Effect of decontamination procedures on marginal and internal adaptation in saliva contaminated resin composite restorations

Eftychia Pappa (effiepappa84@yahoo.com)
National and Kapodistrian University of Athens https://orcid.org/0000-0002-1631-9871
Konstantinos Masouras
National and Kapodistrian University of Athens
Vasileios Margaritis
Walden University
Afrodite Kakaboura
National and Kapodistrian University of Athens

Research article

Keywords: saliva, marginal adaptation, adhesive, microgap, contamination.

Posted Date: June 10th, 2020

DOI: https://doi.org/10.21203/rs.3.rs-16038/v2

Abstract

Background: The aim of this study was to evaluate the effect of natural saliva contamination of dentin, on marginal and internal adaptation of resin-composite restorations performed with an etch-and-rinse adhesive and determine the efficiency of various decontamination techniques.

Methods: A hundred and twenty human molars and premolars were randomly distributed into six groups (n=20). Standardized dentin cavities (diameter: 3 mm, depth: 2 mm) were filled with a two-step etch-and-rinse adhesive and resin-composite under six different surface treatments: (1) adhesive application following manufacturers' instructions (control); (2) dentin etching, 5-s saliva, 5-s air-dry, adhesive; (3) dentin etching, 5-s saliva, 10-s waterrinse, 5-s air-dry, adhesive; (4) adhesive application/light-cure, 5-s saliva, 5-s air-dry; (5) adhesive application/light-cure, 5-s saliva, 10-s water-rinse, 5-s air-dry; (6) adhesive application/light-cure, 5-s saliva, 10-s water-rinse, 5-s air-dry, adhesive reapplication. Wall adaptation was evaluated at the upper surface and at two consecutive depth levels of 0.5 mm each, by measuring the length of debonded margins relative to the cavity periphery (%DM) and the width of maximum marginal gap (MG). Kruskal-Wallis and Mann-Whitney U were applied for statistical analysis.

Results: Statistically significant differences occurred among groups, presenting inferior adaptation after contamination took place (0.05 significance level). Group (1) at the upper surface presented the most satisfactory adaptation, whereas, groups (4), (5) had the significantly poorest adaptation in all surfaces. Groups (2), (3) and (6) revealed no statistically significant differences in comparison to group (1).

Conclusion: Saliva contamination resulted in deterioration of marginal and internal adaptation when it occurred after the adhesive application. Reapplication of the adhesive restored DM and MG values.

Background

A reliable and stable bond between resin-composite and tooth structure is needed in order to achieve an optimal clinical performance. The importance of moisture and contamination control is highlighted throughout the dental literature and all efforts are focused on keeping the adhesive substrate free of oral contaminants such as saliva, blood, intersulcular fluid and handpiece oil. Inadequate isolation of the operating field jeopardizes the restoration quality and longevity and can possibly lead to consequences such as microleakage, marginal discoloration, postoperative sensitivity, caries and pulpal irritation. [1]

The use of rubber dam is recommended for proper isolation and prevention of cavity contamination, among many other benefits (improved visibility, prevention of aspiration, cross-infection control, retraction and protection of soft tissues). Nevertheless, carious lesions are often located in areas where it is difficult to achieve proper moisture control, especially when the site is near or at the gingival margin. Clinicians often encounter difficulties in adequate isolation of the operating field in cases when the cavity margins are located in dentin such as the cervical margins of class V and class II restorations, in pediatric patients with poor cooperation and when seating indirect restorations with subgingival margins. [2]

The effect of enamel saliva contamination on the bond strength of adhesive systems has been previously investigated and studies concluded that oral fluids significantly reduce the bond strength. [3] Compared to enamel, the dentin structure is more complex and despite many investigations have evaluated the effect of saliva contamination on the adhesive interface, results are considered to be inconclusive. A number of studies reported a significant reduction of bond strength between dental adhesives and saliva-contaminated dentin substrate [4, 5, 6] whereas others found no significant differences between contaminated and non-contaminated dentin substrate. [7]

