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Mutans streptococci and lactobacilli in plaque on a leucite-reinforced dental ceramic and on a calcium aluminate cement

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Abstract In this in vivo study, the proportions of mutans streptococci and lactobacilli in plaque were examined (1) on proximal surfaces of bonded, leucite-reinforced ceramic crowns and (2) on class V restorations of calcium aluminate cement (CAC). The examined proportions were intraindividually compared with those of resin composite and enamel. Mutans streptococci and lactobacilli in samples from plaque that was accumulated for 10 days on the following surfaces were determined by cultivation on blood agar plates and species-selective plates: (1) proximal leucite-reinforced ceramic crown, class II composite and enamel ($n=11$); and (2) class V restoration of CAC and composite, and enamel ($n=17$). Mutans streptococci and lactobacilli in the samples were distributed in three groups: 0, >0–1, and >1% of total bacteria. The surfaces with detected mutans streptococci were similarly distributed between the materials and enamel. The highest proportion of mutans streptococci and lactobacilli were observed on ceramic followed by composite and enamel. A higher proportion of lactobacilli, but not of mutans streptococci, was detected on enamel compared to CAC and composite. However, no significant differences were found between the surfaces. Conclusively, the materials investigated did not show different relative proportions of mutans streptococci and lactobacilli in plaque, compared to enamel.

Keywords Dental plaque · Viridans streptococci · *Lactobacillus* · Ceramics · Dental materials

Introduction

A naturally protective microflora exists in the oral environment, with a specific composition on the different surfaces of the oral tissues. Colonization of bacteria occurs by adhesion, interaction, and growth. Dental plaque, a biofilm of bacteria, their metabolic products, and saliva components [30], is a prerequisite to develop dental caries [11]. Cariogenic bacteria, such as mutans streptococci and lactobacilli, efficiently degrade fermentable carbohydrates to acids, which can demineralize tooth tissue [12]. They are aciduric; thus, their proportions in dental plaque increase at low pH levels [29]. Mutans streptococci adhere to the oral hard tissues and take a major part of initiation of caries [15, 26], while increasing proportions of lactobacilli are considered to support a caries-inducing environment and attend in the proceeding demineralization of the caries lesion [5, 44].

A wide variety of dental materials is used to restore the anatomy and function of the tooth surfaces affected by caries. The use of resin composites has increased because of their esthetics and physical properties and tissue preservative preparation technique.

Despite promising clinical durability, several disadvantages exist. The release of unpolymerized monomers and additives indicates a cytotoxic potential [13, 39]. Allergic reactions to acrylates have been reported [46]. Moreover, polymerization shrinkage of resin composites and secondary caries, as the main reason for replacement [6, 33], raise the demands for alternative materials. The caries-preventive effects of fluoride have stimulated the use of fluoride-releasing materials. Glass ionomer cements can be used in a wide range of clinical applications; however, they are contraindicated in loaded posterior cavities [32].

The advantages of ceramic restorations over direct-placed restorative materials have improved marginal adaptation and anatomic form. Ceramics can be defined as inorganic, nonmetallic materials, mostly as a result from high-temperature reactions. A wide definition of ceramics, without the demand for high temperature reactions, comprises also metal oxides and cements. The specific

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properties of ceramics are hardness, porosity, and brittleness. Early ceramics showed a low-fracture resistance, which was improved in leucite crystals reinforced feldspathic porcelain. Ceramic onlays/crowns of the leucite-reinforced glass, luted with a dentin bonding adhesive system and a resin composite material, showed satisfactory clinical performance [10]. The concept of bonding of ceramics has been applied during the last decades for the manufacture of veneers, inlays/onlays, and full crowns. Dental ceramics are considered to be one of the most biocompatible materials [40] that do not favor plaque accumulation [23, 38]. To our knowledge, the proportions of mutans streptococci and lactobacilli in plaque on resin-bonded ceramic crowns have not been assessed.

