Bacterial growth on dental restorative materials in mucosal contact

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Test specimens of amalgam, gold, porcelain, heat-cured acrylic resin, and human enamel were made to fit into cavities prepared in acrylic pontics of maxillary bridges. One side of the specimen was in close contact with the mucosa. Plaque was allowed to accumulate on the specimens for 27 to 48 days, and adherent bacteria were then quantified by cultivation on selective and non-selective media. The inflammatory status of the mucosa overlying the specimens was monitored by exudation measurements. Lower total numbers of bacteria were recovered from acrylic specimens than from the other materials used, including enamel. There were also significant subject differences in bacterial recovery. Test specimens in contact with inflamed mucosa yielded less total numbers of bacteria, but higher numers of *Streptococcus mutans*, than did specimens exposed to mucosa with no or negligible exudation.

Key-words: Exudation; *Streptococcus mutans;* experimental technique; *in vivo;* dental plaque

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The prognosis of fixed dental restorations depends on the physical properties of the materials used, as well as on the biological effects of the restorations (5, 6). The biological effects are frequently associated with the formation of dental plaque, which may cause inflammation or secondary caries. While the development of dental plaque on enamel surfaces has been studied extensively (9), far less is known about plaque on restorative materials.

Previous studies have indicated, however, that plaque on dental materials may vary with respect to quantities of plaque formed (20), biochemistry (16) and microbiological composition (15). Moreover, recent work with artificial substrates for adhesion of plaque bacteria (18) and for bacterial growth (11) have demonstrated that the nature of the solid surface may influence both the quality and the quantity of the plaque formed.

The microbial composition of plaque on dental restorative materials may be of importance for its pathogenicity. Thus, *Streptococcus mutans* is associated with the development of dental caries (10). Periodontal disease has been primarily related to the quantity of plaque (14), although certain species have

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received special attention as specific pathogens (8, 21).

Several previous studies have indicated that the formation and the bacterial composition of plaque vary with its location: fissure plaque differs from smooth surface supragingival plaque which in turn is different from subgingival plaque (21, 22, 24, 26). In conjunction with fixed restorations, plaque development at or below the gingival margin is considered to be of particular clinical importance (17).

In the present investigation, plaque composition on restorative dental materials in mucosal contact was studied. Test specimens retained in acrylic bridge pontics were placed in contact with the alveolar mucosa, avoiding direct exposure to saliva. The degree of inflammation of the mucosa in contact with the plaque was monitored by exudation measurements. The bacteriological analyses placed particular emphasis on *Strep. mutans*.

MATERIALS AND METHODS

Test subjects and specimen application

Five patients at the Department of Prosthetic Dentistry, scheduled for fixed bridge constructions, volunteered for the study. Maxillary gold/acrylic bridges with saddle-formed, acrylic pontics were constructed. Test pieces of human enamel or restorative materials (Table 1) were made to fit into cavities prepared in the pontics. A lingual hole in the pontic giving access to the specimen cavity facilitated aseptic removal of the specimen which was subjected to bacteriological examination (see below). The test pieces were sealed in place with chloropercha (Kloroperka N-Ø, N-Ø Therapeutics, Oslo) and had their free side adapted to the mucosa of the alveolar ridge. Care was taken to minimize pressure of the test specimens against the mucosa. For each pontic, two test pieces of different materials were made to constitute an interchangeable pair for consecutive tests with the same individual as shown in Table 2. An example is illustrated in Fig. 1.

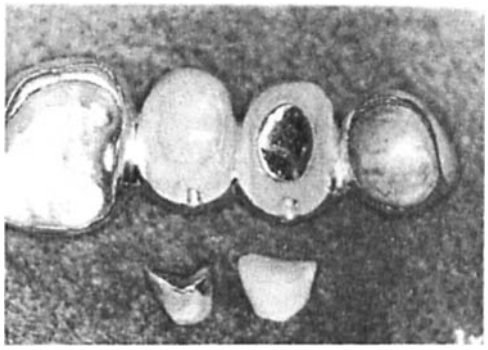

Fig. 1. Bridge with test specimens (enamel, amalgam) sealed in the pontics. Corresponding specimen pairs are shown below. Subject M.