Shear and microtensile bond tests have been usually performed in order to demonstrate the effect of saliva contamination on the quality of adhesive bonding. [4, 7] Additionally, the effect of contamination on the longevity of bonded restorations has been evaluated through microleakage tests. [6] Despite the significant number of studies, there is no clear conclusion whether contamination negatively affects the adhesive interface or not. A variety of influencing factors seems to complicate the interpretation of the results. Such factors are the composition of adhesive systems, the duration of the contamination and the decontamination treatment of the substrate. Moreover, to the best of our knowledge, there is only one study on how saliva contamination affects the adaptation of adhesive restorations and the formation of microgaps in the adhesive interface. [5]

The aim of this study was to evaluate the effect of natural saliva contamination of dentin, on marginal and internal adaptation of resin-composite restorations performed with a two-step etch-and-rinse adhesive, before and after adhesive curing and also to determine the efficiency of various decontamination techniques. The null hypothesis tested was that the adaptation of resin-composite restorations on dentin walls would not be affected by saliva contamination or by the decontamination techniques applied.

Methods

The experimental protocol applied has been approved by the local Committee of Ethics according to the guidelines provided by the World Medical Association Declaration of Helsinki (Ref 180A/23.3.2018). Written informed consent was obtained from all participants whose teeth were used in the study. One hundred and twenty freshly extracted caries-free human molars and premolars were selected and stored at 4 °C in 0.5 % chloramine-T containing distilled water. The teeth were examined to reassure absence of cracks, fissures or any type of restoration. Afterwards, they were embedded in epoxy resin (Epofix; Struers, Denmark) and subsequently, occlusal enamel was removed with a low speed diamond saw under copious water cooling (Isomet; Buehler ~~Ltd~~, Lake Bluff, IL, USA) and exposed dentin was ground wet with a 600 grit SiC paper.

Standardized cylindrical dentin cavities (diameter: 3 mm, depth: 2 mm) were prepared using a 3-mm-diameter diamond bur (Proxxon, Niersdorf, Germany) on a micro-milling machine (MF70 Micro-Miller; Proxxon, Niersdorf, Germany) under copious water cooling. Dentin cavities were examined under 200× magnification to ensure absence of pulp exposure. All cavities were filled with a two-step etch-and-rinse adhesive and resin-composite (Table 1, **Fig. 1**). The resin-composite was placed in a single layer, covered with a mylar strip, pressed against a transparent cover slip and photopolymerized for 40 seconds with a light-curing unit of 850 mW/cm^2 light intensity (Elipar Trilight 3M/ESPE; Seefeld, Germany). The restorations were ground gradually with a series of silicon carbide papers of 320-1000 grit size (Struers, Denmark) under wet conditions until exposure of the dentin cavity margins. Inspection was made after removing the surface debris with copious amounts of water.

Fresh natural saliva was collected near the time of contamination, provided by a single donor, after brushing, flossing and chewing a paraffin gum for 5 min prior to saliva collection. Saliva was collected in a sterile beaker and was used immediately. Each time, 15 μl of saliva were collected using a micropipette and applied on the substrate. The saliva was left undisturbed for 5 seconds (s) before each decontamination technique was applied.

Teeth were randomly distributed into six groups of 20 each and a different surface treatment was accordingly applied:

- Group 1: adhesive application following manufacturers' instructions (control);
- Group 2: dentin etching, 5-s saliva, 5-s air-dry, adhesive;
- Group 3: dentin etching, 5-s saliva, 10-s water-rinse, 5-s air-dry, adhesive;
- Group 4: adhesive application/light-cure, 5-s saliva, 5-s air-dry;
- Group 5: adhesive application/light-cure, 5-s saliva, 10-s water-rinse, 5-s air-dry;
- Group 6: adhesive application/light-cure, 5-s saliva, 10-s water-rinse, 5-s air-dry, adhesive reapplication.

