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CLINICAL AND LABORATORY INVESTIGATIONS ON CERVICAL MARGIN RELOCATION

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PhD PROGRAM:

Medical Biotechnologies
- Section of Dental Biotechnologies -

PhD THESIS of:

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TITLE:
Clinical and laboratory investigations on cervical margin relocation

ACADEMIC YEAR 2019 / 2020

February 12th , 2020
Siena, Italy

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INDEX

Chapter 1:

Introduction

Introduction

There have been always demands made of new dental materials to create solutions for existing problems in the oral environment. Dental composites were one of these promising materials and are still in the focus of the modern dental industry. One of the most popular use of resin composites lies in restoring missing dental substrates namely: enamel & dentin, in a minimal invasive manner.

When there are small or medium sized Class I and II cavities, direct resin restorations are indicated (1, 2). In larger cavities the effects of polymerization shrinkage are a prominent factor for the longevity of the restorations (3, 4). Incremental layering (5) or low light intensity curing light~~s~~ (6) can minimize the effect of polymerization shrinkage. Potential problems due to polymerization shrinkage could promote failures in the marginal adaptation on the adhesive interfaces of the restoration, such as micro-leakage and fracture (3, 4). Micro-leakage could result in clinical symptoms such as postoperative sensitivity, marginal staining and secondary caries (7, 8). Possible ways to overcome the negative effects of polymerization shrinkage of resin composite include restoring the tooth structures with semidirect (9, 10) and indirect (11) techniques, whereby a small amount in a thin thickness of resin needs to be cured when acting as an adhesive luting material (12).

The application of adhesive indirect restorations is technique sensitive and requires precision. The procedure becomes more complicated when the location of proximal margin is below the surrounding gingival margin and is close to or below the cementoenamel junction (CEJ), often with deep cervical cavities in the proximal area (4). In the presence of these cervically located deep margins, impression taking and rubber dam placement to facilitate luting and moisture control can be difficult and sometimes impossible. In order to avoid these problems, surgical crown lengthening (13) or orthodontic extrusion (14) are viable options.

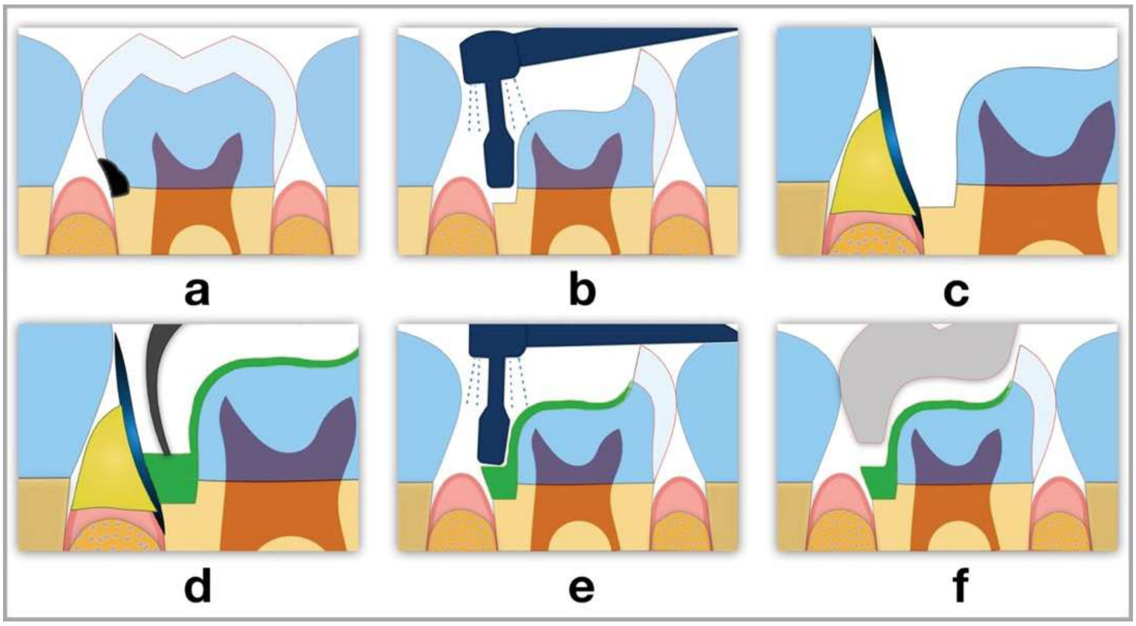

Fig 1: Schematic drawings of the CMR process. **a)** Presence of a cervically located proximal caries. **b)** Cavity preparation for an indirect adhesive restoration, the apical extent of the cavity is located below the CEJ. **c)** Matrix and wedge placement. **d)** Elevation of cervical margin coronally with a resin composite material **e-f)** Elevated cervical margin facilitates the further processes.

The use of a small amount of composite in the proximal area was proposed as an alternative procedure first by Dietschi and Spreafico (15). This clinical procedure had been later referred to in the literature as: as Proximal Box Elevation (PBE) (16-20), Deep Margin Elevation (DME) (12), Cervical Margin Relocation (CMR) (21-23), Coronal Margin Relocation (13).

Figure 1

Dietschi and Spreafico, proposed to extend the base liner composite layer to the cervical margins (15). Then, others have made investigations and published; invitro studies (16, 17, 20, 23-27), articles with the descriptions of the CMR technique (12, 18, 21, 22) and a review article (19), in order to understand the influence of an additional composite layer on the success of indirect restorations.

The application of composite base-liner has been accepted as a stress absorbing layer (24, 25, 28) and advantageous in closing under-cuts and preventing the removal of sound tooth structure (21, 22). In addition, the initial coverage of dentin structure with composite resin can be considered an advantage of the CMR technique, which is also known as dual bonding (29) or immediate dentin sealing (IDS) is usually made simultaneously with the CMR procedure (30).

REVIEW OF EXISTING LITERATURE ABOUT CMR

Review of Clinical Reports:

In the current literature a clinical article described the principles of this technique (12), and others on indirect adhesive restorations in the posterior region (13, 21, 22) primarily described the clinical procedures of the CMR technique.

Review Articles

In the literature, one paper is claimed to be a literature review–(19). Kielbassa and Philipp focused on the elevation of the cervical margins placed below the CEJ, not only for indirect but also for direct restorations. In addition, a clinical case was also documented (19).

Review of *in vitro* studies:

In the current scientific literature, a few studies investigating CMR are available (16, 17, 20, 23, 25-28, 31). The most frequently investigated parameter was the marginal adaptation of the indirect restoration at relatively low magnifications (16, 17, 20, 23, 25, 26, 28, 31). Only one study assessed the influence of CMR on the fracture behavior of the restored teeth (26) and another study evaluated the bond strength of the indirect composite restoration to the proximal box floor (27).

Marginal Adaptation:

The influence of CMR on the marginal quality of adhesively luted restorations was evaluated in eight studies utilizing the SEM and observed margins on gold-sputtered epoxy replicas of the teeth at 50x (23) and 200x magnification (16, 17, 20, 25, 26). The marginal integrity evaluation was performed in a manner consistent with previous studies: the calculation of the percentage of gap-free margins in relation to the individual assessable margin (17, 32, 33). Before and after thermo-mechanical loading (TML), the quality of the marginal adaptation was assessed (16, 17, 20, 23, 25, 26).

It has been shown in most studies that no differences existed between the marginal quality of the restorations placed directly on dentin, following a conventional luting procedure, or on composite restorations used for CMR (16, 20, 23, 25, 26). One study revealed that only after being subjected to TML, the conventional technique, which is performed directly luting on dentin, showed superiority in marginal adaptation when compared to the CMR technique (17). Additionally, in most studies, it was shown that the integration at the enamel and dentin margins (16, 17, 25) and at the onlay/luting composite interface (26) was significantly deteriorated by TML. Moreover, it was also revealed that TML did not result in any inferiority in the quality of the marginal adaptation, regardless of the materials tested (20, 23). However, it must be pointed out that the microscopic studies that evaluated the margins' integrity were performed at low magnification, too low to make final conclusions.

Why we studied CMR. What was missing in the literature?

CMR is well-known by clinicians, however it is a technique sensitive procedure. Dentists may prefer to utilize CMR instead of surgical crown lengthening or orthodontic eruption. The small amount of composite applied to deep cervically located margins has the goal to facilitate impression taking and luting procedures. Nevertheless, the literature supported CMR is scarce, especially regarding clinical studies. Managing the direct composite build-up in cervical areas requires perfect handling of matrix and wedges. A tight matrix adaptation with a well-positioned wedge should prevent the overhanging or underhanging of the composite and result in an appropriate emergence profile (12, 34). Working in this small, deep area, direct composite placement should be performed under absolute moisture control, and the dentist should carry out this process under rubber dam isolation (12, 22, 35). Any contamination with blood and saliva would hamper the clinical success (36, 37).

There is also a confusion among clinicians concerning selection of the correct viscosity, application thickness, and application method of the composite material. Some researchers advocated highly filled flowables (13, 19), while others recommended highly filled flowable in combination with paste-like resin composite (21). *In vitro* studies have demonstrated that application of composite masses in small increments have been favored over mono layering (16, 17).

Another important consideration is the polymerization shrinkage property of resin composites. Unfortunately, there is no existing unshrinkable composite material in the market yet. Some studies revealed the superiority of incremental placement of CMR composite versus single increment (16, 17), owing to the fact that the smaller mass of composite would result in a lower amount of polymerization shrinkage.

Marginal leakage had not been studied.

Marginal adaptation was one of the earliest investigations in CMR studies. Internal and marginal adaptation was examined (16, 17, 20, 25, 26). Gap free margins observed through SEM in 50X (23) magnification showed that CMR technique is comparable to directly luting the dentin margin without CMR. However in some SEM studies, marginal adaptation at 200x magnification demonstrated significant differences between single increment versus three increments of composite placement (16, 17) and CMR with high viscosity composites versus directly luting on dentin (17).

However, in the literature missing points are still existing, and two of them are:

1) *In vitro* evaluation of leakage of margins when proximal cavities were extended below CEJ.

a) The effect of CMR on deep cervical margins using leakage test.

b) Comparison of leakage tests versus SEM investigation on analyzing the effect of CMR on cervically located margins below CEJ.

2*)* *In vivo* researches, such as randomized controlled trials as the most valuable source of evidence.

For that, the aim of this Ph D thesis was to make:

1) A literature review of cervical margin relocation in indirect adhesive restorations (38), selecting *in vivo* studies with CMR placed to luted indirect restorations.

2) An *in vivo* study, a Randomized Controlled Trial investigating the possible influence of CMR on periodontal health after 12- month of clinical service (39).

3) An *in vitro* study about on the Marginal sealing of relocated cervical margins of mesio-occluso-distal overlays (40).

4) An *in vitro* study on the Influence of cervical margin relocation and adhesive system on microleakage of indirect composite restorations (41).

5) An *in vitro* study to evaluate the possible correlation between two methodological approaches applied to evaluate cervical margin relocation(42).

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Chapter 2:
Literature Review

CERVICAL MARGIN RELOCATION IN INDIRECT ADHESIVE RESTORATIONS: A LITERATURE REVIEW

Juloski J, Köken S, Ferrari M (2018) J Prosthodont Res. 62: 3;273-280.

1. Introduction

Restoring large posterior defects with proximal caries extending below the cemento-enamel junction (CEJ) and cavity margins located beneath the gingival tissues represents a very common clinical situation. Due to advances in adhesive technology, development of modern materials and increasing aesthetic requests, a treatment plan in such cases often includes indirect adhesive restoration (1, 2). Unfortunately, when restoring cavities with deep cervical margins two major clinical problems may occur: problems of biological nature and technical-operative problems (3).

The biological problems refer to the possible violation of the “biological width”, a recommended distance of 3mm or more between the restorative margins and the alveolar crest that is considered necessary in order to avoid detrimental effects on neighboring soft and hard periodontal tissues (4). If the principle of biologic width is not respected, it is suggested to obtain the necessary space in one of two ways: surgically, by surgical crown lengthening (5) or orthodontically, by tooth extrusion (6).

The technical-operative problems start with difficulties in tooth preparation in subgingival areas and are followed with a series of challenges in the impression taking, the adhesive cementation of the restoration and the successive phases of finishing and polishing of the margins (7). Most of the above mentioned issues are related to inferior insight and access to the deep parts of the cavity and impossible or inadequate isolation of the operating field with a rubber dam, which leads to inappropriate moisture control and blood and/or saliva contamination throughout the clinical procedures (8).

To make the clinical procedures simpler and less fault-prone, Dietschi and Spreafico in 1998 introduced a technique named “cervical margin relocation” (CMR) (9). In 2012 Magne and Spreafico referred to the same technique as “deep margin elevation” (DME) (10). Similar names, such as “coronal margin relocation” and “proximal box elevation”, could also be heard among the practitioners and found in the literature. This technique proposes application of composite resin in the deepest parts of the proximal areas in order to reposition the cervical margin supragingivally, which is supposed to facilitate the isolation and improve impression taking and adhesive cementation of indirect restorations (9, 10). The CMR technique could be considered, to a certain extent, as a noninvasive alternative to a surgical crown lengthening.

The problem of extensive subgingival defects that still remains, regardless of the technique applied, is limited or no enamel present at deep cervical margins, leaving only dentin and cementum as the main substrates for adhesion. Adhesive bonding to the etched enamel is proved to be efficient and stable (11). Adhesion to dentin, on the other hand, depends on numerous factors related to the substrate morphology (12), on the type of the adhesive (13) and on the sensitive application technique (14). Therefore, adhesive bonding to deep cervical dentin and maintaining the margins of the adhesive restoration sealed throughout the time could not be considered entirely predictable and safe.

Whether the CMR technique is the most optimal treatment option for the restoration of deep cavities reaching below CEJ, how the proposed advantages and possible disadvantages could affect the clinical performance of the indirect restorations and which are the most appropriate materials and techniques that should be applied in such situations are the topics extensively discussed among clinicians. Nevertheless, not much scientific support could be found in the currently available literature. Therefore, the aim of this review was to summarize the existing scientific knowledge on CMR technique performed prior to the adhesive cementation of the indirect restorations.

2. Study selection

2.1. Search strategy

For the identification of the studies to be included in this review, an electronic search with no date restriction was conducted in the MEDLINE database, accessed through PubMed. The following main keywords were used: “cervical margin relocation”; “coronal margin relocation”; “deep margin elevation” and “proximal box elevation”. A further manual search was performed as well; checking for eligible papers in the bibliographies of the initially retrieved articles and exploring the websites of the relevant journals.

2.2. Inclusion criteria

The present review sought only for articles where composite resin materials were used as materials for relocation of the cervical margin. Only articles considering indirect adhesive restoration, as a type of final restoration, were included. Because no randomized controlled clinical trials (RCTs) existed in this field, descriptive studies that made reference to the CMR clinical procedure were also included. The search was limited to the articles published in English language.

2.3. Exclusion criteria

Publications focusing on relocation of the cervical margin using glass ionomer cements were not included in the present review. Studies that used teeth without a restoration or teeth restored with a direct composite restoration with subgingival cervical margin were also excluded. Articles in any language other than English were left out.

The search and the selection process carried out by two review authors independently finished on 1st June 2017. After the screening of the titles and the abstracts, full texts of all reviewed articles were obtained and carefully read. Upon the discussion between the authors, on the basis of the reported inclusion and exclusion criteria, 7 *in vitro* studies, 1 review article with a case report and 4 articles describing the CMR technique were selected for the present literature review.

3. Results

The results of the reviewed studies were categorized and presented in two main parts: **(1)** review of *in vitro* studies and **(2)** review of clinical reports.

Table 1. Overview of the *in vitro* studies.

Author, Year, Reference	Groups (study design)	CMR adhesive/composite material	Number of CMR composite layers	Luting agent of final restoration	Type of final indirect restoration
Marginal adaptation					
Roggendorf et al, 2012 [15]	5 groups: (1) and (2) CMR with self-adhesive cements (3) CMR in 1 layer; (4) CMR in 3 layers; (5) without CMR.	2 self-adhesive resin cements: (1) Maxcem Elite (Kerr), (2) G-Cem (GC Corp.); (3) and (4) self-etch adhesive AdheSe (Ivoclar, Vivadent) + Clearfil Majesty Posterior (Kuraray)	1 layer (3mm); 3 layers (3 x 1mm)	Total-etch adhesive Syntac Primer, Syntac Adhesive, Heliobond (Ivoclar Vivadent) + resin cement Variolink II (Ivoclar Vivadent)	Laboratory made composite MOD inlay Clearfil Majesty Posterior (Kuraray)
Frankenberger et al, 2013 [16]	6 groups: (1), (2) and (3) CMR with self-adhesive cements (4) CMR in 1 layer; (5) CMR in 3 layers; (6) without CMR.	3 self-adhesive resin cement: (1) Maxcem Elite (Kerr), (2) G-Cem (GC Corp.), (3) RelyX Unicem (3M ESPE); (4) and (5) self-etch adhesive AdheSe (Ivoclar, Vivadent) + Clearfil Majesty Posterior (Kuraray)	1 layer (3mm); 2 layers (2 x 1.5mm)	Total-etch adhesive Syntac Primer, Syntac Adhesive, Heliobond (Ivoclar Vivadent) + resin cement Variolink II (Ivoclar Vivadent)	CAD/CAM-fabricated MOD inlay: leucite-reinfirced glass-ceramic IPS Empress CAD (Ivoclar Vivadent)
Zaruba et al, 2013 [17]	4 groups: (1) margin in enamel; (2) margin in dentin, CMR in 1 layer; (3) margin in dentin, CMR in 2 layers; (4) margin in dentin, without CMR.	Total-etch adhesive Syntac Primer, Syntac Adhesive, Heliobond (Ivoclar Vivadent) + fine hybrid composite Tetric A2 (Ivoclar Vivadent)	1 layer (3mm); 2 layers (2 x 1.5mm)	Total-etch adhesive Syntac Primer, Syntac Adhesive, Heliobond (Ivoclar Vivadent) + fine hybrid composite Tetric A2 (Ivoclar Vivadent)	CAD/CAM-fabricated MOD-inlay: feldspatic ceramic (Vitablocs Mark II, Vita)
Spreafico et al, 2016 [19]	4 groups: 2 groups based on restorative material used for CMR and 2 subgroups based on material used for fabrication of the crowns.	(1) 3-step total-etch adhesive Optibond FL (Kerr) + Filtek Flow Supreme XTE (3M ESPE); (2) 3-step total-etch adhesive Optibond FL (Kerr) + Filtek Supreme XTE (3M ESPE)	2 layers (2 x 1mm)	Total-etch adhesive Optibond FL (Kerr) + RelyX Ultimate (3M ESPE)	CAD/CAM-fabricated crowns: (1) resin composite with nanoceramic fillers (Lava Ultimate, 3M ESPE); (2) lithium disilicate (IPS e.max CAD, Ivoclar Vivadent)
Müller et al, 2017 [21]	3 groups: based on material used for luting the inlays	Scotchbond Universal Adhesive (3M ESPE) in total-etch mode + Filtek Supreme XTE (3M ESPE)	not specified (layers of 2 mm)	(1) Scotchbond Universal Adhesive (3M ESPE) in total-etch mode + RelyX Ultimate (3M ESPE); (2) total etch adhesive Syntac Primer, Syntac Adhesive, Heliobond + Variolink II (Ivoclar Vivadent); (3) self-adhesive resin cement Panavia SA Cement (Kuraray)	CAD/CAM-fabricated MOD-inlay: resin composite with nanoceramic fillers (Lava Ultimate, 3M ESPE)
Marginal adaptation and fracture behaviour					
Ilgenstein et al, 2015 [18]	4 groups: 2 groups based on presence or absence of CMR and 2 subgroups based on material used for fabrication of the onlays.	3-step total-etch adhesive Optibond FL (Kerr) + Tetric EvoCeram (Ivoclar Vivadent)	2 layers (2 x 1mm)	Scotchbond Universal Adhesive (3M ESPE) in total-etch mode + RelyX Ultimate (3M ESPE)	CAD/CAM-fabricated MOD-onlay: (1) feldspatic ceramic (Vitablocs Mark II, Vita): (2) resin composite with nanoceramic fillers (Lava Ultimate, 3M ESPE)
Microtensile bond strength					
Da Silva Gonçalves et al, 2016 [20]	4 groups: 2 groups based on presence or absence of CMR and 2 groups based on resin cement used for luting the inlays.	Total-etch adhesive system Adper Scotchbond 1 XT (3M ESPE) + Filtek Z250 (3M ESPE)	2 layers (2 x 1mm)	(1) total-etch adhesive system Adper Scotchbond 1 XT (3M ESPE) + Relyx AEC; (2) self-adhesive resin cement G-Cem (GC Corp.)	Indirect laboratory made composite inlay (Gradia Indirect; GC Corp.)

3.1. Review of *in vitro* studies

In the current scientific literature 7 *in vitro* studies investigating on CMR are taken into consideration for the present review (15-21). The most frequently investigated parameter in almost all of the studies was the marginal adaptation of the indirect restorations (15-19, 21). Only one study additionally assessed the influence of CMR on the fracture behavior of the restored teeth (18) and one study assessed the bond strength of the indirect composite restoration to the proximal box floor (20). The overview of the main characteristics, the materials employed and the designs of the reviewed studies are reported in **Table 1**.

3.1.1. Marginal adaptation

All 6 studies that evaluated the influence of CMR on the marginal quality of the adhesively luted restorations performed the analysis using the scanning electron microscopy (SEM), observing the margins on gold-sputtered epoxy resin replicas of the teeth at 50 (19) and 200 magnification (15-18, 21). The marginal integrity was calculated in the same way, as the percentage of continuous margin in relation to the individual assessable margin, following the well-established protocol consistent with previous studies (22). The quality of the marginal adaptation was assessed before and after thermo-mechanical loading (TML) (15-19, 21). However, the adhesive interfaces that were observed and analyzed were not always the same in all of the studies.

Most of the studies supported the fact that no differences existed in marginal quality of the restorations placed directly on dentin, following the conventional luting procedure, or on composite restorations used for relocation of the cervical margin (15, 17-19, 21). One study recorded that, only after being subjected to TML, conventional technique showed superior marginal adaptation compared to CMR technique (16). Moreover, in most of the studies TML was found to significantly deteriorate the integration at enamel and dentin margins (15-17) and at onlay/ luting composite interface (18). But it was also recorded that TML did not result in inferior marginal quality, regardless of the materials tested (19, 21).

