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Clinical and microbiological effects of different restorative materials on the periodontal tissues adjacent to subgingival class V restorations

1-year results

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Abstract

Objectives: The relationship between subgingival dental restorations and periodontal health has been thoroughly investigated for many years. However, longitudinal data on the subgingival microflora features after the placement of well-finished subgingival restorations are still lacking. Therefore, this study compares the short-term clinical and microbiological features occurring in the gingiva after the completion of different subgingival restorations.

Material and Methods: Sixteen systemically healthy subjects, 10 males and six females (ages: 31.7–45.8 years; mean age 39.3 ± 5.1 years), who were non-smokers and were positive for the presence of three cervical abrasion/erosion defects to be restored in three different adjacent teeth were enrolled in this study. The cervical abrasion/erosion defects were each restored by using one of three different materials: amalgam, glass ionomer cement, or composite resin. Immediately before class V cavity preparations and restorations (baseline), clinical monitoring and subgingival plaque sampling were performed in the mid-buccal aspect of each experimental restored tooth and in one adjacent sound, non-treated, control tooth. These procedures were repeated every 4 months over the following 1 year.

Results: Throughout the study, the clinical parameters recorded did not change significantly in any of the experimental groups, and no differences were detected among them at each clinical session. Over this time, no significant changes in the composition of the subgingival microflora were observed in amalgam, glass ionomer cement, and control groups. Conversely, in the composite resin group, there was a significant increase in the total bacterial counts, and a significant ($p < 0.05$) decrease in Gram-positive, aerobic bacteria, which was associated with a significant ($p < 0.05$) increase in the Gram-negative, anaerobic microbiota.

Conclusions: Over a 1-year observation period, amalgam, glass ionomer cement, and composite resin subgingival restorations do not significantly affect the clinical parameters recorded. However, composite resin restorations may have some negative effects on the quantity and quality of subgingival plaque.

Key words: amalgam; composite resin; glass ionomer cement; subgingival microflora; subgingival restoration

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As the close association of iatrogenic factors with periodontal breakdown was originally recognized in 1912 (Black 1912), the relationship between dental restorations and periodontal health has been thoroughly investigated for many years. Studies have focused their attention on different aspects of the periodontal–restorative interaction, such as position of the restoration with respect to the gingival margin, presence of overhangs, presence of marginal leakage, roughness of the surfaces (Waerhaug 1956, Larato 1972), and the type of restorative material. Considering that there has been an increasing demand for aesthetic restorations in the posterior regions of the oral cavity in recent years, the type of restorative material is becoming of paramount importance. As a consequence, aesthetic materials, such as composite resins and glass ionomer cements, are more likely to face gingival tissues and to be placed in a subgingival location. Several studies have focused their attention on the effects of different aesthetic restorative materials on gingival tissues, and conflicting results have been reported in the literature; indeed, some of them have reported that composite resins cause gingival inflammation when placed in a subgingival position (Larato 1972, Hammer & Hotz 1979, Willershausen et al. 2001), while others have observed that well-finished composite resins may not adversely affect gingival health (Blank et al 1979, van Dijken et al. 1987a). The type of composite resin (conventional, hybrid or microfiller) seems not to have clinical effects on the periodontal margin (van Dijken et al. 1987a, b), while, conversely, the ageing of the composite resin restorations may negatively interfere with gingival health (van Dijken et al. 1987a). On the contrary, only a few studies have investigated the effects of subgingival glass ionomer cement restorations on periodontal marginal health (Garcia et al 1981, van Dijken & Sjostrom 1991). The most important mechanism by which dental restorations may affect marginal health is the enhancement of plaque accumulation; however, modifications of the composition of the subgingival microbiota have also been reported in the presence of overhanging metal restorations (Lang et al. 1983, Paolantonio et al. 1990) with an increase in putative periodontopathic flora. Cross-sectional data on the microbiological features occurring in the subgingival flora after the placement of well-finished subgingival restorations performed with different materials were reported by van Dijken et al. (1991), whereas longitudinal observations are still lacking. Moreover, van Dijken et al. (1991) monitored *Streptococcus mutans* and lactobacilli on 1-year-old glass ionomer cement and composite resin fillings, and no amalgam restoration was considered. Therefore, the aim of this longitudinal study was to compare the short-term clinical and microbiological features occurring in the gingiva after the placement of amalgam, glass ionomer cement, and composite resin in subgingival restorations.

