Clinical and microbiological effects of subgingival restorations with overhanging or clinically perfect margins

Niklaus P. Lang, Robert A. Kiel and Katharina Anderhalden

University of Berne, School of Dental Medicine, Berne, Switzerland

Abstract. The close association between restorations with overhanging margins and chronic destructive periodontitis has been known for many years. However, the mechanisms by which overhanging restorations will interact in the pathogenesis of periodontal disease are still unknown. Generally it is accepted that overhanging restorations contribute to the promotion of the disease process by virtue of their capacity to retain bacterial plaque. The purpose of the present study was to determine if the placement of subgingival restorations with overhanging margins results in changes in the subgingival microflora.

9 dental students with clean teeth and clinically healthy gingivae (GI<0.1) gave their consent to participate in the study. 5 MOD cast gold onlays with 1 mm proximal overhanging margins were placed in mandibular molars for 19-27 weeks. They were replaced in a cross-over design by 5 similar onlays with clinically perfect margins which served as controls. Another 5 onlays were placed in reverse order in the remaining patients.

Prior to and every 2-3 weeks after insertion, subgingival microbiological samples were obtained by inserting a fine sterile paper point for 30 sec into the gingival sulcus subjacent to the restoration. The predominant cultivable flora was determined using continuous anaerobic culturing techniques. Following the placement of restorations with overhanging margins, a subgingival flora was detected which closely resembled that of chronic periodontitis. Increased proportions of Gram-negative anaerobic bacteria, black-pigmented Bacteroides and an increased anaerobe: facultative ratio were noted. Following the placement of the restorations with clinically perfect margins, a microflora characteristic for gingival health or initial gingivitis was observed.

Black-pigmented Bacteroides were detected in very low proportions (1.6-3.8%). These changes in the subgingival microflora were obvious irrespective of whether the restorations with the overhanging margins were placed in the first period of the experiment or following the cross-over. Clinically, increasing gingival indices were detected at the sites where overhanging margins were placed. Bleeding on gentle probing always preceded the peak level of black-pigmented Bacteroides. Loss of attachment was not detected in any site. Changes in the subgingival microflora after the placement of restorations with overhanging margins document a potential mechanism for the initiation of periodontal disease associated with iatrogenic factors.

The close association between iatrogenic factors such as overhanging restorations and destructive periodontitis has been recognized since the early 1900's (Black 1912). Epidemiological (Wright 1963, von Fitchner 1964, Alexander 1967, 1968, Gilmore & Sheiham 1971) as well as clinical experimental studies (Renggli & Regolati 1972, Rodriguez-Ferrer et al. 1980) have repeatedly documented these relationships. Only one recent study (Than et al. 1982) on extracted teeth challenged the close association between overhanging restorations and the promotion of the periodontal disease process. However, also in this study a greater loss of •
attachment was found associated with ill-fitting and overhanging amalgam margins in molar teeth where these lesions are known to be located most frequently (Alexander 1967, 1968, Gilmore & Sheikh 1971).

In daily practice overhanging margins of dental restorations present a very frequently observed problem (Björn et al. 1969, 1976) which may greatly impinge on the maintenance of gingival and periodontal health (for review see Leon 1977). Consequently overhanging restorations will result in excessive loss of the alveolar bone support if they are not recognized and removed for a few years (Hakkarainen & Ainamo 1980, Jeffcoat & Howell 1980).

Although the contributory factor of overhanging restorations in the etiology of periodontal disease has been generally accepted by the dental profession, the mechanisms by which overhanging margins interact in the pathogenesis of periodontal disease are still not completely understood. Some of the irritant effects proposed included the physical and/or chemical properties of dental materials used in restorative procedures (Waerhaug 1956, Waerhaug & Zander 1957, Zander 1957, App 1961). Furthermore, the surface characteristics of dental materials which may lead to a greater tendency of plaque retention (Wise & Dykema 1975, von Rothen et al. 1978) have been mentioned.

