Carlo Bertoldi[1], Emanuela Monari[1], Pierpaolo Cortellini[2], Luigi Generali[1], Andrea Lucchi[1], Sergio Spinato[3], Davide Zaffe[4]

Clinical and histological reaction of periodontal tissues to subgingival resin composite restorations

[1]Department of Surgery, Medicine, Dentistry and Morphological Sciences with Transplant Surgery, Oncology and Regenerative Medicine Relevance, University of Modena and Reggio Emilia, Modena, Italy;
[2]European Research Group on Periodontology (ERGOPERIO), Bern, Switzerland;
[3]Private Practice, Sassuolo MO, Italy;
[4]Department of Biomedical, Metabolic and Neural Sciences, University of Modena and Reggio Emilia, Modena, Italy.

*Corresponding author: Luigi Generali, Dipartimento Chirurgico, Medico, Odontoiatrico e di Scienze Morfologiche con Interesse Trapiantologico, Oncologico e di Medicina Rigenerativa, Università di Modena e Reggio Emilia, Via del Pozzo 71, 41124, Modena, Italy.
Email: luigi.generali@unimore.it; Phone: ++39 0594224324 - Fax: ++39 0594224795.

ABSTRACT

Objectives To compare the clinical and histological response of supracrestal periodontal tissues to subgingival composite restorations versus natural root surfaces.
Material and Methods In 29 subjects with a single tooth requiring subgingival restorations, a deep margin elevation (DME) procedure with composite resin was applied. Full mouth plaque score (FMPS), full mouth bleeding score (FMBS) and focal probing depth (PD) were measured at baseline, before DME, and after 3 months. The distance between the coronal marked point (CM) to the apical margin of the composite reconstruction (AMR), at baseline, and to the tip of the periodontal probe inserted to reach the bottom of the sulcus (APP), 3 months later, were measured. An all-around secondary flap, harvested to ensure the subsequent single crown prosthetic rehabilitation was histologically processed. The histological inflammation degree was evaluated in areas of gingival tissues adjacent to the composite (B-group) and adjacent to the natural surface of each single tooth (A-group).
Results Significant FMPS, FMBS and PD decreases were observed ($p<0.05$). CM–AMR and CM–APP were significantly different ($p<0.05$), suggesting an attachment gain after 3-months. The inflammation level of gingival tissue was similar in A- and B-groups ($p>0.05$).
Conclusions For the first time, this topic was clinically and histologically studied in humans. Subgingival restorations resulted compatible with gingival health, with levels similar to that of untreated root surfaces.
Clinical Relevance Deep margin elevation procedure produces favorable clinical and histological outcomes allowing a routinely utilization in reconstructive dentistry.

Keywords
Deep margin; Gingiva; Inflammation; Histology; Periodontium; Dental restoration.

Introduction

Direct restoration of teeth with composite is a simple, cost-effective and reliable solution to many clinical conditions and, due to advances in adhesive technology, development of modern materials, and increasing aesthetic requests ending, and the need to implement minimally invasive treatment, it is frequently applied in modern dentistry [1,2]. The application of adhesive material is especially challenging when it occurs in the subgingival area but overhanging margins being especially critical for health of periodontal tissues [3-5]. This is a critical area for placement of the cervical margin of restorations because of the potentially negative impact on biofilm accumulation, the possible direct irritation of the gingival tissues, and the possible invasion of the biological width. All of these might influence the inflammatory response of the marginal periodontal tissues even though it has been clearly demonstrated that a key issue is the precision of the subgingival margin, overhanging margins being especially critical for health of periodontal tissues. Given these premises, well-refined, smooth subgingival direct-adhesive restorations represent an important clinical resource in dental practice for treatment of various conditions. They are routinely used for treatment of large Black Class II or Class V, and also Black Class III/IV cavities to correct discolorations or to modify the emergence profile of the clinical crown. However, subgingival restorations are also increasingly applied to treat non-carious cervical lesions (NCCLs), frequently in combination with root coverage procedures. Root coverage is facilitated by the restoration of the emergence profile of the clinical crown to provide a stable, smooth, and convex surface for the positioning of the surgical flap [6-9,10]. Another important area for the subgingival application of composite is the deep margin elevation (DME) or coronal margin relocation (CMR), techniques frequently applied when there is a need for endodontic therapy in teeth with massive subgingival crown destruction to allow for a stable isolation of the unit with a rubber dam [2,6-9,10-14]. They are largely used for treatment of large Black Class II or Class V, and also Black Class III/IV cavities to treat discolorations or to modify the emersion shape of the clinical crown or to manage indirect restorations [1,2,6,7,8].

Dental restorative materials have been extensively studied, particularly with respect to adhesion [15], usage, finishing [16,17], and aesthetics [18]. An area of particular interest is the biocompatibility of composites, especially in conditions in which these materials are placed at the gingival margin or within the gingival sulcus.

Few in vivo studies have evaluated the biocompatibility of these materials with the marginal periodontal tissues exhibiting contradictory clinical outcomes [19-22]. Some studies on animals have also been histologically performed showing biocompatibility of composite materials [23,24]. It would be interesting to analyze the response of human gingival tissues to composites better; however, no human studies comparing clinical and histologic outcomes have been performed so far.