Material and Methods

Patients and study design

Sixteen non-smoking subjects, 10 males and six females, aged between 31.7 and 45.8 years (mean 39.3 ± 5.1 years), participated in this study. They all met the following criteria: (1) positive for the presence of three cervical abrasion/erosion defects in three different adjacent teeth in the cuspid-first molar region (experimental sites); (2) of good general health; (3) no use of anti-inflammatory drugs in the month preceding the beginning of the study; (4) probing depth (PD) values not exceeding 4 mm in the whole dentition and 3 mm in the experimental sites; (5) no radiographic evidence of periodontal bone loss after a full-mouth radiographic periapical examination; and (6) a full-mouth plaque score (FMPS) and a full-mouth bleeding score (FMBS) $\leqslant 20\%$. FMPS and FMBS were recorded as the % of tooth surfaces with the presence of supragingival plaque or bleeding within 15 s after probing with a 20 g controlled-force probe (Vivacare TPS Probe, Vivadent, Schaan, Lichtenstein). Four weeks before the beginning of the study, all the patients underwent a session of professional oral hygiene, including thorough scaling with an ultrasonic device (Cavitron, Dentspy, Mildford, DE, USA) and detailed oral hygiene instructions (OHIs), including the Bass technique of brushing and the use of dental floss. The cervical abrasion/erosion defects were restored by using each of one of three different materials: amalgam (permite C®, Bayer, Germany), glass ionomer cement (Fuji Bond II Capsule, GC Corporation, Tokyo, Japan), or composite resin (Scotchbond Multi-Purpose®, 3M Dental Products, St Paul, MN, USA). Immediately before cavity preparation and restoration (baseline), clinical and microbiological monitoring was performed in the mid-buccal aspect of each experimental (test) tooth to be restored and in one adjacent, sound, non-treated control tooth. These procedures were repeated every 4 months over the following year. Each participant signed a consent form acknowledging their voluntary and non-prejudicial participation in the study, and the protocol was reviewed and approved by the Ethical Committee of the Faculty of Medicine of the G. D'Annunzio University.

Dental restoration procedures

The teeth to be restored were cleaned with a prophylaxis paste on a rubber cup and rinsed with water. A class V cavity was prepared on the abrasion/erosion defects of each experimental tooth with diamond burs (Bayer) using a high-speed handpiece (Kavo Dental GmbH, Verticbsgesellschaft, Germany) under water coolant. If the defects permitted, an attempt was made to prepare cavities of comparable sizes of 3–4 mm occluso-gingivally and 4–6 mm mesio-distally, with their apical margins not beyond the cemento-enamel junction, and placed 1 mm within the gingival sulcus. After cavity preparation, isolation was made using cotton rolls and a saliva ejector. Moreover, a retraction cord containing adrenaline was placed into the gingival sulcus to avoid bleeding in the restoring area. The procedures used for each type of restorative material were as follows:

(i) The amalgam used in this study was supplied by the manufacturers in direct placement capsules that were mixed in an amalgamator (Promix, Dentspy) at 4000 revolutions/min for 10 s. The dental abrasion/erosion defects were packed with encapsulated amalgam; moreover, deep defects received a base of calcium hydroxide (Dycal, Dentspy). Before carving, the restorations were burnished to maximize the adaptation of the material to the teeth. Care was taken in order to avoid marginal excess of amalgam during the carving stage. Forty-eight hours later, these restorations were finished and polished with low-speed plug finishing burs and rubber

polishing points, respectively (Shofu, Dental Corporation, Menlo Park, CA, USA).

(ii) The cavities restored with glass ionomer cement were conditioned on dentin and enamel with a solution of 20% polyacrylic acid and aluminium chloride (GC Cavity conditioner, GC Corporation) for 20 s, washed for 20 s, and gently air dried for 5 s without desiccation of the dentine. The glass ionomer cement was mixed in the amalgamator (Promix) at 4000 revolutions/min for 10 s and the mixture applied in an average of two increments/defect, and light cured for 20 s/increment. The final restoration was light cured for another 20 s.