Experimental design

The test specimens, sealed in the pontics, were polished with abrasive rubber cups (Aaba Dental, Identoflex, Buchs, Switzerland) and rinsed with water prior to inserting the bridges using temporary cement (Kerr Temp-Bond, Kerr Mfg. Co., Romulus, Mich.). They were carried by the patients for periods ranging from 27 to 48 days. During this time the free surface of the specimens was not subjected to any kinds of oral hygiene. At the end of each experimental period, the bridge and alveolar mucosa were isolated with cotton rolls, before spraying the pontics and surrounding mucosa with water for 5 sec. The bridge was then removed from the abutments, and the pontics with the test specimens were immediately rinsed in reduced transport fluid (RTF) (25). The material sealing the access hole was removed with a red-hot amalgam plugger, and the test specimen was loosened with a

Table 1. *Brands of restorative materials used*

Material	Brand name	Manufacturer	Abbreviation
Amalgam	a) Royal dental alloy 5	Guld- & Amalgambolaget, AB, Stockholm, Sweden	A
	b) New True Dentalloy	S. S. White Ltd. London, England	
Gold	Delta (type IV)	K. A. Rasmussen, Hamar, Norway	G
Acrylin resin (heat-cured polymetamethacrylate)	Vita K. + B	Vita Zahnfabrik, Säckingen, FRG	Ac
Porcelain	Vita-VMK	Idem	P

Table 2. *Bridge constructions and specimens pairs**

Subject		Bridge construction												No. of tests
B ♀, 67 yrs	Abutments	17		15		13			22					6
	Pontics		16		14		12	11	21					
	Materials*		A E**		A E		Ac G	P Ac	P G					
C ♂, 56 yrs	Abutments		16			13								3
	Pontics			15	14									
	Materials			G Ac	G Ac									
S ♀, 45 yrs	Abutments		16		14	13	12		21		23		25	4
	Pontics			15				11		22		24		
	Materials			Ac P				A G		A G		P Ac		
A ♀, 67 yrs	Abutments		16				12							4
	Pontics			15	14	13								
	Materials			P Ac	P G	G Ac								
M ♀, 32 yrs	Abutments										23		26	4
	Pontics											24	25	
	Materials											A E	A E	

* Abbreviations for materials as given in Table 1.
** E: enamel test pieces.
Total no. of specimens tested: 72

sterile amalgam plugger, then transferred to a glass tube containing 4 ml RTF and 0.25 ml glass beads with a diameter of 0.5 mm for the subsequent bacteriological analyses (see below).

The oral mucosa was then dried for 30 sec with a cotton roll, and a filter paper strip was placed on the area which had been in contact with the test specimen. The amount of mucosal exudate collected on the strip during the 30 sec was measured in a gingivo-fluid meter (HAR-600, Harco Ltd., Winnipeg, Canada). Finally, the alternating

specimens were sealed in the pontics, polished and rinsed before the bridge was reinserted for another experimental period.

Bacteriological examination

The test specimens in RTF were brought to a bacteriological laboratory and handled within 3 hours. In a few instances, handling was delayed up to 20 hours. After shaking for 30 sec on a Whirlimixer WM/250 T (Fisons, Loughborough, Eng.) to suspend and disperse the plaque, the plaque suspensions were 10-fold diluted in RTF and cultured in duplicate on the following, solid media: horse blood agar (BA); mitis-salivarius agar (MS) (Difco, Detroit, Mich.); mitis-salivarius agar supplemented with bacitracin and sucrose for selective growth of *Strep. mutans* (MSB) (12); the *Actinomyces*-medium of Beighton and Colman (AM) (2); and *Veillonella* agar (VA) (Difco). The BA and VA plates were incubated for 24 to 48 h in an atmosphere of 90 % N_2, 5 % CO_2 and 5 % H_2, whereas the MS, MSB and AM plates were incubated for 48 h in 10 % CO_2 in air.

