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ISBN 91-628-4761-9
ABSTRACT

Screw-shaped titanium implants are today routinely used in the substitution of lost teeth. In this thesis some of the biological factors related to the long-term survival and maintenance of dental implants were studied.

The first aim of these studies was to evaluate the neutrophil activation around teeth and dental implants (Papers I & II). Secondly we wanted to evaluate the clinical, radiographic and microbiological status of implants after long-term function in partly edentulous patients (Paper III). The long-term treatment outcome of implant treatment in fully edentulous was also compared to that in partially edentulous patients (Papers III & IV). The third and final purpose of these studies was to investigate the influence of smoking, a history of periodontitis and a specific host-response pattern on the occurrence of late fixture loss and marginal bone loss around dental implants (Papers IV & V).

Papers I and II showed that the inflammation around implants in partly edentulous patients induced a stronger neutrophil reaction than did the inflammation around implants in edentulous patients albeit similar clinical appearance and absence of significant differences in the microbiota.

Paper III showed that marginal bone loss around implants after ten years of function in partly edentulous patients was limited and comparable to that in edentulous jaws. There was no major difference in the microbiota colonising teeth and implants.

In Paper IV, 143 consecutively treated patients were evaluated retrospectively after five years of function of implants. Only 2% of the fixtures were lost during function. No correlation was found between bone loss around implants and teeth. A history of periodontitis did not influence marginal bone loss around implants. Smoking was not found to correlate with marginal bone loss at neither implants nor teeth.

In Paper V a site-specific inflammatory reaction around implants with peri-implantitis rather than a patient-associated host-response was found in patients with failing implants. Patients with peri-implantitis harboured high levels of periodontal pathogens, *Actinobacillus actinomycetemcomitans*, *Porphyromonas gingivalis*, *Prevotella intermedia*, *Bacteroides forsythus* and *Treponema denticola*.

In conclusion

Although the inflammation around implants in partly edentulous patients induces a stronger neutrophil reaction, than that in the edentulous ones, the marginal bone loss after long-term function in the former is limited and similar to that in edentulous jaws. In patients treated for periodontal disease stable periodontal and peri-implant conditions can be maintained during long term function.

The periodontally-associated microbiota constitute a risk for future development of peri-implantitis. In patients with a history of periodontitis i.e. individuals who previously have shown a tissue-destructive inflammatory response, this risk is more pronounced.

Key words: Dental implants, long-term maintenance, microbiota, inflammation, periodontitis, peri-implantitis, smoking, crevicular fluid.
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PREFACE

This thesis is based on the following papers, which are referred to in the text by their Roman numerals:


V. Hultin M, Gustafsson A, Hallström H, Johansson L-Å, Eklundt A, Klinge B. Microbiological findings and host response around failing implants. Submitted

Papers I and III have been reproduced with the kind permission of Munksgaard, Denmark. Paper II has been included with the permission of the American Academy of Periodontology. BC Decker has kindly given permission to reproduce Paper IV.
INTRODUCTION

BACKGROUND

History

The first evidence of the use of implants dates back to 600 A.D. in the Mayan population where pieces of shell implanted into extraction sockets of lost mandibular teeth have been found (Ring 1985). Although titanium was found to bind to living tissues and bone in animal experiments in the early 1950s (Leventhal 1951), it was not until the 1980s that the concept of bone-anchored oral implants was introduced in the dental community. Osseointegration - i.e., the establishment and maintenance of a direct bone-to-implant anchorage arose from the experimental and clinical work by P.-I. Brånemark and collaborators during the 1960s (Brånemark et al. 1969, Brånemark et al. 1983). In 1977, they described their findings in the first long-term clinical report of tissue-integrated prostheses in completely edentulous mandibles (Brånemark et al. 1977). Characterisation of the direct functional and structural connection between living bone and the surface of a load-bearing implant was confirmed by morphological studies (Schroeder et al. 1981, Albrektsson et al. 1981, Albrektsson et al. 1985, Albrektsson et al. 1986).

Since then, favourable long-term results of tissue-integrated prostheses have been found in partly and completely edentulous cases (Adell et al. 1981, Adell et al. 1990, Albrektsson et al. 1988, Jemt & Lekholm 1993, Nevin & Langer 1993, Lindqvist et al. 1996, Buser et al. 1997). Screw-shaped titanium implants are now routinely used as substitutes for lost teeth. Predictable and comparable results with the commonest and best-documented Brånemark System® (Nobel Biocare, Göteborg, Sweden) and other implant systems - i.e., Astra Tech® (Astra Tech, Södertälje, Sweden), ITI® (Strauman Institute AG, Waldenburg, Switzerland) - have been reported (Arvidsson et al. 1998, Bhenke et al. 2000).

Although rare, failures occur during the initial healing period and after osseointegration. It is not until recently that the aetiology and aetopathogenesis of biological implant failures have been extensively reviewed in the literature (Esposito et al. 1998 I and II). Using a meta-analytic approach comprising a sample of 2812 Brånemark implants, including all clinical indications except bone grafts, they found that biologically related failures occurred in 7.7% of installed implants. On the basis of 16935 implants, early failures accounted for 3.6% - i.e. - the percentage of implants in which osseointegration never developed (before loading). Late failures - i.e. failure to maintain osseointegration (after loading) - were noted in 4.2% of implants over a 5-year period. Factors such as surgical trauma, impaired healing ability, bone volume, jaw bone quality and the positioning of implants in the maxilla compared to the mandible affect the occurrence of early implant failures (Friberg et al. 1991). Excessive occlusal load and infection are possible aetiological factors in consecutively retrieved late failures of Brånemark implants (Esposito et al. 1997, 2000).

Peri-implantitis, defined as an inflammatory reaction with loss of supporting bone in the tissues surrounding a functioning implant (Albrektsson & Isidor 1994), has been found in clinical studies (Sanz et al. 1991, Rosenberg et al. 1991, Åstrand et al. 2000). The average prevalence of fixture losses after the first year of loading attributable to peri-implantitis accounted for 10% with the Brånemark system and 50% of losses with the ITI system (Esposito et al 1998 I). A site-specific infection comparable to chronic adult periodontitis, possibly related to implant design and surface characteristics, may have caused the difference in prevalence of peri-implantitis with the various implant systems.
Implant loss has been found to cluster in a small subset of patients (Weyant & Burt 1993, Weyant 1994), indicating the existence of a high-risk group for implant failure. In a study of partly edentulous patients, van Stenberghe et al. (1993) found a higher number of late fixture losses in those with larger amounts of plaque accumulation. Prospective longitudinal data show that the incidence and prevalence of radiographic bone loss vary between patients (Stenberghe et al. 1993, Bheneke et al. 1997, Bheneke et al. 2000, Ellegård et al. 97a, Ellegård et al. 1997b). In view of the chronic nature of periodontal disease the knowledge of long-term results of peri-implant tissues is limited. At present, insufficient data are available as to which factors such as inflammation, a history of periodontitis, the remaining natural dentition, microbiota and smoking affect the long-term results of dental implants.

**Histological characterisation of the soft tissue around implants**

The soft tissue surrounding healthy osseointegrated dental implants share anatomical and functional features with the gingiva around teeth. The microstructure has been described in dog models and human tissues (Arvidsson et al. 1990, Fartash et al. 1990, Berglundh et al. 1991, Buser et al. 1992, Listgarten et al. 1992, Abrahamsson et al. 1996, Moon et al. 1999). The outer surface of the peri-implant mucosa is lined by a stratified keratinised oral epithelium continuous with a junctional epithelium attached to the titanium surface by a basal lamina and hemidesmosomes (Hansson et al. 1983, Gould et al. 1984, McKinney et al. 1985, Arvidsson et al. 1996). The 2mm long non-keratinized junctional epithelium is in the apical portion only a few cell layers thick, separated from the alveolar bone by 1-2mm of collagen-rich connective tissue. This 3–4mm “biological barrier”, formed irrespective of the original mucosal thickness, protects the zone of osseointegration from factors released from plaque and the oral cavity (Berglundh et al. 1996).

Unlike the gingiva around teeth, the connective tissue compartment between the junctional epithelium and the alveolar bone consists of a scar like connective tissue almost devoid of vascular structures, greater amounts of collagen and fewer fibroblasts (Berglundh et al. 1991,1994). However, more recently the same group examined a 40-μm-wide zone of connective tissue immediately lateral to the implant surface and found that it had many fibroblasts with a relatively low proportion of collagen (Moon et al. 1999). This may indicate that the fibroblast-rich barrier next to the titanium surface has a high cell turn-over and that fibroblasts play an important role in establishing and maintaining the mucosal seal.

The inflammatory infiltrate in peri-implant tissue and the response to plaque accumulation have been described in animal models (Berglundh et al. 1992, Eriksson et al. 1992, Abrahamsson et al. 1998) and humans (Seymor et al. 1989, Arvidsson et al. 1996). As in gingivitis around natural teeth, an inflammatory infiltrate forms in the connective tissue, in response to the microbial colonisation of the titanium surface (Berglundh 1992, Pontoriero et al. 1994). The infiltrate represents the local host-response to bacterial accumulation and proliferates in an apical direction when the time for plaque accumulation is prolonged (Eriksson et al. 1992). The peri-implant mucosa is similar to the gingiva around teeth as regards of function and immunology (Seymore et al. 1989, Tonetti et al. 1993, Tonetti et al. 1994, Tonetti et al. 1995, Romanos et al. 1996, Liljenberg et al. 1997). An inflammatory cell infiltrate of equal size and composition has been found in clinically healthy tissues of gingiva and peri-implant mucosa (Seymore et al. 1989, Liljenberg et al. 1997). Immunohistochemical and immunological analysis show that the inflammatory infiltrate consists of neutrophils,
lymphocytes, macrophages and a few plasma cells. Intraepithelial antigen-presenting cells and adhesion molecules, such as ICAM-1 are expressed in epithelia adjacent to implants in a similar fashion as around teeth (Tonetti et al. 1993). The distribution of inflammatory cell phenotypes in healthy gingiva and peri-implant keratinised mucosa is also similar (Tonetti et al. 1995, MacKenzie et al. 1995). Functional adaptation of the junctional epithelium occurs although its origin differs from that around the teeth (Schmid et al. 1992).