(iii) The cavities restored with composite resin were etched on enamel and dentine with a 37% phosphoric acid gel for 20 s, washed for 20 s, and gently air dried for 5 s without desiccation of the dentine. Primer was then applied and dried gently for 5 s. Subsequently, a layer of adhesive was applied and light cured for 10 s. The composite resin was then placed in the cavities in an average of two increments/defect, and light cured for 20 s/increment. The final restoration was light cured for another 20 s. For all light curing, the Max Polymerisation Unit (Dentsply) was used. For both the glass ionomer cement and the composite resin restorations, the finishing procedures were performed with the Enhance finishing and polishing system (Dentsply) immediately after the completion of restorations. This system is consistent with a sequence of the use of a point and gloss paste and an extra fine paste on a polishing cup, each used for 10 strokes of 10 s/stroke under dry conditions. A new bur/cup/point was used for each restoration. Finally, all cavities, restorations, and finishing/polishing were carried out by two expert operators (authors D. T. and M. P.).

Clinical monitoring and subgingival plaque collection procedures

At each experimental and control tooth, the following parameters were recorded at the mid-buccal aspect: (i) presence or absence of supragingival plaque (PL+); (ii) PD using a 20 g controlled-force probe; and (iii) presence of bleeding within 15 s after the PD measurement. Subgingival plaque samples were obtained prior to PD measurements following isolation of the area using cotton rolls and removal of any supragingival plaque with a sterile curette (Asadental, Bozzano, Italy). The gingival surface was dried with a gentle, sterile oxygen-free-CO_2 gas flow. Three sterile paper points (#30) were inserted into the gingival crevice at each experimental and control site and left in situ for 15 s. At the end of each visit, OHI were reinforced in all patients. The same two operators always collected the clinical data and subgingival plaque (authors C. B. and E. S.). Moreover, the patients were not allowed to take antibiotics or chemotherapeutic agents from at least 1 month preceding each clinical session subsequent to the baseline session.

Microbiological procedures

The three paper points from each tooth in each patient were pooled in 2 ml of 0.1 M phosphate reduced buffer, maintained at a temperature of $+8^{\circ}C$ ($\pm 1^{\circ}C$) in a portable electric refrigerator (International PBI SpA, Milan, Italy) and processed within 60 min from sampling. Patient samples were dispersed by vortexing for 30 s, and each sample was subjected to a series of 10-fold dilutions (to 10^{-4}) in 0.1 M phosphate buffer. Aliquots of 100 μl from each dilution were spread onto Columbia blood agar (CBA) plates (Oxoid Italia SpA, Garbagante Milanese, Milan, Italy) for total anaerobic viable count. Generally, isolation of micro-organisms was carried out by methods previously reported (Finegolds & George 1989, Drasar & Roberts 1991, Levet 1991). In particular, for some bacterial strains, special microbiological procedures were applied. In brief, the following plates were inoculated and incubated at $37^{\circ}C$ for 7 days in an anaerobic chamber (80/10/10, $N_2/H_2/CO_2$; Don Whitley Scientific Ltd, Shipley, UK; International PBI SpA): trypticase soy crystal violet erythromycin (4 mg/l) (CVE) (Walker et al. 1979), to assess *Fusobacterium* spp.; Brucella agar (BA) plates enriched with 5% defibrinated horse blood, 0.5% haemolysed blood and 5 mg/l menadione, to assess *Peptostreptococcus micros* and the black-pigmented *Porphyromonas gingivalis* and *Prevotella intermedia* (Dahlen et al. 1993); and veillonella agar vancomycin (1 mg/ml) (VAV) plates, for assessment of *Veillonella parvula* (Drasar & Roberts 1991). Plates containing between 30 and 300 colony-forming units (CFUs) were selected for quantitative estimation of bacterial growth. The purification and characterization of clinical isolates was carried out essentially as described previously (Dahlen et al. 1993, Piccolomini et al. 1998). Finally, a definitive identification of all representative isolates listed in Table 3 was then obtained by subculturing onto Brucella blood agar (Oxoid) followed by inoculation of purified cultures onto a commercially packaged automated system (bioMérieux Italia SpA, Marcy-l' Etoile, France). For each microbial species, data were recorded as the count of CFUs/ml on the growth plate. The total bacterial counts, obtained by adding data of each microbiota, and the frequencies of detection for each microbiota were also reported. Finally, two blinded operators (authors G. C. and S. D'E.) performed all microbiological examinations.