The amount and the relative distribution of bacteria of each specimen were calculated from the total number of colony-forming units (CFU). *Strep. mutans* was identfied by its colonial morphology on MSB plates.

Treatment of data

Bacterial counts were transformed to logarithmic figures, which showed near-normal distributions according to chi-square analyses (7) of sub-sets of the data. The statistical tests used were Student's *t* test, the rank sign test, and regression analysis by the method of least squares (23). The level of significance was chosen at $\alpha = 0.05$ for all statistical analyses.

RESULTS

Recovery of bacteria

The average total recovery obtained from the specimens (BA count) was 5.3 x 10^5 bacteria. Recovery on MS averaged 9.4 %, on MSB 0.06 %, on AM 3.7 % and on VA 0.03 % of the total recovery on BA. The range of bacterial numbers recovered on all media was considerable, as illustrated in Figs. 2 & 3. Recovery on MS and AM was positively correlated with BA recovery ($p < 0.01$, $n = 69$), whereas the recovery on MSB appeared statistically unrelated to the bacterial growth on BA. A tendency for skewed distribution of the *Veillonella* data restricted their inclusion in statistical comparisons.

Bacterial growth related to test material

Fig. 2 summarizes the bacterial recovery obtained from the various materials on the different growth media. Only small differences among the materials could be seen. On the average, acrylic specimens harbored less bacteria, as measured by total growth on BA, than the other materials tested. In comparison with enamel specimens, which showed the highest total growth, this difference was statistically significant ($p < 0.005$, $n = 27$; *t* test). Growth of streptococci on MS was less from gold than other specimens; when gold and enamel specimens were compared, this difference was statistically significant ($p < 0.05$, $n = 25$; *t* test).

Comparison of paired samples from the same pontic

Intra-pair comparisons of the total growth on BA revealed one combination with significant differences: acrylic specimens had less growth than gold specimens ($p < 0.05$, $n = 12$; rank sign and *t* test). Acrylic specimens also

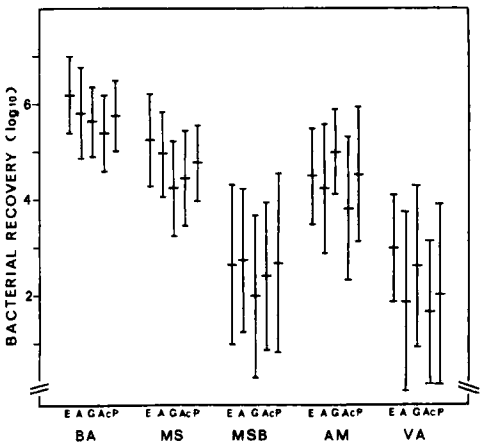

Fig. 2. Total recovery of bacteria obtained from the test specimens. The means and standard deviations are given. E, enamel; A, amalgam; G, gold; Ac, acrylic resin; P, porcelain. BA, blood agar; MS, mitis-salivaris agar; MSB, medium selective for *Strep. mutans;* AM, *Actinomyces* medium; V, Veillonella agar.

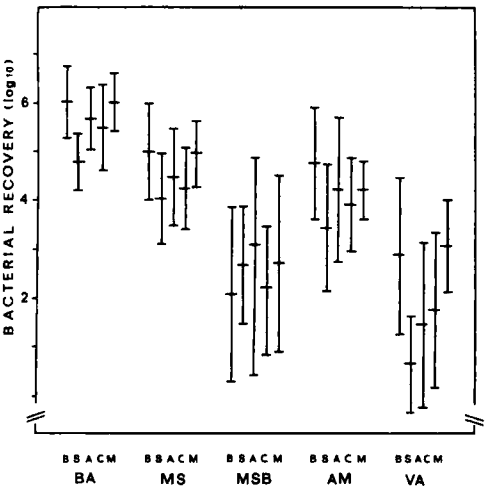

Fig. 3. Total recoveries pooled for the subjects. The means and standard deviations are given. Media abbreviations are as given in Fig. 2.

tended to have less growth than corresponding porcelain specimens, but this difference was not statistically significant. Other intra-pair differences among the materials could not be observed.