Calcium aluminate cement (CAC), intended to be used in class I, II, and V cavities, was introduced a few years ago in Sweden as a “bioceramic” restorative. The direct restorative material is based on two essential constituents: alumina and calcium oxides. The hardening is initiated by an acid–base reaction when calcium aluminate tablets are brought into contact with the supplied liquid, which contains water and small amounts of Li as accelerator. The mechanical properties and the clinical durability have been studied [42], as well as the effect of the CAC on adjacent gingiva [25]. However, the influence of the CAC on the cariogenic microflora has not yet been investigated.

The objectives of this study were to examine the proportions of mutans streptococci and lactobacilli in plaque (1) on proximal surfaces of bonded leucite-reinforced ceramic restorations and (2) on class V restorations of CAC, and to compare the proportions with those on resin composite and enamel.

It was hypothesized that the proportions of mutans streptococci and lactobacilli in dental plaque would be similar between the surfaces of (1) leucite-reinforced ceramic, resin composite, and enamel, and (2) CAC, resin composite, and enamel.

Materials and methods

Individuals participating in two longitudinal follow-ups, the first including enamel–dentin bonded ceramic onlays/crowns [10] and the second including class V CAC restorations [7], were asked to participate in the present study. Exclusion criteria were use of antibiotics, anti-inflammatory drugs, and/or oral antimicrobial agents within the preceding 3 months. To be included, surfaces of bonded ceramics or CAC, resin composite, and enamel should be localized in the same jaw with no detectable dental caries. The restorations should be >3 month old, well-polished, and in gingival touch. The individuals gave informed consent to participate. The ethics committee of the University of Umeå approved the study.

Enamel/dentin-bonded ceramic onlays/crowns

All patients participating in a clinical follow-up study of extensive enamel/dentin-bonded ceramic crowns, fulfilling the inclusion criteria were asked to participate, at their yearly recalls, during a 4-month period. Eleven individuals (seven women and four men, mean age 56.0 years, range 40 to 85) were included. The DMFS index ranged from 32 to 80. In each subject a set of three proximal surfaces, one extensive leucite-reinforced enamel/dentin-bonded ceramic restoration (IPS Empress, Ivoclar, Liechtenstein), one class-II resin composite, and one nonfilled enamel control surface, were available to be intraindividually compared. In one subject, two sets were available. A total of 12 sets were included, and their intraoral distribution on different tooth types is shown in Table 1a. The neighboring proximal surfaces did not have any restoration or carious lesion.

Calcium aluminate cement class V restorations

During a 6-month period, all recall patients, fulfilling the inclusion criteria, in an ongoing clinical follow-up of class V restorations of a CAC were asked to participate. Fifteen individuals (4 women and 11 men, mean age 63.0 years, range 40 to 85) were included. The DMFS index ranged from 35 to 99. In each subject, at the minimum one set of two class V restorations, one of CAC (Doxa Certex, Uppsala, Sweden) and one of hybrid resin composite, and one nonfilled enamel control surface were available to be intraindividually compared. The intraoral distribution on different tooth types of the 20 sets included is shown in Table 1b.

Sampling and bacteriological analysis

Plaque was accumulated for 10 days of no oral hygiene. At baseline (day 0), after a period of strict oral hygiene, no visible plaque was allowed to be present on the experi-

Table 1 Intraoral distribution of leucite-reinforced bonded ceramic (1a), calcium aluminate cement (CAC; 1b), resin composite, and enamel on different tooth types

	Molars	Premolars	Canines	Incisors	Number of surfaces
1a					
Ceramic	10	2	0	0	12
Composite	2	8	1	1	12
Enamel	2	7	3	0	12
1b					
CAC	5	14	1	0	20
Composite	2	10	8	0	20
Enamel	3	5	4	8	20

mental surfaces [25]. The accumulated, supragingival plaque from each cervical surface of the restorative material or enamel was collected after water irrigation of the teeth. The sampling was only performed on the visible parts of the included surfaces to prevent contamination by plaque from adjacent surfaces and to control saliva contamination.