Data analysis

The Statistical Package for Social Sciences (SPSS) software (SPSS® Inc., Chicago, IL, USA) was used to perform the data analysis. The number of tooth sites PL+ and BOP+ were processed as dichotomous data, while the PD was considered as continuous data, after required assumptions needed for parametric methods were verified. A Cochran test was used to evaluate the statistical significance of the differences for PL+ and BOP+ over time within each group, and among the different groups within each time point. A one-way repeated measures analysis of variance (one-way ANOVA) was performed to evaluate the changes in the PD over time within each group, and among the groups within each time point. The statistical significance of the differences in the total bacterial counts and in the proportion of the different bacterial colonies, clustered as: (i) aerobic Gram-positive; (ii) aerobic Gram-negative; (iii) anaerobic Gram-positive; and (iv) anaerobic Gram-negative, within each group over time, and within each time point among the groups, were evaluated by means of a Friedman test; when significant interactions were found, a Bonferroni-cor-

rected Wilcoxon paired signed-rank test was employed as pairwise comparisons. Moreover, the changes in the frequencies of detection of each predominant cultivable microbiota were also tested. A Cochran test was employed to assess differences within each group over time and within each time point among the groups. When significant interactions were found, a Bonferroni-corrected McNemar test was used as pairwise comparisons.

A probability of $p<0.05$ was accepted for rejection of the null hypothesis.

Results

FMPS and FMBS from all subjects participating in the study remained $\leqslant 20\%$ throughout the study.

Table 1 shows the number of sites PL+ and BOP+ and the mean PD from each filled tooth at each time point. No significant changes ($p>0.05$) in the number of PL+ and BOP+ sites and in the mean PD were observed, irrespective of the presence of a restoration or of the material used.

Fig. 1 shows the total bacterial counts in each group over multiple time points. No statistically significant differences were found among the groups at any time point, nor within the amalgam, glass ionomer cement, and control groups over time; whereas, within the composite resin group a significant increase ($p<0.05$) in the total bacterial counts was recorded without significant differences at the pairwise comparisons being found.

Table 2 reports the modifications of the proportion of the different bacterial colonies associated with each type of restorative material over time. No statistically significant differences were found among the groups at any time point, nor within the amalgam, glass ionomer cement, and control groups over time. Conversely, within the composite resin group and over time, a significant ($p<0.05$) decrease in Gram-positive, aerobic bacteria was associated with a significant ($p<0.05$) increase in the Gram-negative, anaerobic microbiota; however, no significant differences were found at the pairwise comparisons.

Table 3 shows the frequencies of detection of the predominant cultivatable microbiota from the groups at the different time points. No statistically significant differences were found among the groups at any time point,

Table 1. Number of sites with the presence of supragingival plaque (PL+) and bleeding on probing (BOP+), and the mean probing depth (PD) (± SD) in the different experimental groups over time

	Baseline				4 months				8 months				1 year			
	A	GIC	CR	C	A	GIC	CR	C	A	GIC	CR	C	A	GIC	CR	C
PL+	1	2	2	1	3	3	5	2	4	2	2	1	2	3	4	2
BOP+	1	0	2	0	2	2	1	0	0	0	2	1	1	0	1	1
PD	1.9 (± 0.7)	1.5 (± 0.6)	1.5 (± 0.7)	1.4 (± 0.5)	1.7 (± 0.6)	1.6 (± 0.7)	1.7 (± 0.6)	1.5 (± 0.6)	1.4 (± 0.5)	1.9 (± 0.9)	1.8 (± 0.8)	1.6 (± 0.4)	1.7 (± 0.5)	1.8 (± 0.7)	1.8 (± 0.6)	1.7 (± 0.6)

No statistically significant differences found.
A, amalgam group; GIC, glass ionomer cement group; CR, composite resin group; C, control group (each group contained $n = 16$).