**Ligature induced peri-implantitis**

Experimental models with dogs and monkeys have been used to study the ability of the peri-implant mucosa to handle plaque-associated lesions. These studies have shown that peri-implantitis lesions can be induced by the insertion of submarginal ligatures and plaque accumulation. The inflammatory lesion after plaque accumulation involves not only the supracrestive mucosa, but also the alveolar bone (Lindhe et al. 1992, Lang et al. 1993, Schou et al. 1993). Resolution after ligature induced-lesions shows exposure of the threaded surface of the fixture and occasional loss of implants (Marinello et al 1995). In animal experiments Isidor (1997) found that ligature-induced infection and biomechanical overload lead to clinical and histopathological changes in the peri-implants tissues. He concluded that excessive occlusal load and marginal infections can result in marginal bone loss. Six of the 8 excessively-loaded implants became mobile, but none of the fixtures subjected to ligature-induced peri-implantitis lost osseointegration.

**Periodontitis a cause of tooth loss**

Epidemiological studies show that although the incidence of periodontitis increases with age, only a limited number of persons develop the more severe forms (Brown et al. 1990, Hugosson et al. 1992). Several studies report that, 5-10% of the adult population have severe disease, which is unaffected by oral hygiene habits and the prevalence is similar in various parts of the world (Baelum et al. 1986, Yoneyama et al. 1988, Baelum et al. 1988). The number of persons developing periodontitis seems to be consistent over time (Hugosson et al. 1998).

The relative contribution of periodontitis as a reason for tooth extractions has been studied in various populations (Klock & Haugejorden 1991, Klock & Haugejorden 1993, Reich & Hiller 1993, Corbet & Davis 1991, Heft & Gilbert 1991). The role of periodontitis on total extractions and the risk factors of tooth loss have also been evaluated (Eklund & Burt 1994, Krall et al. 1994, Hunt et al. 1995). These studies show that caries is the main reason for tooth extractions up to 40 years of age. In older age groups tooth loss is due equally to periodontitis and caries. In general, periodontitis accounts for about 30-35% and caries and caries-related reasons for 50% of tooth extractions above the age of 40 years. The main risk factors for tooth loss include age, smoking, socio-economic behavioural traits and periodontitis scores. It therefore seems reasonable to assume that in partly edentulous patients 30-40 % of those given dental implants has lost their teeth due to periodontitis. In entirely edentulous patients caries and caries-related problems are probably the underlying cause to a greater extent. However, since the number of such cases has declined over the past 20 years
by about 50% and those with severe periodontal disease seem to remain the same, those who have lost their teeth due to periodontitis may comprise a greater proportion of the edentulous cases than previously (Hugosson et al. 1995, Hugosson et al.1998)

FACTORS OF IMPORTANCE FOR PERI-IMPLANT TISSUE REACTIONS

Microbiology of the peri-implant sulcus

The transmucosal abutment of osseointegrated dental implants serves as a surface for bacterial colonisation of microbial biofilms. Like the gingival crevice around the natural tooth, the peri-implant mucosa covering the alveolar bone is closely adapted to the osseointegrated implant and forms a sulcus coronal to the supporting bone. The opportunity for microbial colonisation and thus provoking an inflammatory reaction, possibly leading to tissue destruction, might be analogue with the key events in the pathogenesis of periodontitis. According to Esposito et al. (1988 II) biological failures can be divided into early / late and non-infectious (overload) / infectious (peri-implantitis).

In partly edentulous, the developing microbiota around implants closely resembles the microflora of naturally remaining teeth (Leonhardt et al. 1993, Mombelli et al. 1995). A history of periodontitis- i.e., individuals susceptible to periodontal disease and the presence of putative periodontal pathogens- are factors that can influence the maintenance and long-term prognosis of peri-implant tissues in the partly edentulous. Quirynen & Listgarten (1996) using phase contrast microscopy, examined partly edentulous subjects and evaluated the impact of periodontitis around remaining teeth and of probing depth around the implants on the composition of the peri-implant subgingival flora. They found that the subgingival microflora around implants harboured more spirochetes and motile rods when there were teeth present in the same jaw. The patients were deemed healthy, or as having chronic or refractory periodontitis. Samples from deep peri-implant pockets (≥4mm) in patients with chronic or refractory periodontitis showed significantly higher proportions of spirochetes and motile rods than those with comparable probing pocket depth in periodontally healthy patients.

Papaioannou et al. (1996) using phase contrast microscopy and DNA probes, determined the prevalence of putative periodontal pathogens in partly edentulous and edentulous patients with a history of periodontal disease. Their microbiological profiles were similar around teeth and dental implants of equal pocket depth, which confirmed the hypothesis that pockets around teeth can act as a reservoir for putative periodontal pathogens. This finding has been confirmed by several clinical studies of partly edentulous patients (Apse et al. 1989, Mombelli et al. 1995, Gouvoussis et al. 1997, van Winkelhof et al. 2000). As early as one month after implantation, putative periodontal pathogens can be detected around the implants of partly edentulous patients (Leonhardt et al. 1993).

Implant failures due to infection are characterised by a complex peri-implant microbiota resembling that of adult periodontitis (Rams et al. 1984, Becker et al 1990, Augthun et al. 1997). In edentulous subjects A. actinomycetemcomitans and P. gingivalis are not as frequently associated with peri-implant infection as in dentate subjects (Mombelli et al. 1987). Danser et al. (1994, 1995) reported that after full-mouth extraction in patients with severe periodontitis, they could no longer detect the latter bacteria on the mucosal surface of edentulous patients which shows that a shift in the microflora had occurred after total
extraction. *A. actinomycetemcomitans* or *P. gingivalis* could not be isolated at the peri-implant pockets in these patients after insertion of implants (Danser et al. 1997).

Apart from dark-pigmented Gram-negative anaerobic rods, other bacterial species that associated with peri-implant infection include *B. forsythus, F. nucleatum, Campylobacter, P. micros* and *S. intermedius* (Tanner et al. 1997). Other organisms not primarily associated with periodontitis, such as *Staphylococcus* spp, enterics and *Candida* spp have also been found in peri-implant infections (Slots & Rams 1991, Leonhardt et al. 1999). The longitudinal data available on the microbial colonisation of implants in partly edentulous persons with a history of periodontal disease have shown no association between periodontal pathogens around teeth and implants with loss of attachment during 36 months function of implants (Leonhardt 1993, Sbordone et al. 1999).

To ensure maintenance and long-term stability of osseointegrated dental implants, it is essential to study the relation between microbial provocation and the inflammatory reaction. The inflammation caused by the microbiota probably varies between subjects, as shown in patients with different types of periodontal disease. Individuals positive for the gene encoding for interleukin-1β (allele 2 of IL-1β at +3953) produce up to four times more IL-1β (Pociot et al. 1992). Patients with failing implants have been shown to have a “hyperinflammatory trait” unlike those with only successful and clinically healthy implants (Salcetti et al. 1997). Therefore, the same bacterial stimuli may cause greater tissue destruction in persons with an aberrant host response.

**Inflammation**

Inflammation is a complex reaction of the body in response to an infectious agent, antigen challenge / or injury. An accumulation of microbes at the peri-implant/ mucogingival margin is followed by a local inflammatory response. Within 10-20 days of plaque accumulation, clinical signs of inflammation can be seen (Löe 1965). Even during an early stage of inflammation, considerable tissue damage occurs. The collagen content of the inflammatory lesion decreases by approximately 30% after 28 days of undisturbed plaque accumulation in dogs (Lindhe & Rylander 1975). Thus, the cells in the inflammatory lesion, which are mainly neutrophilic granulocytes, will cause considerable tissue damage in their effort to combat the invading microorganisms. At this stage accumulation of plaque in the crevice aggravates the inflammatory reaction which can also be detected clinically. Until a lesion becomes “established” (Page & Schroeder 1976), this pattern of tissue damage and repair will continue. At some time in this process, marked tissue destruction becomes irreversible. Degradation of connective tissue is followed by epithelial migration and bone resorption. This stage is the borderline between gingivitis/mucositis and periodontitis/peri-implantitis.