Even though it is now generally accepted that overhanging restorations may contribute to more accentuated gingival inflammation by virtue of their retentive capacity for bacterial plaque rather than as a result of mechanical irritation (Ramfjord et al. 1966; World Workshop in Periodontics), the specific aspects of this local bacterial accumulation have never been elucidated. This concept suggests that the plaque mass per se may be responsible for the inflammation of the gingiva and the loss of the periodontal supporting tissues. However, recent studies on the microbiota associated with various forms of periodontitis suggested a more specific approach. The fact that the total mass of bacteria may be less important than its composition has led to our current concept of periodontitis being an opportunistic infection (Loesche 1976, Socransky 1977, Kornman et al. 1981) with specific periodontopathic microorganisms. In view of these recent observations in oral microbiology earlier conclusions concerning the role of overhanging subgingival margins and periodontal disease may in fact be inaccurate. No investigation has made an attempt to assess the individual components of the plaque associated with these restorations.

Hence, it was the purpose of this study to examine the clinical and microbiological effects of the placement of subgingival restorations with either clinically perfect or overhanging margins in patients with periodontal health.

**Material and Methods**

9 patients in excellent systemic health, aged between 20 and 30 years, requiring molar MOD cast gold restorations for the treatment of caries volunteered and gave their informed consent to participate in this study. One restoration was placed in a mandibular molar tooth of each patient with the exception of one patient who received two such restorations. Hence, a total of 10 teeth with 20 sites (mesial and distal) was available for evaluation. Prior to the preparation of the test teeth, all patients received comprehensive dental treatment for all other dental problems including oral hygiene instructions and thorough scaling and root planing. None of the patients required surgical periodontal therapy. At the beginning of the study all patients, therefore, presented with clean teeth and healthy gingivae with mean Plaque and Gingival Indices approaching 0. Furthermore, it was ascertained that there was no history of administration of antibiotics for at least 6 months prior to the study.

**Clinical procedures**

MOD onlays were prepared with an approximately 1 mm subgingival location of the prepa-
rational margin following which conventional impression and temporization techniques were applied. Two cast gold onlays were constructed from the same die with either a clinically perfect or a 0.5–1.0 mm overhanging margin. The patients were randomly assigned to two equal groups. One group received the onlays with clinically perfect subgingival margins while the other group received onlays with overhanging margins. All the restorations were cemented temporarily. After a 3–7 month observation period, the onlays were removed and replaced with onlays of the other type (Fig. 1). The second set of onlays was also left in place for 3–7 months, after which all onlays with overhanging margins were removed and replaced by restorations with clinically perfect margins. During the course of the study, the patients were instructed to continue their normal oral hygiene procedures except that the proximal surfaces of the onlays were to be left uncleansed. At the conclusion of the study all patients received scaling and root planing to restore the experimental teeth to optimal health.

Prior to the onlay preparation, prior to the placement of the cast onlay and every 2–3 weeks thereafter the following clinical parameters were obtained from the experimental sites:

Plaque deposits were assessed using the Plaque Index System (Silness & Löe 1964).
Gingival health or inflammation was scored according to the criteria of the Gingival Index System (Löe & Silness 1963).

Sulcus probing depth was measured to the nearest mm and in relation to the location of the subgingival margin of the restoration using a calibrated M 1 periodontal probe with a point diameter of 0.4 mm.

**Microbiological sampling and processing**

At each clinical evaluation, microbiological samples were obtained for the determination of the predominant cultivable flora associated with each proximal margin. The samples were taken after assessment of the PI. but before assessing the GI and probing depth.

Just prior to sampling, supragingival plaque was removed with a sterile curette. Subsequently, a sterile fine endodontic paper point was gently inserted into the gingival sulcus for 30 sec and removed. The portion of the paper point that had been located subgingivally was cut off with sterile scissors and dropped into a vial containing 5 ml of Reduced Transport Fluid (RTF) without ethylenediamine tetraacetate (Loesch et al. 1972). The samples were brought into an anaerobic incubation chamber (Araniki et al. 1969) within 15 min after sampling. After the dispersion by micro-ultrasonic disruption for 10 sec with a Kontes Cell Disruptor Model K-881440 (Syed & Loesche 1978a), the samples were diluted in RTF and plated on selective and non-selective media using an automatic diluting and plating device (Spiral Systems, Bethesda, MA, U.S.A.). Samples were plated on Enriched Trypticase Soy Agar (ETSA) (Syed et al. 1979).

Some of the samples were also plated on ETSA with 50 µg/ml Kanamycin sulfate (Kornman & Loesche 1980). After 7 days of anaerobic incubation at 35°C, representative colonial morphologies from the non-selective media were quantitated, subcultured, and subjected to a limited series of biochemical tests, as described by Kornman & Loesche (1980) to permit identification as to the genus or species level. Black-pigmented *Bacteroides*-like colonies were also quantitated from the Kanamycin enriched media but were not subcultured.