Table 2. Median (25th;75th percentiles) of the proportions of bacterial group colonization from the subgingival plaque in the different experimental groups over time

	Baseline				4 months				8 months				1 year			
	A	GIC	CR	C	A	GIC	CR	C	A	GIC	CR	C	A	GIC	CR	C
aerobic Gram-positive	44.4 (17;59)	59.0 (48;68)	45.1 (38;63)	48.9 (30;61)	42.5 (28;61)	32.3 (19;60)	33.3 (25;60)	42.5 (34;49)	36.3 (25;49)	31.4 (15;47)	26.5 (12;48)	39.2 (28;52)	40.5 (27;46)	40.5 (23;50)	28.9 (20;37)	49.3 (41;53)
aerobic Gram-negative	0 (0;5)	0 (0;0)	0 (0;1)	0 (0;1)	0 (0;1)	0 (0;1)	0 (0;1)	0 (0;1)	0 (0;1)	0 (0;0)	0 (0;0)	0 (0;1)	0 (0;1)	0 (0;2)	0 (0;1)	0 (0;4)
anaerobic Gram-positive	48.5 (24;76)	38.2 (28;49)	41.0 (34;53)	47.9 (35;63)	46.1 (35;67)	51.5 (40;79)	50.7 (31;70)	45.8 (35;65)	53.0 (40;72)	63.4 (45;72)	52.4 (41;84)	51.2 (42;69)	48.0 (43;57)	46.7 (37;71)	53.1 (40;70)	47.6 (41;51)
anaerobic Gram-negative	0.4 (0;7)	0 (0;3)	2.9 (0;14)	2.7 (0;8)	2.5 (0;9)	2.4 (0;14)	1.7 (0;5)	0.3 (0;8)	3.3 (0;10)	2.3 (0;12)	3.4 (0;7)	4.4 (0;15)	8.3 (3;19)	5.8 (0;12)	10.7 (6;19)	3.0 (0;6)

A, amalgam group; GIC, glass ionomer cement group; CR, composite resin group; C, control group (each group contained $n = 16$).
Statistically significant differences in the proportions of bacterial colonization over time within the CR group (Friedman test): aerobic Gram-positive ($p<0.05$) and anaerobic Gram-negative ($p<0.05$).

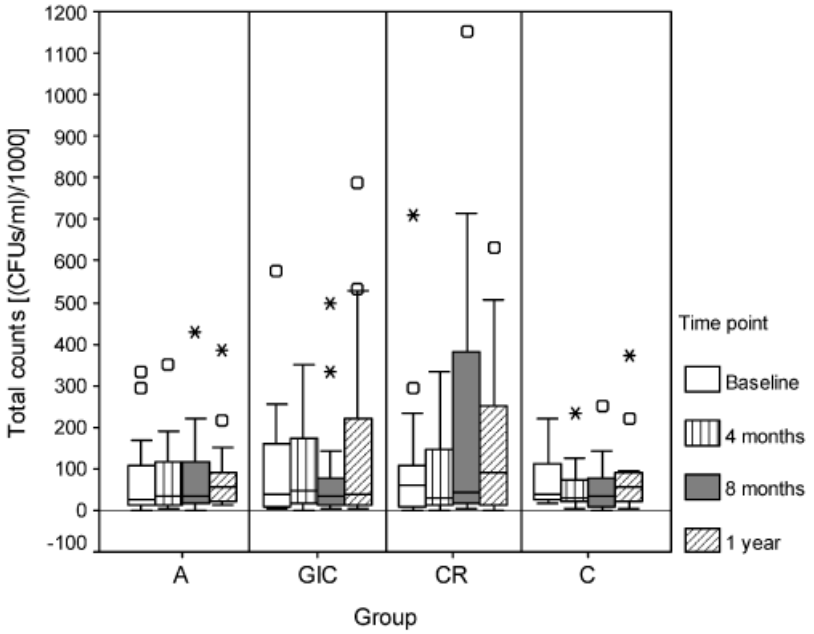

Fig. 1. Boxplots of total bacterial counts (colony-forming units (CFUs)/ml) from the subgingival plaque in the different experimental groups over time; statistically significant increases in the total bacterial counts over time within the CR group (Friedman test, $p < 0.05$). A, amalgam group; GIC, glass ionomer cement group; CR, composite resin group; C, control group (each group contained $n = 16$).

nor within the amalgam and control groups over time. In the glass ionomer cement group, *Bacteroides capillosus* showed a significant increase in the frequency of detection over time ($p < 0.05$). Finally, in the composite resin group, *Actinomyces israelii*, *B. capillosus*, *Fusobacterium mortiferum*, *Peptostreptococcus oralis*, and *P. gingivalis* all underwent a significant increase in the frequencies of detection over time ($p < 0.05$ at least). No significant differences were found for the pairwise comparisons in any of these bacteria.