The neutrophilic granulocytes are rapidly mobilised to the site of injury or bacterial invasion (within 30min) and they constitute the first line of defense against infectious agents. About 50-60% of the total circulating leukocytes are neutrophils. Development in the bone marrow of the myeloid precursor cells to segmented neutrophils takes about 2 weeks. The bone marrow of a healthy adult produces more than 10^{11} neutrophils a day, which can be accelerated to 10^{12} a day during an acute inflammation. The neutrophilic granulocyte is also the main leukocyte in the gingival crevice in health and disease (Attström & Egelberg 1970, Attström 1971). They are recruited from the circulation via chemotactic stimuli released from the bacterial plaque. Adhesion molecules control migration from the blood vessels to the
inflammatory lesion via adhesion molecules by binding selectins (P and E selectin on the endothelial wall and L-selectin on the neutrophil) and their counter receptors. This causes the neutrophil to roll along the vessel walls bringing it into close contact with chemoattractants expressed by the endothelium. By binding integrins on their membranes (LFA-1, Mac-1, VLA-4) with the intercellular adhesion molecules on the endothelial cells (ICAM-1, ICAM-2, VCAM-1) the neutrophil can penetrate the vessel cell wall. The chemoattractants direct the migration of the neutrophil up a chemoattractant gradient.

In the gingival sulcus, the neutrophils form a “leucocyte wall” between the subgingival plaque and the junctional epithelium (Frank 1980). The neutrophils can recognise and combat foreign substances by their receptors for several surface structures such as the Fc-receptors to IgG antibodies, complement receptors, capsule reactive proteins and LPS-complex. The neutrophil delivers antimicrobial substances by four mechanisms: 1) respiratory burst, 2) cytolysis, 3) phagocytosis and 4) extracellular degranulation. The degranulation leads to the release of anti-bacterial substances extracellularly (Wright 1988) from the primary (azurophil) granule (defensin, lysozyme, myeloperoxidase, cathepsin G and elastase) and the secondary (specific) granule (lactoferrin, collagenase, lysozyme).

Elastase is a neutral serine protease (33kDa), stored in the azurophil granule of the neutrophil in amounts up to 3 picograms per cell (Janoff 1985). Elastase can degrade important proteins in the extracellular matrix, such as elastin (Janoff 1968), laminin (Heck et al 1990), fibronectin and collagens (Janoff 1985, Owen et al. 1995). The destructive capacity of the proteases released is usually offset by the proteinase inhibitors α-1-antitrypsin (A1AT) and α-2-macroglobulin (A2MG). The inhibitors are present in such abundance that all active proteases are inhibited within milliseconds, but the release of proteinases in closed compartments and/or oxidative inactivation of A1AT can give the proteinases an opportunity to cause tissue damage. The respiratory burst generates reactive oxygen radicals that are released extracellularly. These radicals are tissue destructive per se (Weiss 1989), but can also act in concert with simultaneously released proteases. Extracellularly released oxygen radicals oxidatively inhibit A1AT and thus allow the proteases to degrade matrix proteins close to the neutrophils (Weiss 1989).

Several morphologic studies of the gingiva have shown that the numbers of neutrophils increases in proportion to the inflammation. Attström (1970) studied healthy and chronically inflamed sites in dog and man. The leukocytes were present in healthy and inflamed sites. Differential counts showed 95-97% neutrophils, 1-2 % lymphocytes and 2-3% monocytes. During inflammation, the proportions were the same but the number of leukocytes increased with the inflammation. The number of neutrophils recovered from crevicular fluid washings double during experimental gingivitis in man (Kowashi et al. 1979). Several studies have shown that the levels of elastase activity are high in periodontitis (Ingman et al 1994, Murray et al 1995). Gustafsson et al. (1992) compared elastase activity and antigenic elastase in patients with gingivitis alone and those patients with periodontal tissue destruction and found higher levels of elastase activity in the latter group. Elastase activity has also been proposed as a predictor for periodontal disease progression (Palcanis 1992, Armitage 1994).

In crevicular fluid from teeth and implants in partly edentulous patients, levels of acute-phase reactants (α2-macroglobulin, α1-antitrypsin, transferrin and lactoferrin) and IgG against P. gingivalis, correlate with clinical inflammation (Adonogianaki et al. 1995). The absolute amounts of acute-phase proteins and IgG against P.gingivalis around teeth and implants are higher around clinically inflamed than healthy sites. Eley & Cox (1991) studied
total enzyme activity and concentrations of cathepsin B/L, elastase activity, dipeptidyl peptidase IV and trypsin-like activity in crevicular fluid of patients with successfully-osseointegrated Brånemark implants. Total enzyme activities correlated with clinical indices- e.g., bleeding and probing pocket depth. During 18-42 months when the implants functioned, elastase activity was found to correlate with marginal bone reduction.

These findings show that the tissues surrounding osseointegrated titanium implants are affected by the inflammatory reaction. Since the neutrophilic granulocyte is not only the primary defender against bacterial invasion, but also a mediator of tissue destruction it is important to determine weather neutrophil activation around teeth differs from that around implants with clinically comparable levels of inflammation. Several studies have shown no essential difference in the microbiota between teeth and implants in partly edentulous persons. Therefore, in partly edentulous the microflora around the remaining dentition may affect the inflammatory reaction around implants as well as the maintenance and long-term prognosis of peri-implant tissues in such patients. Another question is whether the inflammatory reaction around implants in partly edentulous patients resembles that around implants in edentulous.

**Periodontitis**

The tissue destruction in periodontitis may be regarded as a specific host reaction, mediated by the host’s cells in response to an accumulation of bacteria. In periodontitis, the neutrophilic granulocytes are probably mediators of tissue destruction. This cell is associated with tissue destruction in several chronic inflammatory diseases, including periodontitis. Emphysema and rheumatoid arthritis are examples of non-infectious conditions in which tissue damage may partly be mediated by neutrophils (Malech et al. 1987, Janoff 1983, Nurcombe et al. 1991).

Neutrophil hyperreactivity, with increased release of oxygen radicals and proteolytic enzymes is thought to contribute to the tissue degradation. Several studies have shown a greater neutrophil activation in patients with periodontitis (Lamster 1992, Gustafsson et al. 1992, Murray et al. 1995). Gustafsson et al. (1994) compared the elastase activity and lactoferrin concentration between three types of inflamed sites: inflamed sites in patients with chronic gingivitis, inflamed sites with or without tissue destruction in those having periodontitis. Similar numbers of neutrophils were found at the 3 types of sites but neutrophil activation was greater in patients with periodontitis. This would suggest that hyperreactivity of neutrophils may distinguish patients with chronic inflammation from those with tissue destruction- i.e., periodontitis. Higher levels of elastase activity may also predict progression of periodontal disease (Palcanis 1992, Armitage 1994). Peripheral neutrophils from patients with periodontitis are hyperreactive in vitro after Fcγ-receptor stimulation (Gustafsson & Åsman 1996, Fredriksson et al. 1998).

Other explanations of an aberrant host response that contributes to tissue degradation in patients with periodontitis include overreacting monocytes with increased production of PGE2, IL-1β and TNFα (Offenbacher et al. 1993, Shapira et al. 1994, Tokoro et al. 1996), genetically- induced increased production of IL-1β (Kornman et al. 1997, Gore et al. 1998) and PMN cell deficiency with impaired chemotaxis and phagocytosis (Van Dyke et al. 1994, Shapira et al. 1994). It can therefore be concluded that neutrophilic granulocytes play a major role in tissue destruction in periodontitis. Hyperreactive neutrophils seem to participate as effector cells in this destruction.
Although severe types of periodontitis are rare, it is still one of the main causes of tooth loss in adults (Klock & Haugejorden 1991, Reich & Hiller 1993). It can therefore be assumed that a great number of patients receiving dental implants have a history of periodontal disease. When replacing lost teeth with implants it is important and necessary to determine whether a history of periodontitis will affect the prognosis and maintenance of implants. The question can be divided into two parts. First, do patients with periodontal disease lose more implants in the early healing period and secondly is the long-term prognosis and maintenance of implants affected. The similarity of microbiota at teeth and implants in partly edentulous patients and a hyperinflammatory phenotype in patients with periodontitis may contribute to the occurrence of periodontal conditions (peri-implantitis) or longitudinal bone loss around implants in patients with a history of periodontitis.

In the available literature concerning implant treatment of periodontally compromised patients, case reports show that implants are lost in those with severe forms of periodontitis (Malmström et al 1990, Fardal et al 1999). Clustering of implant losses in certain individuals usually indicates systemic or host-related factors of importance for fixture losses (Weyant & Burt 1993). However, early failure rates of implants in patients treated for periodontitis are similar to those in partly edentulous patients in general (Ellegård 1997a, b, Buchmann 1999, Sbordone 1999). A hyperinflammatory phenotype has been found in patients with failing implants compared to those with successful and clinically healthy implants. A positive correlation was found between higher levels of IL-1β in patients with both failing and stable implants versus those with stable implants alone (Salcetti et al 1997).

Only a few studies have evaluated attachment loss and marginal bone loss around implants in patients treated for periodontitis. Ellegård et al. (1997) in a retrospective study of periodontally-treated patients receiving implants reported that the incidence of bone loss during 5 years follow-up increased with 45% of all implants displaying marginal bone loss of 1.5mm or more. Bheneke et al. (1997, 2000) also found gradual loss of a small amount of bone during follow-up after loading. Obviously an important question to further elucidate is if marginal bone loss around implants is associated with bone loss in the naturally remaining dentition and to history of periodontitis.