The aim of this study was to compare the clinical and histological response of supracrestal periodontal tissues to subgingival composite restorations versus natural root surfaces. The null hypothesis of this study is that there are not differences in time, or between control and treated sites, in clinical or histological data.

Materials and methods

Study population and design

This clinical study was performed at the Modena University Hospital (Periodontology Unit of Dentistry and Oral-Maxillofacial Surgery). All procedures were approved, and supervised by the local ethical committee of the Health Service of the Emilia-Romagna region (University-Hospital of Modena- protocol nr.3968/2017, registration nr. 315/17). Enrolled subjects signed informed consent detailing all procedures of the study, as requested by the Helsinki protocols [25].

Subjects referred for dental and periodontal therapy were selected between October 2017 and August 2018. Patients in good general health and without relevant oral or periodontal diseases were screened for the presence of at least one tooth presenting with deep intrasulcular caries requiring endodontic treatment, post-and-core direct reconstruction with composite, and, after the follow-up period of observation, indirect reconstruction with a single prosthetic crown. At screening, inclusion criteria were: 18 years or older patients, non-pregnant or lactating, nonsmokers and without history of alcohol abuse, medical history of good health. Exclusion criteria were: absence of bone disease (metabolic, endocrine, infectious, tumoral, developmental pathologies), anti-inflammatory drugs assumption, uncontrolled or poorly controlled diabetes, unstable or life-threatening conditions, or requiring antibiotic prophylaxis [26, 27]. At the end of cause related therapy, inclusion criteria were: full-mouth plaque score (FMPS) ≤15%, full-mouth bleeding score (FMBS) ≤15%, high levels of compliance [28]. Additional inclusion criteria were: presence of one tooth presenting with extensive caries of the clinical crown extending into the sulcus, requiring endodontic treatment and final reconstruction with a crown, presence of at least 3mm of distance between the cervical margin of the restauration and the bone crest, as

measured with bone sounding after caries removal. Additional exclusion criteria were: presence of detectable plaque and bleeding on probing (BoP) at experimental teeth, as detected at the end of cause-related therapy.

After removal of the decayed dentin, the position of the intrasulcular cervical margin of the reconstruction was carefully inspected. Two conditions had to be satisfied at this point: (1) position of the cervical margin had to be within the sulcus and at a distance of at least 3 mm from bone, as detected through bone sounding; (2) horizontal extension of the intrasulcular cervical margin had to be at least 5mm to have a predictable sample.

The selected experimental dental unit, one tooth per patient, was reconstructed with a pre-endodontic DME to allow for endodontic treatment with the due isolation of the field. The cervical margin of the reconstruction was allocated within the gingival sulcus 3mm apart from bone, thereby respecting the so-called biological width [29, 30]. Endodontic treatment and post-endodontic direct reconstruction were provided. Three months afterward, crown lengthening was planned increasing the distance from the cervical margin of the reconstruction to bone by 2mm to allow for the placement of a crown with the necessary ferrule effect [2, 31-33].

Dental-periodontal procedures and clinical measurements

The study was designed in four steps.

The first step included the subjects' evaluation, case history, dental screening, patient motivation, and professional oral hygiene. Periodontal condition was carefully evaluated and treated with cause-related therapy when needed. Emergencies, such as pain or acute dental-periodontal infections were also treated to establish stable dental-periodontal conditions.

The second step, after re-evaluation following the completion of cause-related therapy, consisted in the complete removal of the decayed dentin, the definition of the intrasulcular cervical margin and the pre-endodontic reconstructive therapy [13,14] using the DME technique [7,11], followed by endodontic therapy. FMPS, assessed by plaque disclosing gel, was recorded as the percentage of total surfaces (6 aspects per tooth) displaying the presence of plaque. Using a periodontal probe with 1-millimeter marks (modified Click-Probe, Kerr Corp., Bioggio, Switzerland), BoP was assessed dichotomously and FMBS (6 aspects per tooth) were then calculated [34]. The following clinical measurements were also taken at the experimental site before DME: (1) probing depth (PD) measured at 6 aspects per tooth and, additionally, PD at the most apical site of the cervical preparation for the pre-endodontic reconstruction; (2) bone sounding measured at the same apical site to verify the presence of a distance of at least 3 mm between cervical margin and bone. These data were considered patient' baseline data, and patients were enrolled and signed the informed consent if met all requirements.

The pre-endodontic restorative therapy was performed under the isolation provided by a rubber dam using Esthet-X HD (DENTSPLY Int., York PA, USA) as composite; surfaces were thoroughly finished and polished to eliminate composite roughness and obtain surfaces as smooth as possible [16, 35, 36]. A marking was made at a coronal point of this reconstruction to serve as a reference for measurements. After completion of the reconstruction, the distance between the coronal marked point (CM) and the apical margin of tooth reconstruction (AMR) was measured joining the CM point to the AMR point (both built with a blue-color composite), parallel to the long axis of the tooth (CM-AMR, Fig. 1).

At the end of step 2, each experimental tooth was partitioned into two sites: the test site (B group), presenting a portion of the subgingival hard tissue replaced with composite (at least 5 mm in horizontal extent) and the rest of the tooth surface, (control site - A group). All patients were entered in a very stringent supportive periodontal care (SPC) program with weekly recalls for three months, with the aim to limit as much as possible plaque accumulation and the possible subsequent, plaque-associated gingival inflammation at the experimental units. The experimental unit was carefully examined at each recall visit for the presence of plaque and BoP: when bacterial plaque or BoP was detected around the dental unit, the patient was dropped out of the study.