Discussion

The results obtained in this study show that after 1 year, well-finished subgingival restorations performed with different materials do not produce significant changes in the PL, BOP, and PD in the adjacent gingival tissues. Indeed, restoration of the test teeth did not cause a greater supragingival plaque accumulation as compared with non-restored, control, sound tooth surfaces (Table 1). However, within the composite resin group only, microbiological data demonstrated a significant increase in the total bacterial counts (Fig. 1) with a decrease in the proportion of aerobic Gram-positive concomitant to an increase in the proportion of anaerobic Gram-negative bacteria (Table 2).

Considering that all dental restorations evaluated in the present study had their apical border in the subgingival area, our results appear not to agree with those obtained from other studies where dental restorations placed below the gingival margin are detrimental to gingival health (Jansson et al. 1997, Schatzle et al. 2001). This inconsistency of data may be explained by the fact that the present study was carried out on a small number of subjects who were highly motivated towards oral hygiene rather then on a large cohort of individuals, as in an epidemiological study. Indeed, clinical results carried out in the present study (Table 1) are probably a direct consequence of the high standard of oral hygiene obtained by the subjects participating in the study. Evidence of this is that the subjects did not show significant modifications in either FMPS or FMBS, which were always less then 20% throughout the experimental term. Moreover, all restorations were performed on the buccal aspects of the teeth, where a correct sulcular brushing is capable of interfering with plaque accumulation (Grant et al. 1988). Furthermore, the data from this study refer to 1 year of observation, which may be insufficiently long to evaluate the effects of deterioration of the restorations that can occur over longer time periods (van Dijken et al. 1980, 1983, van Noort & Davis 1984). Finally, all restorations considered in the present study, irrespective of the material used, were accurately contoured and finished. In connection with this, an accurate contouring and finishing of subgingival restorations has been described to be consistent with clinical gingival health (Gorzo et al. 1979, Laurell et al. 1983). This may further explain why in the present study neither the differences in the nature of the restorative materials nor the presence of a restoration itself clinically affected gingival health.

The findings regarding the clinical effects of the amalgam restorations carried out in the present study are also consistent with several previous investigations (Gorzo et al. 1979, Laurell et al. 1983, Paolantonio et al. 1990) that have documented well the good bio-compatibility of well-finished amalgam. The results regarding the glass ionomer cement restorations obtained in the present study are in agreement with those from other investigations (Garcia et al. 1981, van Dijken & Sjöström 1991). Garcia et al. (1981) reported the absence of detrimental effects on the gingiva by glass ionomer cement restorations if they were carefully contoured and finished, and similar results have also been reported by van Dijken & Sjöström (1991). The absence of damage to the gingiva by glass ionomer cement restorations has been related to the good marginal adaptation of this material to the tooth structure, resulting in less marginal leakage and retention of bacteria (Hembree & Andrews 1978). Controversial data are available on the effects of composite resin restorations on gingival health. Larato (1972) reported gingival inflammation adjacent to subgingival class V composite resin restorations. More frequent inflammatory processes of the gingiva in the presence of composite resin restorations, than those with metal restorations, have been reported by Hammer & Hotz (1979). Similarly, Willershausen et al. (2001) reported a high prevalence of gingival bleeding and an increased PD in association with resin-based restorations, as compared with other restorative materials. Conversely, other studies have shown results similar to our findings: Blank et al. (1979) observed

Table 3. Number of sites positive for the presence of each bacterial species over time in the different experimental groups