**Peri-implantitis**

Peri-implantitis is defined as an inflammatory reaction with loss of supporting bone in the tissues surrounding a functioning implant (Albrektsson & Isidor 1994). It is also been described as “a site specific-infection yielding many features in common with chronic adult periodontitis” (Mombelli et al. 1987) or “an inflammatory, bacterial-driven destruction of the implant-supporting apparatus” (Tonetti et al. 1996). The view that microorganisms play a major role in the development of peri-implantitis is supported by several clinical findings. A cause-related effect between plaque accumulation and peri-implant mucositis has been shown in animals and humans (Berglundh et al. 1992, Pontoriero et al. 1994). Moreover the microbial colonisation of implants follows the same pattern as around teeth (Leonhardt et al. 1992, Leonhardt et al. 1993). During peri-implant breakdown a complex microbiota is established closely resembling that found in adult periodontitis (Rams et al. 1983, Rams et al. 1991, Mombelli et al. 1987, Becker et al. 1990, Rosenberg et al. 1991, Alcoforado et al. 1991, Augthun et al. 1997). When peri-implant tissue breakdown is induced by placing plaque-retentive ligatures submarginally in animals, a shift in the microflora occurs (Leonhardt et al. 1992, Lang et al. 1993, Tillmanns et al. 1998). Resolution of the inflammatory lesion,
improvement in the clinical condition of the peri-implant mucosa and a limited bone fill of the bone defect occurs after treatment (Mombelli & Lang 1992, Jovanovic et al. 1992). Although the microbiological profile at failing implants differs from that in peri-implant health, it is not known whether the host response to the bacterial challenge is similar to that seen in periodontitis. A microbial challenge in conjunction with an aberrant host response has been reported as a possible etiological factor of failing implants (Salcetti et al 1997).

Analysis of inflammatory mediators in crevicular fluid has been used to compare peri-implant tissue health and disease. Neutrophil-derived enzymes such as neutral protease, neutrophil elastase, myeloperoxidase and β-glucuronidase has also been found in association with failing implant sites (Butros et al 1996). Increased levels of IL-1β have been found around implants with peri-implantitis (Kao et al. 1995, Panagakos et al. 1996, Curtis et al. 1997). In periodontitis, host-related factors can be used to distinguish between persons with or without tissue destruction. Neutrophil hyperreactivity contributes to tissue destruction in periodontal disease. Polymorphism of the gene encoding for interleukin-1β appears to be related to an increased risk of severe periodontitis (McGuire et al 1999). Elucidation of the aetopathogenesis of peri-implantitis requires determination of the relationship of an aberrant host response to the development of peri-implantitis lesions.

**Loading**

An association between implant failures and excessive occlusal load has been found in experimental studies in dogs (Isidor et al. 1997). The term overload usually refers to a situation where the functional load of an implant exceeds the capacity of the bone that anchors the implant (Tonetti & Schmid 1994, Esposito et al. 1999). During the early healing phase, jaw bone quality and the use of short implants in an atrophic maxilla have been associated with early implant failures (Friberg et al. 1991). Failures caused by premature or unfavourable loading conditions during healing may therefor be due to overload when the loading forces disrupt a weak bone-to-implant interface or disturb healing (Esposito et al. 1999). The cellular and morphological composition of the tissues surrounding late failures of Brånemark implants suggest that overload occurs when the supporting ability of the bone is exceeded. Of 9 late failures (11 months-6 years after loading), epithelial down-growth surrounded by a dense fibrous tissue capsule with minimal inflammation was seen around 4 implants. Rosenberg et al. (1991) divided patients with failing implants into two groups: suspected infection and trauma (overload). In the trauma group, patients had no pain or suppuration and the failed implants had a microbiological profile similar to that at healthy implant sites. In the infected group implants were colonised by microbiota similar to that found in periodontitis. An indirect association between late failures and overload has also been found in long-term follow-up studies where higher failure rates are seen in areas with poor bone quality (Jemt & Lekholm 1993).

Contradicting results in the literature exist of to what extent overload or excessive physiologic stress may lead to marginal tissue destruction. Marginal bone loss has been reported in association to grinding of teeth and prosthesis wear (Lindqvist et al. 1988). Quirynen et al. (1992) reported a correlation between marginal bone loss and occlusal overload after the first year of function of implant supported prosthes. These authors also reported a higher incidence of failures when shorter implants were used (Naert et al. 1992).
Few animal experiments have studied the effect of load on the peri-implant bone. Isidor (1997) using a monkey model demonstrated that excessive occlusal load in lateral direction resulted in loss of fixtures during an observation period of 18 months. 6 of 8 excessively loaded implants became mobile. This was interpreted by the author as fatigue microfractures in the bone, due to a force exceeding the repair potential of the bone. The fixtures became mobile without preceding marginal bone loss.

No marginal bone loss was observed during 24 weeks of lateral static load placed on ITI implants in the dog (Gotfredsen et al. 2001). The bone density and the mineralised bone-to-implant contact were found to be higher adjacent the lateral loaded implants than at the control implants in the dog model used. Furthermore, experimental studies have not been able to demonstrate marginal bone resorption induced by orthodontic load (Asikainen et al. 1997) or occlusal load (Barbier & Schepers 1997). The mechanisms on how excessive occlusal load and parafunction affect the tissues surrounding implants is still not fully elucidated.

**Smoking**

The possible relation between of smoking and implant failures has been evaluated in several retrospective and prospective clinical studies (Gorman et al. 1994, De Bruyn & Collert 1994, Bain&Moy 1993). These findings show that although implant failure is rare, smokers have significantly higher failure rates. In a retrospective analysis of the outcome of 2194 implants placed in 540 subjects, Bain & Moy (1993) observed that a significantly greater percentage of implant failures occurred in smokers than non-smokers. Smokers had an overall failure rate of 11.3% whereas only 4.8 % of the implants placed in non-smokers failed. Gorman et al. (1994) found that implant failures were twice as common at second-stage surgery in smokers as in non-smokers. In general, it can be concluded that smoking has a negative effect on implant survival, especially during the early healing after implant installation. Smoking seems closely related to fixture losses in the maxilla, possibly in combination with poor bone quality. Impaired wound-healing, excessive bone loss around implants or loss of fixtures was found in bone grafts in the maxilla of smokers (Jones & Tripplett 1992, Kan et al. 1999, Keller et al. 1999).

The effects of smoking on marginal bone loss and peri-implant tissue have also been evaluated. Cigarette smoking was associated with significantly greater marginal bone loss in the treatment of edentulous mandibles (Lindqvist et al. 1996, Lindqvist et al 1997, Carlsson et al. 2000). The 10 and 15-year follow-ups of these edentulous patients showed that bone loss, although limited, was related to several factors, smoking and oral hygiene being the most important.

Haas et al. (1996) compared the association between smoking and peri-implantitis of 366 implants in 107 smokers compared to 1000 implants in 314 non-smokers. Smokers had higher bleeding scores, greater clinical inflammation, deeper probing pocket depth and more radiographic bone loss around implants especially in the maxilla than did non-smokers. It was concluded that the effect of smoking on peri-implant tissue health is greater in the maxilla than in the mandible. Poor healing of surgical wounds is a well-known complication in heavy cigarette smokers (Silverstein et al. 1992).

Several epidemiological studies have shown the negative influence of smoking on periodontal status (Bergström et al. 1989, Bergström et al. 1991, Haber et al. 1993). Its role a risk factor for periodontal disease progression has recently been confirmed (Norderyd et al.}
Current data suggests that smokers have at least a three fold increased risk of developing periodontitis (Papapanou et al. 1996). They also do not respond as well to non-surgical (Preber & Bergström 1985) as well as surgical treatment (Preber & Bergström 1990). Since several studies have shown the negative effects of smoking including a higher early implant failure rate the long-term effects on marginal bone loss around dental implants need further evaluation.
GENERAL AIMS

The general objective of this thesis was to evaluate factors of importance for the long-term survival and maintenance of dental implants.

Aims of studies:

• To evaluate the neutrophil activation around teeth and dental implants and relate these findings to the microbiota (Papers I and II)

• To evaluate the clinical, radiographic and microbiological status of implants after long term function in partly edentulous patients (Paper III).

• To evaluate the influence of smoking and a history of periodontitis on the occurrence of late fixture loss and marginal bone loss around dental implants (Paper IV).

• To compare the long-term treatment outcome of implant treatment in fully edentulous to partly edentulous patients (Paper III, IV).

• Characterise the microbiota and inflammatory host response around implants with marginal tissue destruction, peri-implantitis (Paper V).
MATERIAL
PATIENT SELECTION

In Papers I and II we selected consecutive patients treated with dental implants having regular check-ups in a maintenance programme. They were divided into two groups according to their dental status: edentulous and partly edentulous. All were clinically successful implant cases with stable peri-implant tissues. None of them developed marginal bone loss exceeding 0.2mm annually after the first year of function of their implants on clinical and radiographic follow-ups.

Paper I included 19 patients, 10 completely edentulous and 9 partly edentulous, treated with single crystal sapphire implants 7-13 years previously. These patients, 10 women and 9 men, aged 43-87 years, had been treated for mandibular edentulism by an overdenture retained by sapphire implants or fixed bridges supported by sapphire implants (Fartash et al. 1996, Fartash et al. 1997).

Paper II included 31 patients treated for partial or total edentulism with titanium implants. The 15 edentulous patients, 8 men and 7 women, with a mean age of 71.4 (SD 9.4, range 51-84) years had been treated for total edentulism with 4 to 12 titanium implants. The 16 partly edentulous patients, 12 women and 4 men, with a mean age of 53.6 (SD 16.6, range 24-74) years had been treated with 2 to 4 implants in free-standing tissue-integrated prostheses. The implant-supported prostheses had functioned between 1 and 11 years.