The plaque was sampled with a tip of a sterile applicator (Applicator Tips; Dentsply/De Trey, Konstanz, Germany) and immersed in a 0.5-ml salt buffer [36]. Each sample was homogenized by pulsed ultrasonic oscillation (10 1-s pulses, Sonifer B-30, Branson Ultrasonic, Danbury, CT, USA) and then diluted serially in the salt buffer. Aliquots of the samples were cultured on agar plates, which were incubated in 5% CO₂ and 95% air at 37°C for 2 days. Blood agar was used to determine the total counts of bacteria. *mitis salivarius* agar (Difco, Becton Dickinson, Sparks, MD, USA) supplemented with bacitracin [16] and Rogosa selective lactobacilli agar (Merck, Darmstadt, Germany) were used to estimate mutans streptococci and lactobacilli, respectively. The numbers of bacteria were counted as colony forming units (CFUs) and the relative proportions (percentage of total bacteria) were calculated.

Statistical analysis

The data were processed in SPSS (Statistical Package for the Social Sciences, version 10.0). The relative proportions were not normally distributed, although logarithm transformations were applied. Therefore, the relative proportions were categorized in groups, and nonparametric tests were chosen. Frequency distributions of the relative proportions of mutans streptococci and lactobacilli were described in three groups: 0, >0–1, and >1%. Intraindividual differences between the various materials and enamel were tested with the use of Wilcoxon's signed rank test, where a two-sided *p* value <0.05 indicated statistical significance, and Exact test (Monte Carlo) with a 99% confidence interval (99% CI).

Results

Enamel/dentin-bonded ceramic onlays/crowns

Eleven of 12 sets including a proximal surface of bonded ceramic, resin composite, and enamel were evaluated. The distributions of the relative proportions of mutans strepto-

cocci and lactobacilli are shown in Table 2. Regardless of material or enamel, the number of surfaces with detected mutans streptococci was similar (± 1 surface) but with higher relative proportions on ceramics, followed by resin composites. The min–max value of the relative proportions of mutans streptococci >1% was 1.1–8.6% for ceramic and 1.2–3.6% for resin composite. No enamel surface showed a relative proportion of mutans streptococci >1%. The relative proportions of lactobacilli observed were generally low (<0.1%) and not detected on enamel. The only high relative proportion of lactobacilli (6.2%) was observed on a ceramic surface. No significant differences regarding the presence of mutans streptococci or lactobacilli between the proximal surfaces of leucite-reinforced enamel/dentin-bonded ceramics, resin composite, and enamel were found.

Calcium aluminate cement class V restorations

Two individuals discontinued the study, both because of infectious disease. Eighteen sets comprising one surface each of CAC, resin composite, and enamel were evaluated in the remaining 13 individuals. One set was discarded because of nonoptimal handling. The distributions of the relative proportions of mutans streptococci and lactobacilli are shown in Table 3. Regardless of material or enamel, less than one-fourth of the surfaces demonstrated a relative proportion of mutans streptococci >1%. Among these, the min–max value was 1–55% for CAC, 3.5–37% for resin composite, and 1.5–2.9% for enamel. No lactobacilli were detected in plaque from the major part of the surfaces. The two highest relative proportions of lactobacilli (1 and 18.6%) were observed on the enamel surfaces. No significant differences were observed between the surfaces of CAC, resin composite, and enamel with respect to the relative proportions of mutans streptococci or lactobacilli.

Discussion

Plaque accumulation on tooth surfaces and the composition of the dental biofilm are important factors in the development of caries and periodontal diseases [11, 28]. Dental caries is discussed in terms of the dynamic relationship among the dental plaque microbiota, dietary carbohydrates, saliva, and the pH-lowering and cariogenic potential of dental plaque. Well-known cariogenic bacteria, such as mutans streptococci and lactobacilli, are selectively enhanced at a low pH [45]. These microorganisms are

Table 2 Cariogenic bacteria, in vivo, on leucite-reinforced ceramic, resin composite, and enamel