Bacterial growth related to test subjects

Fig. 3 summarizes the bacterial recoveries pooled for the subjects. Generally, total bacterial recovery was similar for all subjects, but differences were detected. Test specimens from subject S harbored less bacteria than did specimens from the other subjects. This difference was statistically significant ($p < 0.05$; *t* tests) in comparison with subjects B, A and M, but not with subject C. A tendency was observed for a higher recovery of *Veillonella* from subjects B and M than from subjects C, S and A. However, the skewed distributions of the *Veillonella* values precluded statistical evaluation of these differences.

No relationship was found between the total recovery of bacteria and the location of the specimen in the tooth arc. Thus, subjects with a low total average on BA tended to have low numbers recovered from all specimens irrespective of the location in the arc (e.g., subject S), whereas subjects having a high total average tended to have high numbers recovered from all their test sites (e.g., subject B).

Exudation measurements

Generally, visible signs of inflammation (redness) of the mucosa overlying the specimens were reflected in exudation measurements in excess of 10 on the gingivo-fluid-meter. The areas with exudation measurements in excess of 10 at the start of the experiment ($n = 7$) tended to exhibit persisting inflammation. In one case only was healing in terms of substantially decreased exudation observed. On the other hand, areas with low exudation (meter reading < 10, $n = 8$) at the start of the experiment showed consistently low readings throughout the study.

Relationship between mucosal exudation and bacterial growth

Fewer bacteria were recovered from test specimens exposed to mucosa with exudation compared with specimens from non-exuding sites. This negative correlation between exudation and bacterial recovery was statistically significant ($p < 0.01$ by regression analysis; $n = 61$). Moreover, a negative correlation of borderline significance ($p = 0.05$) was also found in 2 subjects when the material was split up and analyzed for each of the subjects separately. For the other 3 subjects, the data were too few to warrant statistical treatment.

Aspects of Strep. mutans' colonization

Strep. mutans was recovered from 54 out of 69 specimens tested. The frequency of *Strep. mutans* was slightly higher on enamel than on the other materials, but no statistically significant differences could be found. Moreover, the total number of *Strep. mutans* appeared unrelated both to test materials (Fig. 2) and subjects (Fig. 3).

When a given test specimen was analyzed for *Strep. mutans* at different times, large fluctuations were observed. These fluctuations occurred in parallel on most specimens within the same mouth (Fig. 4). Concomitant analysis of *Strep. mutans* counts from different test subjects indicated that these variations were not caused by variations in the MSB medium.

The incidence and total number of *Strep. mutans* increased with increasing degree of exudation. When sites with exudate measurements in excess of 10 were compared with sites with no exudation, *Strep. mutans* was found with significantly higher prevalence and also in higher total numbers ($p < 0.05$, $n = 46$; *t* test) from sites where exudate was present.

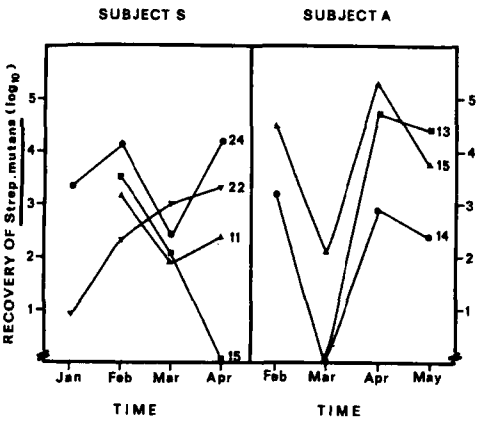

Fig. 4. Variation with time in the recovery of *Strep. mutans* from individual sites (designated as tooth numbers) in separate subjects. Measurements were not obtained for sites 11 and 15 (subject S) in January, or for site 13 (subject A) in February.