With regard to the materials employed for CMR, one study investigated on the performance of flowable and conventional restorative composite materials when used for CMR (19). No significant differences in the marginal integrity were found between traditional or flowable composite, before or after TML, for either ceramic or composite CAD/CAM crown (19). The potential use of self-adhesive resin cements as material for CMR, although deviant from their original indication spectrum, has been explored due to their easy clinical manipulation that could be appealing for dental practitioners (15, 16). Based on the discouraging results obtained and significantly inferior quality of the marginal adaptation to dentin, they were not recommended for this indication in clinical practice, although differences were noticeable among various self-adhesive cements (15, 16).

Moreover, in order to evaluate whether polymerization shrinkage of composite material used for CMR could affect the quality of the margins, 3 investigations were performed applying one or more layers of composite on the cervical margin of the proximal boxes (15-17). Two 1.5-mm increments of a fine hybrid composite (Tetric A2; Ivocalr Vivadent, Schaan, Liechtenstein) applied for CMR did not perform any better then did one 3-mm increment, in terms of marginal adaptation of the final restoration (17). On the contrary, results of another two studies (15, 16) showed that marginal integration to dentin of a restorative composite (Clearfil Majesty Posterior; Kuraray, Noritake Dental Inc., Tokyo, Japan) is improved when composite is layered in three consecutive 1-mm increments than in one 3-mm increment for CMR. Although initially no difference existed among group without CMR and groups where composite was layered in one or three increments, after TML significant degradation of the interface was noticed and statistically significant differences emerged among the groups (15, 16). In particular, bonding directly to dentin without CMR was found to be comparable to CMR composite layered in 3 increments but significantly better than CMR applied only in one layer (15). In a subsequent study of the same group of authors (16), the results were slightly different. Conventional luting technique directly to dentin performed significantly better even than dentin covered with 3 layers of composite, which was also significantly better than CMR applied only in one layer. Therefore, the authors concluded that, although conventional luting procedure is considered as the most effective over time, the CMR technique could be accepted a valid procedure and application of composite in more layers achieved better performance in terms of marginal quality to dentin compared to a single layer application (15, 16).

The influence of CMR on the marginal integrity of indirect restorations made of different materials was the issue investigated and discussed in two studies (18, 19). In both studies the restorations were prepared by CAD/CAM technology. One study tested onlays milled of feldspathic ceramic (VITABLOCS Mark II, Vita Zahnfabrik, Bad Säckingen, Germany) and composite resin blocks with nanoceramic fillers (LAVA Ultimate; 3M ESPE, St. Paul, MN, USA) (18), while another study used the same composite blocks (LAVA Ultimate) to fabricate crowns and compared their behavior to crowns made of lithium disilicate (IPS e.max, Ivoclar Vivadent, Schaan, Liechtenstein) (19). These two studies reported, to a certain extent, conflicting results. While Spreafico et al. (19) found no significant differences in marginal integrity between margins with and without CMR for both types of crowns, before or after TML, Ilgenstein et al. (18) revealed that composite inlays exhibited overall better marginal integrity compared to ceramic inlays. In particular, at the tooth/composite interface after TML, composite inlays luted directly to dentin measured significantly higher percentage of continuous margins than any other group. In addition, comparing only the groups with CMR at the onlay/luting composite interface, before and after TML, a significant reduction in marginal quality was detected in specimens restored with ceramic onlays, while degradation of the margin was not observed for teeth restored with composite onlays. It should be mentioned that the restorative material LAVA Ultimate CAD/CAM used for fabrication of crowns (19), the manufacturer no longer indicates for crowns. The material continues to be indicated only for restorations with an internal retentive design element (such as inlays and onlays) and veneer restorations. This is to be considered when interpreting the results of the studies investigating on crowns made of this material.

The study by Müller et al. focused on the material for luting the inlays, when bonded directly to dentin of deep proximal cavities and when bonded to restorative composite material used for CMR (21). No difference was observed in terms of marginal integrity for luting the inlays directly to dentine or composite used for CMR. Also, no significant reduction of integrity was found after TML and all investigated materials showed promising results for luting the indirect restorations. Therefore, this study suggests that there is no difference in bonding the inlay to dentine or composite used for CMR. However, the critical interface between the dentin below CEJ and CMR composite was the matter of interest of this study.

3.1.2. Fracture behavior

The study by Ilgenstein et al. (18) additionally investigated the impact of CMR and material of CAD/CAM onlays on the fracture behavior of endodontically treated molars. After TML the teeth were subjected to load until failure in order to determine the resistance to fracture and the fracture pattern. The lowest mean fracture value was recorded for group without CMR and feldspathic ceramic onlay and the highest mean value for group without CMR and composite resin onlay. Between these two values there were the two groups that have undergone cervical margin relocation, which both revealed similar fracture resistance regardless of the material used for the onlay restoration. The only statistically significant difference in load to fracture was noticed between two groups without CMR. Additionally, the study demonstrated that ceramic restorations tend to have less severe fractures that do not involve tooth itself, whereas composite restorations transfer more stress to tooth structure causing catastrophic fractures below the bone level.

3.1.3. Bond strength

One study aimed to evaluate the influence of CMR on the microtensile bond strength (MTBS) of composite inlays to the proximal box floor (20). The groups with proximal cervical margin located in dentin 1 mm below the CEJ were compared with those where cervical margins were relocated 1mm above CEJ using a restorative composite (Filtek Z250; 3M ESPE) applied in two 1-mm thick increments. Further aim of this study was to compare the bond strength of inlays luted with two different cements: resin cement used with total-etch adhesive (RelyX ARC used in combination with Adper Scotchbond 1XT; 3M ESPE) and self-adhesive resin cement (G-Cem; GC Corp., Tokyo, Japan).

The results showed that MTBS values increased when the proximal cavity floor was elevated with a composite. However, this difference in bond strength was statistically significant only when self-adhesive resin cement was used for the cementation of the inlay. When resin cement with a total-etch adhesive was used for luting there was no significant difference between the groups with and without CMR. According to the authors, the main reason for such a result could be the good interaction between the resin composite used for CMR and the self-adhesive resin cement. When two luting cements were compared within the same location of the cervical margin, no significant differences in bond strength were recorded, regardless of their different mechanism of adhesion to dentin. However, one should also take into consideration that the failures during MTBS testing happened at the different interfaces. In both groups with CMR the most frequent mode of failure was adhesive failure between dentin and composite used for CMR, which supports the fact that good bonding is achieved between resin cement and CMR composite, as well as the fact that bonding to cervical dentin still remains challenging and unpredictable (11). Also, noticeable difference in failure modes was reported in groups without CMR. While 60% of specimens cemented with self-adhesive resin cement failed adhesively between dentin and resin cement, the same percentage of specimens luted with a total-etch adhesive and a resin cement showed mixed adhesive failures.

3.2. Review of clinical reports

In the current literature no randomized controlled clinical trials or prospective or retrospective clinical studies on CMR technique could be found. One review article that specifically concentrated on this topic and that reported a clinical case was identified (23). One article presented the principles of the technique (10). In addition, several review articles on indirect adhesive restorations in posterior areas looked back also on the CMR technique (3, 24, 25). These articles provided clinical documentation with a detailed description of the treatment protocol. The protocols suggested and described in the above mentioned articles became the matter of this part of the present review (**Table 2**).

3.2.1. CMR material

With regard to the most appropriate adhesive system and composite material employed for the supragingival relocation of the cervical margin, various recommendations were found in the current literature. Most of the reviewed articles consider a traditional 3-step total-etch adhesive as the preferred adhesive system (10, 23, 25), such as OptiBond FL (Primer and Adhesive, Kerr Corp., Orange, CA, USA) (10) or Syntac (Primer, Adhesive and Heliobond, Ivoclar Vivadent, Lichtenstein) (23).

Table 2. Overview of the clinical reports.

Author, Year, Reference	CMR adhesive	CMR composite material	Thickness, number of CMR composite layers	Rubber dam isolation	Matrix and wedge application	Finishing of CMR composite	Treatment prior to bonding of final restoration	Luting agent of final restoration	Type of final indirect restoration	Follow-up period
Veneziani, 2015 [3]	Not specified	Flowable composite	Flowable composite 1 to 1.5 mm thick	Yes	Circumferential stainless steel matrix and wooden wedge	Not specified	Not specified	Not specified	Composite onlays	Not applicable
Magne and Spreafico, 2012 [10]	3-step total-etch adhesive (eg, OptiBond FL; Kerr)	Flowable or traditional restorative materials; microhybrid or nanohybrid restoratives should be preheated	2 mm thickness of the CMR composite (1 or 2 increments)	Yes	Modified curved Tofflemire matrix, matrix height reduced to 2 to 3 mm; if necessary matrix-in-a-matrix technique; wedging is typically not possible	Elimination of excess with no.12 blade or a sickle scaler	Cleaning with airborne-particle abrasion	Not specified	Indirect ceramic onlay	Two cases at 9 and 12 years follow-up
Kielbassa and Philipp, 2015 [23]	3-step total-etch adhesive Syntac Primer, Syntac Adhesive, Heliobond (Ivoclar Vivadent)	Flowable composite (Gaenial Universal Flo, GC Corp.), followed by small portions of filled viscous composite resin (Gaenial, GC Corp.)	Not reported	No	Circumferential stainless steel matrix fixed in Tofflemire retainer and wooden wedge	Bucket-shaped diamond burs, flexible discs and polishing strips	Application of a primer (GC Corp.)	3-step total-etch adhesive Syntac Primer, Syntac Adhesive, Heliobond (Ivoclar Vivadent) and Variolink II resin cement (Ivoclar Vivadent)	CAD-CAM-fabricated ceramic inlay (IPS Empress CAD, Ivoclar Vivadent)	3 months
Dietschi and Spreafico, 2015 [24]	Type of adhesive system not specified	Highly filled flowable composites are recommended (Premise Flow, Kerr) or a bulk fill flowable (eg, SureFil SDR Flow)	The use of flowable up to 1 to 1.5mm; if more material is needed a combination of flowable and restorative composite is recommended	Yes	Full stainless steel or clear matrix and a wedge	Not specified	Not specified	Highly filled light-curing restorative composite material (eg, microhybrid Tetric, Ivoclar; or a homogenous nanohybrid (Inspiro, EdelweissDR)	CAD/CAM-fabricated restorations made of resin composite with nanoceramic fillers (Lava Ultimate, 3M ESPE)	Not applicable
Rocca et al, 2015 [25]	Total-etch or 2-step self-etch adhesive system	Highly filled flowable or hybrid composite	Limit to the minimum (1 to 1.5mm) needed to bring the preparation supragingivally (at least 0.5mm over the free gingival margin)	Yes	Curved matrix, full or sectional and a wedge (wedge when possible)	Fine diamond instruments to remove the excess	Sandblasting	Light-curing restorative material	In-lab composite resin onlays (Tetric Evo Ceram, Ivoclar Vivadent)	Not applicable

In order to avoid over-etching of dentin in the subgingival area where enamel is usually very thin, if any is present, Rocca et al. suggest simultaneous etching of thin interproximal enamel in this area together with dentin only for 5–10 s, or, as an alternative, 2-step self-etch adhesive systems can be used without performing selective enamel etching (25).

Furthermore, both flowable as well as traditional viscous restorative composites could be selected for CMR technique, according to the mentioned studies. Specifically, in a case presented by Kielbassa and Philipp, a base of flowable composite (Gaenial Universal Flo, GC Corp.) was applied, followed by small portions of filled viscous composite resin (Gaenial, GC Corp.) (23). On the other hand, flowable composite in 1–1.5-mm thickness was proposed by Veneziani (3) and up to 2mm thickness of the flowable or traditional restorative composite, in 1 or 2 increments, was suggested by Magne and Spreafico (10). Besides, it was also noted that, if microhybrid or nanohybrid restoratives are to be used, they should be preheated, to facilitate placement and minimize the risk of interlayer gaps (10). Moreover, two articles specify that highly filled flowable composites (e.g, Premise Flow; Kerr Corp.) or bulk fill flowables (e.g, SureFil SDR Flow; Dentsply Pty. Ltd., Victoria, Australia) are highly recommended for CMR, due to its consistency and ease of use (24, 25). Flowables should, however, not be used in thick layers, and their thickness should be limited to 1–1.5 mm (24, 25). As the margin should be relocated to at least 0.5 mm over the free gingival margin (25), if more material is needed, a combination of flowable and traditional restorative composite is proposed (24). The light curing of the final composite increment should be protected by a thick layer of glycerin gel (10, 25), as to eliminate the superficial oxygen inhibition layer, which can interfere with the setting of some impression materials (26).

3.2.2. Application technique

According to Veneziani, three different clinical situations can be identified, based on technical-operating and biological parameters (3). Only in Grade 1, when rubber dam can be correctly placed in the sulcus sufficiently to show the cervical margin, the coronal relocation of the margin could be carried out. In the other two clinical situations, surgical exposition of the margin (Grade 2) or surgical lengthening of the clinical crown (Grade 3) is necessary in order to allow for correct isolation of the operating field. In accordance to these recommendations, the CMR technique is indeed contraindicated if the cervical preparation is not perfectly isolated with a rubber dam and a matrix (10, 24, 25). Nevertheless, the case reported by Kielbassa and Philipp showed a clinical protocol where rubber dam was not used for relocation of the cervical margin (23). The isolation with rubber dam did not prevent bleeding and therefore it was removed and the isolation was done using cotton rolls, dry-angles and saliva ejectors under utmost attention to prevent contamination (23).

The use of either circumferential or sectional matrix, either stainless steel or clear matrix is strongly advocated (3, 10, 23-25). Specifically, curved matrices are recommended, as the curvature allows convergence, adequate emergence profile and a tight subgingival fit (10, 25). Moreover, reducing the height of the matrix to 2–3 mm is suggested, so it is slightly higher than the height of the CMR and in that way the matrix could slide subgingivally and seal the margin more efficiently (10). In addition, in cases of extremely deep and localized subgingival cavities, the final option could be the matrix-in-a-matrix technique (10).

Regarding the wedging, although it was reported that wedging is typically not possible (10) or that it is not always possible (25), in most of the clinical cases presented in the published articles, matrix in combination with a wooden wedge was anyway applied (3, 23, 24).

3.2.3. Treatment of CMR prior to bonding of final restoration

In order to obtain well-defined margins, finishing and polishing of the restorative material placed at the cervical margin are required as the last step before impression taking. Elimination of the excess composite material could be done using a scalpel or a sickle scaler (10). Fine diamond rotary instruments are suggested for removing the excess material, for finishing and polishing, as well as for obtaining the optimal cavity design (23, 25). Besides, finishing of the relocated cervical margins was also completed using flexible discs of decreasing grit and polishing strips (23). However, the question remains how deeply subgingival interproximal margins could be reached by any of the mentioned materials and instruments. Before proceeding with the final impression, Magne and Spreafico advise a bitewing radiograph to evaluate the adaptation of the CMR composite resin in the subgingival area and to make sure that there are no gaps or overhangs (10).

After taking digital or analog impression and fabrication of the final restoration, different protocols for treatment of the relocated margin were described. Most of the articles suggest cleaning the composite by sandblasting, using airborne-particle abrasion (10, 25). In one case the CMR composite resin was primed (GC Primer, GC Corp.) prior to adhesive luting of the final restoration, in order to achieve safe bonding, as reported by the authors (23).

3.2.4. Follow-up

The clinical case of left second maxillary molar restored with the CMR and CAD/CAM ceramic inlay presented by Kielbassa and Philipp (23) was followed up for 3 months. Clinical appearance showed no signs of inflamed papilla, probing did not reveal increased probing depth, no bleeding was observed on probing and no discomfort was reported by the patient.

Long-term clinical view and corresponding radiographs of two different cases, 9 and 12 years after treatment with CMR and indirect ceramic restorations, are available in the article by Magne and Spreafico (10).

4. Discussion

The idea to overcome the difficulties associated with the placement of restorations in the areas difficult to access by applying a base that is open to the oral environment underneath, originates from the “open sandwich technique” in direct composite Class II restorations. Initially, glass-ionomer cements (GICs) were proposed as a base material (27) and later, with advancements in dental material technology, resin-modified GICs (28), polyacid-modified resin composites (29) and flowable composites (30) were investigated. Frese et al. (31) described the restoration of extensively damaged teeth in two clinical steps and called it the “two-step R2-technique”. In the first step layers of flowable and viscous composites were applied to relocate the gingival margin supragingivally and in the second step a direct composite restoration was placed (31). Similarly, CMR technique was proposed as an analog approach to be applied underneath the indirect adhesive restorations and for that purpose only composite resin materials were indicated (9).

The *in vitro* investigations analyzed in this review used composite materials of various manufacturers, chemical compositions and viscosities (Table 1). Based on the reported findings, the overall conclusion could be that the marginal integrity of the indirect restorations was not significantly influenced by the application of CMR. Also, the viscosity of the composite resin was not found to be crucial for the quality of the margins, whereas the application of composite in several thinner layers could be considered advisable. Regarding the influence of the restorative material used for fabrication of final indirect restoration on the integrity of the relocated cervical margins, no conclusive evidence could be found.

Nevertheless, the quality of marginal adaptation, as observed under a microscope, does not necessarily have to correspond to the quality of the marginal seal of the adhesively bonded composite material. Inadequate sealing ability may cause leakage of oral fluids and microorganisms along the tooth/composite interface, which represents one of the major causes of failure of composite restorations (32). Microleakage at the gingival margins of direct Class II composite restorations (33, 34), as well as of indirect restorations, both ceramic (35) and composite (36), has been well documented. In addition, application of flowable composite on the gingival margin as a liner in direct composite restorations did not reduce microleakage or improve clinical performance of the restorations (37).

Based on the available evidence, it could be assumed that applying a layer of composite underneath an indirect restoration would not prevent leakage. However, no study so far assessed the leakage at the gingival margins that have been relocated above CEJ with a composite resin. This would certainly be worth investigating in order to obtain more relevant information regarding the *in vitro* performance of the CMR.

It should also be noticed that in 6 laboratory studies investigating on the marginal integrity the interfaces that were examined were not always the same. As a matter of fact, in some of the studies it was not completely clearly described which of the margins were evaluated. Besides, the material adaptation to the enamel margins, judged in some of the studies, could not be considered essential for assessing the influence of the CMR technique on the marginal adaptation of indirect restorations. In the upcoming research more attention should be given to the most critical margin, the one in dentin, below the CEJ. Different aspects related to the adhesion of the composite material used for CMR to dentin and to indirect restorations, as well as to the analysis of the fracture behavior, the expected fracture pattern and the stress distribution could be the matter interest of future laboratory research.

Moreover, the recent meta-analysis indicates that the survival rate of ceramic inlays, onlays and overlays remains high (91%) after 10 years of follow-up time, regardless of the ceramic material, study design and study settings (2). Another literature review reported 94 % average success for ceramic and composite indirect restorations, concluding that the low failure rate prove them to be an excellent choice in treatment of both Class I and II lesions (1). Nevertheless, the extent of the cavities below gingival margins was not included in any of the analysis and no distinction was made between cavities with proximal cervical margins in enamel and those in dentin, which could considerably affect the clinical outcome. The main problem discovered by the present review is that strong evidence on the clinical performance of teeth restored with CMR technique and an indirect adhesive restoration still does not exist. Only several presentations of the clinical cases could be found (**Table 2**), which is hardly sufficient for drawing any conclusions on the potential beneficial or harmful effects of this technique. Therefore, upcoming clinical research should focus on important issues related to restoration of Class II cavities extending below CEJ with indirect adhesive restorations and, specifically, to the application of CMR technique in such cases.

One of the possible concerns worth investigating is the response of the periodontal tissues to the coronally displaced margins following the CMR technique, as healthy periodontium is a prerequisite for a successful outcome of any kind of prosthetic or restorative therapy (38). Although the margins of the final indirect restoration would be positioned supragingivally, another restorative margin, the one between the tooth and the composite used for CMR, remains deep below the gingival margin. This could still produce detrimental effects, such as gingival inflammation, loss of periodontal attachment and bone resorption (39). Therefore, it is necessary to scientifically prove, through clinical studies, if the CMR technique could indeed represent the alternative to surgical crown lengthening or orthodontic extrusion if there is no compliance with the biological width.

Furthermore, reviewing clinical reports on CMR technique, it was noticed that there is a lack of consensus on the isolation and the application technique. According to several studies, CMR technique should be performed only if the rubber dam, correctly sheathed in the sulcus, is sufficient to show and isolate the cervical margin. Nevertheless, in the clinical cases reported in the literature CMR was also performed without rubber dam isolation. This should, however, not be a matter of discussion, as placing CMR composite material in deep subgingival areas without rubber dam isolation, could seriously threaten the quality of adhesion, particularly having in mind the fact that adhesion to dentin is not as strong and durable as adhesion to enamel (11, 40). In addition, the meta-analyses on clinical outcomes of direct Class II (41) and Class V (42) direct restorations demonstrated that the use of rubber dam significantly influenced the clinical performance and longevity of the restorations. Hence, if it is not possible to isolate the subgingival margins with a rubber dam, an important question that arises is whether or not the CMR technique is indicated.

Finally, after the restoration has been placed and the periodontal tissues have healed, the patient needs to be able to adequately maintain the oral hygiene. If the margins between the tooth and the restoration could not be reached and cleaned in daily oral hygiene routines, no technique could be considered appropriate and successful outcome could not be expected. Therefore, apart from defining more precisely the indication area and strict clinical protocol, future well-designed randomized controlled clinical trials should concentrate on verifying the claimed advantageous features of CMR technique on the long-term clinical outcome of teeth restored with indirect adhesive restorations.

5. Conclusions

On the basis of the reviewed literature, it can be concluded that currently there is no strong scientific evidence that could either support or discourage the use of CMR technique prior to restoration of deep subgingival defects with indirect adhesive restorations. Randomized controlled clinical trials are necessary to provide the reliable evidence on the influence of CMR technique on the clinical performance, especially on the longevity of the restorations and the periodontal health.

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Chapter 3:

Randomized Clinical Trial

INFLUENCE OF CERVICAL MARGIN RELOCATION (CMR) ON PERIODONTAL HEALTH: 12- MONTH RESULTS OF A CONTROLLED TRIAL

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(2018) J Dent. 69;70-76.

1. Introduction

The Cervical Margin Relocation (CMR) was proposed more than 15 years ago, and in the last decade became more and more popular among dental practitioners (1, 2).