	Baseline				4 months				8 months				1 year			
	A	GIC	CR	C	A	GIC	CR	C	A	GIC	CR	C	A	GIC	CR	C
Actinomyces israelii	0	0	1	0	1	0	0	0	4	3	2	4	3	1	5	3
Actinomyces naeslundii	0	1	3	2	4	4	4	5	3	1	3	2	1	1	2	1
Actinomyces odontolyticus	6	3	8	3	5	3	6	7	5	2	5	3	9	5	10	8
Actinomyces spp.	1	1	2	2	3	3	0	3	0	2	1	1	1	2	1	4
Actinomyces viscosus	5	3	4	4	8	6	8	10	6	5	7	7	8	5	9	7
Bacteroides capillosus	4	4	3	5	5	2	5	7	4	8	10	9	8	9	9	9
Bacteroides gracilis	1	1	1	1	2	4	2	4	1	1	2	1	1	0	4	1
Bacteroides spp.	0	0	1	0	2	0	0	0	1	0	1	0	1	0	2	1
Bacteroides uroliticus	0	0	0	0	1	0	2	0	0	1	0	1	0	0	0	0
Bifidobacterium spp.	0	0	0	1	2	3	3	5	1	1	3	2	3	2	5	2
Eubacterium lentum	0	1	2	0	1	0	2	0	0	1	0	0	0	0	2	0
Fusobacterium mortiferum	0	2	0	3	3	2	5	1	5	1	2	1	2	2	3	4
Fusobacterium nucleatum	3	2	1	4	3	2	2	3	1	2	4	4	3	2	7	2
Fusobacterium spp.	0	1	2	1	0	0	0	0	1	0	2	0	0	1	3	3
Fusobacterium varium	2	1	3	1	1	2	2	4	1	1	1	0	0	2	0	2
Gemella morbillorum	3	0	2	2	4	3	3	4	4	2	4	3	1	1	2	0
Lactobacillus acidophilus	1	3	2	4	3	3	4	5	4	1	1	2	3	2	3	1
Lactobacillus fermentum	0	0	0	0	0	1	1	3	0	1	1	1	0	0	2	0
Lactobacillus spp.	0	0	1	1	0	0	0	0	2	0	0	0	2	0	1	0
Peptostreptococcus micros	0	0	0	0	1	1	0	0	0	0	0	0	1	0	0	0
Peptostreptococcus spp.	2	4	1	3	3	4	3	5	1	0	0	0	1	2	4	2
Peptostreptococcus oralis	4	1	2	4	6	1	5	2	2	2	2	1	7	5	9	6
Porphyromonasas asaccharolytica	0	0	0	1	0	0	0	0	0	1	1	1	0	0	1	0
Porphyromonas gingivalis	0	0	0	0	3	1	2	2	2	2	0	2	4	2	5	3
Prevotella intermedia	0	0	0	0	2	0	0	0	0	0	0	0	0	0	0	0
Prevotella melaninogenica	2	1	0	2	3	1	3	1	0	2	2	2	1	1	3	1
Prevotella spp.	0	0	1	0	0	1	1	2	0	1	1	1	1	1	2	0
Propionibacterium granulosum	0	1	1	1	0	0	0	0	0	0	1	0	0	0	0	0
Propionibacterius acnes	2	1	0	0	3	3	1	2	2	1	2	1	1	1	2	2
Streptococcus constellatus	1	2	0	2	2	2	3	3	1	0	1	0	1	0	2	0
Streptococcus intermedius	13	11	11	13	16	15	16	16	16	15	15	14	16	14	16	16
Streptococcus mitis	4	6	5	7	7	6	10	10	8	9	5	6	10	3	7	8
Streptococcus mutans	8	9	9	11	7	6	8	10	9	9	10	12	10	8	9	10
Streptococcus salivarius	2	2	1	2	2	1	1	1	0	0	1	0	1	3	5	5
Streptococcus sanguis	5	1	4	2	8	4	6	7	4	3	5	3	5	5	8	6
Veillonella parvula	0	0	0	0	2	1	1	1	1	0	1	0	0	0	0	0
Veilonella Spp.	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0

A, amalgam group; GIC, glass ionomer cement group; CR, composite resin group; C, control group (each group contained $n = 16$).
Statistically significant increases in the frequencies of detection over time within the GIC group (Cochran test): *B. capillosus* ($p < 0.05$); and within the CR group: *A. israelii* ($p < 0.05$), *B. capillosus* ($p < 0.05$), *F. mortiferum* ($p < 0.05$), *P. oralis* ($p < 0.05$) and *P. gingivalis* ($p < 0.01$).

that well-finished and contoured composite resin restorations do not affect the health of the gingiva, and Van Dijken et al. (1987a) reported that 1-year-old subgingival composite resin restorations are not associated with gingival inflammation. However, when observing 3–4-year-old composite resin restorations, van Dijken et al. (1987a) reported a significantly higher rate of gingival inflammation. This observation was explained by the surface deterioration that occurs in composite resin restorations after in vivo wear, with a consequent increase in plaque accumulation. This observation can explain the heterogeneity of the data from the literature on the relationship between subgingival composite resin restorations and gingival health. In the present study, the last clinical evaluation was performed 1 year after the completion of the restorations, and this may explain why the composite resin restorations did not show adverse effects on the gingival tissues.