**Data analysis**

The cross-over design of the study yielded two groups of 10 sites: one group received restorations with overhanging margins followed by clinically perfect subgingival margins; the other group received the same two types of onlays in the opposite sequence. The results from these groups were designated as follows:

\(+\)/\(-\): Results from overhanging restorations (+) in patients with overhanging restorations placed first

\(+/(-)\): Results from clinically perfect restorations (−) in patients with overhanging restorations placed first

\((-/+)\): Results from clinically perfect restorations (−) in patients with clinically perfect restorations placed first

\(-/+)\): Results from overhanging restorations (+) in patients with clinically perfect restorations placed first.

The results from each of the 4 groups represent multiple samples taken between 4–21 weeks after placement of each restoration. The mean and standard error of the mean (SEM) have been presented as descriptive statistics only. Tests of statistical significance for all values except probing depth were performed using non-parametric analyses (Siegel 1956). Differences in the PI, GI and media comparisons were analyzed using the Chi-square analysis. All other microbiological data were analyzed using the Kruskal-Wallis 1-way analysis of variance and the Mann-Whitney U-test. Correlation analyses employed the non-parametric Spearman Rank Correlation Coefficient. Probing depth was analyzed using the unpaired t-test.
Results

Clinical parameters
All the patients presented with plaque-free tooth surfaces (P1 = 0) at the site of the carious lesion which was to be restored with the cast gold onlay. Following tooth preparation and onlay placement, these indices gradually increased. Almost 100% of the surfaces scored P1 = 2 after 2–3 weeks, owing to the fact that interproximal cleaning was omitted in these experimental areas. At baseline approximately 60% of the gingival surfaces adjacent to the onlay preparation scored GI = 0 in the patient group which first received the onlay with overhanging margins. Bleeding on probing at the experimental sites was completely absent in all patients at this time. Following the placement of the restorations with overhanging margins the percentage of surfaces which bled on probing gradually increased to 44% after 7–11 weeks, to 75% after 12–21 weeks and to 100% after 22 weeks (Fig. 2). At that time 23% of the surfaces showed spontaneous bleeding (GI = 3). When the onlays were replaced by restorations with clinically perfect margins, the frequency of GI = 2 or 3 scores gradually decreased and reached preexperimental levels after 20–27 weeks. In the patient group which first received the onlays with clinically perfect margins, 100% of the surfaces scored GI = 1 at all times and bleeding on probing was absent. However, when the restorations with overhanging margins were placed, this group also gradually developed bleeding on probing at the experimental sites. After 12 weeks, GI = 2 was scored in 100% of all surfaces adjacent to the restorations (Fig. 2).

![Graph A](image1)

![Graph B](image2)

Fig. 2. Frequency distribution of Gingival Index scores at all observation periods for both groups of patients who received either first (A) an overlay with (+) an overhanging margin and then an onlay with clinically perfect margin (−) or vice versa (B).
Clinical probing depths were generally 2–3 mm at baseline. Concomitantly with increasing Gingival Index scores, clinical probing depths also increased by 1–2 mm during the periods when the onlays with overhanging margins were placed. However, the distance from the bottom of the probeable sulcus to the apical margin of the restoration remained the same for all sites at all observation times.

**Microbiological parameters**

The predominant cultivable subgingival microflora at some of the sites at the beginning of the experimental period is shown in Table 1. The samples were predominantly facultative (anaerobe; facultative ratio = 0.4) and Gram-positive (67.9%). Gram-positive cocci composed 61.1% of the flora, consisting of predominantly Streptococci, Staphylococci, Peptostreptococci and Peptococci. Gram-positive rods composed 15.8% of the flora at this time. *Actinomyces naeslundii* was the major component of this group (6.6%). Gram-negative cocci represented 7.4% of the flora, whereas Gram-negative rods comprised 10.1%. At baseline the level of black-pigmented *Bacteroides* was 1.0%.

The microbiological findings from the group of patients that received restorations with overhanging subgingival margins (+) / (−) followed by restorations with clinically perfect subgingival margins (+ / (−)) are shown in Table 2. The placement of restorations with overhanging subgingival margins (+) / (−) resulted in an increase from the baseline values in the anaerobe: facultative ratio, indicating an increase in the proportion of anaerobic species. This increase was primarily in Gram-negative anaerobic rods which now represented approximately 24% of the total cultivable flora. The major component of this group of organisms was black-pigmented *Bacteroides* (BPB) which represented 18.2% of the total cultivable flora. Gram-negative facultative rods had decreased from 6.7 to 2.5%. There was also a decrease in the levels of *Streptococcus mitior* from 29.8% at baseline to 16.5%. There were no significant changes in either Gram-positive rods or Gram-negative cocci.

