MICROBIOLOGY OF THE DENTAL IMPLANT

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Abstract—Longitudinal studies have shown that successful implants are colonized by a predominantly Gram-positive, facultative flora, which is established shortly after implantation. Repeated microbiological sampling in patients with clinically stable implants showed no significant shifts in the composition of this flora over five years. In patients with bone loss and pocket formation around implants, however, a significantly different flora was found: Gram-negative anaerobic bacteria, particularly fusobacteria, spirochetes, and black-pigmenting organisms such as *Prevotella intermedia* were often present in high proportions. Antimicrobial treatment with agents specifically active against anaerobes could halt progression of peri-implant infections in such cases. Although there may be non-microbial primary causes for implant failure, these studies show that Gram-negative anaerobes may play a rôle in peri-implant infections, and that their elimination leads to improvement of the clinical condition.

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Terms such as "failing implant", "early" and "late failure", and "peri-implantitis" have been proposed for pathological states of osseointegrated implants and their surrounding tissues. There is no terminology which has generally been agreed upon: Definitions and etiologies of these conditions are still a matter of dispute. Essential information on the biology of peri-implant tissues is still lacking. Diagnosis and treatment of the different conditions are based primarily on mechanical contemplations; force distributions, load and physical stress, properties of materials, implant design, the macroscopic arrangement and appearance of tissues, and surgical skills are the traditionally considered factors. There is no doubt that mechanical problems, *e.g.*, the fracture of an implant, can give rise to biological problems such as a secondary bacterial infection in the peri-implant tissues. However, there is also the possibility that bacterial factors *initiate* tissue changes without an underlying mechanical cause.

Tissue integration depends on eukaryotic cell compatibility and adhesion to the implant surface. Fundamental physical and molecular principles of cell attachment and adhesion apply to microbial colonization and host-tissue integration (Gristina, 1987). Thus, it is conceivable that implant materials, which are chosen because of their "friendliness" to tissue cells, offer particularly favorable grounds for bacterial adhesion. Pocket formation and loss of bone in the peri-implant area indicate detachment of host tissue cells and availability of "cell-friendly" surfaces for microbial colonization. Adhesion-mediated infections developing on permanently or temporarily implanted biomaterials, such as heart valves, vascular prostheses, or intravascular catheters, respond poorly to antimicrobial treatment and often require that the device be removed. Dental implants, however, can be designed in such a way that the surfaces on which bacterial colonization occurs may be reached from the exterior. Thus, in contrast to internal implants, there is a possibility for control of bacterial colonization on exposed surfaces and a potential for treatment of infectious conditions of peri-implant tissues.

INITIAL COLONIZATION AND BACTERIOLOGY OF STABLE IMPLANTS

Microbial adhesion and aggregation have been studied on different substrata, *in vivo* and *in vitro* (for review, see Mergenhagen and Rosan, 1985). Few publications, however, have addressed the interactions between bacteria and oral implant materials such as titanium (Nakazato *et al.*, 1986; Fujioka-Hirai *et al.*, 1987; Joshi and Eley, 1988; Wolinsky *et al.*, 1989). Likewise, general growth and maturation patterns of bacterial plaque have been studied by light and electron microscopy and bacterial culture (for review, see Theilade and Theilade, 1985), but, so far, only two investigations have focused on the development of plaque on newly inserted implants (Nakou *et al.*, 1987; Mombelli *et al.*, 1988). In