Wall adaptation was determined at the upper surface and at two consecutive depth levels of 0.5 mm each, after gradual grinding of each sample (**Fig. 1**); the dimensions were checked with a digital caliper. The specimens were observed under an optical microscope at 200x magnification and the parameters measured were the length of the debonded margins (DM) relative to the cavity periphery (%DM) and the width of the maximum marginal gap (MG) (**Fig. 2**). The microscope used was a reflected light optical microscope (ME 600 Eclipse; Nikon-Kogakou, Japan) and the measurements were carried out by an image analysis software (Sigma Scan 4; Jandel Scientific, CA, USA). Images were recorded with parallel and crossed polarizers, to differentiate the presence of entrapped debris at the interface which might impose a bias on the results.

Since %DM and MG values were not normally distributed, Kruskal-Wallis and Mann-Whitney U post-hoc tests were used to investigate the possible influence of saliva contamination and the different decontamination techniques before and after the adhesive polymerization on the marginal and internal adaptation. All reported probability values (p-values) were compared to a significant level of 0.05. The analyses of coded data were carried out using IBM SPSS software version 21.0.

Results

Kruskal-Wallis test revealed statistically significant differences among the six groups, for both parameters tested at the 0.05 significance level. Mann-Whitney U multiple comparison tests were used to identify the relations among the experimental groups.

Group 1 was the only one that exhibited excellent marginal adaptation with absolute absence of microgaps at the upper surface of the restoration (level 1). However, no statistically significant differences were revealed between group 1 and each of groups 2, 3 and 6 for %DM and MG values per depth level. Concerning the comparison among groups 2, 3 and 6, no differences were noticed for all recordings. Groups 4 and 5 showed statistically the highest %DM and MG values, at all three depth levels, without differences between them.

Fig. 3 and 4 show representative images of restorations with perfect marginal adaptation and debonded margins, respectively.

Results from the measurement of the length of the debonded margins relative to the cavity periphery (%DM) and the width of the maximum marginal gap (MG) are summarized in Tables 2 and 3, respectively.

Discussion

A gap-free restoration is considered as a key factor for the optimal clinical performance of resin-composite restorations. [8, 9, 10, 11, 12] Despite the fact that there is no threshold gap-size for the occurrence of secondary caries, the presence of marginal defects provides potential pathways for bacteria penetration and as a consequence, jeopardizes longevity and clinical success of adhesive restorations. [13]

According to the results of the present study, the null hypothesis was rejected since both marginal and internal adaptation of resin-composite restorations on dentin walls were found to be negatively affected by the presence of saliva, when contamination took place after the adhesive application. Reapplication of adhesive restored adaptation and could be considered as the decontamination technique of choice. On the other hand, the saliva contamination that occurred after acid-etching and before adhesive application did not adversely affect the adaptation in a significant level. Air-drying for 5 seconds appears to be sufficient in such cases, with no need for water-rinse of the operation field.

An important outcome of the present study was that %DM and MG values were not significantly affected when saliva contaminated the etched dentin and following removed either with air or with water. These findings are in agreement with other studies which showed that adhesion was not negatively affected when the duration of contamination was between 5 and 15 seconds. [5] [7] [14] [15] [16, 17, 18] Notably, in 2-step etch and rinse adhesives, saliva contamination did not prevent hybrid layer formation or the resin penetration into the dentin tubules. [7] For extended contamination time (60 seconds), re-etching of the contaminated dentin effectively removes contamination, but it appears to also deteriorate the quality of adhesion due to the collapse of denatured collagen fibrils, increased thickness of the collagen layer and inadequate hybridization. [19] [20]

Original and internal adaptation. These results are consistent with a similar study on contamination, where it was shown that salivary contamination after light-curing of the adhesive reduced the shear bond strength of a two-step etch-and-rinse adhesive system to about 50% of control values. [21] It was hypothesized that the absorbed glycoproteins onto the poorly polymerized adhesive surface, inhibit adequate copolymerization. Another possible explanation suggests that rinsing and air-drying provides a collapsed layer of resin deprived collagen which cannot be effectively penetrated. [20, 22]

Conversely, several studies concluded that there was no adverse effect on bond strength values of two-step self-etching adhesives when contamination occurred after light-curing. [23, 24] This can be possibly explained by the simultaneous demineralization and infiltration achieved by these systems. Self-etching adhesives contain acidic monomers combined with hydrophilic monomers that simultaneously etch and prime dentin. Hydrophilic and acidic dentin adhesives are considered to be less sensitive to the presence of saliva and researchers have concluded that bond strength values to contaminated dentin are not negatively affected. [25] [7]