When these restorations were replaced with onlays, the margins of which were subgingival but clinically perfect (+ / (−)), there was a change in the associated subgingival microflora. The anaerobe: facultative ratio decreased to 0.3 and was not statistically different from baseline levels. This increase in facultative organisms appeared to be due to an increase in Gram-positive facultative streptococci (*S. mu-
Table 2. Predominant cultivable subgingival microflora from 10 sites in 5 patients in whom restorations with overhanging subgingival margins (+/−) followed by clinically perfect subgingival margins (+/+−) were placed. Results represent mean percent of total cultivable flora ± standard error of the mean (SEM) for all samples from 4−21 weeks after placement of each restoration

<table>
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<th>(+)/−</th>
<th>+/+−</th>
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<tbody>
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<td>No. of samples</td>
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<td>log CFU/ml</td>
<td>6.2±5.6</td>
<td>6.0±5.5</td>
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<td>Gram + cocci</td>
<td></td>
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<tr>
<td>Anaerobic</td>
<td>42.6±3.7</td>
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<td><em>P. micros</em></td>
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<td>12.5±2.9</td>
<td>21.3±4.1</td>
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<td>Gram + rods</td>
<td>16.5±3.0</td>
<td>26.4±6.4</td>
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<tr>
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<td>17.7±2.7</td>
<td>24.6±3.3</td>
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<td><em>A. viscosus</em></td>
<td>3.3±1.2</td>
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<td><em>A. naeslundii</em></td>
<td>0.1±0.1</td>
<td>1.9±1.3</td>
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<tr>
<td>Faculative</td>
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<td>20.1±3.3</td>
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<td><em>A. s. viscosus</em></td>
<td>5.3±1.8</td>
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<tr>
<td>Gram − cocci</td>
<td></td>
<td></td>
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<tr>
<td>Anaerobic</td>
<td>11.6±2.5</td>
<td>11.3±2.3</td>
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<tr>
<td><em>V. alcocecaens</em></td>
<td>5.7±1.7</td>
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<tr>
<td><em>V. parvula</em></td>
<td>1.4±0.8</td>
<td>2.2±1.3</td>
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<tr>
<td><em>V. parvula</em></td>
<td>3.2±1.2</td>
<td>0.8±0.5</td>
</tr>
<tr>
<td>Faculative</td>
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<td>7.8±2.6</td>
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<tr>
<td><em>Neisseria sp.</em></td>
<td>2.5±0.9</td>
<td>3.7±2.3</td>
</tr>
<tr>
<td>Gram − rods</td>
<td></td>
<td></td>
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<tr>
<td>Anaerobic</td>
<td>26.1±2.9</td>
<td>6.7±2.3</td>
</tr>
<tr>
<td><em>B. nucleatum</em></td>
<td>23.6±2.9</td>
<td>6.2±2.2</td>
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<tr>
<td><em>F. nucleatum</em></td>
<td>18.2±1.9</td>
<td>1.6±0.8</td>
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<tr>
<td><em>F. nucleatum</em></td>
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</tr>
<tr>
<td>Faculative</td>
<td>2.5±1.1</td>
<td>0.5±0.3</td>
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</table>

*Anaerobe: facultative ratio
*Logarithm colony forming units/ml ± SEM
*Black-pigmented Bacteroides
*bDifferent from baseline values (P<0.05)

— Statistically significantly different at P<0.05

tans and *S. sanguis*: 12.5−21.3%) and *Actinomyces naeslundii* (5.3−14.5%). There was a decrease in Gram-negative anaerobic rods (23.6−6.2%) which reflected the major decrease in BPP (18.2−1.6%) to near baseline levels. Also, a small but statistically significant increase in the levels of *F. nucleatum* (0.1−0.7%) was noted at this time. The flora now somewhat resembled that seen at the experimental baseline, except that there were decreased levels of Gram-positive anaerobic cocci, Gram-negative facultative rods, increased levels of *S. mutans* and *S. mitior*, and increased Gram-positive facultative rods when compared to the baseline data.