The third step, three months after the second step, ended in the surgical lengthening of the clinical crown of each experimental unit to increase the space for the allocation of the prosthetic cervical margin of the crown. The following clinical measurements were taken at the experimental sites before crown lengthening: (1) FMPS and FMBS (2) PD measured at 6 aspects per tooth and additionally at the most apical site of the cervical reconstruction as at baseline; (3) Distance from the CM of the reconstruction to the tip of the probe inserted to reach the bottom of the sulcus (APP); bone sounding to determine the position of the bone crest with respect to the crown margin, considering the ferrule effect. The measurement (CM-APP) was taken to compare with the baseline CM-AMR taken at baseline.

After these recordings and before the fourth step, the crown lengthening procedure was planned. The surgical procedure was performed tracing para-marginal incisions at buccal and lingual surfaces and regarded the whole dental element that had to be reshaped as hard and soft tissue to avoid an unfavorable architecture and considering the necessity to provide the biologic width all around the crown [37-41]. Moreover, the flap had to be mesio-distal extended also to control flap tension and mobilization [39-42]. So, an all-around secondary flap (that should be eliminated), extended to the attached and interproximal gingiva [39-41] was harvested for the histologic examination of both control and test sites. (A and B group).

The gingival samples of each dental unit were carefully laid and fastened with sutures to rigid cotton strips,

maintaining the orientation of the secondary flaps to discriminate the gingival tissues facing the patient's natural root surface (group A, control sites) from that facing the composite reconstruction (group B, experimental sites) (Fig. 2). The investigators do not assign the intervention act that is only accordingly to the dental damage.

The fourth step, after the crown lengthening, consisted of the prosthetic reconstruction of the experimental teeth with crowns (Fig. 3). Subjects were then included in a supportive periodontal care program.

Subjects who did not meet the inclusion criteria or did not follow the protocol during the full study were dropped out.

The 3-months primary clinical outcome of this study was the evaluation of the position of the clinical attachment with respect to the cervical margin of the reconstruction. Secondary outcome was the evaluation of PD changes.

Histology

The gingival samples (secondary flaps on cotton strips) were paraformaldehyde fixed (4% in pH 7.2 phosphate buffer) for 2 hours. Samples were methyl methacrylate embedded them after positioning between two transparent poly methyl methacrylate (PMMA) blocks (1 cm thick), as previously described [43]. PMMA transparent blocks were orthogonally sectioned along an axis (drew by the surgeon on the sample scheme), joining the control and the test areas of the gingival samples. Four-micron seriated sections were hematoxylin and eosin or toluidine blue stained, then microscopically photographed (Eclipse Ni with DS-U3 digital camera - Nikon, Tokyo, Japan). The amount of inflammation in the connective tissue underneath the epithelium (papillary layer of dermis) was manually counted on images obtained by ordinary light microscopy (x40 objective field), using a score criterion (Table 1) as indicated by Martins et al. [24]. In each gingival sample (one sample per subject), the analysis was performed on 10 seriated sections per sample, evaluating (in a blind way) the area of gingival tissues adjacent to the composite (group B) and the area adjacent to the natural surface of the tooth (group A).

The 3-months histologic outcome of this study was the comparison of inflammation of group A versus group B.

Statistical analysis

The 3-months primary outcomes of this study were: (1) histologic comparison of inflammation of group A versus group B, and (2) position of the clinical attachment with respect to the cervical margin of the reconstruction. Secondary outcome was the evaluation of PD, FMBS and FMPS changes.

Sample size was calculated for ANOVA test of histological data (considering these data normally distributed). A sample 17 patients was necessary for alpha = 0.5, difference = 0.25 ± 0.25 and statistical power 0.8. If parameters will be not normally distributed, the sample size for nonparametric tests will be 15% larger than that required for a parametric test [44].

Statistical analysis was performed by Primer of Biostatistics [45], using the paired t test or the Wilcoxon nonparametric signed-rank test for the clinical data taken before and after the clinical treatment, and the ANOVA test or the Mann-Whitney nonparametric rank-sum test for two groups of different individuals.

For all measured variables, the null hypothesis (H_0) of no difference among groups was rejected for a critical significance level of $p < 0.05$.

Results

A total of 48 patients were screened for the presence of at least one instance of subgingival caries. The number of screened patients exceeded by far the necessary sample size to compensate for the exclusion or dropout of patients during the study. Five were excluded after caries removal because the cervical margin of the preparation resulted in a distance <3 mm from the bone crest (invasion of the biologic width). Fourteen of the 43 enrolled patients were excluded during the follow-up period they because did not comply with the very stringent rules of the SPC protocol. Twenty-nine subjects (Table 2), without periodontal disease diagnosed (that is 29 dental units), 12 men and 17 women, aged 24-70 years (mean ± SD, 45.3±12.4 years) fulfilled the stepwise entry criteria of the study. Samples for histologic examination (secondary flaps harvested during the crown lengthening procedure) were collected from the 29 subjects who completed the experimental protocol, maintaining the stringent SPC protocol.