As far as the width of the formed gaps is concerned, mean values ranged between 0 and 30.74 μm, which are similar to the ones reported elsewhere. [10] At 0.5 mm in-depth level, groups 2 and 4 present particularly high SD values. At those groups, two extreme gaps were measured, which could be possibly attributed to false technique application. The presence of too much moisture on the substrate can create small, blister-like voids which appear like gaps in the interface and which have an impact on the performance of the adhesive material. Incomplete hybridization of the demineralised dentin leaves exposed collagen fibrils and causes nanoleakage through gaps of 20-100 nm size. [6]

Numerous factors are involved to the debonding phenomenon either during bond development or during the oral service of a restoration. Although several studies [4, 7] [6] [5, 14] have previously investigated the effect of saliva contamination on the adhesive interface, there is no sufficient evidence regarding the effect of saliva contamination on the adaptation of adhesive restorations. Parameters such as the extent of the debonded margin and the width of the marginal gap determine the quality of the adhesive interface and the ability of the adhesive system to prevent marginal debonding. [26] The marginal adaptation of the restorations is not necessarily similar to the internal adaptation to the cavity walls; therefore, in the present study, the internal adaptation was additionally evaluated as a factor with crucial contribution to the long term good clinical performance of the restorations. [12] In the current study, the cavity configuration factor was the same for all samples and only one adhesive system and resin-composite were used, to avoid interference by the aforementioned factors. Consequently, differences could be attributed to the different stages of contamination and decontamination techniques applied.

As already stated, the vast majority of previous studies on saliva contamination evaluate the effect of contamination on bond strength. [4, 7, 11] In the present study, marginal adaptation and gap formation were evaluated in order to obtain information concerning the sealing ability of the adhesive system on the contaminated dentin. Under the clinical point of view, saliva contamination is possible to occur either after the etching procedure and before the adhesive application or after the completion of the adhesive placement. Both conditions were investigated in the current study. As an experimental procedure, marginal and internal adaptation is considered to be laborious and time-consuming technique. It often yields false negative results and requires a large number of specimens in each group because of the high variability of the values. Despite the above limitations, it is thought to be more clinically relevant to evaluate the capability of an adhesive to maintain the tooth-restoration interface sealed, rather than to compare bond strength values. [27, 28]

Direct optical microscopic observation of the specimens was preferred to evaluation of replicas by a scanning electron microscopy (SEM) since the first method allows measurements directly on dentin specimens instead of replicas, providing reliable phase identification and allowing evaluation of the same specimen at different levels. It is a simpler, less time-consuming and less destructive method, which avoids any potential drawbacks of the replica technique. [29]

In this study, human saliva was chosen as the contaminant, in order to achieve clinically relevant conditions, contrary to artificial saliva and substitutes which were used in other studies. [19, 24] SEM observations have shown that high-molecular-weight proteins diffuse into dentin tubules, altering the surface characteristics and competing with hydrophilic monomers during the hybridization process. [5, 25] Fresh whole saliva is considered an acceptable material to be used for contaminating testing as artificial saliva, which is deprived of macromolecules, may confound the results. [30]

Saliva was left undisturbed for 5 seconds prior to the application of any decontamination technique, with the hypothesis that this is an average time for the clinician to notice and deal with the contamination. The time span during which the contaminant interacts with the dentin surface is of significant importance, as longer contamination time results in lower bond strengths than drying the saliva quickly. [22] This observation was related to evaporation of water and formation of a thick film of glycoprotein on the contaminated surfaces. The duration of contamination varies significantly among studies and it is a factor that should be particularly considered when comparing their findings.

Conclusions

Under the limitations of this study, the following conclusions can be drawn:

- Saliva contamination negatively affects adaptation of the adhesive and resin-composite in dentin, when contamination occurs after the adhesive application. Reapplication of adhesive restores adaptation and is considered to be the decontamination technique of choice in such cases.
- When saliva contamination occurs after acid-etching and before adhesive application, there is no adverse effect on the adaptation of adhesive and resin-composite in dentin.