When restorations with clinically perfect sub-
Table 3. Predominant cultivable subgingival microflora from 10 sites in 4 patients in whom restorations with clinically perfect subgingival margins (−/+) followed by overhanging subgingival margins (−/+ ) were placed. Results represent mean percent of total cultivable flora ± standard error of the mean (SEM) for all samples from 4–21 weeks after placement of each restoration.

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<tr>
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<th>(−)/+</th>
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<tr>
<td>No. of samples</td>
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<td>A/F ratio</td>
<td>0.2±0.1b</td>
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<tr>
<td>log CFU/ml</td>
<td>5.8±5.4</td>
<td>6.5±5.6b</td>
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<tr>
<td>Gram + cocci</td>
<td>60.6±7.0b</td>
<td>34.2±3.7b</td>
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<td>Anaerobic</td>
<td>0.4±0.4b</td>
<td>2.2±0.9b</td>
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<tr>
<td><em>Ps. micros</em></td>
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<td>0</td>
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<tr>
<td><em>Ps. anaerobius</em></td>
<td>0.4±0.4</td>
<td>2.0±0.8b</td>
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<tr>
<td><em>Pep. no. microsp.</em></td>
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<td>0.2±0.2b</td>
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<td>Facultative</td>
<td>60.2±7.0b</td>
<td>32.0±3.8b</td>
</tr>
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<td><em>Staphylococcus</em></td>
<td>15.0±4.0</td>
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<td><em>S. mutans + sanguis</em></td>
<td>23.0±4.9b</td>
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<td><em>S. mitior</em></td>
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<td>Gram + rods</td>
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<td><em>A. viscosus</em></td>
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<td>Gram - cocci</td>
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<td>16.5±3.3b</td>
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<td>Anaerobic</td>
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<td><em>V. alcohescens</em></td>
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<td><em>V. parvula</em></td>
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<td>4.9±1.5</td>
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<td><em>Neisseria</em></td>
<td>1.4±1.1</td>
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<td>Gram - rods</td>
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<td><em>BPB</em></td>
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<td>Facultative</td>
<td>0.1±0.1b</td>
<td>0.9±0.6b</td>
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</table>

*Anaerobe: facultative ratio
Logarithm colony forming units/ml ± SEM
* Black-pigmented* Bacteroides
* Different from baseline values (P<0.05)

Statistically significantly different at P<0.05

Gingival margins (−/+/+) were placed in the second group of patients, only a few minor changes from the baseline levels were observed (Table 3). A small decrease in the anaerobe: facultative ratio (0.4–0.2) reflected a decrease in Gram-positive anaerobic cocci (11.8–0.4%) and an increase in S. mutans and S. sanguis (6.4–23.0%). A decrease in Gram-negative facultative rods (6.7–0.1%) was also observed.

When these restorations were replaced with those with overhanging subgingival margins, the associated microflora changed to a predominantly anaerobic one (anaerobe: facultative ratio = 1.1). This increase was primarily due
to increases in the levels of Gram-negative anaerobic cocci (0.1–9.3%) and rods (7.4–26.3%). The single group of organisms that appeared to increase most were the BPB (3.8–23.2%). However, there were also increases in Veillonella, facultative Gram-negative cocci and A. naeslundii. A decrease in Gram-positive facultative cocci (60.2–32.0%) was noted. The microflora at this time was distinctly different from the baseline values and that associated with the clinically perfect restorations in most of the groups of organisms detected with the exception of the Gram-positive rods. The levels of this group of organisms were not different from those at baseline. There was a significant increase in the total number of organisms recovered from these sites at this time, relative to both baseline and to clinically perfect restorations.

When the results from the sites with overhanging subgingival margins from both groups were compared, only 3 parameters showed statistically significant differences (Table 4). The group of patients that received the onlays with overhanging margins secondly (i.e. −/ (+)) had greater proportions of A. naeslundii, Gram-negative anaerobic cocci, and more colony forming units per ml than the patients who received the onlays with the overhanging margins first.