CMR is indicated when the gingival margin of a Class II inter- proximal cavity cannot be isolated with rubber dam alone, in alternative to perform surgical crown lengthening. CMR consists on placing a base of direct resin composite using a metal interproximal matrix to elevate the interproximal underneath indirect bonded restorations. Consequently, margins can be predictably caught by a conventional impression and/or intraoral optical scanning (IOS) (3).

A few trials described clinical steps of CMR (4-7) and others mainly evaluated 'quality margins' through SEM observations of the external margins relocated coronally at lower magnifications (8-10).

The clinical success in restorative/prosthodontic dentistry can be based on different technical parameters, such as esthetics, precision of the margins, proper function on occlusion, preservation of vitality and fractures of the abutments (11, 12). It seems mandatory, but beside this feature, healthy periodontal tissues, defined by a Probing Pocket Depth (PPD) less/equal than 4 mm without Bleeding on Probing (BoP). It might be argued that even slightly subgingival located margins may affect the periodontal health (13); and therefore, subgingivally located margins should be avoided whenever possible. Therefore, it has to be emphasized that the extent of the biological width between the cervical aspect of the interproximal composite box and the alveolar bone should be respected (14).

Recently, Paniz et al. (15) evaluated in a 12 month clinical trial, the periodontal response (BoP and gingival recession) of different full crowns placed with subgingival margins, with teeth prepared alternatively with feather edge or chamfer finishing lines. After one year, both experimental groups displayed more deep inflammation (BoP) in respect to baseline.

Unfortunately, the literature does not report any clinical trial evaluating periodontal tissue response on indirect adhesive restorations placed on posterior teeth with CMR (16).

Differently, the literature reports about the influence of approximal restorations extension on the development of secondary caries, showing that restorations ending below the CEJ showed significantly increased risk for failure (17, 18).

The primary aim of this clinical trial was to evaluate BoP on single adhesive indirect restorations made on posterior teeth with one interproximal margin relocated cervically; and secondary, to analyse the correlation between depth of the interproximal margins and BoP. The null hypothesis tested was that there is no statistically significant difference between margins with or without CMR regarding periodontal tissue inflammation (BoP).

2. Materials and methods

A consecutive sample of 35 restorations in 35 patie4nts (**Table 1**) in need of one single partial crown (onlay) on posterior teeth was placed between January and April 2016. A partial restoration was performed from the pool of patients accessing the Department of Prosthodontics and Dental Materials of the University of Siena, Italy. All of them had an old restoration and some carious tissue to be replaced.

Age 42 (± 16.5) years	Sex (19F, 16M)

Table 1. Demographic data of the included study participants.

Patients written consent to the trial was obtained after having provided a complete explanation of the aim of the study. Ethical approval was achieved beforehand by the University of Siena, Italy (ClinicalTrials.gov #NCT01835821).

2.1. Inclusion criteria

A total of 35 patients (19 men/16 women, aged 27–54 years, mean age of 45.1 years) received 35 partial-coverage restorations. All patients were periodontally healthy or have been treated successfully before rehabilitation with indirect restorations on posterior teeth (molars or premolars).

Positive response to vitality testing by a one second application of air from a dental unit syringe (at 40–65 p.s.i. at approximately 20° C), directed perpendicularly to the root surface at a distance of 2 cm and by tactile stimuli with a sharp #5 explorer.

2.2. Exclusion criteria

Patients with the following factors were excluded from the clinical trial: 1) not proper age (< 18years); 2 pregnancy; 3) disabilities; 4) potential prosthodontic restoration of the tooth; 5) pulpitic, non-vital or endodontically treated teeth; 6) (profound, chronic) periodontitis; 7) deep defects (close to pulp, < 1 mm distance) or pulp capping;8) heavy occlusal contacts or history of bruxism; 9) systemic disease or severe medical complications; 10) allergic history concerning methacrylates; 11) rampant caries; 12) xerostomia; 13) lack of compliance; 14) language barriers; 15) plaque index higher than 20.

2.3. Patient selection

After recruitment, oral hygiene instructions were given to the patients and prophylaxis was performed by a Periodontist to establish optimal plaque control and gingival health. After 1 week, the following periodontal measurements were registered by two experienced operators: PPD at two different facial sites (mesial and distal) with a periodontal probe (UNC periodontal probe, Hu-Friedy), rounding the measurements to the nearest millimetre, plaque index (PI), according to Löe and Silness (19); gingival index (GI), according to Löe and Silness (20); gingival bleeding on probing (BoP), according to Ainamo and Bay (20). Intra-examiner calibration took place before initiation of the study by examination of ten patients twice, hours apart (20). The sequence of examiners was random. Measurements were accepted as calibrated if 90% of the recordings could be reproduced within a difference of 1 mm.

The inter-examiner agreement for the assessment of the variables was determined with the intra-class correlation coefficient (ICC). For the two examiners, t-test ($\alpha = 0.05$) revealed no statistically significant differences. All restorative procedures were performed under local anaesthesia (Articaine with 1:100.000 epinephrine) by a single experienced prosthodontist (Faculty member, MF). Intraoral X-rays were made before starting the treatment.

Following anaesthesia, rubber dam was placed, caries detector was applied and all detected carious structures were excavated, and any restorative material was removed.

The preparation was performed using conventional diamond burs in a high-speed hand piece, with no bevel on margins. The preparation design was dictated by the extent of decay, pre-existing restorations and the preparation guidelines define by the manufacturer of the restorative materials (**Fig. 1a and d**). Cavities' preparation must provide at least 1 mm space at the cervical margin and 1.0–1.5 clearance occlusaly. At least one occlusal cusp was covered. The Residual Dentin Thickness (RDT) was evaluated on a periapical radiograph, and teeth with RDT thinner than 0.5 mm were excluded. Interproximal margins were located below cementum-enamel junction into cementum-dentin. The decision where to place CMR was taken flipping a coin for each tooth.

Consequently, two groups were allocated: Group 1 corresponded to the interproximal margin in which CMR was performed and Group 2 to the other interproximal margin in which the crown was luting directly to dental structures.

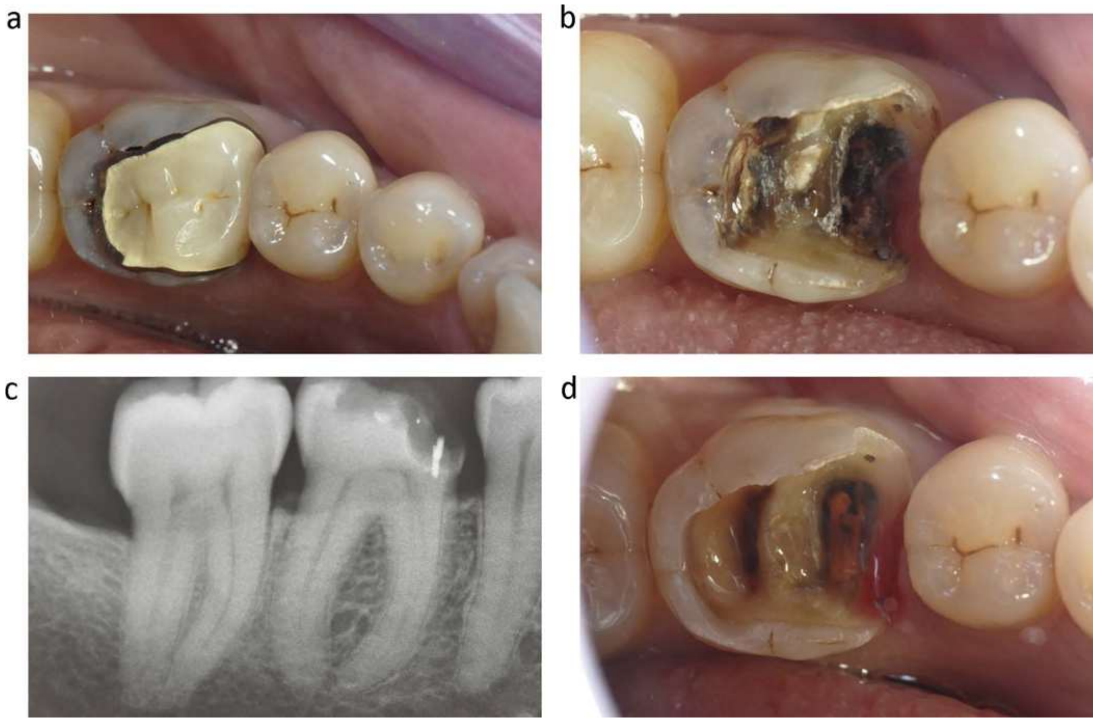

Fig. 1. **a, b.** Old indirect restoration made with porcelain fused to metal in need to be replaced because secondary decay. **c.** X-ray of the cavity after the old restoration was removed. **d.** The cavity after decay removal. The application of a metal matrix protected the soft tissue, although after removing the matrix the tissue is slightly bleeding.

Caries cleaning of the affected area was performed after placing a first matrix band to retract and simultaneously protect the soft tissue, the curvature of the metal matrix was properly adapted to the curvature of the tooth to achieve the best cervical fit was possible (5). In one proximal box CMR procedure was performed using G-Premio Bond, simultaneously used to perform for hybridization of entire exposed dentin of the entire cavity, and universal flow resin composite applied in two or three thin layers depending the depth and size of the cavity (GC Co. Tokyo, Japan) (**Fig. 2a and d**). After final cavity's preparation, an impression was taken (Exa'lence, GC Co., Tokyo, Japan) (**Fig. 2e**) and sent to the laboratory in order to make the restoration using lithium disilicate (LS2) press material (LiSi Press, GC Co. Tokyo, Japan) (**Fig. 3a**). A temporary restoration was made with heat-polymerizing polymethylmethacrylate (PMMA) acrylic resin and luted. Patients were instructed to use a 0.2% chlorhexidine gluconate solution for 7 days until they could perform regular oral hygiene and returned 12 weeks later for the impression procedures, giving enough time for soft tissue adaptation and maturation after teeth preparation. The restorations were milled made in the laboratory, then tried-in, and margins were examined and carefully verified for fit and extension. Rubber dam was always placed to isolate the abutment (**Fig. 3b**). The restorations were luted following manufacturer's instructions using proprietary's cement (Link Force, GC Co., Tokyo, Japan) after being sandblasted, etched with fluoridric acid at 5% for 60 s and a coat of multi primer being applied and left to evaporate for 1 min.

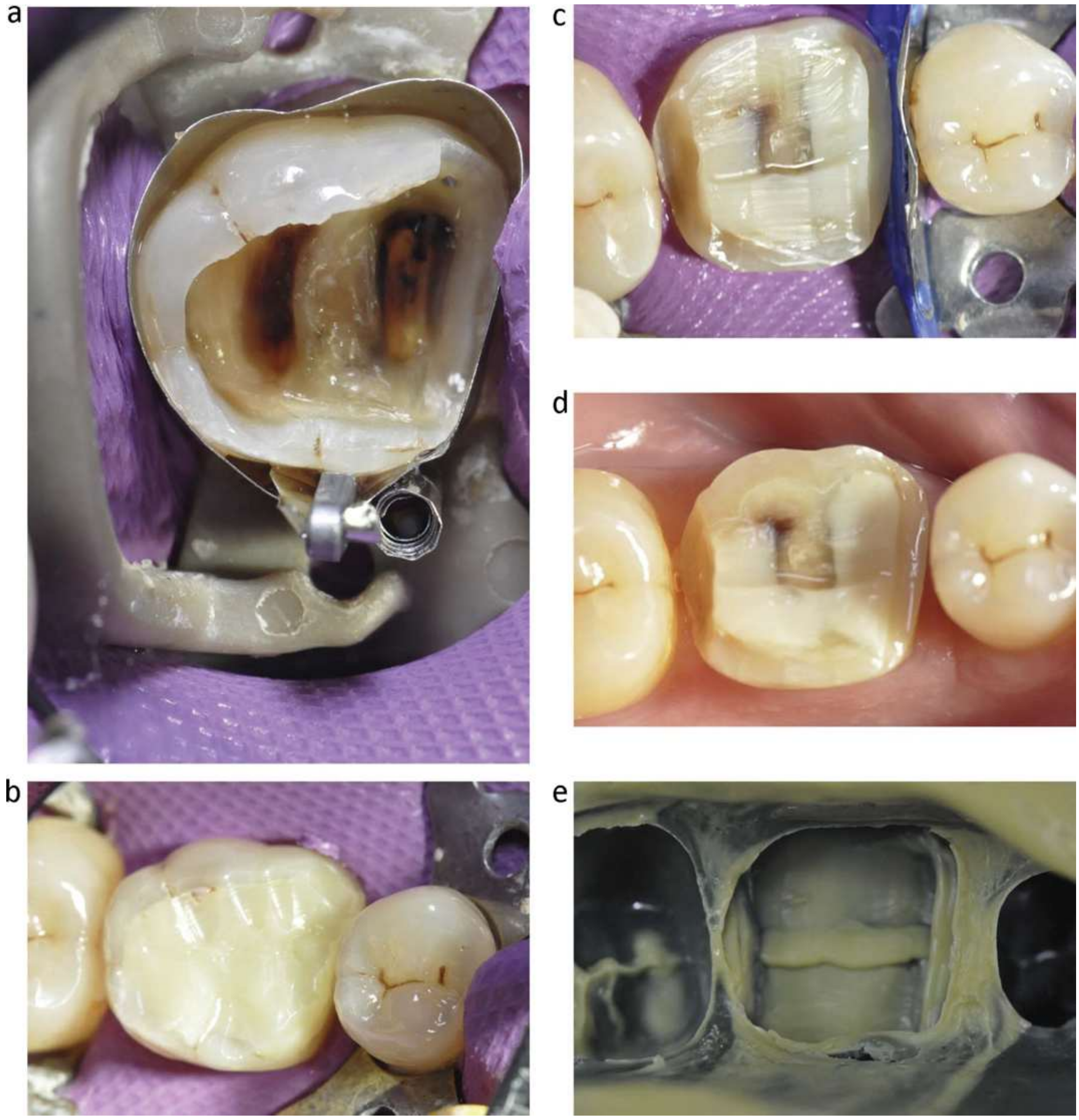

Fig. 2. a. Under rubber dam and after adaptating metal matrix and wedge to the emergence profile of the tooth, the procedure of immediate dentin sealing and cervical margin relocation are perfomed: the first layer of flowable resin composite is already light-cured. **b.** Complete build-up of the cavity. **c.** Immediately after the build up, still under rubber dam, the final preparation was made. **d.** The final preparation. **e.** The traditional impression.

Cement excess was carefully removed, and occlusion was slightly adjusted when needed. Intra-sulcular margin position was verified, and oral hygiene instructions were given to the patients. Patients were recalled 2 weeks later and then 3 months after for evaluation and oral hygiene measures reinforcement.

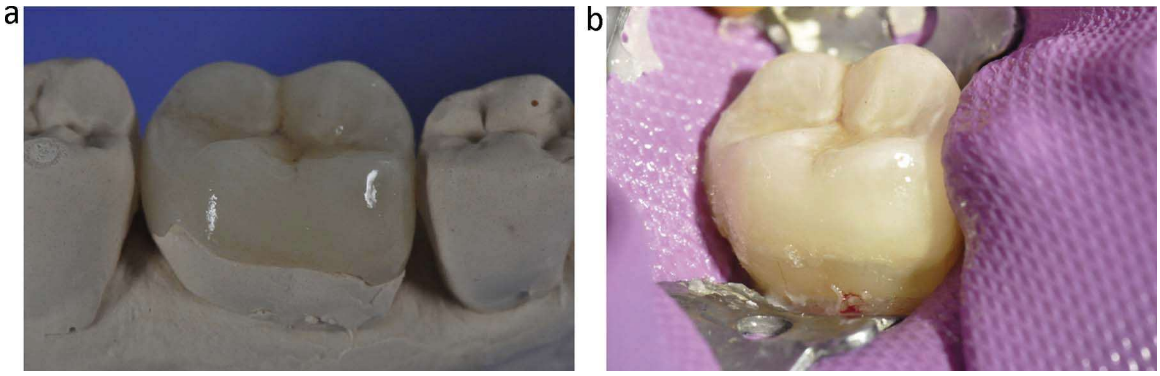

Fig. 3. a. The final LiSi Press partial crown. **b.** The crown after being luted under rubber dam.

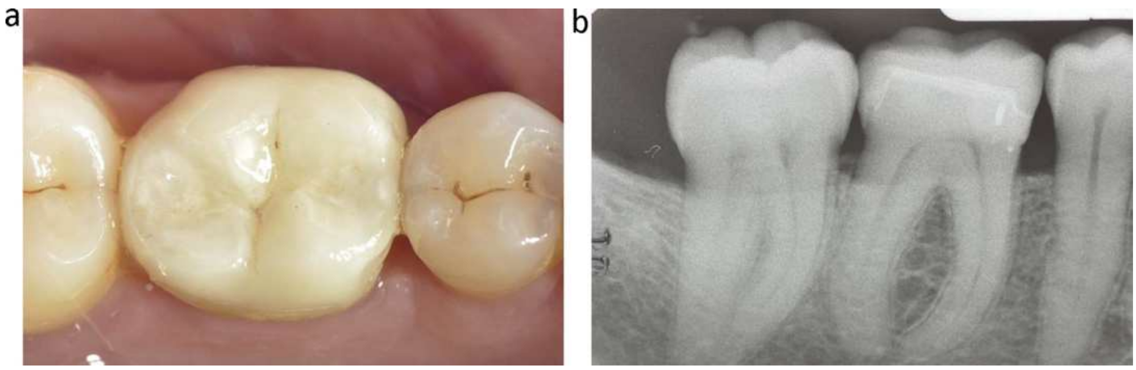

Fig. 4. a, b. Recall after 12 months; clinical and radiographic views.

The restorations were placed in the time period between January 2016 and April 2016 and examined for (BoP) at baseline (cementation of the restorations), and after 12 months by two calibrated operators (EFC, ND) (**Fig. 4a and b**).

At baseline, the restorative margin position in relation to the gingival margin was recorded quantifying by probing in mm (20), and the linear distance from the bone crest was calculated in mm by intraoral x- ray. In addition, intraoral x-rays were made at the 12 month recall as well.
All clinical procedures were made using ∼3.5/4.5 magnification.

2.4. Data analysis

Descriptive statistics were expressed as mean (SD) and valid percentage for continuous and categorical data, respectively. The baseline comparisons between study groups were performed using chi-square test (Fisher exact test with observed frequencies < 5) for categorical variables whereas continuous variables were tested using t-test (U- Mann Whitney test if the variables were not normally distributed).

Outcomes were analysed using analysis of covariance (ANCOVA), once assumptions for the convenience were confirmed, with baseline values and age as covariates and study group as independent variable. Least square (LS) mean ± standard error (SE) was calculated for variables involving each outcome. Paired t-test or McNemara test (if applicable) was used to compare outcomes at baseline and 12 months. Level of significance was set at 0.05. SPSS version 21 software (IBM) was used for all calculations.

3. Results

Included participants completed the 12 month follow-up (**Table 2**).

At 12 months follow-up, changes from baseline were observed in GI, PI, and BoP: 20% of the sites of Group 1 (CMR) and 8.5% of Group 2 (shoulder preparation) presented dental plaque (PI), while at baseline dental plaque was not present. Teeth at baseline did not show any degree of gingival inflammation (GI) or BoP, while at 12 months 31.5% of Group 1 and 18.5% of Group 2, the GI scoring ranged from 1 to 3, and BoP was presented in 53% of Group 1 and 31.5% of Group 2, respectively.

Statistically significant differences existed for PPD at mesial and distal sites at baseline (P = .001) (**Table 2**). Considering the two different groups, differences were identified for PPD from baseline to 12 months (P = .340). PI and GI at 12 months were similar in both groups (P = .250 and P = .465), respectively. Significantly more sites in Group 1 had BoP (53%) compared with group 2 (31.5%) (P = .010) (**Table 3**).

Evaluating Group 1 cases with positive BoP at one year recall, the recorded margin-bone crest distance was mainly 2 mm (13 of 19 margins) and similarly in 6 cases of 11 in Group 2.

No evident radiographic anomalies of recurrent decay were found after 1 year of clinical service.

Table 2

Sample characteristics at Baseline and 12 Months.

Variable	Baseline	12 mo follow-up	P
Age (y)[x]	45.1 (7.6)		
Sex (men)	19		
GI (n[%])			
0	35	24	NA
1	/	7	
2	/	4	
3	/	0	
PI (n[%])			NA
0	35	28	
1	/	7	
BoP (n[%])			NA
0	35	16	
1	/	19	
PPD mesial/distal, mm[a]	2.3 (0.25)	3.1(0.70)	0.001[b]

NA = not applicable; GI = Gingival Index; PI = Plaque Index; BoP = bleeding on probing; PPD = periodontal probing depth.

[a] Mean (SD). [b] Paired t-test (quantitative variables).

Table 3 Pre-Post analysis by Study Group.

Variable	Baseline Group 1 (CMR)	(n = 35) Group 2 (below CEJ)	P[b]	12 months Group 1 (CMR)	(n = 35) Group 2 (below CEJ)	P
Age (y)[x]	43.2(5.3)	48.5(3.7)		.001		
GI (n[%])						.465[c]
0	35	35	NA	24	29	
1				7 (20%)	6 (18.5%)	
2				4 (11,5%)	0	
3				0	0	
PI (n[%])	35	35	NA			.250[c]
0	35			28	32	
1	/			7 (20%)	3 (8.3%)	
BoP (n[%])	35	35	NA			0.10[d]
0				16	24	
1				19 (53%)	11 (31.5)	
PPD mesial/distal, mm[a]	2.3 (0.40)	2.4 (0.25)		3.1(0.25)	3.2 (0.35)	.340[d]

NA = not applicable; GI = Gingival Index; PI = Plaque Index; BoP = bleeding on probing.
PPD = Periodontal probing Depth.

[a] Mean (SD).

[b] Nonpaired Student t-test was used for comparisons between groups in baseline measures.

[c] Chi-square test was used for comparisons between groups at 12 months.

[d] ANCOVA (LS mean) was used for comparison of 12 months vs baseline (mean adjusted by baseline value and age).

4. Discussion

The CMR procedure is a popular restorative procedure but in need to be validated scientifically and clinically by randomized clinical trials. Because the CMR technique is usually made on posterior teeth that had an interproximal decay and/or an existing restoration to be replaced and in need to receive an adhesive partial restoration the present clinical trial focused on BoP of subgingival margins in the interproximal area.