In Paper III we evaluated implant treatment after 10 years of functional load. Fifteen patients were included, 8 women and 7 men, with a mean age of 65.5 (SD 12.0, range 39-76) years treated for partial edentulism with implants ad modum Brånemark. During the first 5 years after bridge connection the patients were part of a prospective multicenter study (Van Steenberghe et al. 1990, Henry et al. 1993, Lekholm et al. 1994). In these patients, 55 implants were assessed clinically, radiographically and microbiologically after 10 years of function.

Paper IV consisted of 143 consecutively treated patients, in a Swedish dental specialist centre. The study comprised of all patients given a fixed implant-anchored bridge during one year with implants ad modum Brånemark and who completed a clinical and radiographic 5-year follow-up. These patients were divided into two groups: (1) 59 partly edentulous patients with implants and teeth in the same jaw: mixed jaw group (MJG) and (2) 84 patients edentulous in one or both jaws: full jaw group (FJG). All patients were treated with a freestanding implant-supported fixed prosthesis as a partial bridge or as a full jaw-anchored bridge. The MJG consisted of 12 men and 47 women with a mean age of 64.9 (SD 10.3) year at the 5-year follow-up. The FJG, 36 men and 48 women had a mean age of 66.3 (SD 9.6) year. In the FJG, 58 were edentulous in one jaw and 26 edentulous in both. A total of 239 implants in partly edentulous and 505 implants in edentulous jaws were evaluated retrospectively after 5 years of functional load.

Paper V included 17 patients and 19 controls. The patients, 8 women and 9 men, had a mean age of 62.8 (SD7.7) years and 2-6 titanium implants. They had been selected on basis of the radiographic findings of marginal bone loss and clinical signs of inflammation around the implants. All patients were treated for partial edentulism with osseointegrated titanium implants, 14 with implants ad modum Brånemark and 3 with ITI solid-screw implants. Compared to the one-year intraoral radiographic examination, patients had at least one
implant showing radiographic marginal bone loss of ≥ three fixture threads (1.8mm) mesially or distally. The fixture was integrated in the apical portion. The controls consisted of 19 persons, 10 men and 9 women, with a mean age of 65.1 (SD 6.7) years, and no clinical or radiographic signs of marginal tissue destruction around implants. The patients and controls were of the same age (± 5 years), had similar smoking habits and had implants placed in similar positions of the jaws. They were all selected from the regular maintenance programme for patients treated with osseointegrated implants.

Crevicular fluid and microbiological samples were taken from five types of sites: 1) failing implant sites- i.e. implants with marginal bone loss and inflammation, peri-implantitis (FI), 2) stable implant sites (SI) in patients with both stable and failing implants, 3) control implant sites (CI) in patients with stable implants alone, 4) teeth in patients (TP) and 5) controls (TC).

**METHODS**

**CLINICAL EXAMINATION**

*Plaque and clinical inflammation*

Supragingival plaque (PI) at teeth and implants was evaluated by using the criteria of Silness & Löe 1964 (*Papers I and II*) or by scoring in a binomial fashion (0=no plaque, 1=plaque, *Papers III and V*). Clinical inflammation around teeth and implants was evaluated with the gingival index by Löe (1967) and a corresponding index for the peri-implant mucosa (*Papers I, II and V*). In *Paper III*, clinical inflammation was assessed by evaluating redness, swelling and bleeding with the sulcus bleeding index by Mühlemann & Son (1971), but keeping the parameters separate. The Mühlemann index of inflammation was used in order to compare clinical data with those reported elsewhere for the same patients (van Steenberghe et al. 1993, Lekholm et al. 1994).

*Probing pocket depth*

Probing pocket depth (PPD), was calculated by measuring the distance from the gingival / peri-implant mucosal margin to the bottom of the pocket with a calibrated periodontal probe, 0.4 mm in diameter (*papers I, II*). In *Papers III and V* a force-controlled calibrated periodontal probe (Florida probe®, Computerised Probe Inc, Gainesville, FL, USA), with a constant probing force of 0.20 N and a probe-tip diameter of 0.4mm, to determine PPD was used. Tissue inflammation, probe-tip diameter and probing force are factors affecting probing pocket depth (PPD). The Florida probe was used to reduce the variation in probe pressure shown to affect the probing pocket recording of inflamed peri-implant sites (Ericsson & Lindhe 1993, Lang et al. 1994).
SAMPLING AND METHODS

**Subgingival plaque**

After gently drying of the area to be sampled with an air syringe supragingival plaque was removed with sterile cotton pellets. Two sterile paper points (Johnsson & Johnsson, Windsor, NJ, USA) were inserted in the crevice and left for 10 seconds. The paper points were placed in a vial containing anaerobic transport medium VMGA III (Dahlén et al. 1993) for anaerobic cultures (Paper II) or in a sterile transport vial for DNA-probe analysis (Papers III and V).

In paper II bacterial culturing was used to for the identification and enumeration of *Porphyromonas gingivalis* (Pg), other black pigmenting bacteria (BPB), *Actinobacillus actinomycetemcomitans (Aa)*, *Eikinella corrodens (Ec)*, *Fusobacterium* spp, *Streptococcus mitis* (Sm) and *Streptococcus sanguis* (Ss). Samples for culturing were mixed and dilutions of $10^{-2}$ and $10^{-4}$ cultured anaerobically on brucella agar, supplemented with horse blood and menadion, for identification and enumeration of *P. gingivalis* and other black pigmenting bacteria (BPB, Slots 1986).

Undiluted samples were cultured in CO$_2$ (5%) on trypticase soy agar containing horse serum, bacitracin and vancomycin (TSVB) for identification of *A. actinomycetemcomitans* (Slots 1982). A dilution of $10^{-2}$, cultured anaerobically on blood agar containing kanamycin and vancomycin (BVK, Columbia II Agar Base, BBL) for identification and enumeration of *E. corrodens* by colony morphology. Anaerobic cultures on blood agar, containing vancomycin and neomycin (BVN, Columbia II Agar Base, BBL) was used for identification of *Fusobacterium* spp by their typical morphology and on mitis salivarius agar (MS) for identification and enumeration of *S. mitis* and *S. salivarius*. The frequency of each species was expressed as a percent of the total viable count of colony forming units on the brucella agar plates.

In paper II, we found no significant difference in the microbiota between stable edentulous and partly edentulous patients although this has been shown by others (Apse et al 1989, Quirynen et al 1990). Consequently, we used the chequerboard DNA-DNA hybridisation method (Socransky et al 1994) in papers III and V to detect periodontal pathogens. This method has shown a higher prevalence and significantly higher bacterial counts of periodontal pathogens when compared to cultures (Papapanous et al. 1997). The twelve micro-organisms in subgingival samples from teeth and implants tested with the DNA probe were: *Porphyromonas gingivalis*, *Prevotella intermedia*, *Prevotella nigrescens*, *Bacteroides forsythus*, *Actinobacillus actinomycetemcomitans*, *Fusobacterium nucleatum*, *Treponema denticola*, *Peptostreptococcus micros*, *Campylobacter rectus*, *Eikenella corrodens*, *Selenomonas noxia* and *Streptococcus intermedia*. Standardised procedures of the checkerboard DNA-DNA hybridisation method was used (Papapanou et al. 1997 a, b).

The hybrids formed between the bacterial DNA and the probes are detected by applying an anti-digoxin antibody conjugated with alkaline phosphatase and incubated with a chemiluminescent substrate. Evaluation of the chemiluminescent signal is performed by comparing the signals obtained with those of pooled standard samples containing $10^6$ or $10^5$ of each of the 12 species. The chemiluminiscent units obtained are transformed into a scale from 0-5, where 0 indicates no signal, 1, a signal weaker than that of the low standard ($10^5$ bacteria) 2, a signal equal to the low standard 3, a signal higher than that of the low standard but lower than that of the high standard ($10^6$ bacteria) 4, a signal equal to that of the high standard, and 5 a signal higher than that of the high standard. Thus, 6 scores (0-5) can be used for each species.
**Crevicular fluid**

The area was gently dried with an air syringe (Papers I, II and V). Thirty seconds later, crevicular fluid was collected with a prefabricated paper strip (Peripaper®, Pro Flow, Amityville, NY, USA) inserted into the crevice until mild resistance was felt, and left there for 30 seconds. Strips macroscopically contaminated with blood or saliva was discarded. To calculate the concentration of inflammatory markers in crevicular fluid we measured the volume of collected fluid. Immediately after sampling, the fluid the volume was measured with a Periotron® 6000 (Papers I and II) or with a Periotron® 8000 (Paper V) GCF metre (IDE Interstate, Amityville, NY, USA).

The Periotron measures the electrical capacitances since the dielectric insulating properties of the filter paper vary with the quantity of fluid absorbed by the paper. Differences in the composition of the various crevicular samples do not affect the measurements (Hinrichs et al 1984). Before each study, the instrument was calibrated, using saline administered with a Hamilton syringe. They were placed in 500 or 1000µl phosphate buffered saline (PBS), pH 7.4. The strips were left in PBS during gentle horizontal agitation at room temperature for 60 minutes. After removing of the strips, the samples were centrifuged for 10 minutes at 3000g. The supernatants of the eluted samples were stored at -70°C, pending analysis.

**RADIOGRAPHIC EXAMINATION AND EVALUATION OF MARGINAL BONE LOSS**

**Method, Paper III**

Intraoral radiographs of all implants were taken using the long-cone paralleling technique with a focus-film distance of 20 or 25 cm. A dental x-ray machine (Siemens Heliodent, Erlangen, Germany) was used together with films of speed group E (Ektaspeed, Eastman Kodak Co., Rochester, NY, USA). Exposure time was 60 kVp, 0.5s in the upper jaw and 0.25s in the lower jaw. At 70 kVp, the exposure time was 0.5s in the upper jaw and 0.32s in the lower jaw.