Since FMBS and FMPS were not normally distributed, non-parametric tests were used for all statistical comparisons.

Clinical outcomes

At baseline, FMPS and FMBS were 13.01±1.12% (mean ± SD), range 10.6-14.7%, and 9.27±2.1% (mean ± SD), range 6.3-14.4%, respectively (Table 3). An average probing depth (PD) of 1.95±0.64 mm (mean ± SD), range 1-3 mm, was recorded at sites facing the natural root surface (group A), whereas at sites facing the area of tooth preparation for caries removal (group B), the average PD was 2.57±0.61mm (mean ± SD), range 1-3 mm (Table 3). The difference in PD between groups A and B was statistically significant ($p<0.01$, Mann-Whitney); the

average PD recorded at the most apical site of the cervical preparation for the pre-endodontic reconstruction was 2.86±0.35 mm (mean ± SD), range 2-3 mm (Table 3). Average CM-AMR was 5.59±2.04 mm (mean ± SD), range 4-12 mm, as measured at the end of the restorative procedure (Table 3).

Three months afterward (step 3), FMPS and FMBS were 11.48±1.89% (mean ± SD), range 8.3-14.9%, and 7.48±2.24% (mean ± SD), range 0-12.2% (Table 3). An average PD of 1.66±0.56 mm (mean ± SD), range 1-3 mm, and 2.21±0.68 mm (mean ± SD), range 1-3 mm, was recorded at group A and group B sites, respectively (Table 3). The PD recorded at the most apical site of the cervical preparation was 2.45±0.63 mm (mean ± SD), range 1-3 mm (Table 3). The average CM-APP was 5.14±2.10 mm (mean ± SD), range 4-11 mm (Fig. 1) (Table 3). The difference between the baseline and 3-month measurements of FMPS and FMBS was statistically significant for both parameters ($p<0.01$, Wilcoxon). After 3 months, the PD comparison between groups A and B was statistically significant ($p<0.01$, Mann-Whitney). The difference in PD between the baseline and 3-month after was statistically significant ($p<0.01$, Wilcoxon) in both group A (control sites) and group B (experimental sites). Similarly, the difference in PD, recorded in correspondence of the most apical site of the cervical preparation, between the baseline and 3-month measurements, was statistically significant ($p<0.02$, Wilcoxon). The difference between the CM-AMR (baseline) and the CM-APP (3-month after) was statistically significant ($p<0.05$, Mann-Whitney), indicating that the cervical edge of the composite was within the clinical attachment (Fig. 3).

Histologic outcomes

Gingival samples in both groups were characterized by epithelium that frequently formed a thick rete ridge with well-developed papillae, sometimes very thin and elongated (Fig. 4). Inflammatory cells were normally found at the deeper part of the papillae, starting from 100-200 μm from the lower portion of the rete ridge. Sometimes, inflammatory cells were located more superficially, reaching the base of the papillae in both group A and group B specimens (Fig. 4). Inflammatory infiltrate was mainly composed of macrophages, lymphocytes, and mastocytes, whereas polymorphonuclear leukocytes were found less frequently. When the inflammation level was very low, such as inside the papillae, the presence of some mastocytes was almost always a regular occurrence (Fig. 5). In the 29 treated subjects, the average grade was 3.71±0.36 in group A (mean ± SD), range 2.8-4.1 mm, and 3.83±0.25 (mean ± SD), range 3.2-4., in group B. Difference in inflammation grade between groups A and B was not statistically significant ($P = 0.36$, Mann-Whitney).

Discussion

The study was undertaken under a very stringent, almost experimental protocol to create a plaque-free environment to evaluate the direct effect of composite on gingival tissues eliminating the confounding factor mainly represented by bacterial plaque-associated gingival inflammation.

Several in vitro studies have evaluated the cytotoxicity of composites on cell cultures or on epithelium or connective-tissue cultures [23, 46, 47]. The presence of monomer eluates in the culture medium or interactions with composite polymers results in a significant cytotoxicity [47, 48]. A suitable animal model is likely to provide more information than in vitro studies in which tissue physiology and complex cellular interactions are neglected [49-51] even though animal experimental models present ample dissimilarities to human oral tissues and physiology [48, 52]. Few studies that evaluated the effects of composite resin on periodontal tissues histologically in animal models were performed [23, 24, 48-50]. Martins et al. [24] found an apparently marked chronic inflammatory infiltrate and problems with the growth of new bone and the connective tissue attachment associated with subgingival composite restorations in dogs. They used the same inflammation index adopted for our study. The restorations were completely submerged by oral soft tissues after finishing and polishing; as a consequence, monomer overplus that accumulated in the covering oral tissues probably caused the high inflammatory response. Waerhaug [49] found that sub-gingival restorations cause more inflammation than supragingival restorations in monkeys. However, analyzing that data, it is apparent that the irritating effect on gingiva around such restorations was primarily due to bacterial plaque accumulation and to the inability to obtain highly polished restorations [53, 54].

Many clinical studies in humans have shown that the presence of restorations close to the gingival margin or within the crevicular space is associated with gingival inflammation [2, 35, 36, 53, 55]. A 26-year long-term clinical study found that restorations placed below the gingival margin were detrimental to gingival health resulting in a significant loss of attachment which was mainly detected 1 to 3 years after the restorative procedures [55]. However, these negative outcomes were especially found in a group of patients presenting a large number of caries associated with greater plaque retention at baseline [2, 55]. Ferrari et al. showed a higher incidence of BoP around teeth treated with DME and followed by indirect bonded restorations [56]. However, two thirds of these margins were located mainly at a distance of 2 mm from the bone crest. Therefore, bleeding may be related to an invasion of the biological width.