When the results from the sites with clinically perfect subgingival margins from both groups were compared, 3 groups of organisms differed significantly (Table 5). Patients who received

<table>
<thead>
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<th>Group with higher level</th>
<th>(+)/−</th>
<th>−/(+)</th>
</tr>
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<tbody>
<tr>
<td>log CFU/ml</td>
<td>*</td>
<td></td>
</tr>
<tr>
<td>A. naeslundii</td>
<td>*</td>
<td></td>
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<tr>
<td>Gram − anaerobic cocci</td>
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*Significant difference at P<0.05

Table 5. Statistically significant differences between both groups of patients with clinically perfect subgingival margins

the restorations with clinically perfect subgingival margins first had lower levels of Gram-negative cocci and Gram-negative anaerobic cocci but higher levels of BPB in the subgingival microflora associated with these restorations than did patients who received the perfect onlay margins secondly.

All of the sites examined in each group did not respond to the restorations identically. For example, 1 of the 20 sites with overhanging subgingival margins appeared to show no change in the level of BPB at this site. Likewise, half of the sites with clinically perfect subgingival margins showed no detectable levels of BPB whereas the other half showed increases of varying proportions in the level of BPB. Patients who did demonstrate an increase in this group of organisms as a result of the placement of overhanging margins did so at different rates, i.e. the development of peak levels of BPB occurred at different times in some patients (Fig. 3). Many patients also demonstrated a tendency to decreased levels of BPB after this maximum level had been reached. Each patient tended to show similar response times at both the mesial and the distal surfaces of each onlay. The differing response times were observed primarily when different patients were compared.

In order to determine whether inflammation preceded, occurred simultaneously with, or followed the development of high levels of BPB, a comparison of Gingival Indices with the peak levels of BPB was performed (Fig. 4). The
frequency distribution of GI scores at the site of sampling at the time that the highest level of BPB was detected was compared to the frequency distribution of GI scores at the site of sampling both earlier and later in patients with restorations that had overhanging subgingival margins. 1 month prior to the occurrence of the peak levels of BPB, there was no difference in the frequency distribution of GI scores relative to the scores that occurred simultaneously with peak levels of BPB. Likewise, the frequency distribution 1 month after the peak levels of BPB did not differ from that found at the time of the peak level of BPB. However, 2 months prior to the occurrence of the peak levels of BPB the frequency distribution of GI scores was significantly different ($P<0.05$) from that at the time of the peak level of BPB. Significantly more test sites bled on gentle probing (GI=2) 1 month prior to and at the peak level of BPB than 2 months prior to it. Conversely, more GI=0 or 1 scores were attributed to the test sites 2 months prior to the peak level of BPB.

Of the 220 samples examined in this study, 121 were plated on both ETSA and ETSA plus Kanamycin for comparison of the efficiency of the recovery of BPB. The results of this comparison are shown in Table 6. When the levels of BPB were below or equal to 5%, the recovery was greater on the selective than the non-selective media, whereas when the levels of BPB were above 5%, the recovery was greater on the non-selective media ($P<0.001$). Of the remaining 40 samples examined during this study, the recoveries were identical on both media although most of these samples (94%) contained no detectable BPB. The Spearman Rank Correlation Coefficient for the recoveries on the 2 media was $+0.81$ ($P<0.01$). When the level of BPB in the samples was below 5%, the recovery on the non-selective media was usually 0%. When the level of BPB in the samples was above 5%, the recovery of BPB averaged between 10–50% less on the selective medium than the non-selective medium.

Since only a limited series of biochemical tests was used in this investigation, it was not possible to identify BPB to the species or subspecies level. However, it was possible to separate most of the isolates into saccharolytic and non-saccharolytic strains. It was possible to recover saccharolytic strains from each of the 9 patients several times during the experimental period, but it was only possible to recover non-saccharolytic strains from 6 of the 9 patients at any time during the study. There was no consistent pattern in the recovery of saccharolytic or non-saccharolytic strains from sites in patients who had both types of organisms. The frequency with which these 2 types of BPB were isolated was similar.
Discussion
In the present study, the placement of restorations with overhanging subgingival margins resulted in changes in the associated microflora. The altered flora resembled in some ways that which has been observed in adult chronic periodontitis (Slots 1977, Spiegel et al. 1979, Tanner et al. 1979, White & Mayrand 1981). In those studies, increased proportions of Gram-negative anaerobic rods, in particular BPB, were associated with increased inflammation and loss of periodontal attachment. Longitudinal experimental animal studies of ligation induced periodontitis have also reported an association of these bacteria and loss of periodontal attachment (Slots & Hausmann 1979, Kornman et al. 1981).