**Measurement of marginal bone loss around implants, Paper III**

To measure marginal bone height at implants, all radiographs were digitised, using a Hamamatsu L5810 3CCD video camera (Hamamatsu Photonics K.K., Hamamatsu City, Japan) mounted on a macrostand with a Nikon AF Nikkor 85mm, 1:1.8 lens (Tokyo, Japan). No further processing, except magnification was done. Marginal bone height was measured as the distance between the marginal bone crest and the fixture-abutment connection mesially and distally to the nearest 0.1mm at all fixtures, using an image processing system and Semper 6 plus software (Synoptics Ltd, Cambridge, UK).

To evaluate changes in marginal bone height at the mesial and distal surfaces during 10 years of functional load, the marginal bone heights measured to the nearest 0.1mm on radiographs from the time of bridge connection, were compared to those taken at the 10-year
follow-up. Only radiographs with threads clearly seen at both sides of the implant were used for evaluation. One reader evaluated all radiographs. Readings were repeated three times, with one week between readings, in random order. Measuring error was calculated as the mean of the standard deviations of the three measurements at each site. Mean marginal bone height of all 220 sites was 1.6mm and the mean of the standard deviations was 0.1mm.

**Method, Paper IV**

Intra-oral radiographs of implants were taken using the long-cone paralleling technique, with a focus-film distance of 20 cm. A dental x-ray machine (Siemens Heliodent, Erlangen, Germany), operating at 60 kVp, was used with films of speed group E (Ektaspeed, Eastman Kodak Co. Rochester, NY, USA). The exposure time was 60 kVp, 0.32 s in both the upper and lower jaws. A panoramic picture was also taken of the patient’s jaws with a cephalostat (Cranex 3, Orion Corp., Ltd, Helsinki, Finland), using panoramic film (T-Mat L/RA Dental film, Eastman Kodak Co., Rochester, NY, USA).

**Measurement of marginal bone loss around implants, Paper IV**

The marginal bone level at the mesial and distal surfaces of all fixtures was evaluated on the intraoral radiographs taken at the time of bridge connection and at the five-year follow-up. Only radiographs perpendicular to the long axis of the fixtures were used for evaluation—i.e., where the threads of the implants were clearly visible. The marginal bone level was recorded as the number of fixture threads (with a distance between fixture threads of 0.6mm) above the marginal bone crest. Changes in marginal bone level at each fixture were measured as the difference in marginal bone height between radiographs at the time of the bridge connection and the five-year follow-up. The surface, mesial or distal, showing the greatest amount of bone loss was used to calculate the individual mean of bone loss at fixtures in each patient. The measurements were made by one reader using a Mattson viewer, with x 2 magnification (Dental Aps, Copenhagen, Denmark).

**Measurement of marginal bone loss at teeth, Paper IV**

Alveolar bone loss at teeth in the remaining dentition was evaluated at the Ramfjord teeth (Ramfjord 1967) on the panoramic radiographs and expressed as the percentage bone loss of the total root length (RL), measured from the cemento-enamel junction (CEJ) to the apex. To assess total bone loss during the 5 years of observation, the marginal bone loss was measured on panoramic radiographs taken at the time of bridge connection and compared to panoramic radiographs taken at the five-year follow-up. In patients edentulous in one jaw, or if a Ramfjord tooth was missing, the corresponding contralateral tooth was chosen.

To determine the variation of the method for evaluating marginal bone loss at teeth on panoramic radiographs, we randomly selected 15 radiographs from previously-treated
patients. On these, the marginal bone loss was determined at the Ramfjord teeth. Readings of radiographs were repeated three times in a random order with an interval of one week between them. The measurement error was calculated as the mean of the standard deviations of the three measurements at each Ramfjord tooth. Mean marginal bone loss of the 85 evaluated teeth was 20 % of the RL and the mean of the standard deviations was 2 % of the RL.

LABORATORY ANALYSES

Elastase activity

The elastase activity (Papers I, II and V) was measured with a chromogenic, low molecular weight substrate (mw 445.5 Da) L-pyroglutamyl-L-propyl-L-valine -p-nitroanilide. The substrate is highly specific for granulocyte elastase (Kramps 1983, Tanaka et al. 1990), but is also hydrolysed by the elastase-α-2-macroglobulin complex (Wewers et al. 1988). Samples and substrate were incubated at +37°C in a 96-well microtitre plate and the absorbance read after 5 hours (Papers I and II) and 2 hours (Paper V) at 405nm in a spectrophotometer.

ELISAs

The concentration of elastase complexed with α-1-antitrypsin (A1AT), lactoferrin and IL-1β were measured with ELISAs (Papers I, II and V). The wells of a 96-microtitre plate were coated overnight at 4°C with a polyclonal antibody to A1AT or a monoclonal antibody to lactoferrin / IL-1β. After coating, all plates were washed 4 times with PBS + 0.05% Tween® 20. Samples and standards of the three assays were added to the plates and incubated at +37°C for 1 h. All plates were washed once again as above before adding the detection antibody of each assay. An alkaline phosphatase conjugated antibody against elastase and a biotinylated polyclonal goat anti-IL-1β was used to detect of granulocytic elastase and IL-1β. To detect lactoferrin, we used a polyclonal rabbit anti-lactoferrin and an alkaline phosphatase conjugated anti-rabbit IgG antibody. The plates were then incubated again at +37°C and washed four times more. The substrate, p-nitro-phenol-phosphate was added to the wells in the elastase and lactoferrin assays and the absorbance at 405nm was read at 10 and 15 minutes, respectively. In the IL-1β assay, horseradish peroxidase (HRP)-conjugated streptavidin was added to the wells and the plate was incubated 15 minutes at room temperature. After a final washing, we added the HRP substrate, 3,3’,5,5’-tetramethylbenzidine. The reaction was stopped with 1M H₂SO₄ after 10 min and the absorbance read at 450 nm in a spectrophotometer.
**Protein concentration**

In Papers I and II, the total protein concentration of the crevicular fluid samples was measured with a protein-staining method described by Bradford (Bradford 1979) using Bio Rads protein assay. The protein-staining method is particularly sensitive to arginine-rich proteins (Compton & Jones 1985). The protein reagent was added to the samples and the absorbance measured at 595mn. The samples were compared to a standard curve, obtained by serial dilutions of standard serum with including PBS on the same plate. The protein composition of this serum is similar to that of crevicular fluid and GCF.

**Analyses of blood samples**

In paper V, a venous blood sample was taken from each of the patients and controls to assess the general health and systemic markers of inflammation. The number of white blood cells, neutrophilic, eosinophilic and basophilic granulocytes, lymphocytes and monocytes, as well as the number of red blood cells and haemoglobin concentration were analysed with a Coulter STKS Analyser. The acute-phase reactants in C-reactive protein (CRP), α-1-antitrypsin and haptoglobin in plasma were determined with a Nephelometer-Analyser. The limit for the CRP assay was 0.5mg / l.

**STATISTICAL METHODS**

Data from each type of site in patients were averaged so that the patient could be used as a “statistical unit” for comparison. Since the distribution of test values was skewed we used non-parametric statistical tests. The Mann-Whitney U-test was used to calculate differences between edentulous and partly edentulous or between patients and controls. The Wilcoxon signed-rank test was used to calculate individual differences and differences between types of sites in patients. Correlations were calculated with the Spearman rank correlation coefficient.
INVESTIGATIONS AND RESULTS

MICROBIOTA

In *Paper II*, bacterial samples were collected at three types of sites: (1) crevices around teeth (GCF) and (2) implants (PISF-p) in 16 party edentulous patients, and (3) crevices around implants (PISF-t) in 15 edentulous patients. Anaerobic cultures were used to detect the occurrence of periodontal pathogens (Slots 1986). The mean values and standard deviation (SD) of the clinical data are shown in Table 1. Smaller amounts of visible plaque were seen around natural teeth than around implants in the partly edentulous patients ($p=0.0164$). There was no difference in plaque index in partly edentulous patients compared to edentulous patients. Gingival inflammation and probing depth showed no difference between the three types of sites. Probing depth at non-sampled sites did not exceed 4 mm.

No significant differences in the microbiota were found among the three types of sites (Table 2). Some trends were noted, however, in the bacterial counts and these will be described. The main cultivable bacteria at all sites were facultative anaerobic Gram-positive cocci- i.e., 30-40% of the bacteria at teeth and implants in partly edentulous and 55% at implants in the edentulous. Gram-negative anaerobic rods associated with periodontal disease, such as *A. actinomyctemcomitans* and *P. gingivalis*, were found only around the teeth of one patient. Black pigmenting bacteria (BPB) and *Fusobacterium spp* were present around both the teeth and the implants. BPB accounted for 3% of the cultivable microflora around the teeth and 2.5% around the implants in patients with teeth and implants. In patients with implants alone, the mean frequency of BPB was 0.1%. The proportions of anaerobic microorganisms in the cultures were the same in the three types of sites.