Completely different outcomes were reported in other studies in which composite resin and even resin-modified

glass ionomer restorations were used to treat NCCLs associated with gingival recessions [19-22]. Reportedly, in some of these studies the supportive care program was stringent and similar to that of our study [21, 22]. Composite resin has to be accurately managed with respect to preparation, bonding, adaptation of margins, finishing, and finally polishing to reduce surface roughness [7, 11]. It is clear that such a faultless application of composites is much easier when the area to be restored is completely visible. Such a condition is more common when treating NCCLs associated with gingival recessions than when treating deep subgingival cavities [19, 21, 22].

The peculiar design of our study allowed for a direct comparison in terms of inflammatory response between gingival tissues facing restorations and natural root surfaces within the same dental unit. Probing depth was significantly reduced from baseline to follow-up measurements in both group A and group B as well as for PD recorded at the most apical site of the restorations. The statistically significant PD improvement demonstrates that well-shaped and well-refined subgingival restorations are compatible with soft-tissue health similar to natural root surfaces, within a very stringent regimen of periodontal supportive care [55, 57, 58].

In addition, the statistically significant discrepancy between the CM-AMR baseline measurement and the CM-APP follow-up measurement supports the hypothesis of a small clinical attachment level (CAL) gain above the cervical margin of the restoration. This peculiar measurement method was adopted to overcome some problems associated with traditional clinical measurements of clinical attachment and of the position of the gingival margin in sites where the cement-enamel junction (CEJ) is disappeared.

To date, no human studies have investigated the inflammatory response of gingiva to composites combining clinical outcomes and histological evaluations. The histologic and clinical outcomes of this study demonstrate that well-refined composite placed within the gingival sulcus and not invading the biologic width are compatible with gingival health and are associated with an inflammatory state of the gingiva similar to that associated with the natural root surface. The inflammation level revealed by the histological analysis was similar in the control gingiva (group A) and treatment gingiva (group B). The histologic findings corroborate the clinical outcomes supporting the conclusion that the inflammation response of tissues surrounding composite restorations is similar to that of tissues surrounding teeth.

It has to be emphasized that the outcomes of this study were obtained after 3 months from DME in an experimental population of patients and experimental units selected with very stringent stepwise entry criteria. The selected subjects were systemically healthy, nonsmokers and without a history of alcohol abuse, without periodontal diseases or acute oral diseases and with high compliance [59, 60]. The restorations were performed

under best conditions, accurately managed, providing a cervical margin in the sulcus with full respect of the biological width [61]. All subjects received the pre-endodontic restoration after an accurate cause-related therapy and then were enrolled in a stringent supportive care program with weekly recalls for 3 months up to the follow-up examination and periodontal surgery for crown lengthening. This stringent protocol was applied to eliminate as much as possible the confounding variables associated with clinical procedures, biological factors, lifestyle or poor compliance that could compromise the evaluation of the direct impact of composite on gingival inflammation. However, these criteria could also represent a limit to the clinical application of the study results. The results obtained could not be appropriate to all type of subgingival defect or to any sort of patient. In particular, it is necessary that DME could be obtained under rubber dam, and only if the margin can be properly isolated without requiring surgical access at this stage. These results are referred to subjects keeping a degree higher level of compliance above standard. Anyway, the successful long-term therapy requires exceptional patient compliance to periodontal therapy and maintenance program. The maintenance is necessary to retain treatment benefits and to prevent relapse [62, 63]. Moreover, the three-month follow-up could be considered a short term observation. However, this follow-up is comparable to that used in the few available studies with histologic evaluations [23, 24, 48, 49] and a longer follow-up in relation to devital teeth needing indirect restoration, but restored only by direct reconstruction, may be hazardous [64-66]. Besides, the timing has been also conditioned by the instructions of the local public health system, requiring that conservative therapy should be completed in a short time (3 months), without discrimination of enrolled from the rest of the patients.

Within the limits of this experimental sample, the following conclusion may be drawn: subgingival restorations result compatible with gingival health, provided that biological width was respected and a stringent supportive therapy could be performed.

Compliance with ethical standards

Conflict of interest Carlo Bertoldi declares that he has no conflict of interest. Emanuela Monari declares that she has no conflict of interest. Pierpaolo Cortellini declares that he has no conflict of interest. Luigi Generali declares that he has no conflict of interest. Andrea Lucchi declares that he has no conflict of interest. Sergio Spinato declares that he has no conflict of interest. Davide Zaffe declares that he has no conflict of interest.

Funding The Research University Fund (FAR) of the Department of Surgery, Medicine, Dentistry and Morphological Sciences with Transplant Surgery, Oncology and Regenerative Medicine Relevance, University of Modena and Reggio Emilia, supported this investigation.

Ethical approval All procedures performed in studies involving human participants were in accordance with the ethical standards of the institutional and/or national research committee and with the 1964 Helsinki Declaration and its later amendments or comparable ethical standards.