Microbiological examination of the subgingival plaque adjacent to the subgingival restorations yielded different results in the presence of different materials. In the composite resin group, the total bacterial counts underwent a significant increase (Fig. 1) and, in the same group, a significant increase in the proportion of Gram-negative anaerobic bacteria, and a significant decrease in the proportion of Gram-positive aerobic bacteria (Table 2) were seen. Similar effects were not observed in the amalgam or glass ionomer cement groups (Fig. 1 and Table 2). However, cross-sectional analyses show that the composition of the subgingival microflora did not differ significantly among the experimental groups in any of the time point (Fig. 1 and Table 2). Hence, these results are in accordance with those previously reported by van Dijken et al. (1991) at a cross-sectional analysis level only; indeed, van Dijken et al. (1991) described a similar colonization by *S. mutans* and lactobacilli on 1-year-old glass ionomer cement and composite resin restoration by a cross-sectional study design. In the present study, interesting results are obtained by considering the frequency of detection of predominant cultivatable microbiota associated with subgingival restorations. In the glass ionomer cement group, only

B. capillosus showed a significant increase in detection over time, while in the composite resin group, *A. israelii*, *B. capillosus*, *F. mortiferum*, *P. gingivalis*, and *P. oralis* all underwent a significant increase in detection over time (Table 3). These findings are relevant, considering that bacteria such as *A. israelii* and *P. gingivalis* are considered to be putative periodontopathogens (Moore et al. 1985). Microbiological findings regarding the amalgam and glass ionomer cement groups are consistent with previously reported data. It has been reported that amalgam can have an antibacterial activity (Glassman & Miller 1984, Orstavik 1985, Morrier et al. 1989, 1998, Wang & Liu 2000); this activity has been related to the presence of mercury, copper, and zinc (Morrier et al. 1998). Similarly, fluoride, which constantly leaks from the surface of the glass ionomer cement, could interfere with the initial adherence of bacteria to the restoration surface (van Dijken & Sjöström 1991). Fluoride can also inhibit the metabolism and the growth of the bacteria (van Dijken & Sjöström 1991). These considerations may explain why amalgam and glass ionomer cement restorations do not produce any significant changes in the composition of subgingival microflora, as is also observed with the control sites. Although they yielded statistically significant differences, the microbiological data obtained in the composite resin group did not produce relevant effects on the PL, BOP, and PD. However, it is of interest that a potentially pathogenic flora associated with a restorative material is considered to be detrimental to gingival health by several authors (Larato 1972, Hammer & Hotz 1979, Willershausen et al. 2001). In this regard, van Dijken & Sjöström (1991) found significant differences in the amount of gingival crevicular fluid (GCF) around sound dental surfaces and 1-year-old composite resin restorations, suggesting that although not macroscopically evident, the composite resin restorations may produce subclinical gingival inflammation. Potentially limiting, the present study did not include a measure of the amount of GCF in correspondence to the experimental sites; however, we cannot exclude that a subclinical inflammation may also be present in composite resin sites. The reason why composite resin restorations are associated with increases in the total cultivable bacterial counts (Fig. 1) and in the frequencies of detection of some putative periodontopathic microflora (Table 3) may be found in the observation that subgingival composite resin restorations often show marginal leakage (Kohalmi et al. 1999, Demarco et al. 2001), and that such a defect may present a microenvironment that may favour the growth of an anaerobic flora. On the other hand, amalgam and glass ionomer cement are characterized by a better marginal adaptation, which reduces the microgap between the tooth structure and the restoration (Kohalmi et al. 1999, Demarco et al. 2001). Furthermore, the chemical characteristics of amalgam and glass ionomer cement may reduce their bacterial colonization.

In brief, our results suggest that over a 1-year observation period, amalgam, glass ionomer cement, and composite resin subgingival restorations do not affect the PL, BOP, and PD at a clinically detectable level. However, composite resin restorations may have some negative effects on the quantity and quality of subgingival plaque.

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