In *Paper III*, bacterial samples from teeth and implants were collected at the 10-year examination of 15 partly edentulous patients. Microbiological samples were assessed from 41 implant sites and 31 tooth sites. The DNA-DNA hybridisation method was used to detect 12 micro-organisms in subgingival samples from teeth and implants (Socransky et al. 1994). Sixty-five per cent of the tooth surfaces, but only 47% of the implant surfaces had visible plaque. The degree of clinical inflammation around teeth and implants were similar, as judged by redness, swelling and bleeding, using the Mühleman & Son’s index (1971). Table 3 shows the mean probing pocket depth values (PPD ± SD) of six surfaces around teeth and implants. The PPD around implants was also compared to that around teeth, corresponding to fixtures on the opposite side of the jaw (corresponding teeth) in patients edentulous according to Appelgate-Kennedy classes II, and in the opposite jaw in patients in Appelgate-Kennedy classes I and IV. The PPD was significantly greater at implants at the distobuccal, lingual and distolingual surfaces than at the corresponding tooth surfaces.

The frequencies and numbers of the 12 microorganisms are shown in Table 4. There were no significant differences in bacteria present between teeth and implants. The commonest bacterial species isolated from teeth and implants were *T. denticola*, *P. micros* and *S. intermedia*. *A. actinomyctemcomitans* was found in only four samples from implants, but not from teeth. The microbiological analysis showed that 55% of the samples from teeth were positive for one or more of the putative periodontal pathogens evaluated in this study, *P. gingivalis*, *P. intermedia*, *B. forsythus*, *A. actinomyctemcomitans* and *T. denticola* versus 40% of those from implants. The periodontal pathogens analysed were present at sites with marginal bone loss exceeding 2mm.
In paper V we wanted to characterise the microbiota around implants and teeth in patients with peri-implantitis. Seventeen partly edentulous patients with a total of 98 implants, of which 45 showed marginal bone loss of ≥ three fixture threads after the first year of loading were included. Nineteen subjects with stable marginal tissue conditions served as controls. Microbiological samples were collected from teeth and implants in both groups. Clinical characteristics of the five types of sites are shown in Table 5. The amounts of visible plaque were similar at implants in patients and controls. Significantly more clinical inflammation and a greater probing depth were found at failing implant sites. The results of the microbiological analyses are shown in Table 6. The same method as in Paper III, DNA-DNA hybridisation method, was used to detect 12 micro-organisms in subgingival samples from teeth and implants.

All 12 micro-organisms were found around teeth and implants in patients with peri-implantitis. *Campylobacter rectus, Selemonas noxia* and *Eikenella corrodens* were not detected around implants in the edentulous controls nor were *C. rectus* and *S. noxia* found around implants in partly edentulous controls. The specific periodontal pathogens tested-i.e, *Porphyromonas gingivalis, Prevotella intermedia, Bacteroides forsythus, Actinobacillus actinomycetemcomitans* and *Treponema denticola* were present at all types of sites in both groups. In patients with peri-implantitis, bacterial samples with ≥ 10^6 bacteria in each sample were found around stable and diseased implants. High amounts of *A. actinomycetemcomitans* were never detected at stable implants and teeth in peri-implantitis patients, but were present in 23.5 % of the samples from failing implants. Samples with ≥ 10^6 bacteria in each sample were never recovered in controls.

Table 1. Mean (SD) values of clinical variables in the 3 types of sites: crevices around teeth (GCF) and implants (PISF-p) in partly edentulous patients, and from crevices around implants (PISF-t) in totally edentulous patients (Paper II).

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Unit</th>
<th>GCF</th>
<th>PISF-p</th>
<th>PISF-t</th>
</tr>
</thead>
<tbody>
<tr>
<td>No. of patients</td>
<td>n</td>
<td>16</td>
<td>16</td>
<td>15</td>
</tr>
<tr>
<td>Plaque index</td>
<td></td>
<td>0.7 (0.7)</td>
<td>1.0 (0.6)</td>
<td>1.0 (0.6)</td>
</tr>
<tr>
<td>Gingival / peri-implant index</td>
<td></td>
<td>1.5 (0.4)</td>
<td>1.6 (0.4)</td>
<td>1.4 (0.5)</td>
</tr>
<tr>
<td>Probing depth</td>
<td>mm</td>
<td>2.7 (0.7)</td>
<td>2.5 (0.7)</td>
<td>2.5 (0.6)</td>
</tr>
</tbody>
</table>
Table 2. Frequency of bacteria (mean, SD) from crevices around teeth (GCF) and implants (PISF-p) in partly edentulous patients, and from crevices around implants (PISF-t) in totally edentulous patients (*Paper II*).

<table>
<thead>
<tr>
<th></th>
<th>GCF n=16</th>
<th>PISF-p n=16</th>
<th>PISF-t n=15</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Mean (SD)</td>
<td>Mean (SD)</td>
<td>Mean (SD)</td>
</tr>
<tr>
<td><em>A. actinomycetemcomitans</em></td>
<td>% 0.01 (0.03)</td>
<td>0.00 (0.00)</td>
<td>0.00 (0.00)</td>
</tr>
<tr>
<td><em>P. gingivalis</em></td>
<td>% 0.04 (0.15)</td>
<td>0.00 (0.00)</td>
<td>0.00 (0.00)</td>
</tr>
<tr>
<td>Black pigmenting bacteria</td>
<td>% 3.03 (7.48)</td>
<td>2.50 (5.36)</td>
<td>0.12 (0.42)</td>
</tr>
<tr>
<td><em>Fusobacterium spp.</em></td>
<td>% 0.76 (1.12)</td>
<td>1.20 (2.16)</td>
<td>1.14 (5.41)</td>
</tr>
<tr>
<td><em>E. corrodens</em></td>
<td>% 0.00 (0.00)</td>
<td>0.00 (0.00)</td>
<td>0.00 (0.00)</td>
</tr>
<tr>
<td><em>S. mitis</em></td>
<td>% 18.49 (17.35)</td>
<td>17.47 (17.06)</td>
<td>26.68 (26.73)</td>
</tr>
<tr>
<td><em>S. sanguis</em></td>
<td>% 16.33 (17.93)</td>
<td>22.65 (26.61)</td>
<td>28.18 (27.18)</td>
</tr>
</tbody>
</table>

Table 3. Mean (SD) probing pocket depth of all teeth, corresponding teeth and implants, measured ten years after bridge connection (*Paper III*).

<table>
<thead>
<tr>
<th></th>
<th>Db</th>
<th>B</th>
<th>Mb</th>
<th>Ml</th>
<th>L</th>
<th>Dl</th>
</tr>
</thead>
<tbody>
<tr>
<td>All teeth (n=1374)</td>
<td>mean 1.57</td>
<td>1.13</td>
<td>1.92</td>
<td>1.70</td>
<td>1.20</td>
<td>1.51</td>
</tr>
<tr>
<td></td>
<td>SD (0.44)</td>
<td>(0.29)</td>
<td>(0.44)</td>
<td>(0.39)</td>
<td>(0.26)</td>
<td>(0.39)</td>
</tr>
<tr>
<td>Corresponding teeth (n=279)</td>
<td>mean 1.43</td>
<td>1.12</td>
<td>1.89</td>
<td>1.71</td>
<td>1.23</td>
<td>1.47</td>
</tr>
<tr>
<td></td>
<td>SD (0.32)</td>
<td>(0.43)</td>
<td>(0.59)</td>
<td>(0.67)</td>
<td>(0.49)</td>
<td>(0.5)</td>
</tr>
<tr>
<td>Implants (n=279)</td>
<td>mean 2.16</td>
<td>1.77</td>
<td>1.92</td>
<td>2.02</td>
<td>1.61</td>
<td>2.07</td>
</tr>
<tr>
<td></td>
<td>SD (0.80)</td>
<td>(0.69)</td>
<td>(0.68)</td>
<td>(0.69)</td>
<td>(0.49)</td>
<td>(0.62)</td>
</tr>
<tr>
<td><em>p</em> (corresponding teeth/implants)</td>
<td>0.021</td>
<td>ns</td>
<td>ns</td>
<td>ns</td>
<td>0.017</td>
<td>0.004</td>
</tr>
</tbody>
</table>

n=total number of sites. 51 surfaces at fixtures could not be measured because of the prosthetic construction. 6 surfaces were measured at teeth and implants: distobuccal (Db), centrobuccal (B), mesiobuccal (Mb), mesiolingual (Ml), centrolingual (L) and distolingual (Dl).
Table 4. Frequencies and numbers of 12 micro-organisms in subgingival samples from teeth and implants. Each number represents one positive sample. The numbers 1-5 indicate the approximate number of bacteria in each sample. 1<10^5, 2 = 10^5, 3 > 10^5, 4 = 10^6, 5 = > 10^6 (Paper III).