Informed consent Informed consent was obtained from all individual participants included in the study.

References

1 Köken S, Juloski J, Sorrentino R, Grandini S, Ferrari M. (2018) Marginal sealing of relocated cervical margins of mesio-occluso-distal overlays. J Oral Sci 60: 460-468.

2 Juloski J, Köken S, Ferrari M. (2018) Cervical margin relocation in indirect adhesive restorations: A literature review. J Prosthodont Res 62: 273-280.

3 Newcomb GM. (1974) The relationship between the location of subgingival crown margins and gingival inflammation. J Peridontol 45: 151-154.

4 Lang NP, Kiel RA, Anderhalden K. (1983) Clinical and microbiological effects of subgingival restorations with overhanging or clinically perfect margins. J Clin Periodontol 10: 563-578.

5 Flores-de-Jacoby L, Zafiropoulos GG, Ciancio S. (1989) Effect of crown margin location on plaque and periodontal health. Int J Periodontics Restorative Dent 9: 197-205.

6 Pecie R, Krejci I, Garcia-Godoy F, Bortolotto T. (2011) Noncarious cervical lesions--a clinical concept based on the literature review. Part 1: prevention. Am J Dent 24: 49-56.

7 Magne P, Spreafico RC. (2012) Deep margin elevation: a paradigm shift. Am J Esthet Dent 2: 86-96.

8 D'Arcangelo C, Vanini L, Casinelli M, Frascaria M, De Angelis F, Ladini M, D'Amario M. (2015) Adhesive cementation of indirect composite inlays and onlays: a literature review. Compend Contin Educ Dent 36: 570-557.

9 Bertoldi C, D. Bencivenni D, Lucchi A, Consolo U. (2007) Augmentation of keratinized gingiva through bilaminar connective tissue grafts: a comparison between two techniques. Minerva Stomatol 56: 3-20.

10 Zucchelli G, Gori G, Mele M, Stefanini M, Mazzotti C, Marzadori M, Montebugnoli L, De Sanctis M. (2011) Non-carious cervical lesions associated with gingival recessions: a decision-making process. J Periodontol 81: 1713-1724.

11 Dietschi D, Spreafico R. (1998) Current clinical concepts for adhesive cementation of tooth-colored posterior restorations. Pract Periodontics Aesthet Dent 10: 47-54.

12 Spreafico R, Marchesi G, Turco G, Frassetto A, Di Lenarda R, Mazzoni A, Breschi L. (2016) Evaluation of the in vitro effects of cervical marginal relocation using composite resins on the marginal quality of CAD/CAM crowns. J Adhes Dent 18: 355-362.

13 Castellucci A. (2004) Endodontics, Il Tridente, Firenze, Vol. 1, pp 330-350.

14 Nica L, Goguta L, Ianes C. (2007) Pre-endodontic restorative treatment: the first step to success in endodontic therapy. Rev Clín Pesq Odontol 3: 33-36.

15 Van Meerbeek B, Peumans M, Poitevin A, Mine A, Van Ende A, Neves A, De Munck J. (2010) Relationship between bond-strength tests and clinical outcomes. Dent Mater 26: 100-121.

16 Roeder LB, Tate WH, Powers JM. (2000) Effect of finishing and polishing procedures on the surface roughness of packable composites. Oper Dent 25: 534-543.

17 Kwon Y, Ferracane J, Lee IB. (2012) Effect of layering methods, composite type, and flowable liner on the polymerization shrinkage stress of light cured composites. Dent Mater 28: 801-809.

18 Heintze SD, Rousson V, Hickel R. (2015) Clinical effectiveness of direct anterior restorations-a meta-analysis. Dent Mater 31**:** 481-495.

19 Antico Lucchesi J, Santos VR, Amaral CM, Peruzzo DC, Mendes Duarte P. (2007) Coronally positioned

flap for treatment of restored root surfaces: a 6-month clinical evaluation. J Periodontol 78: 615-623.

20 Santos VR, Lucchesi JA, Cavalca Cortelli S, Amaral CM, Feres M, Mendes Duarte P. (2007) Effects of glass ionomer and microfilled composite subgingival restorations on periodontal tissue and subgingival biofilm: a 6-month evaluation. J Periodontol 78: 1522-1528.

21 Santamaria MP, Ambrosano GM, Casati MZ, Nociti FH Jr, Sallum AW, Sallum EA. (2009) Connective tissue graft plus resin-modified glass ionomer restoration for the treatment of gingival recession associated with non-carious cervical lesion: a randomized-controlled clinical trial. J Clin Periodontol 36: 791-798.

22 Santamaria MP, da Silva Feitosa D, Nociti FH Jr, Casati MZ, Sallum AW, Sallum EA. (2009) Cervical restoration and the amount of soft tissue coverage achieved by coronally advanced flap: a 2-year follow-up randomized-controlled clinical trial. J Clin Periodontol 36: 434-441.

23 Gociu M, Pătroi D, Prejmerean C, Păstrăv O, Boboia S, Prodan D, Moldovan M. (2013) Biology and cytotoxicity of dental materials: an in vitro study. Rom J Morphol Embryol 54: 261-265.

24 Martins TM, Bosco AF, Nóbrega FJ, Nagata MJ, Garcia VG, Fucini SE. (2007) Periodontal tissue response to coverage of root cavities restored with resin materials: a histomorphometric study in dogs. J Periodontol 78: 1075-1082.