Scientific publications available on CMR are mainly based on 'marginal quality' (21-25); however, neither leakage tests under laboratory conditions nor clinical investigations, such as randomized controlled trials, evaluating CMR are available yet.

In vitro studies were mainly performed with thermal and/or mechanical-occlusal stress (9, 10, 22-24) and the main findings concluded that the quality of the external margins, under scanning electron microscopic observations, were very good but a significant decrease of margins' quality after thermal and mechanical stress was observed (10, 22, 23).

However, the evaluation of margins' quality under scanning electron microscopy (SEM) when conducted at low magnification can not clarify if the margins sealed efficiently: leakage to be present does not require an evident gap visible at low magnifications, and it can be clearly detected only using micro computerized tomographic analysis and/or cutting the samples after being processed for marginal leakage. Recently, no correspondence between SEM quality margin assessment and presence of nano-leakage was found (25).

From a clinical point of view, the effectiveness and the “bio-integration” of CMR of posterior indirect restoratives should be related to both BoP, as a measure of periodontal tissue stability, and to a radio-graphic examination, able to assess the marginal bone stability.

In the present trial, two different margin designs were compared in regard to periodontal tissue response in the same sample tooth (resin composite where cervical margins were relocated coronally and margins located in the root below the cementum-enamel junction cementum-dentin).

At the 12 month evaluation, PI and GI were increased as in the previous literature with no statistically significant differences between the two types of margins (**Table 2**). This data is in agreement with previous investigations (15, 26). It must be noted that BoP refers to deep probing whilst GI to superficial probing and PI to the presence of plaque superficially.

When the two experimental groups were compared in terms of Bop, statistically significant differences were found, and consequently, the null hypothesis was rejected. Recent articles confirm how the presence of a deep subgingival margin is otherwise associated with an increase of bleeding after probing (15, 27-29).

Lang et al. clearly described the periodontal inflammation mechanism that occur when overhanging margins are found interproximally (29). However in the same preclinical model, Lang and co. demonstrated how periodontal inflammation is a reversible process, and *restitutio ad integrum* can be again established if proper margin is present (29). The periodontal inflammation experimentally provoked (29) can be similar to that took placed in the interproximal areas where CMR was applied in this study.

The CMR clinical procedure is advocated to get a better control of margins of the indirect restoration at the time of preparation, impression and luting (1, 2), but cannot improve quality of bonding to cementum-dentin substrates (30, 31), and the progressive degradation of the hybrid layer at the bonding interface can not be avoided (32, 33). The seal of the cervical margins below the CEJ remains an important unsolved issue.

While no differences were present between the groups at baseline, at the 12-month follow-up 53% of sites in Group 1 was positive to BoP versus 31.5% in Group 2 (P = .010) (**Table 2**).

When the margin-bone crest distance was considered, it was noticed that in Group 1 samples, 13 margins of 19 were located at a distance of 2 mm from the bone crest and in Group 2 in 6 of 11 cases. This figure can be prudently evaluated because no attempt was made to standardize the angulation of the x-ray. If it is considered that the recorded distance between the restorative margins and the bone crest of all cases with positive BoP (in both Groups 1 and 2) was between 2 and 3 mm, it can be speculated that one of the reason of the bleeding might be related to an invasion of the biological width (34, 35). This interpretation can be also supported by the fact that any resin composite material can have defects that formed as a result of the inclusion of air are liable to have a negative impact on their properties (36, 37). These defect within the resin composite material can alter its mechanical properties by reducing the conversion rate (38, 39), can be the starting point of fractures that can propagate inside the material itself, with a potential reduction of the resistance to compression, flexion, traction and wear and can also increase the diffusion of water molecules inside them (39-44), It thus appears that porosity is a factor liable to have an impact on plaque retention, the durability and clinical performance of the restoration.

At the authors' best knowledge, the present investigation offers for the first time short-term clinical results about the periodontal tissues response to the CMR procedure. After one year of clinical service, there was no evidence of bone loss (BL) neither pathological interproximal PPD > 5 mm; this can be due to the fact that the time needed to develop an evident bone loss and pathological interproximal probing pocket depth can be longer than the 1 year observation time.

In addition, BoP was mainly related with a deeper radiographic location of the margin and in 9 cases out of 19, BoP was also present in the other marginal side, where the crown margin was luted directly to the sound dental structure.

However, BoP was evident in the majority of relocated margins. Among the clinical reasons that can justify the BoP positive sites in group 1, it's worth to mention the difficulties to keep clean deep margins by the patients, overhangins and/or underhangins of the margins, roughness of the cervical resin margins, incomplete control of adhesive and resin composite flow in between the interproximal margin and the metal matrix in an amount that can not be visible in the x-ray.

However, especially in wide MOD-cavities, which often extend close or below the cemento-enamel junction, rubber dam application as well as the adhesive cementation is often difficult to perform. In these situations, a surgical crown lengthening can be useful to allow proper placement of the indirect restoration, to ensure dry conditions during cementation with supragingival margins and to make the restorations more easily home maintainable by the patient (34, 35).

Veneziani recently proposed a new classification based on the depth of the cervical margins related to periodontal tissue: a prudent approach on using CMR when the margins are too deep on the root surface was advocated and a traditional periodontal surgery, based on crown lengthening is still the most reliable procedure when the interproximal margin is placed into the sulcus, in order to expose and make it easily maintainable by the patient (7).

The results of this study and limited information of medium-long term clinical behaviour of CMR procedure suggest a prudent selection of clinical cases in which CMR can be made, and a periodic recall of patients in order to keep under control all periodontal parameters.

However, as increased BoP was observed, long-term data will be needed to rule out the (potential negative) effect of gingival inflammation in terms of tissue stability. For this reason, the results of the present study might be considered preliminary, as longer observational period studies are under evaluation to establish better correlations between the examined parameters.

5. Conclusions

Within the limitations of this study, higher incidence of BoP can be expected around CMR margins and in coincidence with deep margins. CMR of margins is a sensitive-technique, especially when deep subgingival margin is selected and bonding restorative procedures are performed on cementum dentin substrate below the cementum enamel margins.

Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at https://doi.org/10.1016/j.jdent.2017.10.008.

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Chapter 4:

In Vitro Studies

4.1 MARGINAL SEALING OF RELOCATED CERVICAL MARGINS OF MESIO-OCCLUSO-DISTAL OVERLAYS

Köken S, Juloski J, Sorrentino R, Grandini S, Ferrari M (2018) J Oral Sci. 60: 3;460-468.

1. Introduction

The use of adhesive resin restorative materials has improved the aesthetics of dental treatment in the posterior region (1-6). Conventional amalgam restorations have been replaced by minimally invasive adhesive restorations, which protect the intact tooth structure without sacrificing sound tooth structures for mechanical retention (7).

Direct composites are indicated and effective for small and medium-sized Class I and Class II cavities (8, 9). However, in larger cavities, the risk of polymerization shrinkage may cause problems in marginal adaptation, such as fracture and microleakage (10, 11), which can lead to postoperative sensitivity, marginal staining, and secondary caries (12, 13). Because of the lower amount of resin to be cured, semidirect (14, 15) and indirect (16) restorations may improve marginal adaptation by reducing polymerization shrinkage stress. In patients requiring an indirect restoration, the proximal box is often below the surrounding gingival margin and close to or below the cementoenamel junction (CEJ). Subgingivally positioned margins may complicate impression-making and adhesive luting.

Optimal isolation throughout adhesive luting is usually very difficult or impossible to achieve in deep subgingival margins. Surgical margin relocation can address this (17) but is associated with attachment loss and anatomic complications because of the proximity to root concavities and the furcation area (18). As an alternative to periodontal surgical procedures, the cervical margin can be elevated coronally by applying bonding and resin composite materials (19), in accordance with proximal box elevation technique (20-25), also referred to as cervical margin relocation (CMR) (26-28), deep margin elevation (18), or open-sandwich technique (29-32). CMR can be performed with hybrid or flowable composites, after placing the metal matrix and interproximal wedge. Subsequent impression-making is more predictable and luting under rubber dam isolation is more likely to be successful because of the better control during removal of excess cement from the margins.

The absence of enamel at the cervical margin results in areas of weak bonding. Bonding to dentin is not as stable as bonding to enamel (33) and is associated with higher risks of microleakage, bacterial penetration, hypersensitivity, and secondary caries. In addition, resin composite material and its adhesive interfaces in CMR degrade under occlusal loading (34), thus allowing bacterial biofilm penetration at the dentin-restoration margin and, possibly, faster secondary caries development *in vivo (35)*.

This *in vitro* study evaluated the effect of CMR on marginal sealing with two different viscosity resin composites, before adhesive cementation of composite computer-aided design/computer-assisted manufacture mesio-occluso-distal (MOD) overlays. The null hypotheses tested were that the marginal seal would not differ between flowable and hybrid resin composites used for CMR, and that the marginal seal of an MOD overlay would not differ between the enamel and dentin margins.

Materials and Methods Teeth preparation

Thirty-nine intact, healthy, similarly sized human extracted molars without visible cracks, cavities, or restorations were selected for the study after informed consent was obtained from all patients. This study was approved by the Ethical Committee of the University of Siena.

The teeth were mechanically cleaned with hand scalers, brushed with a pumice, and stored in a 0.1% thymol solution for no longer than 3 months. Standardized MOD cavity preparations were created by using water-cooled diamond burs (Komet Burs Expert Set 4562/4562ST, Komet, Lemgo, Germany) in a high-speed handpiece. The remaining axial walls had a thickness of 2 mm and were reduced for a cuspal coverage. Proximal box-shaped preparations were made (1.5 mm in the mesiodistal and 4 mm in the buccolingual direction). The inner angles of the cavities were rounded, and the margins were not beveled. Proximal margins on the mesial side were located 1 mm below the CEJ; on the distal side, tooth margins were located 1 mm above the CEJ.

Teeth were randomly assigned to one of three groups (*n* = 13 specimens each), as follows (**Tables 1, 2; Fig. 1**). Group 1: mesial proximal margins below the CEJ were elevated in two increments of 1 mm with a viscous composite (Essentia; GC Corp., Tokyo, Japan). Group 2: mesial proximal margins below the CEJ were elevated in two increments of 1 mm with a flowable composite (G-ænial Universal Flo; GC Corp.). Group 3 (control): mesial proximal margins were not elevated.

Table 1 Description of the experimental groups

Groups	Restorative material for CMR	Restorative material for overlay	Adhesive system	Resin cement
1. Essentia	GC Essentia MD	GC Cerasmart	GC G-Premiobond	GC LinkForce
2. G-ænial Universal Flo	GC G-ænial Universal Flo A2	GC Cerasmart	GC G-Premiobond	GC LinkForce
3. Control (no CMR)	________	GC Cerasmart	GC G-Premiobond	GC LinkForce

Steel Kerr 2181 Adapt SuperCap matrices (0.038; height, 5.0 mm; Kerr, Orange, CA, USA) were used to create marginal elevation. The circumferential matrix was carefully adjusted to eliminate the risk of overhang of the composite material on the margins, and a 2-mm space was marked on the inner side of the matrix, to avoid overfilling the box. Distal proximal margins were not elevated in any sample. To perform CMR and immediate dentin sealing (IDS), a universal adhesive (GC G-Premio Bond; GC Corp.) was used in selective enamel etch mode. Enamel was etched for 15 s and rinsed for 15 s under laminar water flow. The cavity was gently air-dried, and the bonding agent was applied with a microbrush for 20 s, air blown at maximum pressure for 10 s, and light-cured for 20 s with a BA Optima 10 curing light (B.A. International Ltd, Northampton, UK). In Groups 1 and 2, the cervical margins on the mesial sides were filled with two 1-mm increments of the composite GC Essentia (Group 1) or G-ænial Universal Flo (Group 2). Adaptation of composites was performed with ball-ended hand instruments and a microbrush. Care was taken not to layer the composite at a thickness greater than 2 mm. Water-cooled diamond burs (Komet Burs Expert Set 4562/4562ST, Komet) on a high-speed handpiece were used to create the final shape of each cavity after CMR.

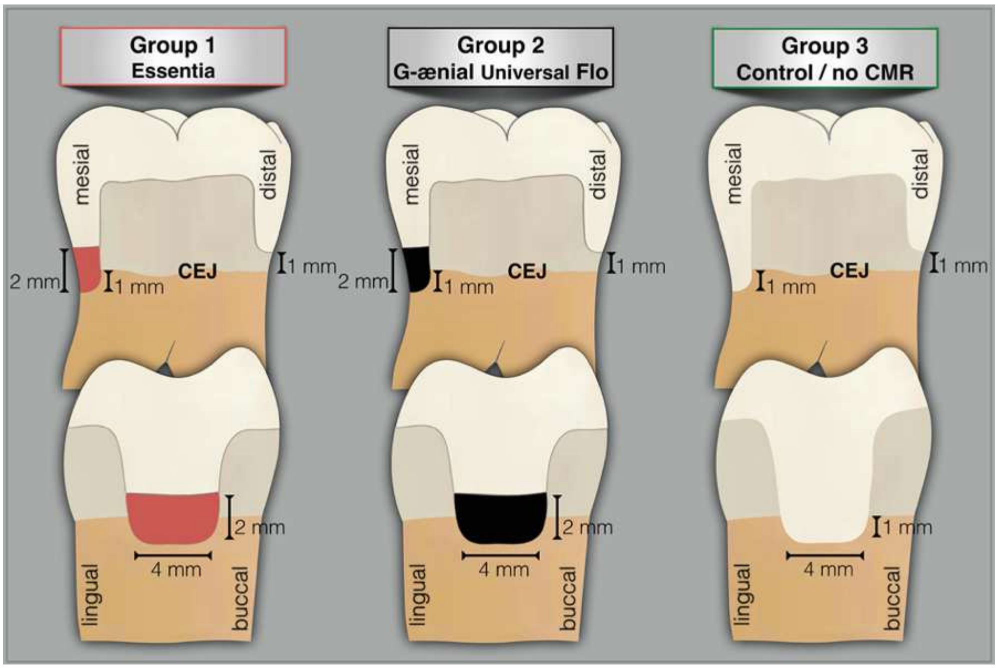

Fig. 1 Illustrations of the techniques used for all experimental groups.

Table 2 Chemical composition and application procedures for the tested materials.

Material (manufacturer)/ Batch number	Type	Application procedure	Composition
G-Premio BOND (GC Corporation, Tokyo, Japan) lot:1606272	Universal adhesive	Selective etching of enamel for 15 s Rinsing for 15 s Air blowing (max pressure) for 10 s Light curing for 20 s	MDP, 4-MET, MDTP, dimethacrylate monomers, acetone, water, silicon dioxide, photoinitiators
Essentia MD (GC Corporation, Tokyo, Japan) lot:1607271	Microhybrid resin composite	Each layer is light cured for 20 s	UDMA, dimethacrylate monomers, silicon dioxide, fillers, pigments, photoinitiators
G-ænial Universal Flo (GC Corporation, Tokyo, Japan lot:1506131	High filled flowable resin composite	Each layer is light cured for 20 s	UDMA, bis-EMA, dimethacrylate monomers, silicon dioxide, fillers, pigments, photoinitiators
G-CEM LinkForce (GC Corporation, Tokyo, Japan) lot:1608231	Dual-cure adhesive luting cement	Mixture is applied on restoration's inner surface and preparation surface. Overlays firmly pressed. Each axial wall is light cured for 60 s	Paste A: UDMA, bis-GMA, dimethacrylate monomers, fillers, pigments, photoinitiators Paste B: UDMA, bis-EMA, dimethacrylate monomers, fillers, photoinitiators
G-Multi Primer (GC Corporation, Tokyo, Japan) lot:1601141	Primer for glass ceramics, hybrid ceramics,zirconia, alumina, composites ,metal bonding.	Applied with a micro brush on restoration's inner surface	Ethanol, Phosphoric ester monomer, γ-Methacryloxypropyl trimethoxysilane, Methacrylate monomer
GC Etchant (GC Corporation, Tokyo, Japan) lot:1610271	Etching gel %37 phosphoric acid	Selective etching of enamel for 15 s	Phosphoric acid (37%), silicon dioxide, colorant
GC Cerasmart (GC Corporation, Tokyo, Japan) lot:1609082	Force absorbing hybrid ceramic CAD/CAM block	Sandblasting and silanization of the inner surface.	Raw materials of the pre-cured composite block: UDMA, dimethacrylate monomers, bis-EMA, silicone dioxide, barium glass powder, pigments, initiator

Impression-making

An extraoral scanner (Aadva Lab Scan, GC Corp.) was used to make digital impressions of the prepared teeth. Scanned files were sent to a milling center (GC Corp., Leuven, Belgium) that created the resin composite overlays (Cerasmart, GC Corp.). The teeth were kept in fresh water for 2 weeks at room temperature until the overlays were luted. The fit of the overlays was examined under a digital microscope (Nikon Shuttle Pix, Tokyo, Japan), and digital photographs were obtained at 10× magnification.

Luting procedure

Before luting, the teeth were cleaned with ethanol, and the enamel was selectively etched for 15 s and rinsed with laminar water flow for another 15 s. Preparation surfaces were gently dried, and G-Premio Bond (GC Corp.) was applied with a microbrush for 20 s, air blown at maximum pressure for 10 s, and light-cured for 20 s (BA Optima 10, B.A. International Ltd.). Cerasmart overlays were sandblasted at approximately 3 bar pressure with 50-µm aluminum oxide particles. Later, G-Multi primer (GC Corp.) was applied to silanize the inner sandblasted surface of the overlays. An adhesive resin cement (G-Cem LinkForce; GC Corp.) was used to lute the overlays in both groups. G-Cem LinkForce (GC Corp.) was mixed with its special mixing tip, and the initial mixture was discarded on clean paper. The subsequent mixture was applied to the inner surface of the restoration and the preparation surface. The overlays were pressed firmly on teeth, and excess luting materials were cleaned with a microbrush and cotton pellets. The restoration margins were covered with a water-based glycerine gel (Airblock, DeTrey-Dentsply, Konstanz, Germany). Each axial wall was light-cured for 60 s, and the occlusal surface was cured for 60 s. Margins were gently finished with flexible disks (SofLex Pop-on, 3M ESPE, St. Paul, MN, USA).

Evaluation of marginal seal

All tooth surfaces were covered with nail varnish. We left exposed the 1 mm around the area of the adhesive interfaces between the overlay and tooth and the CMR on the mesial aspect of the tooth. A diluted ammoniacal silver nitrate solution (1:4 ratio of ammoniacal silver nitrate to distilled water) was prepared, and the diluted solution was filtered with a Millipore filter (0.22-nm filter, Carrigtwohill, County Cork, Ireland) mounted on a syringe. Under laboratory light, each tooth was placed in a test tube with diluted ammoniacal silver nitrate solution. After 24 h, specimens were thrice rinsed in water for 10 min. Nail varnish around the tooth was removed with acetone, and each tooth was placed in a test tube with the diluted photo-developer solution (Kodak, Rochester, NY, USA; 1:10 ratio of photo-developer solution to distilled water). After 8 h, teeth were thrice rinsed in water for 10 min.

Each tooth was embedded in transparent self-curing acrylic resin. The teeth were then sliced with a low-speed diamond saw under water cooling (Isomet; Buehler, Lake Bluff, NY, USA) into three or four 1-mm-thick slices along their long axis and perpendicularly to the proximal margins. Samples were examined with a digital microscope at 1×, 3×, and 6× magnification.

Two observers independently scored the amount of tracer along the interface, by using the scheme follows (36) (**Fig. 2**).

0: no nanoleakage;

1: 0% to 20% of gingival floor interface showing nanoleakage;

2: 20% to 40% of gingival floor interface showing nanoleakage;

3: 40% to 60% of gingival floor interface showing nanoleakage;

4: 60% to 80% of gingival floor interface showing nanoleakage;

5: 80% to 100% of gingival floor interface showing nanoleakage.

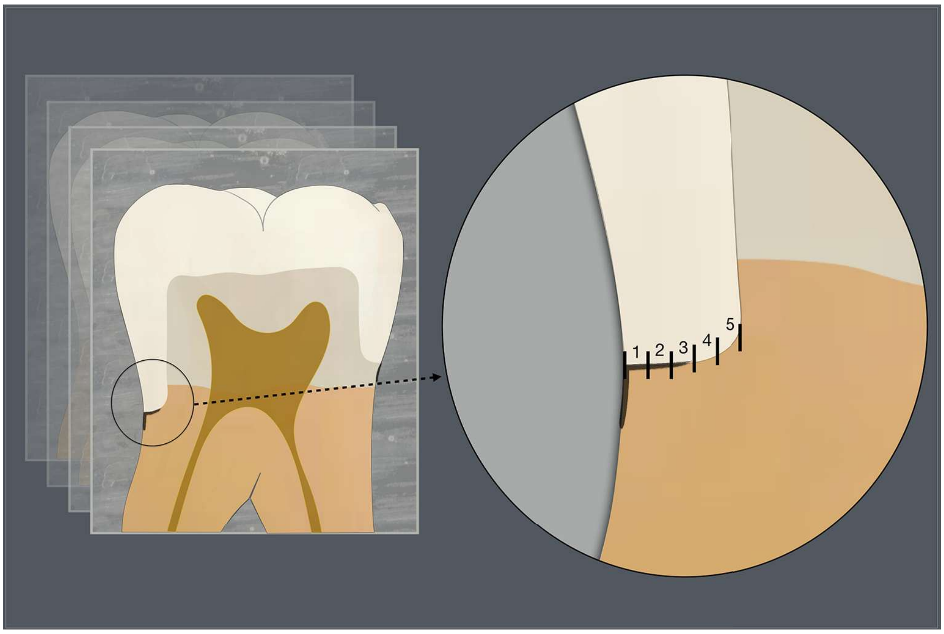

Fig. 2 Illustration of the scoring system.

Fig. 3 Representative sample from Group 1 (Essentia group) with a nanoleakage score of 4 (×6).

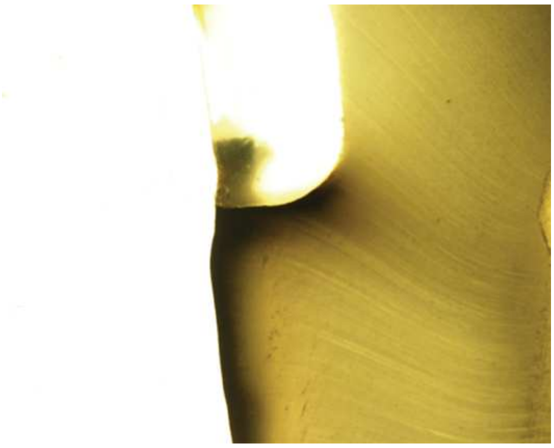

Fig. 4 Representative sample from Group 2 (Universal Flo group) with a nanoleakage score of 5 (×6).