For obvious ethical reasons, the current study was not continued long enough to clinically detect loss of periodontal attachment in these human subjects, and it was not possible to establish whether the flora produced as a sequel to the placement of restorations with overhanging margins would have eventually led to periodontitis, or if it would have in fact spontaneously resolved. Longitudinal experimental animal evidence has suggested that in monkeys ligation induced periodontitis may be associated with an initial loss of periodontal attachment that subsequently becomes quiescent (Kornman et al. 1981).

The placement of restorations with clinically perfect subgingival margins resulted in only relatively few changes in the associated subgingival microflora relative to preexperimental findings. In contrast to the sites with overhanging restorations, the flora at the sites with clinically perfect margins was more characteristic of periodontal health (Slots 1977b, Newman et al. 1978, Syed & Loesche 1978b), in that it was composed of predominantly Gram-positive facultative cocci and rods. A minor increase in BPB was observed at these sites, but was too small to be statistically significant (1.0-3.8%). Since all patients in the present study were instructed to refrain from interproximal cleaning of the proximal margins of the experimental restorations, these observed changes may be explained in this light. However, the changes in the sites with overhanging restorations cannot simply be attributed to the absence of oral hygiene, but rather may be due to an altered bacterial ecosystem.

Experimentally or accidentally produced periodontal breakdown has been created in both animals and humans using elastic or silk ligatures (Dalitsch 1939, Caton & Kowalski 1976, Heijl et al. 1976, Lindhe & Ericsson 1978, Kornman et al. 1981). On the other hand, the placement of ligatures, did not apparently, induce bone loss in germ-free rats (Rovin et al. 1966), suggesting that the mechanical component alone is not sufficient to create periodontitis. It is more likely that experimental subgingival ligation placement causes a change in the subjacent microflora that eventually leads to gingival inflammation and loss of periodontal attachment, although exact cause-and-effect relationships have yet to be established. It is possible that restorations with overhanging margins, whose clinical association with periodontitis has been well documented (for review see Leon 1977), act in a similar manner to
ligature placement in favoring the colonization of more periodontopathic organisms in the subgingival flora at that site by creating an altered ecological environment. The similarities in the microbiological findings from the present study and those of ligature models (Slots & Hausmann 1979, Kornman et al. 1981) support this possibility.

It has been suggested that the mechanism for the increased periodontal disease associated with restorations that have overhanging margins is due to the ability of these restorations to retain bacterial plaque (Ramfjord et al. 1966: World Workshop in Periodontics). The results of the present investigation do not support this concept in that it was not possible to demonstrate an increase in the colony forming units/ml from sites where restorations with overhanging margins were placed first (Table 2). Changes in the percentage distribution of organisms, however, were consistently observed, and it is therefore suggested that these restorations disturb the ecological balance in the periodontal pocket and allow a group of disease associated organisms (i.e. Gram-negative anaerobic species) to increase at the expense of health associated organisms (i.e. Gram-positive facultative species). However, it is possible that the inability to detect increases in the number of organisms at some of these sites reflects the insensitivity or saturation of the sampling technique used in this study.

The observation from the current study that patients can respond to the same stimulus (i.e. an overhanging margin of a restoration) at different rates as shown in Fig. 3, suggests that as yet unidentified differences exist between such patients. Subtle differences in the baseline microflora may effect the susceptibility of individual sites. Host factors, such as age Holm-Pedersen et al. 1975, Matsson 1978), hormones of puberty (Sutcliff 1972), pregnancy (Silness & Löe 1964, Kornman & Loesche 1980), immune status (Patters et al. 1976, Lang & Smith 1977, Smith et al. 1978, Østerberg et al. 1983) and nutrition (Waerhaug 1967) are some of the many potential variables that are commonly offered to explain such differences. The role of these factors and others in the current material can only be assessed to a limited degree.

The longitudinal nature of the current study permitted a determination of the onset of "bleeding on gentle probing" relative to the peak detectable levels of BPB (Fig. 2). It was seen that bleeding preceded these peak levels, which suggests that the highly inflamed nature of the gingival tissues could allow the subsequent increase in BPB. Growth requirements for B. melaninogenicus have been reported to be complex and to include analogs of vitamin K and hemin (Gibbons & McDonald 1960). It is possible that nutrients such as these or analogs could be provided by the gingival crevicular fluid or red blood cells associated with severe inflammation. The observation that inflammation, as defined by bleeding on probing, preceded peak levels of BPB and bone loss has been previously reported in longitudinal primate studies (Kornman & Loesche 1980).