The findings of this study are of significant clinical relevance. The possible negative influence of saliva contamination after the adhesive application can be effectively reversed by reapplication of the adhesive.

Abbreviations

DM: debonded margins

%DM: the length of the DM relative to the cavity periphery

MG: maximum marginal gap

SEM: scanning electron microscopy

Declarations

Ethics approval and consent to participate:

The experimental protocol applied has been approved by the local Committee of Ethics according to the guidelines provided by the World Medical Association Declaration of Helsinki. Written informed consent was obtained from all participants whose teeth were used in the study.

Consent to publish:

Not Applicable

Availability of data and materials:

The datasets analysed during the current study are presented in summary in Tables 1,2,3. Spreadsheets are available from the corresponding author on reasonable request.

Competing interests:

None to declare. All authors declare no financial interest or conflict associated with this work.

Funding:

Not Applicable

Authors' Contributions:

All four authors meet the four criteria described by BMC Oral Health for authorship. EP, KM, VM and AK substantially contributed to the conception and design of the work; the analysis and interpretation of data; the revision of the draft and they all approved the final version of the manuscript to be published. This manuscript has been read and approved by all the authors. The requirements for authorship as stated earlier in this document have been met and each author believes that the manuscript represents honest work.

Acknowledgments:

Not Applicable

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Tables

Table 1: Composition and application mode of the adhesive system and resin-composite used.

	Composition	Application mode
Adhesive ExciTE F (Ivoclar Vivadent, Schaan, Liechnstein)	Phosphonic acid acrylate, HEMA, dimethacrylate, highly dispersed silicone dioxide, initiators, stabilizers and potassium fluoride, ethanol	Phosphoric acid gel was applied on dentin for 10–15 seconds and removed with a vigorous water spray for at least 5 seconds. Excess moisture was removed with an air gun leaving the dentin surface with a glossy wet appearance (wet bonding). Dentin was saturated with a generous amount of ExciTE F for at least 10 second and excess was removed with a weak stream of air, leaving a uniform, glossy appearance. ExciTE F was light-cured for 10 seconds at 850 mW/cm^2.
Resin-composite Tetric EvoCeram (Ivoclar Vivadent, Schaan, Liechnstein)	Bis-GMA, urethane dimethacrylate, ethoxylated Bis-EMA, barium glass filler, ytterbiumtrifluoride, mixed oxide, prepolymers, additives, catalysts and stabilizers, pigments	Resin-composite was placed in a single layer and photopolymerized for 40 s at 850 mW/cm^2 light intensity.

Table 2: Mean values and SD of the %DM parameter for all groups and depth levels*.

Group		DM-level1	DM-level2	DM-level3
Group 1 (Control)	Mean	$0.00^{e,f}$	$0.02^{a,e}$	$0.01^{d,e}$
	SD	0.00	0.04	0.03
Group 2	Mean	$0.01^{a,b}$	$0.02^{b,f}$	$0.02^{a,f}$
	SD	0.02	0.04	0.04
Group 3	Mean	$0.01^{c,d}$	0.03^{c}	$0.02^{g.h}$
	SD	0.03	0.06	0.04
Group 4	Mean	$0.05^{a,c,e}$	$0.06^{a,b}$	$0.08^{b,d,f,g}$
	SD	0.06	0.08	0.08
Group 5	Mean	$0.06^{b,d,f}$	$0.08^{c,d,e,f}$	$0.07^{a,c,e,h}$
	SD	0.07	0.08	0.08
Group 6	Mean	0.02^{a}	0.03^{d}	$0.03^{b,c}$
	SD	0.04	0.05	0.05

**Same letters within the columns indicate statistically significant differences among the Groups (1 to 6) for each level/column (DM1, DM2, DM3), according to post-hoc analysis, Mann-Whitney U test, $p<0.05$.*

Table 3: Mean values and SD of the MG parameter for all groups and depth levels *.