25 American Medical Association (2013) World Medical Association declaration of Helsinki: ethical principles for medical research involving human subjects. JAMA 310: 2191-2194.

26 Duvina M, Barbato L, Brancato L, Glover GD, Amunni F, Tonelli P. (2012) Biochemical markers as predictors of bone remodelling in dental disorders: a narrative description of literature. Clin Cases Miner Bone Metab 9: 100-106.

27 Bertoldi C, Balli F, Tanza D, Bertolani P, Chiarini L. (1995) Experimentation and clinical analysis of the interrelationships between dental damage and celiac disease. Minerva Stomatol 44: 395-105.

28 Eickholz P, Kaltschmitt J, Berbig J, Reitmeir P, Pretzl B. (2008) Tooth loss after active periodontal therapy. 1:subject-related factors for risk, prognosis, and quality of outcome. J Clin Periodontol 35: 2165-2174.

29 Gargiulo AW, Wentz FM, Orban B. (1961) Dimensions and relations of the dentogingival junction in humans. J Periodontol 32: 261-267

30 Schmidt JC, Sahrmann P, Weiger R, Schmidlin PR, Walter C. (2013) Biologic width dimensions--a systematic review. J Clin Periodontol 40: 493-504.

31 Zhang YY, Peng MD, Wang YN, Li Q. (2015) The effects of ferrule configuration on the anti-fracture ability of fiber post-restored teeth. J Dent 43: 117-125.

32 Cakan U, Yuzugullu B, Canay S. (2008) Prosthodontıc and perıodontal reconstructıon of severely damaged endodontıcally treated teeth: a clınıcal report. Internet J Dent Sci 8: 1-6,

33 Savychuk A, Manda M, Galanis C, Provatidis C, Koidis P. (2017) Stress generation in mandibular anterior teeth restored with different types of post-and-core at various levels of ferrule. J Prosthet Dent 119: 965-974.

34 Cortellini P, Pini Prato G, Tonetti MS. (1993) Periodontal regeneration of human infrabony defects. I. Clinical measures. J Periodontol 64: 254-260.

35 Demarco FF, Correa MB, Horta B, Barros AJ, Peres KG, Peres MA. (2013) Multilevel analysis of the

association between posterior restorations and gingival health in young adults: a population-based birth cohort. J Clin Periodontol 40: 1126–1131.

36 Noor E, Al-Bayaty FH. (2015) A review on predisposing and modifying factors of periodontal disease. J Advan Med Res 5: 5-23.

37 Ingber F. J, S., Glover, L. F, & Coslet, J. G. (1977) The "Biologic Width" - A concept in periodontics and restorative dentistry, Alpha-Omegan 10, 62-65,

38 Brägger, U., Lauchenauer, D., & Lang, N.P. (1992) Surgical lengthening of the clinical crown. J Clin Periodontol 19: 58-63.

39 Glover ME (2004) Periodontal plastic and reconstructive surgery. In: Rose LF, Mealey BN (ed) Periodontics: medicine, surgery and implants, Saunders Elsevier, St. Louis, Missouri, pp. 405-487

40 Melnik PR (2006) Preparation of periodontoium for restorative dentistry. In: Carranza's (ed) Clinical Periodontology, 10th edn. Saunders Elsevier, St. Louis, Missouri, pp. 1039-1049

41 Pontoriero R, Carnevale G. (2001) Surgical crown lengthening: a 12-month clinical wound healing study. J Periodontol 72: 841-848

42 Pini Prato G, Pagliaro U, Baldi C, Nieri M, Saletta D, Cairo F, Cortellini P. (2000) Coronally advanced flap procedure for root coverage. Flap with tension versus flap without tension: a randomized controlled clinical study. J Periodontol 71: 188-201.

43 Zaffe D, D'Avenia F. (2007) A novel bone scraper for intraoral harvesting: a device for filling small bone defects. Clin Oral Implants Res 18: 525-533.

44 Lehmann EL (2006) Nonparametrics - Statistical methods based on ranks, Spirnger-Verlag, New York. pp 76-81.

45 Glantz SA. (2007) Primer of biostatistic, 6th ed. Mc-Graw Hill, New York.

46 Moharamzadeh K, Van Noort R, Brook IM, Scutt AM. (2007) Cytotoxicity of resin monomers on human gingival fibroblasts and HaCaT keratinocytes. Dent Mater 23: 40-44.

47 Deviot M, Lachaise I, Högg C, Durner J, Reichl FX, Attal JP, Dursun E. (2018) Bisphenol A release from an orthodontic resin composite: A GC/MS and LC/MS study. Dent Mater 34: 341-354.

48 Goldberg M. (2008) In vitro and in vivo studies on the toxicity of dental resin components: a review. Clin Oral Investig 12: 1-8.

49 Waerhaug J. (1960) Histologic considerations which govern where the margins of restorations should be located in relation to the gingiva. Dent Clin North Am March: 160–176.

50 Perel ML. (1971) Axial crown contours. J Prosthet Dent 25: 642-649.

51 Graves DT, Fine D, Teng YT, Van Dyke TE, Hajishengallis G. (2008) The use of rodent models to investigate host-bacteria interactions related to periodontal diseases. J Clin Periodontol 35: 89-105.