One patient developed an unrelated sinus infection during the final phases of this study and was given a 1-week course of systemic tetracycline HCl (250 mg q. i.d. for 7 days). The results from this patient after commencement of administration of the antibiotic were excluded from the analyses reported here. However, it is of interest to note that at that time, this patient had the overhanging restoration in place. He did not develop increased levels of BPB for at least 6 months after the placement of this restoration or 14 weeks after the beginning of the antibiotic therapy. This patient was clearly the last one to develop increased BPB after the placement of the restoration with overhanging margin. This observation agrees with the results of transient systemic tetracycline reported by Listgarten et al. (1978).

In conclusion, this study has demonstrated that the placement of restorations with overhanging subgingival margins resulted in a change in the composition of the subgingival microflora at that site to one that may be
associated with periodontitis, rather than by harboring increased plaque masses. The obvious potential for iatrogenic initiation of periodontal disease process is hereby documented.

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Zusammenfassung

Klinische und mikrobiologische Folgen subgingivaler Restorationen mit Überschüssen oder klinisch einwandfreien Randschluss


9 Studenten der Zahnmedizin mit gepflegten Gebissen und klinisch gesunder Gingiva (GI<0,1) stellten sich für diese Studie freiwillig zur Verfügung. 5 gegossene MOD-Onlays mit approximal etwa 1 mm überstehenden Rändern wurden 19–27 Wochen lang in Unterkiefermolen eingesetzt. Darauf wurden dann diese Onlays in einem Kreuzversuch durch 5 ähnliche Onlays, dieses Mal mit klinisch perfektem Randschluss, ersetzt. Bei den übrigen Patienten wurden zweimal je 5 Onlays in umgekehrter Reihenfolge eingesetzt.


Résumé

Effets cliniques et microbiologiques des obturations sousgingivales avec ou sans débordements cliniques

L'association étroite entre obstructions avec débordements et parodontite chronique est connue depuis longtemps. Cependant, les mécanismes selon lesquels les débordements influencent la pathogenèse de la maladie parodontale sont encore inconnus. Il est généralement admis que les débordements contribuent au développement de la maladie en retenant la plaque bactérienne. Le but de cette étude a été de déterminer si la pose de restaurations avec débordements sousgingivaux provoque une modification de la flore sousgingivale.

Neuf étudiants en science dentaire ayant les dents propres et une gencive cliniquement saine (GI<0,1) ont accepté de participer à cette étude. Cinq onlays MOD en or avec débordements sousgingivaux de 0,5 à 1 mm ont été placés dans des molaires inférieures de 19 à 27 semaines. Dans cette étude croisée, ils ont été remplacés par 5 onlays temporaires semblables à bords marginaux cliniquement parfaits. Cinq autres onlays ont été placés dans l'ordre inverse chez les autres patients.
Avant l’insertion, puis toutes les 2 à 3 semaines, des échantillons microbiens sousongivaux ont été prélevés en insérant durant 30 sec une pointe de papier stérile dans le sillon gingival sous-jacent à l’obturateur. La flore cultivable prédominante a été déterminée en utilisant des techniques de culture en anaérobie continue. Après insertion des obturations avec débordements, la flore sousongivale découverte ressemblait fortement à la flore observée dans les cas de parodontite chronique. Une augmentation des proportions de bactéries anaérobies Gram négatif, de Bacteroides à pigmentation noire et d’anaérobies vis-à-vis des facultatifs a été constatée. Après l’insertion de restaurations cliniquement parfaitement adaptées, la microflora caractérisant une génèse saine ou un début de gingivite était observable.

La proportion de Bacteroides à pigmentation noire était très faible (1,6-3,8%). Ces modifications de microflora sousongivale étaient évidentes, que les obturations avec débordements aient été placées avant ou après les onlays bien adaptées. Une augmentation de l’indice gingival au niveau des sites avec débordements gingivaux a été enregistrée cliniquement. Un saignement au sondage léger précédait toujours le niveau maximum de Bacteroides à pigmentation noire. Aucune perte d’attache n’a été enregistrée. Les changements de la microflora sousongivale intervenus après l’insertion d’obturations avec débordements révèlent l’existence possible d’un mécanisme d’initiation de la maladie parodontale associé à des facteurs iatrogènes.

References


Address:
Dr. N. P. Lang
Professor & Chairman
School of Dental Medicine
University of Berne
Freiburgstrasse 7
CH-3010 Berne
Switzerland
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