Group		MG-level1	MG-level2	MG-level3
Group 1 (Control)	Mean	$0.00^{e,f}$	$5.26^{a,d}$	$3.59^{c,d}$
	SD	0.00	13.06	9.42
Group 2	Mean	$4.49^{a,b}$	17.05^{e}	15.40
	SD	11.65	51.81	24.91
Group 3	Mean	$4.92^{c,d}$	15.31^{b}	$6.68^{e,f}$
	SD	12.51	26.89	13.95
Group 4	Mean	$29.43^{a,c,e}$	29.89^{a}	$27.89^{a,c,e}$
	SD	38.86	40.44	24.88
Group 5	Mean	$24.10^{b,d,f}$	$30.74^{b,c,d,e}$	$29.34^{b,d,f}$
	SD	28.17	26.81	26.39
Group 6	Mean	11.70	12.07^{c}	$10.52^{a,b}$
	SD	19.11	19.97	20.32

**Same letters within the columns indicate statistically significant differences among the Groups (1 to 6) for each level/column (MG1, MG2, MG3), according to post-hoc analysis, Mann-Whitney U test, $p<0.05$.*

Figures

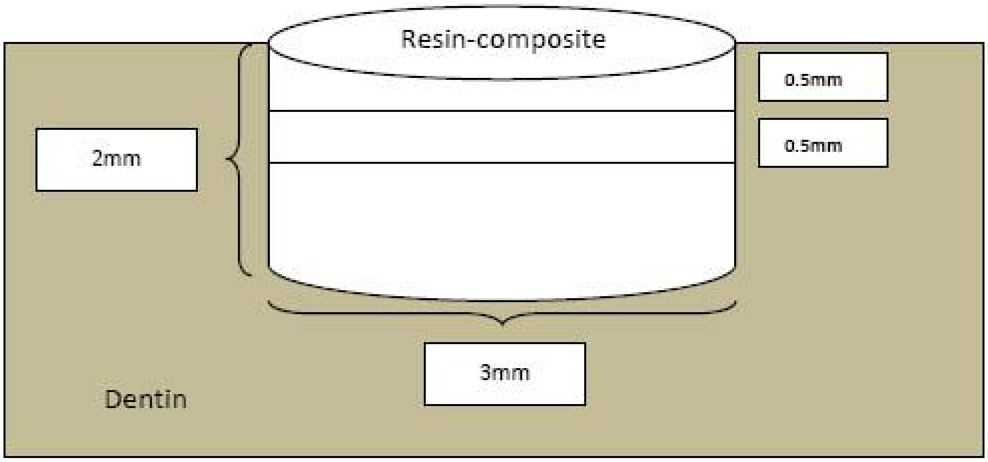

Figure 1

Schematic cross-sectional view of the specimen. Wall adaptation was evaluated at the upper surface and at two consecutive depth levels of 0.5mm each, after gradual grinding of each sample.

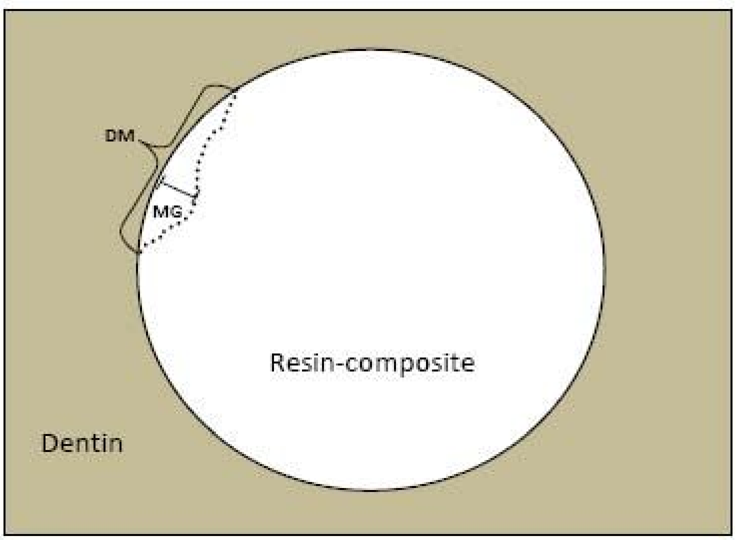

Figure 2

Schematic occlusal view of the specimen. Marginal and internal adaptation was evaluated by measuring the length of the debonded margins (DM) relative to the cavity periphery (%DM) and the width of the maximum marginal gap (MG).