Fig. 5 Representative sample from Group 3 (Control group) with a nanoleakage score of 3 (×6).

Statistical analysis

The Kruskal-Wallis test was used to assess differences between the composite materials in leakage scores recorded at the dentin-composite interface in groups with CMR and to compare those score with scores at the dentin-overlay interface of the control group without CMR. The Wilcoxon signed-rank test was used to determine separately whether leakage significantly differed between the two substrates (i.e, dentin and enamel interface) for the tested CMR composite materials and in the control group.

The significance level was set at $P < 0.05$, and the analyses were performed with the software package SPSS IBM Statistics version 21 for Mac (SPSS Inc., Chicago, IL, USA).

Results

Nanoleakage along the dentin-bonding interfaces significantly differed among the three groups (Kruskal-Wallis, $P = 0.000$; **Figs. 3-5**). The Mann-Whitney U test showed no significant difference in leakage scores at the dentin- CMR composite interface between the two composites ($P = 0.279$); however, the control group showed significantly less nanoleakage. The median leakage score was 2 for both composites and 1 for the control group, with no CMR. Descriptive statistics for the leakage scores are shown in Table 3.

Leakage significantly differed between the two bonding interfaces (enamel and dentin), when analyzed in aggregate, and in the Essentia (*P* = 0.000, Wilcoxon signed-rank test), G-ænial Universal Flo (*P* = 0.000, Wilcoxon signed-rank test), and control groups (no CMR) (*P* = 0.000, Wilcoxon signed-rank test), when analyzed separately. In all three analyses, leakage scores were significantly higher at the dentin interface (median 2, interquartile range 0-3) than at the enamel interface (median 0, interquartile range 0-0). The descriptive statistics are shown in **Table 4**.

Table 3. Descriptive statistics of leakage scores recorded at along dentin/composite interface (Groups 1 and 2) and dentin/overlay interface (Group 3).

Microleakage Score	*n*	Mean	SD	Median	Interquartile range	
					25th percentile	75th percentile
1. Essentia [B]	42	2,40	1,449	2,00	1,00	3,00
2. G-ænial Universal Flo [B]	46	2,04	1,095	2,00	1,00	2,25
3. Control (no CMR) [A]	45	1,18	0,777	1,00	1,00	2,00

n: number of slices; SD: standard deviation. Different superscript letters indicate statistically significant differences among the groups. Kruskal-Wallis, *P* = 0.000

Table 4. Descriptive statistics of leakage scores recorded at dentin/composite (Groups 1 and 2) and dentin/overlay (Group 3) interface and enamel/overlay interface (all three groups).

Microleakage Score	*n*	Mean	SD	Median	Interquartile range	
					25th percentile	75th percentile
1. Essentia						
Dentin [B]	42	2,40	1,449	2,00	1,00	3,00
Enamel [A]	42	0,07	0,261	0,00	0,00	0,00
2. G-ænial Universal Flo						
Dentin [B]	46	2,04	1,095	2,00	1,00	3,00
Enamel [A]	46	0,24	0,480	0,00	0,00	0,00
3. Control (no CMR)						
Dentin [B]	45	1,18	0,777	1,00	1,00	2,00
Enamel [A]	45	0,16	0,367	0,00	0,00	0,00

n: number of slices; SD: standard deviation. Different superscript letters indicate statistically significant differences among the groups. Wilcoxon signed-rank test, $P = 0.000$; three groups tested separately.

Discussion

We evaluated the effects of cervical marginal relocation on marginal sealing when two resin composites with different viscosities were used before adhesive cementation of CAD/CAM MOD overlays. Since the first description of CMR, some researchers have suggested that flowables are the material of choice for elevating the deepest parts of the cavity (17,37). Others, however, support the use of flowable or restorative composite (18, 26, 27) or a combination of both if more material is needed (26, 27). In addition, microhybrid or nanohybrid resin composite should be preheated, to facilitate placement and minimize the risk of interlayer gaps (18). There remains a lack of consensus regarding the preferred material and application technique for this clinical procedure.

The viscosity of flowables makes them favorable for use in CMR because they are easy to apply to deep proximal areas, result in fewer voids, and thoroughly wet the bonded surface (38); however, because of the low viscosity of flowables, excess and overhang are concerns (39).

We studied two resin composites that were used in combination with a proprietary adhesive material. The marginal seal did not differ between the two materials, and the first null hypothesis was therefore accepted. Thus, both flowables and microhybrid resin composites are suitable for CMR. Furthermore, we observed almost no leakage at the enamel-bonding interface, most likely because the cut and etched enamel prisms provide reliable micromechanical interlocking (40), thus preventing adhesive and cohesive fracture at the luting-enamel interface (41). In contrast, leakage was always observed at the dentin-bonding interface, and the second null hypothesis was therefore rejected.

Treatment of posterior proximal cavities with deep cervical margins below the CEJ is usually highly complex when an adhesive indirect restoration is selected. All prosthodontic steps, such as preparation of the cavity and both traditional and digital impression and luting, are difficult to perform properly (24). Therefore, placement of a few composite resin layers (CMR) was proposed as a method to facilitate clinical handling of indirect restorations (19). This procedure should be carried out under rubber dam isolation, followed by matrix placement (18). However, control of interproximal margins is a concern, as it requires both careful consideration of the arrangement of the emergence profile and a perfect subgingival fit for the CMR. Previous studies proposed specific matrix types for CMR, including circumferential and sectional matrices, and stainless steel and clear matrices (17, 18, 24, 26, 27), as well as matrices with curvature that provides an adequate emergence profile and tight subgingival fit (18, 27). In the present study, the circumferential matrix was carefully adjusted to eliminate the risk of composite material overhang on the margins. In addition, a 2-mm space was marked on the inner side of the matrix, to avoid overfilling the box. Thus, polymerization shrinkage was reduced by the controlled thickness of the CMR composite.

In this study, two 1-mm increments of flowable or microhybrid composite were placed, to allow for an overall 2-mm elevation of the cervical margins. Application of CMR with meticulous layering of the two 1-mm increments of flowable or restorative composite had no effect on the quality of cervical margins (28).

Moreover, one-bottle universal self-etch adhesive was used in selective etch mode in combination with proprietary luting material. Universal adhesives are the latest-generation bonding system and reduce sensitivity to the clinical procedure (42). In addition, application of a universal adhesive on dentin decreases the risk of over-etching and ensures that the dentin substrate will not be too dry or too wet (42, 43). To date, universal adhesive syste4ms have yielded promising results (44-46).

This *in vitro* study evaluated all bonding interfaces involved in the CMR procedure, and leakage was always detected at the interface between the root cementum-dentin margin and composites. Analysis of the dentin margin showed that the marginal seal for the two tested materials did not significantly differ when they were used for CMR. However, the performance of the flowable composite was slightly better than that of the hybrid composite. The favorable performance of flowables may be explained by their easier application and adaptation to the cavity bottom (47). The present findings are consistent with those of previous studies (28, 37, 48), which showed that flowable and restorative composites did no differ in marginal quality when applied for a CMR approach on dentin.

This study also showed that direct placement of composite CAD/CAM overlays on dentin (without CMR), with the same luting procedure, resulted in a significantly better marginal sealing than that obtained with a CMR approach and either flowable or hybrid resin composite. In contrast, most previous studies reported no significant difference in marginal quality between restorations placed directly on dentin and those with CMR composite (20, 25, 28, 48-50). However, two studies showed that, after being subjected to thermal and mechanical stress, luting directly to dentin (conventional technique) resulted in superior marginal adaptation as compared with CMR composite on dentin (21, 23). The present findings might have been affected by polymerization shrinkage of the resin composite materials used for making the CMR and luting the overlay (51-54).

The present study used a leakage test to evaluate the marginal seal of restorations; however, previous studies evaluated margin quality by using low-magnification scanning electron microscopy (20, 21, 23, 25, 37, 48-50, 55).

It is possible that previous studies under-evaluated the actual seal of restorations after CMR. Nevertheless, no previous study reported an experimental group with a perfect seal, which indicates that microscope type, technique, and magnification affect evaluation of margin quality. In other words, high-magnification examination of marginal seals, with silver nitrate perfusion testing along the hybrid layer, is likely a more rigorous test.

To date, only a few *in vitro* studies have examined CMR applied in indirect restorations. The investigated variables were marginal (20, 21, 23, 25, 28, 48-50, 55) and internal adaptation (37, 48, 50), bond strength to the proximal box floor (56), and fracture behavior of restored teeth (23). Marginal adaptation was usually evaluated by SEM examination of impression replicas, to determine the percentage of continuous gap-free margins before and after thermal and mechanical stress. Many studies (20, 21, 23, 25, 28, 48-50, 55) reported a consistent decrease of margin quality after exposure to stress. In the present study, teeth were not subjected to mechanical or thermal stress. Such exposure might increase leakage.

From a clinical perspective, CMR does not properly seal the cervical margin in the root cementum-dentin, regardless of the type of resin composite material used, perhaps because of difficulties in isolating the field (57), the presence of cementum-dentin substrate (58, 59), the difficulty in achieving a proper seal on cementum-dentin substrate (60), the effectiveness of bonding procedure and material (43, 61), shrinkage of resin composites (51), operator skill and knowledge and the sensitivity of this technique (62), and occlusal stress transmitted to the margin through the indirect resin restoration (63).

CMR is a relatively new restorative procedure and information on its performance is limited. Future *in vitro* and *in vivo* studies should evaluate the effectiveness of CMR technique and the marginal seal of different bonding systems and luting cements in combination with CMR. In addition, randomized clinical trials should investigate the durability of CMR and the response of periodontal tissues.

In conclusion, the present results indicate that the performance (marginal sealing ability) of flowable and microhybrid resin composites is comparable for CMR. Furthermore, luting overlays directly to dentin, without CMR, appears to be a better method for limiting marginal leakage underneath CAD/CAM overlays.

Acknowledgments

The materials were generously provided by GC Europe.

Conflict of interest

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

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4.2 INFLUENCE OF CERVICAL MARGIN RELOCATION AND ADHESIVE SYSTEM ON MICROLEAKAGE OF INDIRECT COMPOSITE RESTORATIONS

Köken S, Juloski J, Ferrari M (2019) J Osseointegr. 11: 1;21-28.

1. Introduction

Over the last two decades the use of adhesive restorative materials has aesthetically improved the possibilities for dental treatments in the posterior region (1-6). Minimally invasive restorations have gradually replaced conventional amalgam restorations, providing protection of intact tooth structure without sacrificing sound tissue for mechanical retention (7). In small and medium sized Class I and Class II cavities direct composite restorations are indicated and are found to be effective (8, 9). However, when the size of the cavity enlarges, the risk of polymerization shrinkage increases, which may result in marginal adaptation problems, such as fracture of adhesively formed tooth-restoration interface and microleakage (10, 11), possibly leading to postoperative sensitivity, marginal staining or secondary caries (12, 13).

Considering the detrimental effects of polymerization shrinkage and the complexity of placing a direct composite restoration in large posterior cavities, semidirect (14, 15) and indirect (16) restorations were proposed in such cases. Extraorally fabricated restorations that are adhesively cemented are less affected by polymerization shrinkage due to the reduced thickness of the resin to be cured. However, the problem that may often occur is the margin of the proximal box of indirect restorations located below the surrounding gingival margin and close to or below the cemento-enamel junction (CEJ). Impression taking, as well as the adhesive luting procedures are therefore hampered by subgingivally positioned margins. Although the surgical crown lengthening could be performed to overcome those clinical difficulties, elevating cervical margins coronally by adhesively bonding of a small amount of resin composite material on the proximal margin was proposed as an alternative to surgical procedures (17). Cervical margin relocation was first proposed by Dietschi et al. (18), and although it is well known among the clinicians there is a scarce scientific literature about this technique (19). The subgingival location of margins and the absence of enamel at the cervical margin create the weak area for reliable bonding. Bonding to dentin is a sensitive technique and not as stable as that to enamel (20). Due to the degradation of the resin composite material and its adhesive interfaces over time under continuous occlusal loading (21), restoration margins in dentin could be more susceptible to microleakage and bacterial biofilm penetration, possibly leading to hypersensitivity or secondary caries (22).

The aim of this *in vitro* study was to evaluate the influence of the cervical margin relocation and the adhesive system on the microleakage of indirect composite restorations with proximal margins located below the CEJ. The three null hypotheses tested were:

1. no difference would be found in the marginal sealing

between the proximal margins with and without CMR when a universal adhesive was used for composite onlay cementation;

2. no difference would be found in the marginal sealing between the proximal margins with and without CMR when a 3-step total-etch adhesive was used for composite onlay cementation;
3. no differences would be found in the marginal sealing between the two different adhesive systems used for composite onlay cementation when CMR was not performed.

MATERIALS AND METHODS

Teeth preparation

Twenty intact, healthy, similar sized human third molars extracted for therapeutic reasons without any visible cracks, cavities or restorations were selected for the study after informed consent of the patients was obtained. Teeth were mechanically cleaned with hand scalers, brushed with pumice and stored in 0.1% thymol solution for no longer than three months. Standardized MOD cavity preparations were created using water-cooled diamond burs (Komet Burs Expert Set4562/4562ST, Komet, Lemgo, Germany)mountedon a high-speed handpiece. The remaining axial walls had 2 mm of thickness, and they were reduced for a cuspal coverage. Proximal, box-shaped preparations were made 2 mm in the mesio-distal and 5 mm in bucco-lingual direction. The inner angles of the cavities were rounded, and the margins were not beveled. Proximal margins in both mesial and distal sides were located 1 mm below the CEJ. Teeth were randomly assigned to two equal groups of 10 specimens each, as follows (Fig. 1, Table 1, 2).

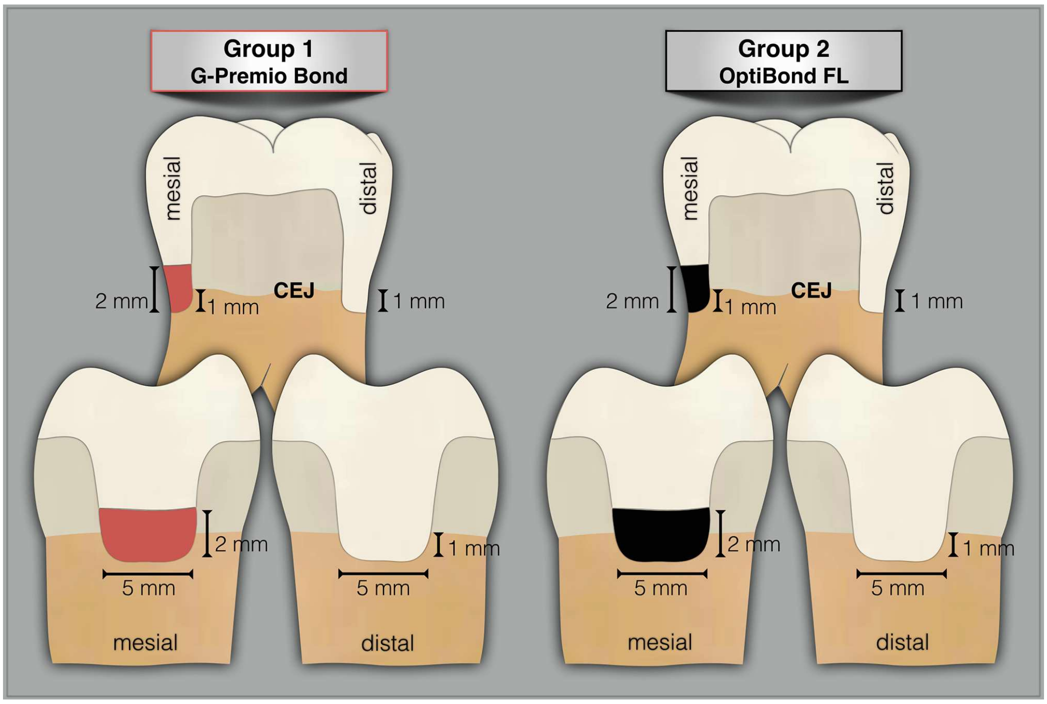

FIG. 1 Schematic description of groups.

- Group 1: mesial proximal margins below the CEJ were elevated with two increments of 1 mm each with G-ænial Universal Flo (GC Corp., Tokyo, Japan), which was bonded with a universal bonding agent G-Premio Bond (GC Corp.). Resin composite overlays were luted with a resin cement (G-Cem LinkForce, GC Corp.) in combination with the same universal bonding agent G-Premio Bond in selective enamel etch mode.
- Group 2: mesial proximal margins below the CEJ were elevated with two increments of 1 mm each with G-ænial Universal Flo (GC Corp.), which was bonded with a universal bonding agent G-Premio Bond (GC Corp.). Resin composite overlays were luted with the same resin cement (G-Cem LinkForce, GC Corp.), but in combination with a gold standard 3-step total-etch bonding system (Optibond FL, Kerr, Orange, CA, USA).

Table 1. Chemical compositions and application procedures of the tested materials.

Material (manufacturer) / Batch number	Type	Application procedure	Composition
OptiBond FL (Kerr; Orange, CA, USA) lot:5866021	Three-step etch-and-rinse adhesive	Etch-and-rinse: 37% phosphoric acid, 30 s enamel, 15 s dentin, primer for 20 s, bonding for 20 s, and light curing for 20 s	Primer: ethyl alcohol, alkyl dimethacrylate resins and water Bonding agent: uncured methacrylate ester, monomers, triethylene, glycol, dimethacrylate, ytterbium trifluoride, inert mineral fillers, photoinitiators and stabilizers
G-Premio BOND (GC Corporation, Tokyo, Japan) lot:1703272	Universal adhesive	Selective etching of enamel for 15 s Rinsing for 15 s Air blowing (max pressure) for 10 s Light curing for 20 s	MDP, 4-MET, MDTP, dimethacrylate monomers, acetone, water, silicon dioxide, photoinitiators
G-ænial Universal Flo (GC Corporation Tokyo, Japan) lot:1506131	High filled flowable resin composite	Each layer is light cured for 20 s	UDMA, bis-EMA, dimethacrylate monomers, silicon dioxide, fillers, pigments, photoinitiators
G-CEM LinkForce (GC Corporation, Tokyo, Japan) lot:1608231	Dual-cure adhesive luting cement	Mixture is applied on restoration's inner surface and preparation surface. Overlays are firmly pressed. Each axial wall is light cured 60 s	Paste A: UDMA, bis-GMA, dimethacrylate monomers, fillers, pigments, photoinitiators Paste B: UDMA, bis-EMA, dimethacrylate monomers, fillers, photoinitiators
G-Multi Primer (GC Corporation, Tokyo, Japan) lot:1703231	Primer for glass ceramics, hybrid ceramics, zirconia, alumina, composites, metal bonding	Applied with a micro brush on restoration's inner surface	Ethanol, Phosphoric ester monomer, γ-Methacryloxypropyl trimethoxysilane, Methacrylate monomer
GC Etchant (GC Corporation, Tokyo, Japan) lot:1610271	Etching gel %37 phosphoric acid	Selective etching of enamel for 15 s	Phosphoric acid (37%), silicon dioxide, colorant
GC Cerasmart (GC Corporation, Tokyo, Japan) lot:1609082	Force absorbing hybrid ceramic CAD/CAM block	Sandblasting and silanization of the inner surface.	Raw materials of the pre-cured composite block: UDMA, dimethacrylate monomers, bis-EMA, silicone dioxide, barium glass powder, pigments, initiator

Table 2. Description of the experimental groups.

Groups	Restorative material for CMR	Adhesive system for CMR	Restorative material for overlay	Adhesive system for luting	Resin cement
Group 1: G-Premio Bond	GC G-ænial Universal Flo A2	GC G-Premio Bond	GC Cerasmart	GC G-Premio Bond	G-Cem LinkForce
Group 2: Optibond FL	GC G-ænial Universal Flo A2	GC G-Premio Bond	GC Cerasmart	Kerr Optibond FL	G-Cem LinkForce

Kerr 2181 Adapt® SuperCap® matrices in steel (0.038, 5.0 mm high) were used to create the cervical marginal elevation. The circumferential matrix was carefully adjusted to eliminate the risk of overhanging of the composite material on the margins and 2 mm space is marked on the inner side of the matrix to avoid overfilling of the proximal box. Distal proximal margins were not elevated in any of the samples. To achieve CMR of mesial proximal margins and immediate dentin sealing (IDS) an universal adhesive G-Premio Bond was used in selective enamel etch mode. Enamel was etched for 15 s and rinsed for 15 s under laminar water flow. The cavity was gently dried, G-Premio Bond was applied with microbrush for 20 s, air blown with maximum pressure for 10 s and light cured for 20 s (BA Optima 10, B.A. International Ltd, Northampton, UK). In all specimens the cervical margins on the mesial sides were filled with two 1-mm increments of flowable composite G-ænial Universal Flo and the adaptation was performed with flowability of the material itself and with a ball ended hand instruments and a micro brush. Special care was taken not to layer the composite more than 2 mm in thickness. Water-cooled diamond burs (Komet Burs Expert Set 4562/4562ST, Komet, Lemgo, Germany) on high-speed handpiece were used to give final shape of each cavity after CMR was performed.

Impression

Extraoral scanner GC Aadva Lab Scanner (GC Corp.) was used for making digital impressions of the prepared teeth. Scanned files were sent to Milling Center (GC Leuven) to create resin composite overlays (Cerasmart, GC Corp.). Teeth were kept in fresh water for two weeks at room temperature until the overlays were luted. The fit of overlays was examined under digital microscope (Nikon Shuttle Pix, Tokyo, Japan) and the digital photographs were taken at a 10x magnification.

Luting procedure

- Group 1: before luting, teeth were cleaned with ethanol and enamel was selectively etched for 15 s and rinsed with laminar water flow for further 15 s. Preparation surfaces were gently dried and GC G-Premio Bond was applied with microbrush for 20 s, air blown with maximum pressure for 10 s and light cured for 20 s (BA Optima 10, B.A. International Ltd, Northampton, UK).
- Group 2: before luting, teeth were cleaned with ethanol, first etchant gel was applied on enamel for 15 s and later etchant gel was applied on dentin further 15 s. All etchant gel was rinsed with laminar water flow for 30 s. Preparation surfaces were gently dried, Optibond FL Primer was applied with a light scrubbing motion using a micro brush for 20 s, and gently air dried for 5 s. Then Optibond FL Adhesive was applied on the preparation surfaces with a light scrubbing motion for 20 s using a micro brush but was

only gently air thinned for 3 s and light cured at the final stage for 20 s (BA Optima 10, B.A. International Ltd, Northampton, UK).