Relative proportions of the total bacteria flora	Ceramic			Resin composite			Enamel		
	0%	>0–1%	>1%	0%	>0–1%	>1%	0%	>0–1%	>1%
Mutans streptococci	5	2	4	4	5	2	4	7	0
Lactobacilli	6	4	1	9	2	0	11	0	0

The relative proportions (percentage of total bacteria) of mutans streptococci and lactobacilli in 10 days accumulated plaque on surfaces of leucite-reinforced ceramic, resin composite, and enamel surfaces (*n*=11)

Table 3 Cariogenic bacteria, in vivo, on CAC, resin composite, and enamel

Relative proportions of the total bacteria flora	CAC			Resin composite			Enamel		
	0%	>0–1%	>1%	0%	>0–1%	>1%	0%	>0–1%	>1%
Mutans streptococci	2	11	4	1	13	3	1	13	3
Lactobacilli	12	5	0	12	5	0	9	6	2

The relative proportions (percentage of total bacteria) of mutans streptococci and lactobacilli in 10 days accumulated plaque on surfaces of CAC, resin composite, and enamel ($n=17$)

usually present in secondary caries contiguous to tooth-colored restorations [17].

Bacterial affinity and plaque formation on material surfaces may be influenced by several material-related factors such as surface roughness, surface energy, and marginal adaptation [37]. Some studies have discussed that different oral bacteria adhere preferentially to either hydrophobic or hydrophilic surfaces [18, 35]. A lower tendency to accumulate plaque on ceramic crowns compared to enamel has been reported [4, 23, 38], and ceramics are considered to be one of the most biocompatible restoratives in operative dentistry. Hahn et al. [19] studied in an intraindividual approach, proximal inlays of cast and sintered ceramics, bonding resin composite and enamel. After 3 days, both ceramics accumulated significantly less plaque compared to enamel, while the resin composite showed no differences compared to enamel. Skjörland [41] postulated that resin composites have a tendency to accumulate more plaque than other filling materials. However, Hannig [20] concluded that early plaque formation (within 24 h) was predominantly influenced by nonmaterial-dependent factors in the oral environment. The proportions of the cariogenic microflora in plaque on resin-bonded ceramic crowns have not been assessed. Recently, it was shown that the counts of the mutans streptococci in early plaque on molar-attached specimens did not differ between dental ceramic and restorative resin composite [43].

The present in vivo study revealed no differences concerning the relative proportions of mutans streptococci and lactobacilli between the resin-bonded ceramic, resin composite, and enamel surfaces. Thus, the first hypothesis was accepted.

Mutans streptococci were recovered from more than half of the surfaces. Some relatively high proportions (>1%) of mutans streptococci were detected on the proximal surfaces of the restorations but not on enamel. Lindquist and Emilson [27] observed that mutans streptococci tended to colonize restored surfaces with composite/silicate, amalgam, and gold/porcelain in a greater extent than on sound surfaces. Furthermore, mutans streptococci were distributed in a decreasing gradient from molars to incisors. Most of the bonded ceramic restorations were placed on molars and resin composites restorations and control enamel surfaces in premolars, which may partly explain the higher proportions of ceramic surfaces with >1% mutans streptococci. However, in contrast to Lindquist and Emilson [27], the counts of mutans streptococci in the present study were related to the total counts (relative proportions) minimizing

the effects of differences in sampled plaque amounts. Besides, the sampled area was limited to the visible part of the proximal surfaces, to secure a similar extent of the sampling area on each of the compared teeth except for the anterior teeth.

In the bonded ceramic group, the relative proportions of lactobacilli observed were generally low (<0.1%) and were detected on a minor part of all proximal surfaces. In a similar study of class III restorations, lactobacilli were also detected at very low proportions on resin composites and enamel [9]. Marsh et al. [31] showed that lactobacilli were rarely isolated and never recovered from interdental plaque on caries-free premolars in schoolchildren. Moreover, lactobacilli were never found interdentally without the presence of mutans streptococci [5].