edentulous patients, the flora developing on successfully integrating one-stage transmucosal titanium implants was found to be very similar to the mucosal flora on the adjacent alveolar ridge (Mombelli *et al.*, 1988). This flora was established shortly after the installation of the implant. Over 85% of the micro-organisms were identified, in the microscope, as coccoid cells, and over 80% of the cultivated bacteria were Gram-positive facultative cocci. During the first six months after insertion, no significant longitudinal changes were noted in these proportions. Spirochetes were never detected; Fusobacteria and black-pigmenting Gram-negative anaerobes were found infrequently. The microflora associated with stable osseointegrated implants serving successfully as abutments for overdentures was investigated in 18 edentulous patients, two years after implantation (Mombelli and Mericske-Stern, 1990). Over 50% of the organisms cultured in this study were facultatively anaerobic cocci, and 17% were facultatively anaerobic rods, while Gram-negative anaerobic rods accounted for only 7%. *Fusobacterium* sp. and *Prevotella intermedia* were both found in 9% of the samples. *Porphyromonas gingivalis* and spirochetes were not found. Repeated microbiological and clinical data were collected in nine patients during the third, the fourth, and the fifth years after implantation. No significant time trends were noted. Separate samples taken within the same patient from different sites showed a similar composition of the microflora. The data of this study are in agreement with results reported by Lekholm *et al.* (1986), Apse *et al.* (1989), and Bower *et al.* (1989) from successful Brånemark-type implants. This indicates that the normal microbial flora of ITI and of Brånemark fixtures are not substantially different from each other. Intra-individual topographical variation of the bacterial flora seems to be more pronounced in partially edentulous patients than in edentates. The microbiota of remaining teeth is probably the primary source of putative pathogens to colonize adjacent implants. Apse *et al.* (1989) found higher percentages of black-pigmenting Gram-negative anaerobes and "wet spreaders" (*Capnocytophaga*) on implants of partially edentulous patients than in edentulous patients. This means that the microbial status of remaining teeth influences the fate of newly incorporated implants.

BACTERIOLOGY OF THE FAILING IMPLANT

The first data on the microbiota associated with unsuccessful implants were presented by Rams and co-workers (Rams and Link, 1983; Rams *et al.*, 1984). These investigators collected samples from 17 implants of various designs (ramus frame assembly, blade implants, carbon and ceramic posts) and analyzed them by phase-contrast and in part by transmission electron microscopy. Among these implants were four failures showing advanced pocket formation. While the samples from the successful implants yielded a predominantly coccoid microbiota, the failures showed significantly elevated levels of spirochetes.

In 1987, we reported data from seven cases with unsuccessful hollow cylinder titanium implants (ITI Type F). Implant sites with pocket formation ≥ 6 mm, suppuration, and radiographic evidence for bone loss were compared with implant sites with no signs of infection in the same individuals and in patients with only successful implants (Mombelli *et al.*, 1987). In the unsuccessful sites, a substantially different distribution of bacterial morphotypes was found in comparison with healthy sites in both the same patients and in the successful patients. While spirochetes were not found in any of the successful cases and in only two samples of the healthy sites of the unsuccessful patients, all but one failing site in these patients harbored spirochetes. Failing sites harbored significantly elevated numbers of motile rods and fusiform bacteria. The total count of colony-forming units, determined by anaerobic culture, was significantly higher in the failing sites than in the healthy sites. In the samples of the failing sites, 41% of the cultivated organisms were Gram-negative anaerobic rods. This number was significantly higher than that of the successful sites, where the group of facultative cocci predominated. Failing sites harbored significantly elevated numbers of *P. intermedia* and *Fusobacterium* sp. *P.gingivalis* was not found in any of the samples investigated in this study, neither culturally nor by indirect immunofluorescence.

In the earlier-mentioned prospective study on the microbiology of newly inserted implants (Mombelli *et al.*, 1988), the development of a peri-implant infection could be documented clinically and microbiologically in one patient. Clinical signs of infection, including pocket development and pus formation, emerged 120 days after implantation. Samples taken before day 120 repeatedly yielded $>10^6$ CFU/mL of anaerobically cultivable bacteria. *Fusobacterium* was detected 42 days after implantation, with increasing numbers in the subsequent samples. In this particular site, from day 21 on, a steady decrease of coccoid cells and a simultaneous increase of rods were observed. *Actinomyces odontolyticus* was first detected after day 21, and fusobacteria were detected after day 42; at day 120, small spirochetes were found for the first time, pus formation was noted clinically, and a pocket probing depth of 6 mm was recorded. None of the other sites showed pus formation or pocket depths of over 3 mm.