Cerasmart onlays were sandblasted at approximately 3 bar pressure with 50-µm aluminum oxide particles. Subsequently, GC Multi Primer (GC Corp.) was applied to silanize the inner sandblasted surface of the overlays. Adhesive resin cement (G-Cem LinkForce GC Corp) was used to lute the overlays for both groups. G-Cem LinkForce was mixed with its special mixing tip, initial mixture was discarded on a clean paper. The latter mixture was applied on restoration's inner surface and preparation surface. The overlays were pressed firmly on teeth and the excess luting materials were cleaned with a microbrush and cotton pellets. The restoration margins were covered with a water-based glycerine gel (Airblock, DeTrey-Dentsply). Each axial wall was light cured for 60 seconds and finally occlusal surface was cured 60 s. Margins were gently finished with flexible disks (SofLex Pop-on, 3M ESPE, St.Paul, USA).

Marginal seal evaluation

All surfaces of the teeth were covered with nail varnish leaving exposed 1 mm around the area of the adhesive interfaces between the overlay and the tooth on the distal aspect and between the tooth and the CMR on the mesial aspect of the tooth. Diluted ammoniacal silver nitrate solution (1:4 ratio ammoniacal silver nitrate and distilled water) was prepared. The diluted solution was filtered using Millipore filter (0.22 nanometer filter, Carrigtwohill, County Cork, Ireland) mounted on a syringe. Each tooth was placed in a test tube with diluted ammoniacal silver nitrate solution in the presence of laboratory light. After 24 hours specimens were rinsed in water for 10 minutes three times. Nail varnish around the tooth was removed with acetone, and each tooth was placed in a test tube with the diluted photo developer solution (Kodak, Rochester, NY, 1:10 ratio photo developer solution and distilled water). After 8 hours teeth were rinsed in water three times for 10 minutes.

2a 2b

FIG. 2 (a) Sample of Group 1; CMR (mesial) side in which microleakage of 2 score is represented, **(b)** Sample of Group 1; non-CMR (distal) side in which microleakage of 1 score is represented.

3a 3b

FIG. 3 (a) Sample of Group 1; CMR (mesial) which microleakage of 3 score is represented, **(b)** Sample of Group 1; non-CMR (distal) which microleakage of 3 score is represented.

Each tooth was then embedded in transparent self-curing acrylic resin. Subsequently, the teeth were sliced using a low-speed diamond saw under water- cooling (Isomet; Buehler, Lake Bluff, NY, U.S.A) into five to six 0.65-mm thick slices along their long axis and perpendicularly to the proximal margins. Samples were examined under digital microscope at 1x, 3x and 6x magnification (Nikon Shuttle Pix, Tokyo, Japan) (**Fig. 2, 3**). Two observers independently scored the amount of tracer along the interface as follows (23) (**Fig. 4**):

- score 0= no microleakage;
- score 1= 0-20% of the interface showing microleakage;
- score 2= 20-40% of the interface showing microleakage;
- score 3= 40-60% of the interface showing microleakage;
- score 4= 60-80% of the interface showing microleakage;
- score 5= 80-100% of the interface showing microleakage.

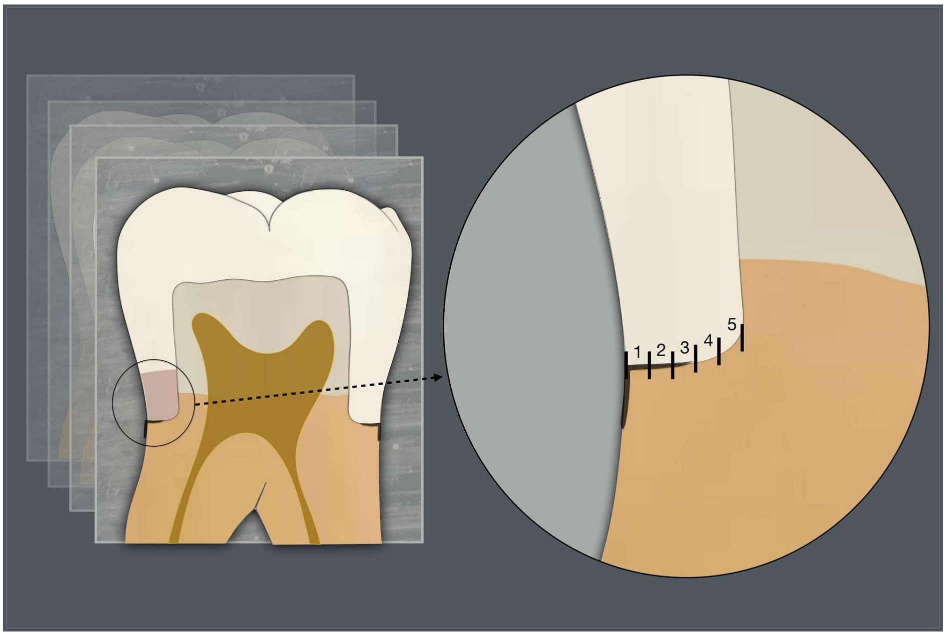

FIG. 4 The schematic drawing of the scoring system used for evaluation of the microleakage.

Statistical analysis

Two separate Wilcoxon signed-rank tests were performed on the data obtained from the two groups separately, in each group comparing the microleakage scores on the CMR site to those on the non-CMR site. These analyses were performed so the effect of the CMR technique on the marginal seal could be assessed for two different adhesive systems.

Additionally, in order to evaluate the influence of the adhesive system on the quality of the seal, Mann-Whitney U test was used to assess the statistical difference in the microleakage scores between the two groups on the distal aspects of the teeth where CMR was not performed.

The significance level was set at $P < 0.05$ and the analyses were performed by means of the statistical software SPSS IBM Statistics, version 21 for Mac (SPSS Inc., Chicago, IL USA).

RESULTS

Descriptive statistics of microleakage scores are reported in **Table 3, 4, 5**.

Wilcoxon signed-rank test performed on data obtained from Group 1, when G-Premio Bond was used for luting the onlays, revealed that statistically significant difference existed in microleakage scores between CMR and non-CMR sites ($P = 0.000$). It showed that significantly higher microleakage was present on CMR sites (**Table 3**).

Group 1: G-Premio Bond	N	Mean	SD	Median	Interquartile range	
					25th percentile	75th percentile
CMR*	53	1,92	0,560	2,00	2,00	2,00
non CMR	53	1,16	0,758	1,00	1,00	2,00

Table 3. Descriptive statistics of microleakage scores recorded in Group 1. Statistically significant difference existed in microleakage between CMR and non-CMR sites (Wilcoxon signed-rank test, P = 0.000). Asterix (*) indicates that significantly higher microleakage was present on CMR site.

The same analysis conducted for Group 2 where OptiBond FL used for luting the onlays, revealed no statistically significant difference in microleakage between CMR and non-CMR margins (**Table 4**, Wilcoxon signed-rank test, P = 0.491).

Group 2: Optibond FL	N	Mean	SD	Median	Interquartile range	
					25th percentile	75th percentil e
CMR	58	2,09	0,830	2,00	2,00	3,00
non CMR	58	2,19	0,974	2,00	2,00	3,00

Table 4. Descriptive statistics of microleakage scores recorded in Group 2. No statistically significant difference existed in microleakage between CMR and non-CMR sites (Wilcoxon signed-rank test, P = 0.491).

Furthermore, when the non-CMR sites were compared between the two groups statistically significant differences emerged in microleakage scores (Mann-Whitney U test, P = 0.000). Significantly lower scores were observed when the universal adhesive G-Premio Bond was used for luting the composite onalys directly to dentine below CEJ without CMR compared to the 3-step total-etch OptiBond FL used in the same conditions (**Table 5**).

MICROLEAKAGE SCORE non-CMR site	N	Mean	SD	Median	Interquartile range	
					25th percentile	75th percentile
Group 1: G-Premio Bond	53	1,16	0,758	1,00	1,00	2,00
Group 2:* Optibond FL	58	2,19	0,974	2,00	2,00	3,00

Table 5. Descriptive statistics of microleakage scores recorded at non-CMR sites in Groups 1 and 2. Statistically significant difference existed in microleakage at non-CMR sites between Group 1 and 2 (Mann-Whitney U test, P = 0.000). Asterix (*) indicates that significantly higher microleakage was observed in Group 2.

DISCUSSION

The aim of this *in vitro* study was to assess the seal of the margins located in the root below CEJ when CMR technique is performed in order to relocate the margins above CEJ. Also, this paper searched for an answer to a question whether the adhesive system used for luting the indirect restoration has an impact on the leakage at the adhesive interfaces. The first null hypothesis was rejected as the results revealed that in Group 1 significantly higher microleakage was scored at the mesial aspects of the teeth where CMR was applied (median value 2) compared to the distal aspects where such treatment was not carried out (median 1). Conversely, in Group 2 no differences were detected between the two aspects of the tooth, as both median values were 2 (**Table 4**) and therefore the second null hypothesis was accepted. From these results it may be assumed that CMR would not be beneficial in terms of microleakage at the adhesive interface when a universal adhesive is used. On the other hand, in case of a total- etch adhesive, the results of the present study indicate that leakage at the margins does not depend on the CMR technique, and at both proximal sites the median score value was 2. These contradictory results obtained evaluating data from Group 1 and Group 2 may suggest that the adhesive system represents a significant factor. As a matter of fact, this observation was confirmed in the third analysis, where the non-CMR sites were compared between two groups and significant differences were detected. Therefore, the third null hypothesis was also rejected.

Several papers described the clinical procedure of CMR (18, 24, 25) and several *in vitro* tests followed in order to evaluate quality of the margins after CMR was performed for reposition of the proximal margins (19). Most of the *in vitro* research investigated the marginal integrity of the relocated margins as seen under scanning electron microscopy (SEM) (26-30). A recently published paper tested the effect of CMR on the microleakage using the same method as described in the present study (31). The focus of that paper was on the type of the composite used for margin relocations and the authors reported that flowable and microhybrid resin composite show comparable performance when used for CMR prior to adhesive cementation of composite CAD/CAM overlays (31). It may be argued that the leakage test performed in both studies might be too stressing for the margins and does not truly simulate the clinical conditions. Also, it would be beneficial to further investigate whether the leakage at the adhesive interfaces recorded with *in vitro* tests corresponds to the quality of the margins as seen at SEM.

Although CMR is a well known technique and often performed among clinicians in order to avoid periodontal surgery and facilitate the restorative procedures, clinical evidence is scarce (19). Unfortunately, it is not yet pointed out what are the real clinical indications for such treatment option and in particular what are its limitations. Only 1 clinical study evaluating the CMR concept is available in the current scientific literature (32). It was shown that after 1 year of clinical service CMR was associated with statistically significant increased bleeding on probing (BoP) scores compared to shoulder preparation without CMR (32). The BoP is one of the most important clinical parameters for the evaluation of periodontal tissue health around any type of restoration and it can be directly influenced by precision of the margin, location of the margin and oral hygiene of the patient (33). The results of the present *in vitro* research could, to a certain extent, be considered in line with this clinical study, as significantly higher leakage was recorded at sites with CMR in Group 1 (**Table 3**). On the contrary, when another adhesive was used in Group 2 no differences between the mesial and distal margins were observed and the negative influence of the CMR could not be confirmed. Nevertheless, in this group microleakage scores recorded at the non-CMR side of the tooth were higher, which led to the absence of difference (**Table 4**). Moreover, the comparison between the two adhesive systems when no prior CMR procedure was performed, clearly showed a statistically significant difference between them (**Table 5**). The result implies that simplified universal adhesive can be more effective in bonding to dentin compared to the most traditional total-etch adhesives, probably due to their well-known issues related to dentin bonding under tested conditions (34). Also, when used in combination with proprietary luting material for luting indirect resin restorations, less compatibility problems may occur. Another possible reason that could be of interest for clinicians is that less bonding steps may lead to less risk of mistakes and at the same time faster application.

Finally, the CMR could represent a clinical procedure that can be of a certain help for the clinicians when indirect adhesive restorations are indicated. However it must be considered that the deeper the location of the proximal margin is, the more difficult the control of the bonding steps will be (32). Also, this clinical procedure can be operator-sensitive. In order to scientifically validate the beneficial or harmful effect of CMR, more *in vitro* and *in vivo* research is needed, in particular randomized clinical trials as the most valuable source of evidence.

From the results of this *in vitro* study the following conclusions can be drawn.

1. Cervical margin relocation could influence the quality of the marginal seal of composite restorations located below CEJ. In particular, when a universal adhesive applied in a selective enamel etch mode was used for restoration luting, CMR had negative influence on the

microleakage, whereas no difference existed in microleakage when a 3-step total-etch adhesive was employed.

2. The adhesive system employed for luting the indirect composite restorations represented a significant factor affecting the microleakage at the adhesive interface below CEJ when CMR was not previously performed. The universal adhesive showed better results than the three steps adhesive system under these experimental conditions.

Acknowledgements

The materials were generously provided by GC Europe.

Conflict of Interest

The authors of this article 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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4.3 NO CORRELATION BETWEEN TWO METHODOLOGICAL APPROACHES APPLIED TO EVALUATE CERVICAL MARGIN RELOCATION

Juloski J, Köken S, Ferrari M (2020). Dent Mater J. (Accepted 27 August 2019)

INTRODUCTION

Cervical margin relocation (CMR) technique has been proposed as a non-invasive pretreatment for the restoration of deep Class II cavities with proximal cervical margins extending below cemento-enamel junction (CEJ) (1, 2). This concept suggests application of a layer of composite in the deepest parts of the proximal areas in order to reposition the cervical margin supragingivally, which would facilitate rubber dam isolation, impression taking and subsequent adhesive cementation of the indirect restoration.

Some of the issues related to CMR technique that concern both the researchers and the clinicians are adhesion to deep cervical dentine, quality and durability of the seal at the subgingival margins, as well as the periodontal tissue response to the performed treatment (3). Scientific evidence on these topics obtained through clinical studies is scarce. So far only one randomized clinical study exists, which investigated the influence of the CMR pretreatment on the periodontal health of posterior teeth restored with indirect restorations (4). The results from that study indicated higher incidence of inflammation of periodontal tissue around teeth that underwent CMR pretreatment prior to definitive restoration compared to teeth without CMR at 12-month follow up. All the other available data on CMR technique is provided by in vitro studies.

Several clinical protocols and case reports suggested the use of various adhesive systems, composite materials and application techniques for the CMR (5-8). In order to evaluate the marginal quality *in vitro*, different methods have been used. The majority of studies used scanning electron microscopy (SEM) to observe the margins and to assess the presence of gaps and irregularities (9-15). Couple of other studies tested the microtensile bond strength of the restoration to the floor of the proximal cavity (16) or the sealing ability of the subgingival proximal margin by performing the microleakage test (17). The above-mentioned studies aimed to compare the performance of teeth with and without cervically relocated gingival margins or teeth where CMR was done with different adhesives and composites, applied in one or more layers, and inconsistent and inconclusive results have been reported. Although each one of these studies provided certain information about the quality and the integrity of the margin, the question that might be posed is which of the methods provide more useful and clinically relevant evidence and even more interestingly, whether or not different *in vitro* tests performed on the same samples would give consistent results. For example, would higher presence of gaps at the margins as observed by SEM also imply higher microleakage scores, and vice versa.

Therefore, the aim of this study was to evaluate the quality of the margins created by the CMR technique using different materials and to analyze the consistency of the results obtained by two *in vitro* methods commonly used for such evaluations. The null hypotheses tested were that: (**1**) there is no significant relationship between the results of the marginal quality obtained from microleakage test and SEM analysis, (**2**)

CMR technique would not affect the microleakage at the cervical margins, and (**3**) the marginal quality would not be influenced by the type of adhesive system and restorative material used for relocation of the cervical margin.

MATERIALS AND METHODS

Teeth preparation

Fourteen intact, healthy, similar sized human molars without any visible cracks, cavities or restorations were selected for the study after informed consent of the patients was obtained. Teeth were mechanically cleaned with hand scalers, brushed with a pumice and were stored in 0.1% thymol solution for no longer than three months. Standardized mesio-occlusal-distal (MOD) cavity preparations were created using water-cooled diamond burs from the set for ceramic inlays and partial crowns preparation (Komet Burs Expert Set 4562/4562ST, Komet, Lemgo, Germany) mounted on a high-speed handpiece. The remaining axial walls had 2 mm of thickness, and they were reduced for a cuspal coverage. Proximally, box-shaped preparations were made, 2 mm in the mesio-distal and 5 mm in bucco-lingual direction. The inner angles of the cavities were rounded, and the margins were not beveled. Proximal margins in both, mesial and distal sides, were located in dentin, approximately 1mm below the CEJ. Distal proximal margins were not elevated in any of the samples. In all the samples the cervical margins on the mesial sides were relocated above CEJ with flowable composite (**Fig. 1**). To achieve CMR of the mesial proximal margins and immediate dentin sealing (IDS) appropriate adhesives were used, according to the assigned experimental groups. The adhesive layer, as well as each 1-mm increment of the flowable composite, was light cured for 20 seconds from the occlusal direction using the light curing unit with a curing output of 1200mw/cm^2 (BA Optima 10, B.A. International Ltd, Northampton, UK). The adaptation of the flowable composites was performed with flowability of the material itself and with a ball ended hand instruments and a microbrush. Special care was taken not to layer the composite more than 2 mm in thickness. Kerr 2181 Adapt® SuperCap® (Kerr Corporation, Orange, CA, USA) matrices in steel (0.038, 5.0 mm high) were used to create the CMR. The circumferential matrix was carefully adjusted to eliminate the risk of overhanging of the material over the margins and 2 mm space is marked on the inner side of the matrix to avoid overfilling of the proximal box. Water-cooled diamond burs (Komet Burs Expert Set 4562/4562ST, Komet, Lemgo, Germany) on high-speed handpiece were used to give final shape of each cavity after CMR was performed.

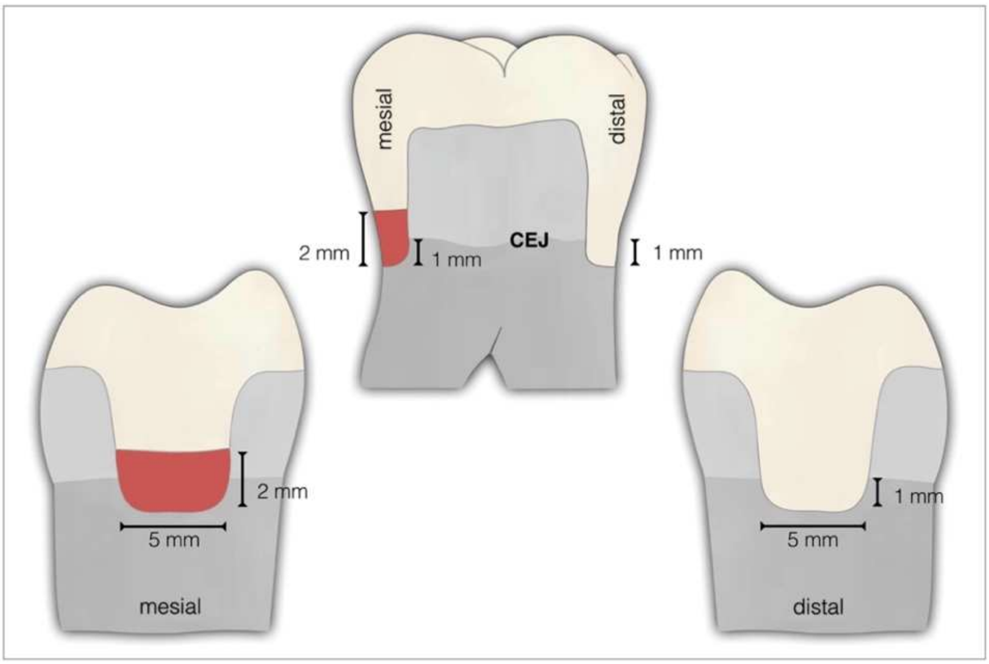


Fig. 1. Schematic representation of the cavity design.

Impression

Extraoral scanner GC Aadva Lab Scanner (GC Corporation. Tokyo, Japan) was used for making digital impressions of the prepared teeth. Scanned files were sent to Milling Center (GC Leuven, Belgium) to create the overlays out of hybrid ceramic computer-aided design/computer-aided manufacturing (CAD/CAM) blocks (Cerasmart, GC Corporation. Tokyo, Japan). The prepared teeth without any temporary restorations were kept in fresh water for two weeks at room temperature until the overlays were luted. The fit of the overlays was examined under the digital microscope (Nikon Shuttle Pix, Tokyo, Japan) and the digital photographs were taken under 10x magnification.

Luting procedure

Before luting, teeth were cleaned and preparation surfaces were gently dried. The overlays were sandblasted at approximately 300 kPa pressure with 50-µm aluminum oxide particles. Inner sandblasted surface of the overlays was silanized: in Group 1 Silane primer (Kerr Corporation, Orange, CA, USA) was applied and in Group 2 Monobond Plus (Ivoclar Vivadent, Schaan, Lichtenstein). Luting procedures were performed with an appropriate resin cement, according to the assigned experimental group. The resin cements were mixed with its special mixing tip, initial mixture was discarded on a clean paper. The latter mixture was applied on restoration's inner surface and preparation surface. The overlays were pressed firmly on teeth and the excess luting material was cleaned with a microbrush. The restoration margins were covered with a water-based glycerine gel (Airblock™, Dentsply DeTrey GmbH, Konstanz, Germany).

Each axial wall was light cured for 60 seconds and finally occlusal surface was cured 60 seconds (BA Optima 10). Margins were gently finished with flexible disks (SofLex Pop-on, 3M ESPE, St.Paul, USA).