The leucite-reinforced ceramic restorations were bonded with resin composite. Previous studies have shown that extracts of resin composites may stimulate [21] or modulate [24] the growth of cariogenic microorganisms. However, in vitro, cured resin composite specimens exhibited no significant effect on the growth of mutans streptococci [3]. Besides, residual unbound monomers, which may influence bacterial growth, are rapidly eluted after polymerization [13]. To eliminate any possible initial leakage, only aged restorations were included in the present study.

The calcium aluminate restorative material was initially introduced as a “bioceramic” filling material. The material does not fulfill the criteria for conventional dental ceramics and should therefore rather be considered as restorative cement. Previously, higher amounts of 10-day accumulated plaque were observed on class V restorations of CAC compared with resin composite and enamel surfaces [25]. In the present study, no differences in the proportions of cariogenic bacteria were detected between those surfaces. Consequently, the second hypothesis was accepted. The results indicated that aged CAC restorations did not contribute to a biofilm, which favors a growth of mutans streptococci or lactobacilli. The proportions of mutans streptococci were similarly distributed on the class V restorations of CAC, resin composite and enamel. Mutans streptococci were found on nine out of ten surfaces and lactobacilli on one-third of all surfaces. Surprisingly, the few samples with high proportions of lactobacilli (>1%) were recovered from the enamel surfaces and not from the class V restorations.

There were no differences found between enamel and resin composites in a similar study of the levels of mutans streptococci and lactobacilli in 14-day-old plaque on

buccal surfaces of restorative materials [8]. The length of the plaque accumulation period may influence the levels of the various microorganisms in plaque. After several days of plaque accumulation, the depth of the biofilm increases, causing a shift in the local environment, indirectly modifying the composition of the microflora [30].

The antibacterial effect of some dental materials is well-known. A short-term reduction of mutans streptococci in plaque on glass ionomer cement has been shown [9]. This inhibitory effect on the cariogenic microflora is ascribed to the initial release of fluoride ions and decreases with aging of the glass ionomers, when the fluoride release reaches the steady-state level. Metal ions included in various dental materials, like amalgam, copper phosphate cement, and glass ionomer-cermet restorations have demonstrated antibacterial effects [1, 14, 34]. In addition, Hayacibara et al. [22] suggested that aluminum release by ionomeric materials may enhance the biological effects of fluoride in inhibiting bacterial metabolism and growth of mutans streptococci biofilms in vitro. The CAC contains aluminates, but without a potential fluoride release. Berglund et al. [2] studied the dimensional change of the CAC and resin composite materials for a duration of 360 days. The CAC was less dimensionally stable than the composites. During the experimental period, a whitish precipitate could be seen in the storage vessels, which was probably also present on the surface of the CAC specimens. Fast erosion of the restorative was confirmed in the clinical follow-up of the material [7]. The contents of the released precipitate and their effect on the microflora are unknown. The restorations studied were at least 3 months old, evading any effect of initial leakage products on the microflora. The present study was focused on ranking the bacterial proportions in plaque on different surfaces, and all the intraindividually compared surfaces were treated in the same way. Intraindividual comparisons eliminate the influence on individual-related factors of the results. Still, the local microflora is unique for every habitat and may differ from various surfaces or part of surfaces. To conclude, the materials investigated did not show different relative proportions of mutans streptococci and lactobacilli in dental plaque, compared to enamel. The novel calcium aluminate cement did not show lower frequencies of cariogenic bacteria in cervical plaque compared to the conventional resin composite surfaces. The resin-bonded ceramic reconstructions also showed a similar presence of cariogenic bacteria compared to resin composite in proximal plaque.