DISCUSSION

The present study of plaque on restorative materials focused on some of the dominant bacteria in the mouth, i.e. streptococci, actinomycetes and veillonellae. With the bacteriological techniques used, a number of genera and species cannot be detected. However, the simplified approach employed was felt justified because it monitored both cariogenic and potentially periodontopathic organisms.

Logarithmic transformations of the bacterial counts facilitated statistical analyses of the data (23). Statistical analyses were necessary for meaningful interpretation of the results from this in vivo study which involved a number of controlled variables (materials, subjects, site, time and exudation). Since the logarithmic transformation yielded distributions which conformed with assumed normal distributions of the data, it was considered justified to draw some conclusions based on the statistical analyses. Even so, a note of caution

is appropriate in that both sampling and bacteriological techniques may have introduced variables unaccounted for (e.g., variations in the bacteriological media with time).

The use of exudation fluid measurements provided some information on the inflammatory status of the experimental sites. Studies by Wærhaug (27) have indicated that bridge pontics in contact with oral mucosa induce a transformation of the epithelium to resemble sulcular epithelium, which may facilitate exudation. Although the exudation measurements were not correlated with histologic parameters of inflammation in the present study, clinical observations confirmed that inflamed areas gave consistently high exudation values.

The various materials differed only slightly with respect to the bacterial composition of their plaques. However, the results indicated that fewer bacteria grew on acrylic specimens than on the other materials. This finding was corroborated by the observation that acrylic resin had less bacteria than gold in intra-pair comparisons. Since the acrylic/gold intra-pair comparison involved 3 subjects and exluded the one subject (S) with significantly less total growth, a subject-dependence of this finding would seem unlikely. The low, specific free energy of acrylic resin may be one factor to consider in this context. The specific free surface energy of materials has been positively correlated with the quantity of plaque that can be retained by dental materials (11). Acrylic resin is the only material in the present study with a free surface energy low enough to be affected by this relationship (11).

An influence of the materials on selected genera or species may be suspected from the data obtained. Thus, gold specimens showed a tendency for higher numbers of actinomycetes and lower numbers of streptococci (15). In the absence of significant differences, however, such an influence remains unsubstantiated.

Subject differences were more pronounced than material differences with regard to the total number of bacteria recovered from the specimens. The nature of this subject-dependence is not known. Mucosal exudation is a factor of possible significance, since the subject with the lowest recovery on blood agar showed regularly high values for exudation (data not shown). Moreover, when the results from all the subjects were pooled, specimens from mucosae with exudation had low total numbers of bacteria. This may seem contradictory to other observations showing that plaque formation is enhanced at the gingival margin (19) and in subjects with gingivitis (13). However, in the absence of direct salivary exposure the mucosal exudate may more effectively exert a growth-restricting influence on plaque bacteria through its antibacterial components. Such an influence was indicated in the study by Attström & Schroeder (1), who suggested that 'normally functioning neutrophils and other exudative phenomena operate to prevent subgingival advancement of micro-organisms'. Similarly, exudation may affect microbial growth on bridge pontics placed in contact with the mucosa.

Exudation was accompanied by increased frequency, prevalence and absolute numbers of *Strep. mutans*. This contrasted with the negative correlation between exudation and total growth, and may point to a particular ecologic role for the exudate. *Strep. mutans* is known to metabolize salivary proteins (4), and it is possible that this species utilizes components of the mucosal exudate relatively more efficient than other species do. Moreover, the placement of the specimens onto the mucosa may have created atmospheric

conditions leading to the proliferation of *Strep. mutans* (3).

The association between mucosal exudation and plaque growth was clear-cut and may have clinical significance. Although the exudate may reduce the total number of bacteria, the higher prevalence of *Strep. mutans* on surfaces exposed to exuding mucosae may impart a particular cariogenic potential to plaque on restorations placed at or below the gingival margin. In fact, increased gingival exudation appears to be inevitable in conjunction with subgingival filling margins (17).

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