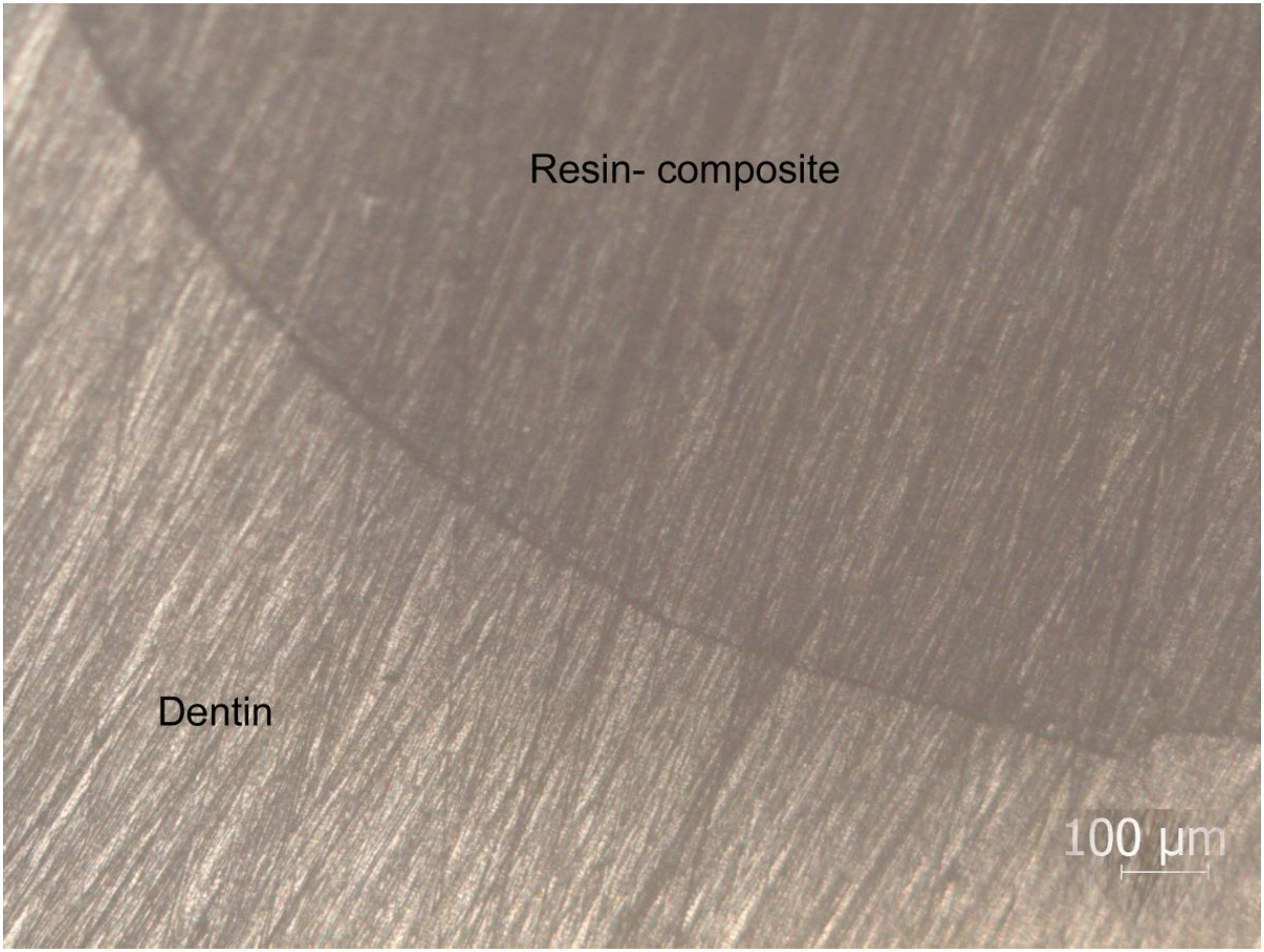

Figure 3

Restoration with perfect marginal adaptation