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References

1. Berg JH, Farrell JE, Brown LR (1990) Class II glass ionomer/silver cermet restorations and their effect on interproximal growth of mutans streptococci. *Pediatr Dent* 12:20–23
2. Berglund A, Hultström A-K, Gruffman E, van Dijken JW (2005) Dimensional change of a calcium aluminate cement for posterior restorations in aqueous and dry media. *Dent Mater* DOI 10.1016/j.dental.2005.04.041 (in press)
3. Boeck C, Schumacher E, Podbielski A, Haller B (2002) Antibacterial activity of restorative dental biomaterials in vitro. *Caries Res* 36:101–107
4. Chan C, Weber H (1986) Plaque retention on teeth restored with full-ceramic crowns: a comparative study. *J Prosthet Dent* 6:666–671
5. Crossner CG, Claesson R, Johansson T (1989) Presence of mutans streptococci and various types of lactobacilli in interdental spaces related to development of proximal carious lesions. *Scand J Dent Res* 97:307–315
6. Davidson CL, de Gee AJ, Feilzer AJ (1984) The competition between the composite-dentin bond strength and the polymerization contraction stress. *J Dent Res* 63:1396–1399
7. van Dijken JWV, Sunnegårdh-Grönberg K (2004) A three-year clinical evaluation of a new calcium aluminate cement in Class V cavities. *Swed Dent J* 28:111–118
8. Dijken JWV van, Persson S, Sjöström S (1991) Presence of *Streptococcus mutans* and lactobacilli in saliva and on enamel, glass ionomer cement, and composite resin surfaces. *Scand J Dent Res* 99:13–19
9. van Dijken JWV, Kalfas S, Litra V, Oliveby A (1997) Fluoride and mutans streptococci levels in plaque on aged restorations of resin-modified glass ionomer cement, compomer and resin composite. *Caries Res* 31:379–383
10. Dijken van JWV, Hasselrot L, Örmén A, Olofsson AL (2001) Restorations with extensive dentin/enamel-bonded ceramic coverage. A 5-year follow-up. *Eur J Oral Sci* 109:222–229
11. Fehr FR von der, Löe H, Theilade E (1970) Experimental caries in man. *Caries Res* 4:131–148
12. Fejerskov O (1997) Concepts of dental caries and their consequences for understanding the disease. *Community Dent Oral Epidemiol* 25:5–12
13. Ferracane JL (1994) Elution of leachable components from composites. *J Oral Rehabil* 21:441–452
14. Foley J, Blackwell A (2003) Ion release from copper phosphate cement and influence on *Streptococcus mutans* growth in vitro: a comparative study. *Caries Res* 37:416–424
15. Gibbons RJ (1984) Adherent interactions which may affect microbial ecology in the mouth. *J Dent Res* 63:378–385
16. Gold OG, Jordan HV, van Houte J (1973) A selective medium for *Streptococcus mutans*. *Arch Oral Biol* 18:1357–64
17. Gonzalez-Cabezas C, Li Y, Gregory RL, Stookey GK (2002) Distribution of cariogenic bacteria in carious lesions around tooth-colored restorations. *Am J Dent* 15:248–51
18. Grivet M, Morrier JJ, Benay G, Barsotti O (2000) Effect of hydrophobicity on in vitro streptococcal adhesion to dental alloys. *J Mater Sci* 11:637–642
19. Hahn R, Weiger R, Netuschil L, Bruch M (1993) Microbial accumulation and vitality on different restorative materials. *Dent Mater* 9:312–316
20. Hannig M (1999) Transmission electron microscopy of early plaque formation on dental materials in vivo. *Eur J Oral Sci* 107:55–64
21. Hansel C, Leyhausen G, Mai UFH, Geurtsen W (1998) Effects of various resin composite (co)monomers and extracts on two caries-associated micro-organisms in vitro. *J Dent Res* 77:60–67
22. Hayacibara MF, Rosa OPS, Koo H, Torres SA, Cosat B, Cury JA (2003) Effects of fluoride and aluminium from ionomeric materials on *S. mutans* biofilm. *J Dent Res* 82:267–271
23. Jensen ØE, Schultes AM, Handelsman SL (1990) Plaque retention on Dicor crowns and gingival health evaluated over a 4-year period. *Int J Periodontics Restorative Dent* 10:454–463
24. Khalicihi P, Cvitkovitch DG, Santerre JP (2004) Effect of composite resin biodegradation products on oral streptococcal growth. *Biomaterials* 25:5467–5472
25. Konradsson K, van Dijken JWV (2002) Effect of a novel ceramic filling material on plaque formation and marginal gingiva. *Acta Odontol Scand* 60:370–374