52 Bracken MB. (2008) Why animal studies are often poor predictors of human reactions to exposure. J R Soc Med 101: 120-122.

53 Larato DC. (1972) Influence of a composite resin restoration on the gingival. J Prosthet Dent 28: 402–404.

54 Reinhardt RA. (1979) Guidelines for locating the cervical margins of dental restorations Oper Dent 4: 90-99.

55 Schätzle M, Lang NP, Ånerud Å, Boysen H, Bürgin W, Loë H. (2001) The influence of margins of

restorations on the periodontal tissues over 26 years. J Clin Periodontol 27: 57–64.

56 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.

57 Romanelli JH. (1980) Periodontal considerations in tooth preparation for crowns and bridges. Dent Clin North Am 24: 271-284.

58 Costalonga M, Herzberg MC. (2014) The oral microbiome and the immunobiology of periodontal disease and caries. Immunol Lett 162: 22-38

59 Bertoldi C, Pellacani C, Lalla M, Consolo U, Pinti M, Cortellini P, Cossarizza A. (2012) Herpes Simplex I virus impairs regenerative outcomes of periodontal regenerative therapy in intrabony defects: a pilot study. J Clin Periodontol 39: 385-392.

60 Bertoldi C, Bellei E, Pellacani C, Ferrari D, Lucchi A, Cuoghi A, Bergamini S, Cortellini P, Tomasi A, Zaffe D, Monari E. (2013) Non-bacterial protein expression in periodontal pockets by proteome analysis. J Clin Periodontol 40: 573-582.

61 Veneziani M. (2010) Adhesive restorations in the posterior area with subgingival cervical margins: new classification and differentiated treatment approach. Eur J Esthet Dent 5: 50-76.

62 Umaki TM, Umaki MR, Cobb CM. (2012) The psychology of patient compliance: a focused review of the literature. J Periodontol. 83: 395-400.

63 Bertoldi C, Venuta M, Guaraldi G, Lalla M, Guaitolini S, Generali L, Monzani D, Cortellini P, Zaffe D. (2018) Are periodontal outcomes affected by personality patterns? A 18-month follow-up study. Acta Odontol Scand. 76:48-57.

64 Fedorowicz Z, Carter B, de Souza RF, Chaves CA, Nasser M, Sequeira-Byron P. (2012) Single crowns versus conventional fillings for the restoration of root filled teeth. Cochrane Database Syst Rev. 16: CD009109. doi: 10.1002/14651858.CD009109

65 Ploumaki A, Bilkhair A, Tuna T, Stampf S, Strub JR. (2013) Success rates of prosthetic restorations on endodontically treated teeth; a systematic review after 6 years. J Oral Rehabil 40: 618-630.

66 Sequeira-Byron P, Fedorowicz Z, Carter B, Nasser M, Alrowaili EF. (2015) Single crowns versus conventional fillings for the restoration of root-filled teeth. Cochrane Database Syst Rev. 2015 Sep 25;(9):CD009109. doi: 10.1002/14651858.CD009109

Figure captions

Fig. 1

Schematic drawing showing the two measurements performed with a probe, positioned parallel to the long axis of the tooth from the coronal marked point (CM) to the apical margin of the composite reconstruction (AMR, solid line) at baseline, and to the tip of the periodontal probe inserted to reach the bottom of the sulcus at the 3-month follow-up (APP, dashed line). CEJ = cement-enamel junction; DP = defect perimeter.

Fig. 2

Secondary flap fastened on rigid cotton strip with sutures, maintaining its orientation to discriminate portions next to hard dental tissues from those next to the composite reconstruction.

Fig. 3

Clinical view of the 1.5 occlusal side (a) and the preoperative X-ray (b) at the initial stage. After rubber-dam placement the root canal orifices and the outline of the defect were visible (c). The most apical part of the residual tooth resulted disto-buccal and corresponded to AMR (c and d - white arrow). A curved matrix was used to carry out the DME to perform the pre-endodontic restorative treatment by placing direct composite resin to allow the root canal therapy (e). The second step was completed by the post-and-core direct reconstruction with composite (f). During the tooth reconstruction, a coronal marked point (CM), coronal to AMR (but also the reconstruction in correspondence to AMR - d), were built with a blue-color composite (f - black arrow). The CM-AMR (at the second step) and CM-APP (at the third step) distances were measured considering CM as a key-point. The buccal (g) and the occlusal (h) side of the prosthetic rehabilitation at the fourth step (after the end of clinical surveying), and the X-ray examination (i) after completing endodontic, conservative and prosthetic therapy were showed.

Fig. 4

Representative histology showing the papillary connective tissue of A group (control group) and B group (experimental group) with inflammatory cells reaching up to the epithelium. Note the epithelium thick rete pegs, the small vessels, and the slightly, lower level of inflammation (statistically non-significant) shown by the control gingiva (a) with respect to the experimental gingiva (b). Toluidine blue stain. Bar = 50 µm.

Fig. 5

Representative histology showing the fibrous tissue of papillae of A group and B group with very low level of inflammation. Note the similar level of inflammation in both groups (a = control group, b = experimental group) and the presence of several metachromatic mastocytes (with dark cytoplasm) inside the soft tissue. Toluidine

blue stain. Bar = 50 μm.