These studies suggested that what was chosen to be called "peri-implantitis" was a site-specific disease process with micro-organisms associated in patterns known from chronic periodontitis of natural teeth. More recent studies have confirmed and extended these earlier findings. Sanz *et al.* (1990) made comparisons between healthy and diseased and between implant and control sites in patients wearing endosteal sapphire ceramic implants. Diseased sites harbored a microbiota with a large segment of Gram-negative anaerobic rods, including black-pigmented organisms and surface translocators. Healthy sites in the same patients yielded small amounts of bacteria which were mainly facultative and Gram-positive. Becker *et al.* (1990) used commercially available DNA probes to test for the presence of the three periodontal marker organisms—*Actinobacillus actinomycetemcomitans*, *P. intermedia*, and *P. gingivalis*—in 36 failing implant sites of 13 patients with different types of implants (blade-type, subperiosteal, and root-form-type). They reported high levels of *P. gingivalis* in one patient with a failing blade implant and high levels of *P. intermedia* in two additional patients with unsuccessful blades. In the other cases, some weak signals were obtained for one or

several of the three tested organisms. Alcoforado *et al.* (1991) examined the subgingival microflora of 18 failing osseointegrated implants of various designs (Brånemark, Core-Vent, Integral, Screw-Vent, and TPS) for potentially pathogenic oral bacteria. *Peptostreptococcus micros* was recovered from 6 failing implants, *Wolinella recta* from 6, *Fusobacterium* sp. from 5, and *P. intermedia* from 4. The authors reported significant numbers of enteric rods or pseudomonads in the microflora of 5 failing implants. *A. actinomycetemcomitans*, non-pigmented *Bacteroides* species, *Capnocytophaga* sp., and staphylococci were also detected in some implant failures. In addition, 5 cases were positive for *Candida albicans*. Based on these findings, the authors suggested that antimicrobial therapies for implant failures should not be implemented without a prior comprehensive microbiological analysis. Rams *et al.* (1990) also suggested that some peri-implant lesions may be dominated by a specific microbiota. A limited number of their patients demonstrated particularly high counts of *Staphylococcus* sp., implying that these organisms are possible pathogens under certain conditions. Rosenberg *et al.* (1991) compared microbiological features of implants suspected of failing from infection or trauma. An infectious etiology was assumed if there was bleeding, suppuration, pain, high plaque and gingival indices, and granulomatous tissue upon surgical removal. Implants were suspected of failing because of traumatic reasons in the absence of these signs. Distinct bacteriologic profiles emerged in the two groups: Implants suspected of failing because of trauma demonstrated microbiological features similar to those of periodontally healthy teeth, while many suspected periodontal pathogens were found if clinical signs suggested infection.

The rôle of micro-organisms in the development of peri-implant pathology has also been investigated in animals. Differences in the presence of putative periodontal pathogens on titanium implants and teeth were determined in beagle dogs in experimental gingivitis and in a ligature-induced peri-implantitis/periodontitis situation (Leonhardt *et al.*, 1992). Similar colonization patterns were seen on implants and teeth. Significant microbiological differences between implants and teeth were found neither with gingivitis nor in ligature-induced peri-implantitis/periodontitis.

To summarize the data available today: There appears to be a very clear microbiological distinction between clinically stable implants and implants with peri-implant pathology. Undoubtedly, Gram-negative anaerobic bacteria are involved in pathological developments in the peri-implant region. These organisms are also suspected pathogens in periodontitis and orofacial infections (for review, see Crawford, 1984; Van Steenbergen *et al.*, 1991). Spirochetes can be perceived as indicators for a flora with anaerobic characteristics. They are evidently not a feature of the physiological flora of successful implants.

