly via a “pumping effect.” No such loading was provided in our study during the biofilm phase. Thus, in a clinical setting, true secondary lesions could be more likely.⁴⁵ In such a setting, long-term margin integrity and secondary lesion induction will differ not only between materials but also between patients due to individually different load strengths and caries risks/⁴⁵⁻⁴⁹

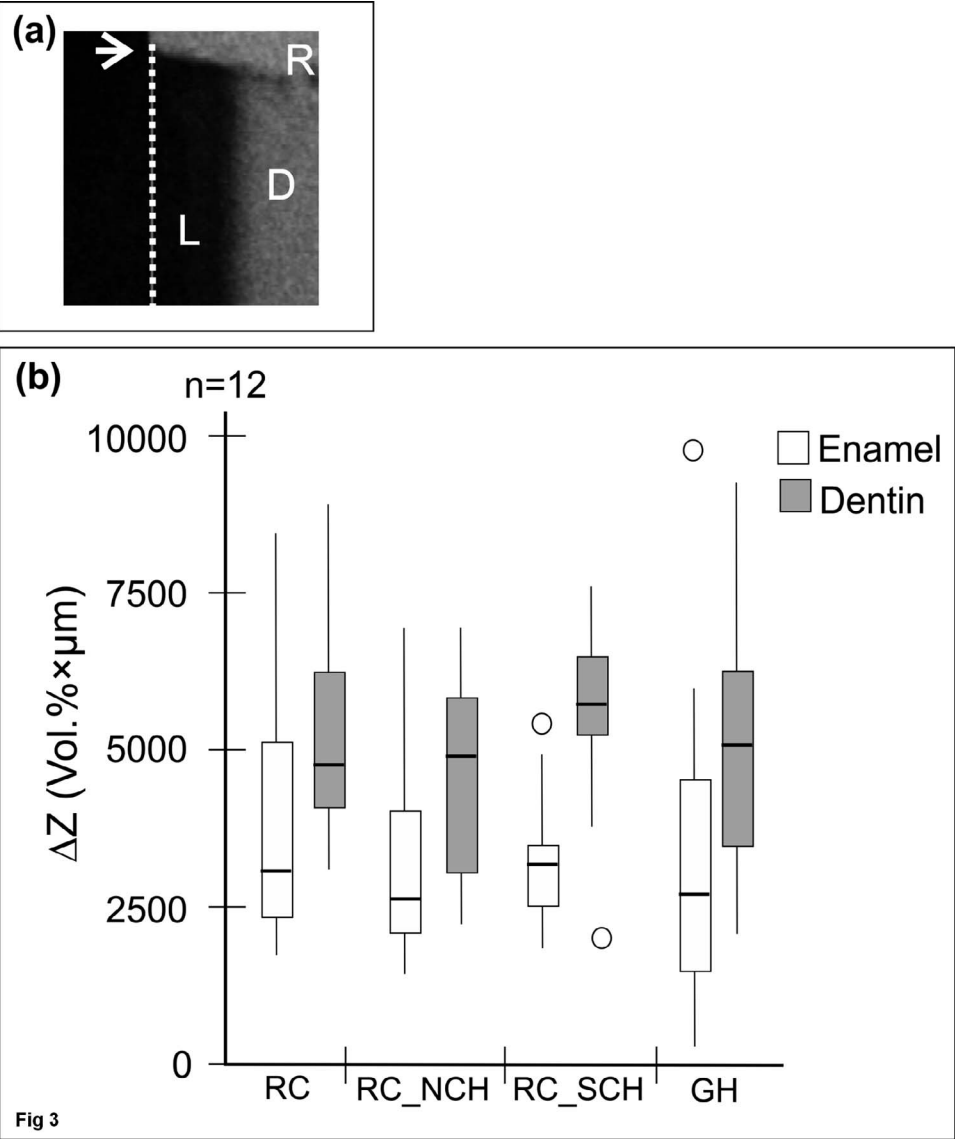


Figure 3. Mineral loss adjacent to restorations. (a): Microradiographs were analyzed for mineral loss adjacent to restorations (R). We analyzed lesions in enamel and dentin (D), the latter being shown here. The dotted line indicates the lesion surface. In no sample was a wall lesion; all lesions were outer lesions (L). (b): Mineral loss (ΔZ) of enamel (white) and dentin (grey) carious lesions adjacent to restorations. No significant differences between groups were identified ($p > 0.05$; Mann-Whitney U test). n =number of specimens per group. Box and line: Interquartile range and median; whiskers: range; circles: outliers. GH, glass hybrid; RC, resin composite restoration without any liner; RC_NCH, resin composite restoration with a non-setting calcium hydroxide liner; RC_SCH, resin composite restoration with a setting calcium hydroxide liner.

The present study has a number of limitations. First, the cavities were standardized via copy-milling, which reduces variability between samples but results in cavities that might be less undermining and extended than real-life deep cavities. Moreover, liner application was not fully standardized, which could have contributed to the observed variance. Second, while the protocol used to induce residual lesions has been found to generate lesions with similar mechanical and mineral loss characteristics as natural residual lesions,²⁵ the experimental setup does not account for any remineralization from the pulp (we did not investigate mineral gains induced by restorative or lining materials in this study). Such remineralization could alter mechanical properties with time (which would not change bond strengths but the support of the restoration against mechanical loading). Thus, the external validity of the chosen setup is limited. Similarly, we simulated premolars, where most lesions occur proximally first. In this case, pulp exposure is most likely at pulpo-axial walls, which is where residual carious dentin was simulated. If lesion location is different, for example, occlusal, or the excavation is terminated earlier than simulated by our protocol (which would result in more soft dentin being left beneath the restoration), one might expect different material behaviors. Our findings thus cannot be transferred to such situations. Third, the used biofilm model was comprised of *L. rhamnosus* mono-species biofilms only, while multispecies biofilms or *in situ* analysis might yield more realistic findings. Additionally, mineral loss was not assessed along the whole margin, only in two sections of each tooth, which do not necessarily represent the area of greatest marginal imperfection. Fourth, our findings of statistical nondifference between materials with regards to secondary caries might be attributed to a lack of power. However, considering that for reaching significant differences in mineral loss in enamel, 4500 samples would be needed according to power calculations, while 87 specimens per group would be required to demonstrate significant differences in dentin, the clinical relevance of these differences might be limited. Last, we only assessed one restorative parameter, margin integrity and the resulting susceptibility for biofilm formation and secondary caries. Future studies should compare differently restored teeth for static fracture or fatigue resistance and complement these analyses with finite element modeling to allow a better understanding of the effects of lining among other issues.

CONCLUSIONS

In conclusion, GH or composite restorations that were lined with non-setting CH showed inferior margin integrity compared with non-lined composites in cavities resulting from selective excavation. No such disadvantage was shown for setting CH. Differences in margin integrity did not translate into different susceptibility for secondary caries. Clinical decision making should weigh the efforts for placing liners and different restoration materials against the resulting pulpal and restorative outcomes.

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Regulatory Statement

This study was conducted in accordance with all the provisions of the local human subjects oversight committee guidelines and policies of Charite Berlin. The approval code for this study is EA4 102/14.

Conflict of Interest

The authors of this manuscript certify that they have no proprietary, financial or other personal interest of any nature or kind in any product, service and/or company that is presented in this article.

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