Groups

Teeth were randomly assigned to two equal groups of 7 specimens each, according to the adhesive materials employed, as follows (Fig. 2):

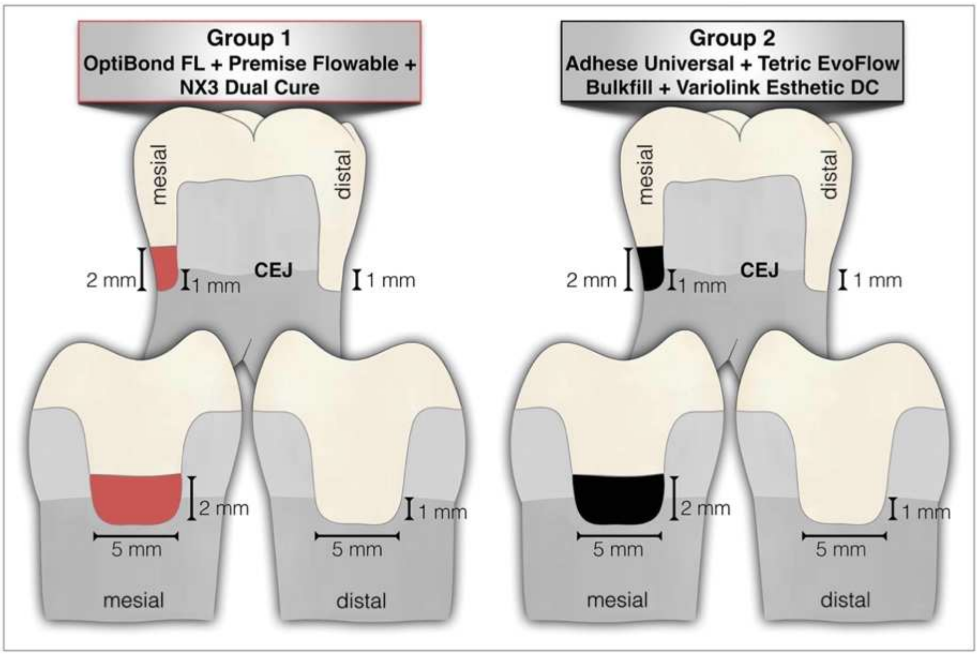


Fig. 2. Schematic representation of the experimental groups tested in the study.

Group 1: Mesial proximal margins below the CEJ were elevated with two increments (1mm each) with a flowable composite Premise flowable (Kerr Corporation, Orange, CA, USA), which was bonded with a “gold standard” 3-step total-etch bonding system (Optibond FL, Kerr Corporation, Orange, CA, USA). Resin composite overlays were luted with the dual cure resin cement NX3 Nexus™ Third Generation (Kerr

Corporation, Orange, CA, USA), in combination with the same adhesive (OptiBond FL).

Group 2: Mesial proximal margins below the CEJ were elevated with two increments (1mm each) with Tetric EvoFlow® Bulk Fill (Ivoclar Vivadent, Schaan, Lichtenstein) which was bonded with a universal bonding agent Adhese Universal (Ivoclar Vivadent, Schaan, Lichtenstein) in selective enamel etch mode. Resin composite overlays were luted with a dual cure resin cement Variolink Esthetic DC (Ivoclar Vivadent, Schaan, Lichtenstein) in combination with the same universal bonding agent (Adhese Universal) in selective enamel etch mode.

List of all materials used in the study, their batch numbers, chemical compositions and application procedures is reported in **Table 1**.

SEM evaluation of marginal quality

Impressions of the finally restored teeth were taken using addition silicone impression material (Elite HD+, Zhermack, Badia Polesine, Italy) and a set of epoxy resin replicas for each tooth (Alpha Die, Schuetz Dental, Rosbach, Germany) was made for SEM evaluation. The replicas were mounted on aluminum stubs, sputter-coated with gold (Polaron Range SC7620; Quorum Technology, Newhaven, UK), and examined under a scanning electron microscopy (SEM) (JSM 6060 LV; JEOL, Tokyo, Japan) at 50× and 200× magnifications. SEM examination was performed by one operator having experience with quantitative margin analysis who was blinded to the restorative procedures. The marginal integrity of the adhesive interface was expressed as the percentage of "continuous margin" in relation to the entire margin length in dentin. Such measurements were made by placing the SEM images over the grip paper for each sample individually (Fig. 3). Marginal qualities were classified according to the criteria "continuous margin," "gap/ irregularity", and "not judgeable/artifact" according to a well-proven protocol relating margins in dentin, resin-resin transitions, and margins in enamel consistent with previous studies (10, 11, 14, 18).

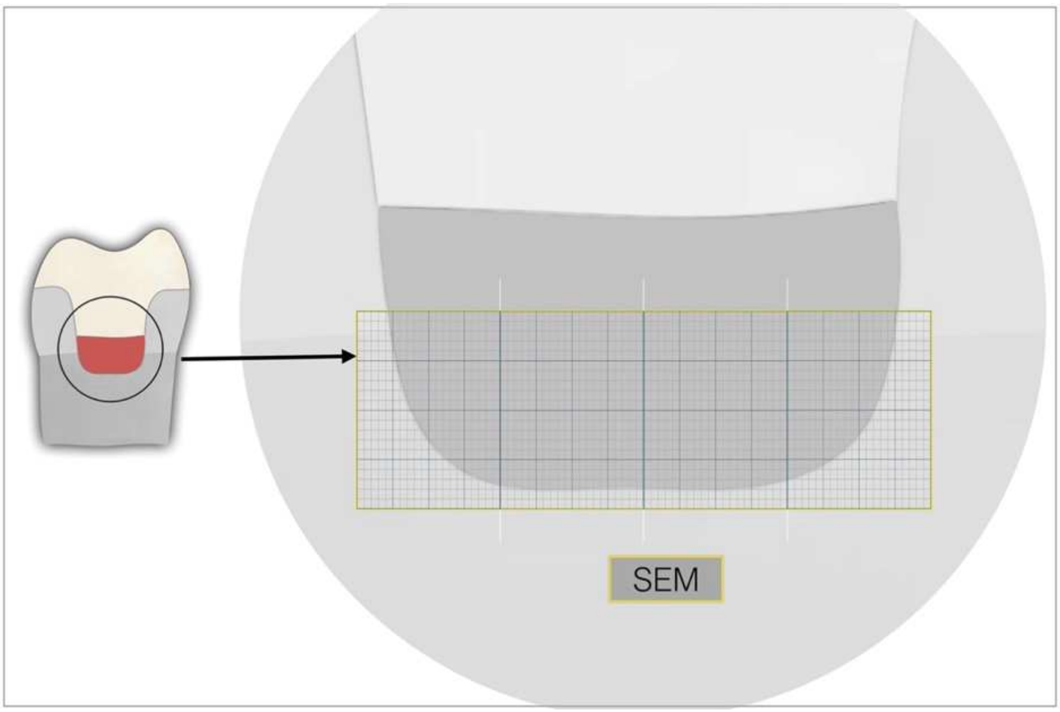


Fig. 3. Schematic representation of SEM marginal quality calculation.

Microleakage evaluation of marginal quality

After the replicas have been made, all surfaces of the teeth were covered with nail varnish leaving exposed 1mm around the area of the adhesive interfaces between the overlay and the tooth on the distal aspect and between the tooth and the CMR on the mesial aspect of the tooth. Diluted ammoniacal silver nitrate solution (1:4 ratio ammoniacal silver nitrate and distilled water) was prepared. The diluted solution was filtered using Millipore filter (0.22 nanometer filter, Carrigtwohill, County Cork, Ireland) mounted on a syringe. Each tooth was placed in a test tube with diluted ammoniacal silver nitrate solution in the presence of laboratory light. After 24 hours specimens were rinsed in water for 10 minutes three times. Nail varnish around the tooth was removed with acetone, and each tooth was placed in a test tube with the diluted photo developer solution (Kodak, Rochester, NY, 1:10 ratio photo developer solution and distilled water). After 8 hours teeth were rinsed in water three times for 10 minutes.

Each tooth was then embedded in transparent self-curing acrylic resin. Subsequently, the teeth were sliced using a low-speed diamond saw under water-cooling (Isomet; Buehler, Lake Bluff, NY, USA) into five to six 1-mm thick slices along their long axis and perpendicularly to the proximal margins. Samples were examined under digital microscope at 1×, 3× and 6× magnification (Nikon Shuttle Pix, Tokyo, Japan). Two observers independently scored the amount of tracer along the adhesive interfaces according to the following scheme (19) (**Fig. 4**):

score 0=no microleakage

score 1=0–20% of the interface showing microleakage

score 2=20–40% of the interface showing microleakage

score 3=40–60% of the interface showing microleakage

score 4=60–80% of the interface showing microleakage

score 5=80–100% of the interface showing microleakage.

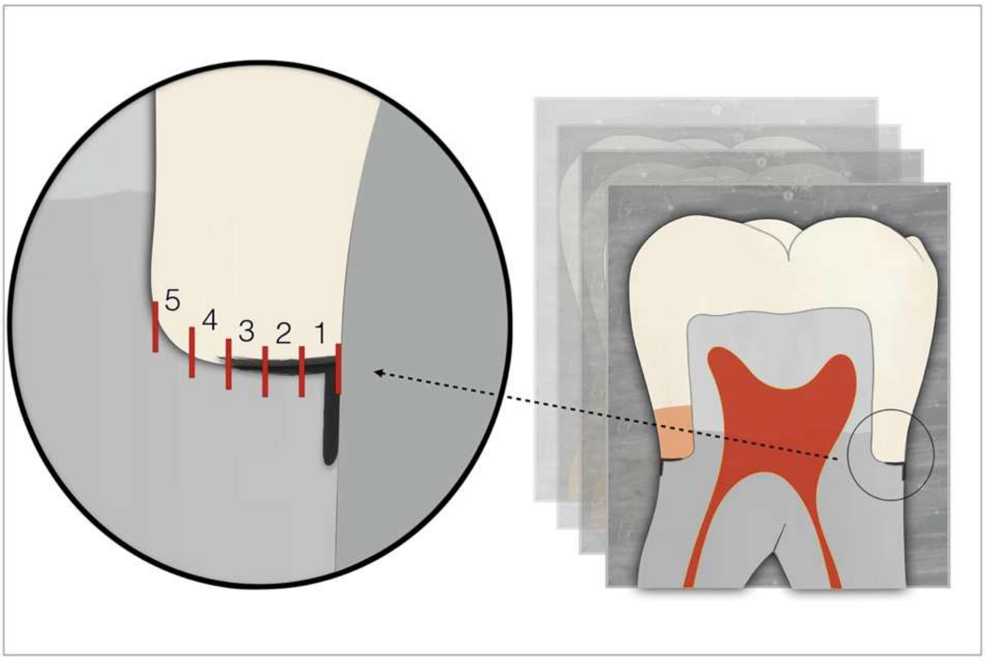


Fig. 4. Schematic representation of microleakage scoring.

Statistical analysis

Firstly, Wilcoxon signed-rank tests were performed on the data obtained from the two groups separately, in each group comparing the microleakage scores on the mesial (with CMR) to those on the distal (without CMR) aspects of the teeth. Also, another Wilcoxon signed-rank test was applied to all the obtained data, in order to compare all CMR scores to all non-CMR scores, regardless of the group.

Then, two separate Mann-Whitney U tests were used to assess the statistical difference in the microleakage scores between the two groups, separately for the mesial (with CMR) and for the distal (without CMR) aspects of the teeth.

Then, the difference in SEM marginal integrity percentage on CMR sides of the teeth between two groups was assesses using Independent T-test, after the normality of data distribution (One-Sample Kolmogorov Smirnov test) and equality of variances (Levene’s test) were confirmed ($p>0.05$).

Finally, in order to verify the correlations between the results related to the quality of the margin obtained from the microleakage test and the SEM observation non parametric correlations (Spearman’s coefficient) were analyzed.

In all the tests, the significance level was set at $p<0.05$ and the analyses were performed in statistical software SPSS IBM Statistics version 21 for Mac (SPSS Inc., Chicago, IL, USA).

Table 1. Chemical compositions and application procedures of the materials used in the study.

Material (manufacturer)/ Batch number	Type of material	Application procedure	Composition
Optibond FL (Kerr Corporation; Orange, CA, USA) lot: 5866021	Three-step etch-and-rinse adhesive	Etch-and-rinse: 37% phosphoric acid, 30s enamel, 15s dentin, primer for 20s, bonding for 20s, and light curing for 20s.	Primer: ethyl alcohol, alkyl dimethacrylate resins and water Bonding agent: uncured methacrylate ester, monomers, triethylene, glycol, dimethacrylate, ytterbium trifluoride, inert mineral fillers, photoinitiators and stabilizers.
Adhese Universal (Ivoclar Vivadent, Schaan, Liechtenstein) lot: W38849	Universal adhesive	Selective etching of enamel 15s Rinsing 15s, dry with oil- and water-free compressed air until the etched enamel surfaces appear chalky white.	Methacrylates, ethanol, water, highly dispersed silicon dioxide, initiators and stabilizers.
Premise Flowable (Kerr Corporation; Orange, CA, USA) lot: 5890564	High filled flowable resin composite	Each layer light cured for 20s.	Dibutyl phthalate, DBP dibenzoyl peroxide titanium dioxide metakrylat ester monomer.
Tetric EvoFlow® Bulk Fill (Ivoclar Vivadent, Schaan, Liechtenstein) lot: V29293	High filled flowable resin composite	Each layer light cured for 20s.	Ethyoxylated bisphenol A dimethacrylate, Bis-GMA, ytterbium trifluoride, tricyclodocane dimethanol dimethacrylate.
Kerr NX3 (Kerr Corporation, Orange, CA, USA) lot: 6315654	Universal adhesive aesin dental cement (dual cure)	Mixture was applied on restoration's inner surface and preparation surface. Overlays firmly pressed. Each axial wall light cured 60s.	Base: 2-hydroxyethyl methacrylate, 2-pyridylthiourea methacrylate ester monomer, titanium dioxide, pigment. Catalyst: 2-hydroxyethyl methacrylate, α,α-dimethylbenzyl hydroperoxide, cumene hydroperoxide, methacrylate ester monomer, titanium dioxide, pigment.
Variolink Esthetic DC (Ivoclar Vivadent, Schaan, Liechtenstein) lot: W35835	Dual-curing luting composite for the permanent cement	Mixture was applied on restoration's inner surface and preparation surface. Overlays firmly pressed. Each axial wall light cured 60s.	Base: ytterbium trifluoride, urethane dimethacrylate, 1,10-decandiol dimethacrylate, acetyl-2-thiourea. Catalyst: ytterbium trifluoride, urethane dimethacrylate, 1,10-decandiol dimethacrylate, α,α -dimethylbenzyl hydroperoxide.
GC Cerasmart (GC Corporation, Tokyo, Japan) lot:1609082	Force absorbing hybrid ceramic CAD/CAM block	Sandblasting and silanization of the inner surface.	Raw materials of the pre-cured composite block: UDMA, dimethacrylate monomers, bis-EMA, silicone dioxide, barium glass powder, pigments, initiator.
Silane Primer (Kerr Corporation; Orange, CA, USA) lot: 6114803	Silane primer	Applied with a microbrush on restoration's inner surface.	Ethyl alcohol, ethylene oxide.
Monobond Plus (Ivoclar Vivadent, Schaan, Liechtenstein) lot: V12120	Universal primer	Applied with a microbrush on restoration's inner surface.	Ethanol, Methacrylated phosphoric acid ester.

MICROLEAKAGE SCORE						
					Interquartile range	
	N	Mean	SD	Median	25th percentile	75th percentile
Group 1						
CMR[B]	43	5.00	0	5	5.00	5.00
non-CMR[A]	43	2.81	1.350	3.00	2.00	4.00
Group 2						
CMR[B]	42	3.60	1.449	4.00	2.75	5.00
non-CMR[A]	42	2.00	1.361	1	1.00	3.00

Table 2. Comparison of microleakage scores between CMR and non-CMR margins within the groups separately. Statistically significant differences exist in microleakage between CMR and non-CMR margins in both groups (Wilcoxon signed-rank test, p=0.000). Different superscript letters indicate statistically significant differences between the sites of the teeth within the groups. N. Number of analyzed slices; SD. Standard deviation.

MICROLEAKAGE SCORE regardless of the material	N	Mean	SD	Median	Interquartile range 25th percentile	Interquartile range 75th percentile
CMR[B]	85	4.31	1.235	5.00	4.00	5.00
non-CMR[A]	84	2.41	1.408	2.00	1.00	3.00

Table 3. Comparison of microleakage scores between CMR and non-CMR margins regardless of the group. Statistically significant differences exist in microleakage between CMR and non-CMR margins (Wilcoxon signed-rank test, p=0.000). Different superscript letters indicate statistically significant difference. N. Number of analyzed slices; SD. Standard deviation.

	MICROLEAKAGE SCORE					
					Interquartile range	
	N	Mean	SD	Median	25th percentile	75th percentile
CMR side						
Group 1[B]	43	5.00	0	5	5.00	5.00
Group 2[A]	42	3.60	1.449	4.00	2.75	5.00
non-CMR side						
Group 1[B]	43	2.81	1.350	3.00	2.00	4.00
Group 2[A]	42	2.00	1.361	1	1.00	3.00

Table 4. Comparison of microleakage scores between groups recorded at CMR and non-CMR margins separately. Statistically significant differences exist in microleakage between the 2 groups on CMR side (Mann-Whitney U, p=0.000) and non-CMR side of the teeth (Mann-Whitney U, p=0.004). Different superscript letters indicate statistically significant differences between the groups. N. Number of analyzed slices; SD. Standard deviation.

	PERCENTAGE OF GAP FREE MARGINS (%)				
	N	Mean	Standard Deviation	Standard Error	*p* value
50× magnification					
Group 1[A]	7	57.50%	18.70	7.07	0.324
Group 2[A]	7	67.59%	17.99	6.80	
200× magnification					
Group 1[A]	7	36.43%	14.88	5.62	0.169
Group 2[A]	7	45.71%	7.77	2.93	

Table 5. Comparison of marginal integrity of CMR sides between two groups observed on SEM images under 50× and 200× magnifications. Same superscript letters indicate lack of statistically significant differences between the groups (Independent T-test, p>0.05). N. Number of analyzed teeth.

RESULTS

In the present study the following adhesive interfaces were observed:

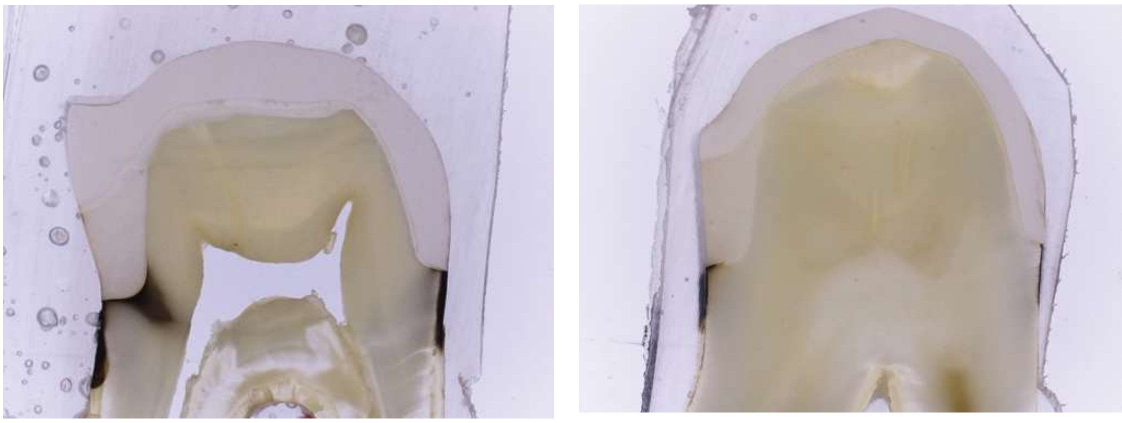
- overlay-luting cement and luting cement-dentin, at the distal side without CMR;
- overlay-luting cement, luting cement-flowable composite and flowable composite-dentin, at the mesial side with CMR.

However, no trace of leakage was noticed at the overlay-luting cement and luting cement-flowable composite interfaces (score 0 was recorded in all the specimens). Only at the dentin interfaces variable leakage was recorded. Therefore, the statistical analyses and all further discussion in the study refer only to the luting cement-dentin interface (non-CMR group) and flowable composite-dentin interface (CMR group) for both, SEM observation and microleakage test.

When microleakage data was analyzed within the groups, the results of the statistical tests (Wilcoxon signed-rank) showed significantly lower microleakage score on distal sides, where CMR was not performed, compared to the mesial sides with CMR (p=0.000), for both groups separately (**Table 2**). Also, when CMR and non-CMR scores were compared between each other, regardless of the groups, significantly lower values were present at distal sides, where CMR technique was not carried out (**Table 3**, Wilcoxon signed-rank test, p=0.000).

The statistical analyses also revealed that significant differences existed in microleakage between two experimental groups on the CMR aspects of the teeth (Mann-Whitney U, p=0.000), as well as on the non-CMR sites (Mann-Whitney U, p=0.004). On both sites in Group 2 significantly lower microleakage scores were recorded than in Group 1 (**Table 4**). One representative slice of microleakage testing from each group is presented in Fig. 5. On the contrary, when the percentage of gap free margins at the CMR aspects of the teeth was statistically analyzed, no significant differences existed between two groups (**Table 5**, Independent T-test, $p>0.05$). Compilations of SEM images of the entire cervical margins of representative specimens from each group are presented in Figs. 6 and 7.

Finally, the results of the statistical analysis (Non Parametric correlations, Spearman's coefficient, ρ) indicated that there were no statistically significant correlations on CMR sites between microleakage scores and percentage of marginal integrity observed under SEM. It was not possible to perform the correlation analysis on the data set from Group 1, since the microleakage scores at the CMR side of all samples from Group 1 was 5, so it was constant (**Table 2**). Lack of strong correlations existed when data were analyzed for Group 2 on both magnifications (for SEM results at 50×: $\rho=0.306$, $p=0.504$; for SEM results at 200×: $\rho=-0.505$, $p=0.248$), as well as when all results for CMR side were used, regardless of the groups (for SEM results at 50×: $\rho=0.115$, $p=0.694$; for SEM results at 200×: $\rho=-0.443$, $p=0.113$).



A B

Fig. 5. Representative slices of microleakage testing from each group. **A.** Group 1: score at CMR side 5, score at non-CMR side 3. **B**. Group 2: score at CMR side 2, score at non-CMR side 1.