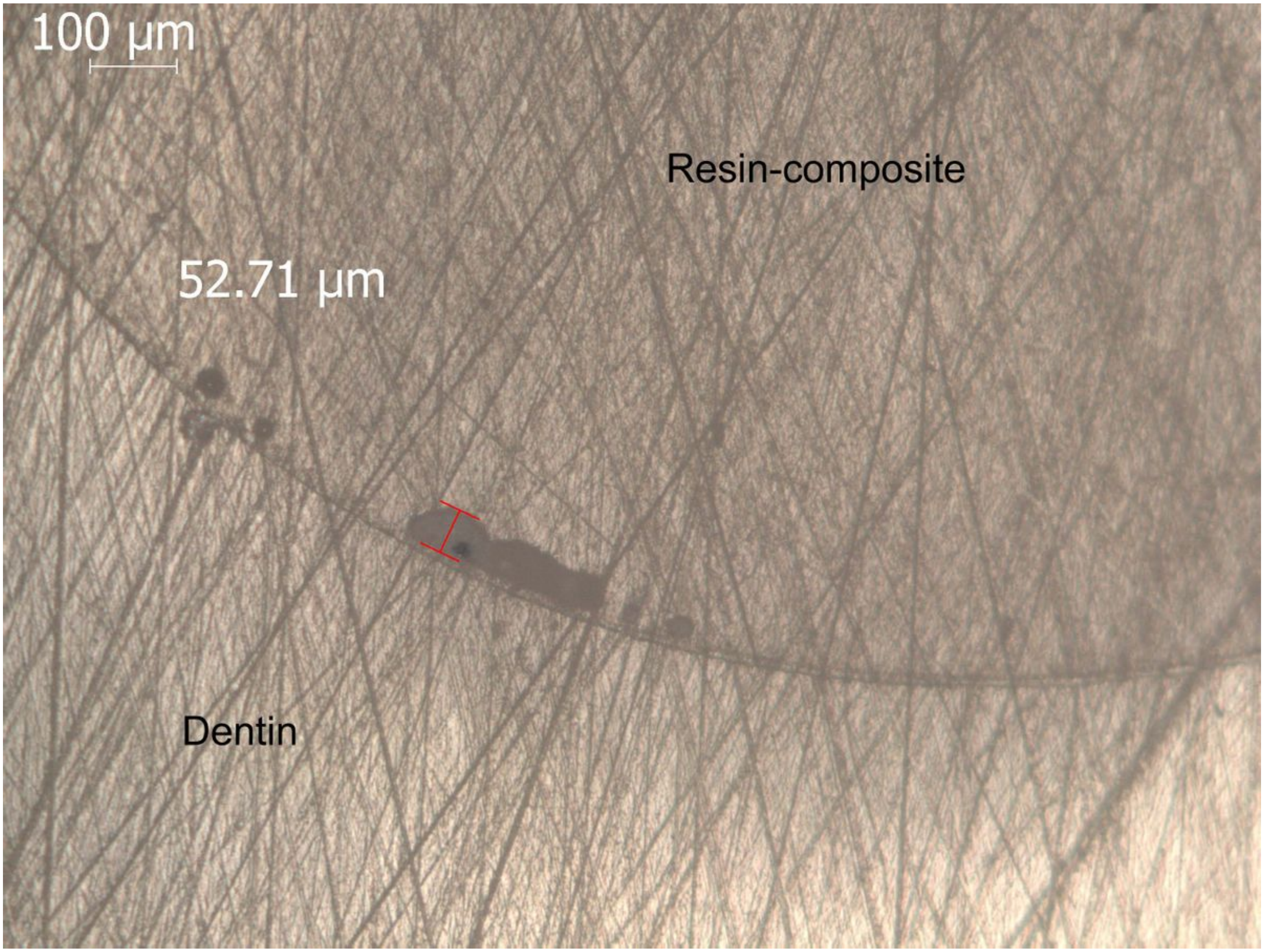

Figure 4

Restoration with debonded margins