TREATMENT OF PERI-IMPLANT INFECTIONS

Since anaerobic bacteria are associated with peri-implant pathology, the question arises of whether clinical conditions can be improved by suppression or elimination of these organisms from affected sites. An antimicrobial therapeutic approach was tested in a study involving nine patients with marked loss of bone and pocket probing depths ≥ 5 mm around implants (Mombelli and Lang, 1992). These patients were selected based on microbiological screening; only individuals with anaerobic cultivable counts ≥ 10^6 CFU/mL, including ≥ 20% Gram-negative anaerobic bacteria, in diseased sites, were considered. The treatment included mechanical debridement, irrigation of all peri-implant pockets > 3 mm with 0.5% chlorhexidine, and systemic antimicrobial therapy with an agent specifically effective against strict anaerobes (ornidazole, 1000 mg for ten consecutive days). After therapy, bleeding scores decreased immediately. Over a one-year observation period, they remained significantly lower than before treatment. A significant gradual reduction in mean probing depths was detected over this one-year period. Only one case showed no improvement of local probing depth. Microbiological parameters indicated an instantaneous quantitative and qualitative change following treatment. Subsequently, several of these parameters tended to shift back toward pre-treatment values. In the second half of the observation period, however, this tendency was reversed, and levels significantly different from baseline were eventually established. Most affected sites harbored large numbers of *Fusobacterium*, and many were *P. intermedia*-positive before treatment. Although these organisms could no longer be detected upon completion of therapy, all patients showed *Fusobacterium* again at some time during the following year, and only three individuals remained *P. intermedia*-negative throughout this period. Similar observations could be made with the other organisms under investigation. Since no organism was consistently present before and uniformly absent after therapy, the clinical success of the treatment cannot be attributed to the elimination of a single pathogen. Rather, it seems that a temporary relief from a massive anaerobic bacterial load allowed local host-response mechanisms to recover so that they could cope with re-emerging opportunistic pathogens. While the immediate effects of chemotherapy may fade away with time, gradual reduction in pocket depth, leading to a rise in oxygen partial pressure (pO_2) and redox potential (E_h) (Loesche *et al.*, 1983), together with a reduced availability of blood products, may exert a long-term ecological pressure to stabilize a less pathogenic subgingival microbiota. Because of the possibility that micro-organisms other than strict anaerobes may be involved in some cases of peri-implant pathology (Rams *et al.*, 1990; Alcoforado *et al.*, 1991), the choice of an antimicrobial drug should be based on a comprehensive microbiological analysis.

RE-CALL

From periodontal research, it is known that the development of a complex microbiota with a large segment of Gram-negative, anaerobic bacteria depends on specific environmental conditions, which are provided in part by facultative initial colonizers (Van Houte, 1982; Socransky *et al.*, 1988; Ter Steeg and Van der Hoeven, 1990). It is also known that the development of a flora with a large proportion of Gram-negative anaerobes can be prevented by oral hygiene (Kornman, 1982; Wolff *et al.*, 1988). Flemmig *et al.* (1989) reported

about the possibility of maintenance of an implant-associated microflora without substantial proportions of suspected periodontal pathogens by recalling patients at three-month intervals. Clinical and microbiological features of Brånemark implants submitted to three-month recalls on a long-term basis (Ericsson *et al.*, 1986; Lekholm *et al.*, 1986) seem to confirm that this form of maintenance is effective. However, it cannot be excluded that three-month intervals mean overtreatment in many cases. In our longitudinal study on the microflora associated with stable osseointegrated implants in edentates (Mombelli and Mericske-Stern, 1990), the data collected up to five years after implantation were analyzed for evaluation of shifts in the composition of the microflora over time and the impact of re-call intervals and selected clinical parameters on microbiological features. In this study, patients on longer re-call intervals showed no significant difference in the composition of the microflora, when compared with patients on short re-call intervals. Significant inter-individual differences were found for proportions of *P. melaninogenica* and *A. odontolyticus*. This means that some individuals were less susceptible to colonization and growth of certain dental plaque micro-organisms than others. No general time trends were observed during the longitudinal monitoring phase in this study. Thus, the microbiological situation could be maintained fairly stable after a successfully completed initial healing phase.

CONCLUDING REMARKS

A review of the research performed so far leads to the conclusion that micro-organisms are involved in peri-implant pathology. There is evidence for an association of certain plaque constituents with tissue breakdown around implants. Antimicrobial treatment aimed at the reduction of anaerobic bacteria leads to improvement of clinical parameters. Nevertheless, it is premature to claim proof for a direct cause-effect relationship between specific pathogens and tissue destruction. It has been repeatedly emphasized that numerous factors may influence the health conditions of peri-implant tissues, particularly in the initial healing phase. Bacterial infection may occur as a secondary phenomenon if osseointegration is not achieved or is lost due to non-microbial reasons. It has been speculated that distinct types of failure may be differentiated based on the presence or absence of the "typical" peri-implantitis flora (Rosenberg *et al.*, 1991), and that in some patients, specific infections may be the reason for implant failure (Rams *et al.*, 1990). These possibilities illustrate that microbiological tests may be valuable tools for the differential diagnosis of problems occurring with osseointegrated implants. Longitudinal, prospective studies are needed for determination of whether microbiological parameters can indicate a risk of peri-implant tissue destruction or allow early disease states to be detected.

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