26. Liljemark WF, Blomquist C (1996) Human oral microbial ecology and dental caries and periodontal diseases. *Crit Rev Oral Biol Med* 7:180–198
27. Lindquist B, Emilson CG (1990) Distribution and prevalence of mutans streptococci in the human dentition. *J Dent Res* 69:1160–1166
28. Löe H, Silness J (1963) Periodontal disease in pregnancy. Prevalence and severity. *Acta Odontol Scand* 21:535–551
29. Marsh PD (1994) Microbial ecology of dental plaque and its significance in health and disease. *Adv Dent Res* 8:263–271
30. Marsh PD, Bradshaw DJ (1995) Dental plaque as a biofilm. *J Ind Microbiol* 15:169–175
31. Marsh PD, Featherstone A, McKee AS, Hallsworth AS, Robinson C, Weatherell JA, Newman HN, Pitter AF (1989) A microbiological study of early caries of approximal surfaces in schoolchildren. *J Dent Res* 68:1151–1154
32. Mjor IA, Jokstad A, Qvist V (1990) Longevity of posterior restorations. *Int Dent J* 40:11–17
33. Mjor IA, Moorhead JE, Dahl JE (2000) Reasons for replacement of restorations in permanent teeth in general dental practice. *Int Dent J* 50:361–366
34. Morrier JJ, Suchett-Kaye G, Nguyen D, Rocca JP, Blanc-Benon J, Barsotti O (1998) Antimicrobial activity of amalgams, alloys and their elements and phases. *Dent Mater* 14:150–157
35. Olsson J, van der Heijde Y, Holmberg K (1992) Plaque formation in vivo and bacterial attachment in vitro on permanently hydrophobic and hydrophilic surfaces. *Caries Res* 26:428–433
36. Persson A, Claesson R, van Dijken JWV (2005) Levels of mutans streptococci and lactobacilli in plaque on aged restorations of an ion-releasing and a universal hybrid composite resin. *Acta Odontol Scand* 63:21–25
37. Quirynen M, Bollen CM (1995) The influence of surface roughness and surface-free energy on supra- and subgingival plaque formation in man. A review of the literature. *J Clin Periodontol* 22:1–14
38. Savitt ED, Malament KA, Socransky SS, Melcer AJ, Backman KJ (1987) Effects on colonization of oral microbiota by a cast glass-ceramic restoration. *Int J Periodontics Restorative Dent* 2:23–35
39. Schedle A, Franz A, Rausch-Fan X (1998) Cytotoxic effects of dental composites, adhesive substances, compomers and cements. *Dent Mater* 14:429–440
40. Schuster GS, Lefebvre CA, Wataha JC, White SN (1996) Biocompatibility of posterior restorative materials. *J Calif Dent Assoc* 24:17–31
41. Skjörland K (1973) Plaque accumulation on different dental filling materials. *Scand J Dent Res* 81:538–542
42. Sunnegårdh-Grönberg K (2004) Calcium aluminate cement as dental restorative. Mechanical properties and clinical durability. Umeå University Odontological Dissertations, No 84, Umeå, Sweden
43. Tanner J, Robinson C, Söderling E, Vallittu P (2005) Early plaque formation on fibre-reinforced composites in vivo. *Clin Oral Investig* 9:154–60
44. Tanzer JM, Livingston J, Thompson AM (2001) The microbiology of primary dental caries in humans. *J Dent Educ* 65:1028–1037
45. van Houte J (1994) Role of micro-organisms in caries etiology. *J Dent Res* 73:672–681
46. Wrangsjö K, Swartling C, Meding B (2001) Occupational dermatitis in dental personnel: contact dermatitis with special reference to (meth)acrylates in 174 patients. *Contact Dermatitis* 45:158–163

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