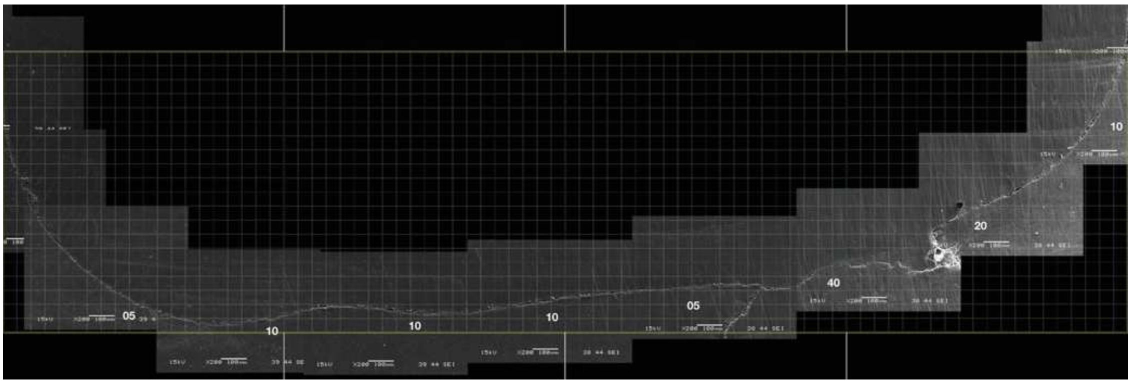


Fig. 6. Compilation of SEM images of the entire cervical margin of a specimen from Group 1.

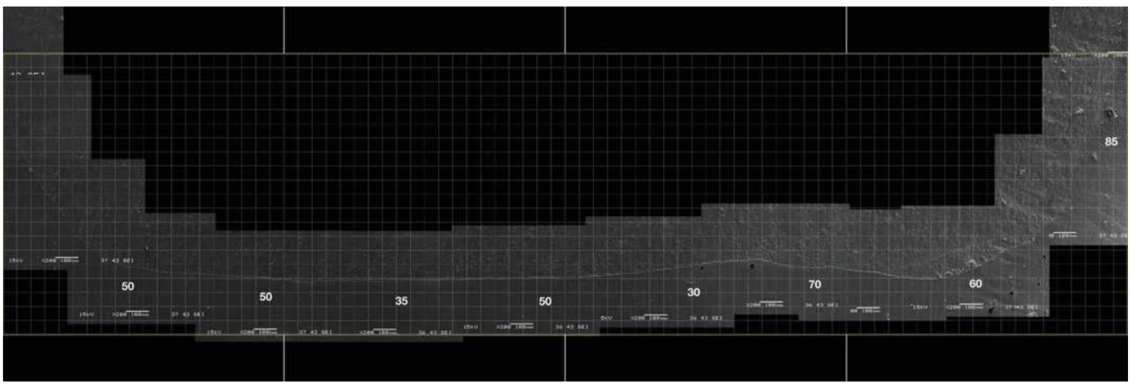


Fig. 7. Compilation of SEM images of the entire cervical margin of a specimen from Group 2.

DISCUSSION

From the results of the present study it emerged that the interfaces between the luting cement and the flowable composite, as well as between the ceramic overlay and the luting cement, should not be a matter of concern for the researchers and clinicians, considering that leakage was not observed in any of the specimens. This could also be due to the maximal precision of the CAD/CAM restorations that provided very good fit of the overlay margins to the preparation margins. Consequently, a good seal without any imperfections or gaps at the interface could be achieved, which did not permit any marginal leakage. It may be assumed that restorations created in a traditional way would have given different results. However, this study confirmed that the only troublesome interface for the adhesion was dentin, which became the matter of further interest of this investigation.

The results indicate that there was not statistically significant relationship between the results obtained from two *in vitro* methods used for evaluation of the quality of the margins. Consequently, the first null hypothesis was accepted. Furthermore, the CMR technique impaired the sealing at the cervical margins, as significant differences in microleakage scores were recorded at sides of the teeth with and without CMR, so the second null hypothesis had to be rejected. And finally, the marginal microleakage was significantly influenced by the type of adhesive system and restorative material used for relocation of the cervical margin, whereas the percentage of the gap-free margin assessed on the SEM images did not differ between the different materials. Therefore, the third null hypothesis was only partially rejected.

The present study showed that direct placement of the restoration on dentin without CMR resulted in significantly lower marginal leakage and therefore better marginal seal than that obtained with CMR technique. It was a case for both materials tested in the study (**Table 2**), higher microleakage scores were recorded on mesial sides with CMR (Group 1: median score 5, Group 2: median score 4) than on distal sides without CMR (Group 1: median score 3, Group 2: median score 1). Also, when marginal leakage was assessed regardless of the materials employed, the same results emerged: median score was 5 at CRM sites and median score was 2 for non-CMR sites (**Table 3**). These findings are in accordance with a previous investigation (17) that compared marginal leakage at the gingival margin in teeth with and without CMR performed prior to cementation of the CAD/CAM overlays. Currently there are no other studies that used the microleakage test to assess the sealing ability at the CMR gingival margin our results could be compared to. If we refer to the *in vitro* study that investigated the impact of CMR on the fracture behavior of endodontically treated molars (13), it could be noted that the fracture resistance did not differ significantly between teeth with and without CMR. Another study (16) showed increased microtensile bond strength of composite inlays to the proximal box floor with CMR, but only when self-adhesive resin cement was used for the cementation. Otherwise, in case of a resin cement with a total-etch adhesive there was no significant difference between the groups with and without CMR. Nevertheless, high occurrence of adhesive failures at the interface between cervical dentin and CMR composite was noticeable. The only available clinical trial (4) reported that CMR was associated with increased bleeding on probing, a significant indicator of jeopardized periodontal health, emphasizing however the importance of "biological width", a necessary distance between the restorative margins and the alveolar crest in order to avoid detrimental effects on neighboring soft and hard periodontal tissues (20).

Moreover, both flowable as well as traditional packable restorative composites could be selected for CMR technique, according to the published clinical reports (5-8). A recent study that examined the performance of a flowable and a conventional packable composite reported no significant difference between two different viscosity composites, in terms of microleakage (17). Hence, for the present research two highly filled flowable composites were selected (Group 1: Premise flowable, 2: Tetric EvoFlow® Bulk Fill) as particularly recommended for CMR, due to their consistency and ease of use (6-8). However, they were used in combination with different adhesives, that may account for the differences in the results: in Group 1 a "gold standard" 3-step total-etch adhesive Optibond FL was applied, whereas for Group 2 an universal adhesive Adhese Universal was chosen. The results from microleakage test imply that significant differences exist between different materials utilized to perform CMR (**Table 4**). In particular, for both sides, CMR and non-CMR, significantly better seal was achieved in Group 2 with Adhese Universal. Since the universal adhesive was used in the selective enamel etch mode, meaning that deep cervical dentin was not etched and in those parts of the cavity the adhesive was practically used in its self-etch mode, it could therefore be assumed that it is preferable not to etch the substrate when bonding to deep cervical dentin and to use a self-etch or an universal adhesive system in the self-etch mode. As a matter of fact, a possible over-etching of subgingival dentin when total-etch adhesives are used for CMR has already been raised as a concern by Rocca et al. (6) Anyway, it is well known that dentin is much more demanding substrate for adhesion than enamel, first of all due to its structure and the sensitivity of the application technique (21-23). Also the effectiveness of the bond was proven to depend on the type of the adhesive (24), so other adhesive systems are worth investigating in relation to the CMR technique. Another important issue one should also take into consideration when it comes to CMR margins is that the presence of a cementum layer at the cervical margin of the cavity may pose a serious clinical problem for reliable bonding (25).

Regardless of the differences in microleakage found between two groups, when same teeth were observed under SEM, significant difference in the quality of the CMR gingival margin could not be determined. Previous studies that used the same method and the same protocol for calculation of marginal integrity observed the specimens at 50× (14) and 200× magnification (9-13, 15). So, in the present study all samples were observed at both of these magnifications. As expected, at higher magnification it was possible to notice more gaps and irregularities than at lower magnification (**Table 5**) and hence, the percentage of continuous margins was lower at 200× (Group 1: 36,43%, Group 2: 45.71%) than at 50× (Group 1: 57,50%, Group 2: 67,59%). However, the adhesive material did not represent a significant factor that would influence the marginal adaptation of the composite, which is contradictory to one of the earlier studies that investigated various adhesives for CMR (9). The main results of the other SEM studies are presented in a recent literature review on the CMR technique (3).

Finally, one of the aims of this study was to compare the results of two different *in vitro* methods and to determine whether significant relationship exists between presence of the marginal gaps seen under SEM and the microleakage scores at CMR sites. To the authors' knowledge, no study so far searched for an answer to this question and the present study represents the first attempt to provide that answer. The results revealed the presence of statistically significant differences in the microleakage analysis (**Table 4**) and the lack of differences in the SEM analyses (**Table 5**) between two groups at CMR sites. This finding could already indicate that these two methods do not provide consistent data. When this assumption was verified statistically, it was confirmed that the correlation between two variables is weak and that the statistical significance was absent. One of the potential explanations for such a result may be that it is not possible to identify if the margin is sealed efficiently by observing it under SEM. Probably much higher magnifications or diverse techniques are necessary to reveal the adhesive interfaces that are not efficiently sealed. One study has proposed microcomputed tomography as a non-destructive technique for inspection of the tooth-restoration interfaces and interfacial leakage (26). However, it would be valuable to investigate more about the most appropriate *in vitro* method to appraise the quality of the CMR adhesive margins. One limitation of the present study is a small sample size, which was used in order to obtain the initial data. Further research should verify the results of this study using larger number of the specimens and clarify which of the *in vitro* tests provide more reliable data and which one could give more accurate predictions for clinical success. Undisputedly, only well-designed clinical trials could provide strong scientific evidence that would allow for definitive clinical recommendations.

CONCLUSION

Based on the results obtained under conditions of the present study, several conclusions could be drawn:

1. CMR seems to provide less adequate seal of the margin than the one achieved by cementing the restoration directly to dentin without CMR.
2. The sealing ability of the marginal interface depends on the adhesive materials used for performing CMR.
3. Differences in the quality of the marginal adaptation between two different materials used for CMR could not be detected by SEM observations.
4. SEM examination of the marginal adaptation does not allow for the predictions of the functional sealing of the margins.

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Chapter 5:

Conclusions and future directions

Conclusions and future directions

Our *in vitro* studies demonstrated that previously existing articles had the bias of the too low magnification used to evaluate the 'margins' precision' (1-10). All our studies showed that it is not possible to seal any restoration placed below the Cementum Enamel Junction (11-13).

In the current literature, various resin composite materials were proposed to relocate cervical margins coronally. The most commonly prescribed materials for the CMR procedure are the flowable and traditional viscous restorative composites (14). In deep cervical areas, creating smooth and void-less margins on the cementum-dentin complex without any overhangs or underhangs is a challenging process. This technique requires clinical skills and meticulous manipulation of the composite in deep, complicated (problematic) areas (11). The physical and mechanical properties of the material should be ideal and able to withstand any potential complications while placing the material and provide function for a prolonged time (1). Highly filled (medium rigid) flowable was advocated as a stress absorber layer under the indirect restoration due to their elasticity modulus and physical properties (1, 3, 5). Additionally, from the *in vitro* existing literature related to CMR, it can be concluded that only one study supported the use of highly filled flowable composite (1). In contrast, others revealed the use of highly flowable composites comparable to traditional viscous restorative resin composites (3, 8, 9). In a study of this thesis, the marginal sealing ability (performance) of a highly filled flowable and microhybrid resin composite for CMR was tested and didn't result in significant differences (11). Besides, in previous *in vitro* studies, self-adhesive resin cements resulted inferior in marginal adaptation and were not recommended as a CMR material (4, 5). Moreover, a recent study by Grubbs et al. (2019) evaluated the effect of different direct restorative materials in CMR on the marginal quality and fracture resistance of molar teeth restored with resin CAD/CAM blocks. Interestingly, in this study, tested Glass Ionomer (GI), Resin Modified Glass Ionomer (RMGI), Resin Based Composite (RBC), and Bulk Fill Resin Based Composite (BF) materials did not influence the results in terms of margin quality and fracture resistance. However, there is no additional study that can support the use of GI and RMGI for CMR, and it was further stated in the study to be cautious in utilizing GI materials for CMR procedure, clinically (15).

In the previous studies related to CMR, the effect of polymerization shrinkage was also analyzed using the incremental layering technique utilizing highly filled composites (5, 6). These studies showed that incremental layering of highly filled composites resulted in significant differences when compared to single increment placement (5, 6). Therefore, incremental layering of resin composites should be preferred to create a CMR material.

From a clinical perspective, in the build-up stage, flowables provide a beneficial environment such as the lessened risk of air voids and perfect wettability of the dental substrates. On the other hand, traditional viscous restorative composites can provide a similar advantage via a heated application (16, 17). However, when used in its normal thermal environment, the application in deep areas is usually difficult and requires meticulous application in small increments. Any overdosed pressure of the instrument might reposition the well-adapted matrix and wedge system and possibly overfill the matrix space, thus overhangs or ledges in neighboring periodontal areas are a risk. However, the same overhang formation on the CMR composite-cementum-dentin margin is also possible when using flowable and an ill-fitting matrix. Clinically, only under higher magnification is this unproper matrix coverage in cervical margins visible (18). Any opening in the matrix-wedge system would lead to an overflow of the low viscous material. When a flowable composite is preferred due to their ease of use, a highly filled flowable, having 65-75% of fillers by weight, should be used in a thickness less than 1.5mm (19). However, the increased thickness of the composite to be cured in the CMR area would create more marginal gaps (6). In the reviewed literature, either flowable (1, 19-22) or restorative paste composites (19, 21, 22) or a combination of both (19, 22) up to 1.5 to 2.0 mm in a total thickness were advocated. However, adaptation problems (gaps) were detected in almost all in-vitro studies (1-8, 13, 15) where the resin replicas of the cervical margins were examined under the Scanning Electron Microscope at 200X magnification.

In previous literature, in regards to the most appropriate adhesive systems to coronally elevate the cervical margins, different prescriptions were mentioned in some limited clinical articles describing the CMR technique. Mostly, it was indicated to use the 3-step total-etch adhesives as the preferred adhesive system (19, 21, 23) or two-step self-etch adhesives in self etch mode (19). In our studies evaluating the microleakage of adhesive interfaces in the margins below the CEJ, it was shown that a universal adhesive system resulted in significantly less microleakage compared to a 3-step total-etch adhesive protocol when the indirect composite restorations were directly luted (in a conventional way) (12). Another study reported that significant differences existed in the microleakage of the adhesive interfaces for both CMR and non-CMR sides, and universal adhesive in selective-etch mode sealed significantly better compared to the 3-step total-etch adhesive. However, the marginal adaptation of the same samples under SEM investigation did not reveal any differences (14).

Another topic discussed in *in-vitro* studies examined when CMR was not applied, and indirect restorations were directly luted on proximal margin below the CEJ. Interestingly, in two previous *in vitro* studies, the quality of marginal adaptation in the presence of the cervical margins located below CEJ was investigated. Margins created by directly luting the indirect restoration on the dentin without any CMR application resulted in significant differences when compared to margins created between the CMR composite and dentin, only after thermomechanical loading (6, 7). This result was also supported by microleakage studies of the thesis (11-13). Similarly, our randomized clinical trial study revealed less bleeding on probing scores on the directly luted side, where CMR was not performed (24).

From the clinical point of view, it must be pointed out that the clinical procedure to relocate the cervical margin coronally is operator dependent, being delicate and requiring complete isolation of the field. Furthermore, isolation from any type of moisture, perfect placement of the matrix, wedge and clamp, etc. is required. An accurate radiographic examination must also be made after preparing the cavity to check the distance between the bone crest and the apical extent of the cavity: when the distance is at least or more than 2 mm, cervical margin relocation will be indicated but when it is less than 2 mm, a periodontal surgery for crown lengthening or/and orthodontic extrusion will be indicated (24).

Clinically the location of deep proximal margins below the gingival crest and CEJ is critical, especially when using a digital intraoral scanner. The inappropriate deep margin location may hamper precise detection of the margins; thus, the CMR technique became critical of today's' digital workflow. Even when an impression will be made via conventional techniques, the luting process under moisture control is challenging, and removal of excess luting resin cement becomes problematic.

Unfortunately, in the current literature, only a few clinical trials were reported. Only one randomized controlled clinical trial on CMR technique is available (24), which is our study, evaluated the periodontal effects of adhesively cemented indirect restorations on deep cervical margins with or without the CMR procedure. In this study, the completed 12-month follow-up results showed that a higher Bleeding on Probing (BoP) score could be expected around CMR margins. In other words, directly luting without any additional material on deep cervical margins were found less detrimental for the periodontal tissue (24). Besides, a clinical study by Bertoldi et al. (2019) evaluated the clinical and histological reaction of periodontal tissues to subgingival pre-endodontic composite restorations. The 3-month primary clinical histologic outcomes were discussed. It was found that subgingival restorations resulted in compatible gingival health, with levels similar to that of untreated root surfaces. However, no indirect restorations luted to CMR composite were made and evaluated (25).

Moreover, a recent clinical study investigated the safety and feasibility of cervical marginal relocation procedures in cases of deep caries involving the supracrestal tissue attachment (STA). In this study, three different CMR approaches were designed. Pocket depth (PD) on probing and residual bleeding on probing (BOP) after 1 year were examined, and no differences were found among the three CMR approaches for PD or residual BOP. The Authors concluded that the periodontal health status of patients was not negatively affected by the (three) different CMR procedures when the connective compartment of the STA is not violated (17). Interestingly, a recent retrospective study revealed that indirect restorations with CMR had a good survival rate after up to 12 years; clinical evaluation of 197 partial indirect restorations was done, with an average evaluation time of 57.7 months was declared. However, the second hypothesis of that study questioning whether differences exist in the quality of survival of the partial indirect restorations in time was rejected, since more degradation of the partial indirect restorations was observed over the time. More margin discoloration, more fracture of the restoration, more fracture of the tooth, and more caries were also detected on older restorations (26).

Another important clinical aspect is related to the home oral hygiene of patients. In prosthodontic therapy, the three parameters that can preserve a crown from complications under clinical service are 1. Margin precision, 2. Margin location, and 3. Maintenance of oral hygiene (27-29).

When a Cervical Margin Relocation is made, we now clearly know that a perfect precision of the cervical margin cannot be achieved. Because of the difficulty to clinically make the CMR, the need to perfectly isolate the margin with rubber dam, which is used by no more than 8-12% of dentists in daily dentistry (30-33); also existing adhesive systems cannot seal cementum-dentin substrate (34, 35). Last but not for sure least, the maintenance of oral hygiene by the patient is probably the most critical aspect. The margin location is into the sulcus, at a different depth, but always in the most difficult periodontal area to be cleaned by the patient, namely, the interproximal area, that can be reached only with a floss. To clean a cervically relocated margin, the patient must use the floss and use it with a proper technique: unfortunately, only 13,2-17,4% of patients use the floss regularly (36-39).

Also, it must be considered that the bonding interface (the hybrid layer) can degrade over time under clinical service (40-42) because of endogenous enzymes such as MMP (43). Resin composite material can degrade because acids produced by bacteria and other acids present in the oral environment (41), and that under occlusal loading resin composites can present microfractures that might lead to failure (44, 45).

Within the limits of clinical studies of the current literature, it can be concluded that CMR application and its resinous compartments would result in periodontal responses that can be examined by periodontal measurements with BoP. Premature failure of the restorations such as margin discoloration, fracture of the restoration, or fracture of the tooth can lead to more caries. Great care must be taken not to violate the biological width. Meticulous placement of CMR composite, under moisture control, without any iatrogenic effects (i.e., overhangs, underhangs, excess, unpolished surface) is essential for the tissue health maintenance.

Regarding the *in vitro* studies, it can be concluded that highly filled resin composites flowable or highly viscous restorative, placed in multi increments, up to 2 mm in thickness, with a universal adhesive in selective-etch mode should be preferred when CMR approach is desired prior to an indirect restoration.

Additional controlled clinical trials are desired for further evaluation of the effects of CMR on surrounding periodontal tissues.

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INTERNATIONAL PUBLICATIONS

1. Ferrari M, Koken S, Grandini S, Ferrari Cagidiaco E, Joda T, Discepoli N (2018) Influence of cervical margin relocation (CMR) on periodontal health: 12-month results of a controlled trial. J Dent 69: 70-76.
2. Juloski J, Köken S, Ferrari M (2018) Cervical margin relocation in indirect adhesive restorations: A literature review. J Prosthodont Res 62(3): 273-80.
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5. Eid RY, Koken S, Baba NZ, Ounsi H, Ferrari M, Salameh Z (2019) Effect of Fabrication Technique and Thermal Cycling on the Bond Strength of CAD/CAM Milled Custom Fit Anatomical Post and Cores: An In Vitro Study. J Prosthodont 28(8): 898-905.
6. Juloski J, Köken S, Ferrari M (2020) No correlation between two methodological approaches applied to evaluate cervical margin relocation. Dent Mater J.(In Press)

List of Abstracts

1. Michele Carrabba, Serhat Koken, Edoardo Ferrari Cagidiaco, Marco Ferrari, Injectable Resin Composite, Preliminary Clinical Report: 1 Year Follow-Up, IADR 97th General Session, June 20 2019, Vancovour,BC, Canada, Poster presentation, #0604
2. Serhat Köken , Jelena Juloski , Roberto Sorrentino , Simone Grandini ,Marco Ferrari, Marginal Seal of Relocated Cervical Margins of MOD Overlays . IADR 96th General Session, July 27 2018, London, England, Poster presentation. #2429
3. Marco Ferrari, Serhat Koken, Edoardo Ferrari Cagidiaco, Simone Grandini, Cecilia Goracci, Tim Joda, Nicola Discepoli. Influence of cervical margin relocation (CMR) on periodontal health: preliminary results of a controlled trial.,XXXVI International AIOP Congress, 2017 November 16-18 2017 AIOP, Bologna, Italy, Poster presentation

Acknowledgements

This thesis is respectfully submitted to Prof. Francesco Frati, Rector of University of Siena. I wish to express my sincere gratitude to my promoter Prof. Marco Ferrari for the opportunities he gave me to develop my PhD program and for his support during these academic years. Futhermore I'd like to express my thankfulness to a group of masters who have been very important in my professional growth: Prof. Simone Grandini, Prof. Jelena Juloski, Prof. Roberto Sorrentino, Prof. Nicola Discepoli. Thank you all. Last, but certainly not least, I would like to thank my family and friends, especially my wife and children, who have been so supportive during this process.