<table>
<thead>
<tr>
<th>Bacteria</th>
<th>Pos. sites (%)</th>
<th>Teeth (n=31)</th>
<th>Implants (n=43)</th>
<th>Pos. sites (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>P. gingivalis</td>
<td>9.7</td>
<td>2 2 1</td>
<td>1 2 2 2 3 4 4</td>
<td>16.3</td>
</tr>
<tr>
<td>P. intermedia</td>
<td>9.7</td>
<td>3 3 3</td>
<td>2 2 2 3 3 4 4</td>
<td>18.6</td>
</tr>
<tr>
<td>P. nigrescens</td>
<td>19.4</td>
<td>4 4 3 3 1 1</td>
<td>2 2 2 3 3 3 3 3 4 4 4</td>
<td>27.9</td>
</tr>
<tr>
<td>B. forsythus</td>
<td>16.1</td>
<td>3 3 2 2 2</td>
<td>2 2 2 3 4 4 4</td>
<td>18.6</td>
</tr>
<tr>
<td>A. a.</td>
<td>0</td>
<td></td>
<td>1 1 2 2</td>
<td>9.3</td>
</tr>
<tr>
<td>F. nucleatum</td>
<td>19.4</td>
<td>4 3 2 2 2 2 2</td>
<td>2 2 2 2 3 3 3 3 4 4</td>
<td>27.9</td>
</tr>
<tr>
<td>T. denticola</td>
<td>48.5</td>
<td>5 4 4 4 4 4 4 3 3 3 3 2 2 2 2 2</td>
<td>2 2 3 4 4 4 5 5 5 5 5 5 5 5 5 5</td>
<td>30.2</td>
</tr>
<tr>
<td>P. micros</td>
<td>29.0</td>
<td>3 2 2 1 1 1 1 1</td>
<td>1 1 1 1 1 1 2 2 2 2 2 2 3</td>
<td>34.9</td>
</tr>
<tr>
<td>C. rectus</td>
<td>9.7</td>
<td>2 1 1</td>
<td>1 2 3 3 3 3</td>
<td>14.0</td>
</tr>
<tr>
<td>E. corrodens</td>
<td>12.9</td>
<td>3 2 2 1</td>
<td>1 1 2 2 2 2 2 2 3</td>
<td>23.3</td>
</tr>
<tr>
<td>S. noxia</td>
<td>0</td>
<td></td>
<td>1 1</td>
<td>4.7</td>
</tr>
<tr>
<td>S. intermedia</td>
<td>35.5</td>
<td>3 3 3 2 2 2 2 2 2 2 2</td>
<td>1 1 1 2 2 2 2 2 2 2 2 3 3 3 3 3 4 4 4 4 5</td>
<td>58.1</td>
</tr>
</tbody>
</table>

No. positive samples: 15, 10, 5, 5, 10, 15, 20, 25.
Table 5. Visible plaque, gingivitis and probing pocket depth at five types of sites: failing implant sites: (FI)- i.e., implants with loss of surrounding bone, stable implants (SI)-in patients with both stable and failing implants, control implants (CI)-in those patients with stable implants alone, and teeth in patients ( TP) and controls (TC) (Paper V).

<table>
<thead>
<tr>
<th></th>
<th>Patients n=17</th>
<th>Controls n=19</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>SI</td>
<td>FI</td>
</tr>
<tr>
<td>No. of implants/teeth</td>
<td>53</td>
<td>45</td>
</tr>
<tr>
<td>Visible plaque Index (%)</td>
<td>31.3</td>
<td>33.7</td>
</tr>
<tr>
<td>Gingival Index</td>
<td>1.1 (0.6)</td>
<td>1.6 (0.4)</td>
</tr>
<tr>
<td>PPD (mm)</td>
<td>2.6 (0.9)</td>
<td>4.3 (1.0)</td>
</tr>
</tbody>
</table>
Table 6. The occurrence of 12 micro-organisms in subgingival samples from six types of sites: stable implants (SI), failing implants (FI), in patients with stable and failing implants and teeth in patients (TP) and controls (TC). Controls have been divided into dentate and edentulous subjects. Control implants in partly edentulous (CI-dent) and control implants in edentulous controls (CI-edent). A= % positive samples at each type of site, B= % samples of each type of site with ≥ 10⁶ bacteria in each sample (*Paper V*).

<table>
<thead>
<tr>
<th>Micro-organism</th>
<th>Partly edentulous patients n=17</th>
<th>Partly edentulous controls n=13</th>
<th>Edentulous controls n=6</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>SI n=14</td>
<td>TP n=17</td>
<td>CI-dent n=13</td>
</tr>
<tr>
<td></td>
<td>A</td>
<td>B</td>
<td>A</td>
</tr>
<tr>
<td><em>P. gingivalis</em></td>
<td>79</td>
<td>7</td>
<td>65</td>
</tr>
<tr>
<td><em>P. intermedia</em></td>
<td>50</td>
<td>21</td>
<td>77</td>
</tr>
<tr>
<td><em>P. nigrescens</em></td>
<td>93</td>
<td>14</td>
<td>100</td>
</tr>
<tr>
<td><em>B. forsythus</em></td>
<td>36</td>
<td>7</td>
<td>59</td>
</tr>
<tr>
<td><em>A. actinomycetemcomitans</em></td>
<td>93</td>
<td>0</td>
<td>94</td>
</tr>
<tr>
<td><em>F. nucleatum</em></td>
<td>100</td>
<td>7</td>
<td>100</td>
</tr>
<tr>
<td><em>T. denticola</em></td>
<td>43</td>
<td>14</td>
<td>41</td>
</tr>
<tr>
<td><em>P. micros</em></td>
<td>50</td>
<td>0</td>
<td>65</td>
</tr>
<tr>
<td><em>C. rectus</em></td>
<td>64</td>
<td>0</td>
<td>71</td>
</tr>
<tr>
<td><em>E. corrodens</em></td>
<td>50</td>
<td>0</td>
<td>53</td>
</tr>
<tr>
<td><em>S. noxia</em></td>
<td>7</td>
<td>0</td>
<td>12</td>
</tr>
<tr>
<td><em>S. intermedius</em></td>
<td>71</td>
<td>0</td>
<td>88</td>
</tr>
</tbody>
</table>
INFLAMMATION

In Papers I and II, crevicular fluid samples were collected from three types of sites: (1) crevices around teeth (GCF) and (2) implants (PISF-p) in partly edentulous patients, and (3) crevices around implants (PISF-t) in edentulous patients. The neutrophilic response, measured as functional and antigenic elastase, was assessed around teeth and implants in 25 partly edentulous and 25 edentulous patients. The mean values and standard deviation (SD) of the clinical data are shown in Table 7.

In Paper I, the amount of visible plaque in the partly edentulous group was very small, with mean plaque indices of 0.7 and 0.5 at teeth and implants, respectively. In edentulous patients, plaque index was higher than at implants in the partly edentulous. Clinical inflammation, probing pocket depth and crevicular fluid volume were similar between the three types of sites (Table 7). The elastase activity was significantly lower in samples from patients with implants alone (PISF-t) than in those from teeth (GCF) (p=0.0025) and implants (PISF-p) (p=0.0034) in partly edentulous patients (Figure 1A). The lactoferrin concentration was significantly lower in samples from implants in patients with implants alone (PISF-p) than in samples from teeth (GCF) (p=0.05) (Figure 1B). The protein concentrations showed no difference between the three types of sites (Table 7).

In paper II, the amount of visible plaque around natural teeth was significantly lower than around implants in the partly edentulous (p=0.0164). No difference in plaque index was detected around implants in partly edentulous compared to implants in edentulous. Gingival index and probing pocket depth were similar in both groups of patients (Table 7). The elastase activity was significantly lower in samples from patients with implants alone (PISF-t) than in those from implants (PISF-p) in patients with mixed dentitions (p=0.04) (Figure 1C). There was no difference between the three types of site in the elastase-α1-antitrypsin complex concentration.

In paper III, the marginal bone loss around 55 Brånemark implants, after 10 years of function in 15 partly edentulous patients, was evaluated on intraoral radiographs taken at the bridge connection and at the 10-year follow-up. The marginal bone level at implants is shown in Table 8. A mean marginal bone loss of 0.55mm (SD ± 0.73) mesially and 0.62 mm (SD ± 0.87) distally occurred during this period. The surfaces of implants close to remaining teeth were assessed separately to determine whether the presence of remaining teeth affected the marginal bone loss at implants. The mean bone loss at implant surfaces facing teeth was 0.58mm (SD±0.71). Figure 2 shows the distribution of radiographic bone change at all mesial and distal surfaces of the implants in the 15 subjects. Seventy-four percent of the surfaces measured remained free of marginal bone loss exceeding 1mm. At 4 implants (4.5% of the sites), the marginal reduction exceeded 2mm.

In paper V, neutrophil activation around implants and teeth in patients with peri-implantitis was compared to that in patients with stable implant conditions. We included 17 partly edentulous patients with a total of 98 implants, of which 45 showed marginal bone loss of ≥ three fixture threads after the first year of loading. 19 subjects with stable marginal tissue conditions served as controls. Crevicular fluid samples were collected from teeth and implants in patients and controls. Clinical characteristics of the site-categories in patients and controls are shown in Table 5. Elastase activity was higher at failing implants (FI) than at stable implants (CI) in controls (Fig. 3). Lactoferrin concentration was higher at failing implants (FI) than at stable ones (SI) in patients with peri-implantitis. Higher levels of both lactoferrin and
elastase activity were found at failing implants (FI) than at teeth (TP) in patients. Stable implants in patients and controls had similar levels of elastase activity and lactoferrin concentrations. The lactoferrin concentration tended to be lower around implants in edentulous patients (CI-edent), but the difference was not significant (Fig. 3).

Table 7. Mean values (SD) of clinical variables at the three types of sites: inflamed crevices around natural teeth (GCF) and implants (PISF-p) in partly edentulous patients. Inflamed crevices around implants in patients with implants alone (PISF-t) (*Papers I and II*).

<table>
<thead>
<tr>
<th>Parameter</th>
<th>GCF</th>
<th>PISF-p</th>
<th>PISF-t</th>
</tr>
</thead>
<tbody>
<tr>
<td>Paper I</td>
<td>9</td>
<td>16</td>
<td>9</td>
</tr>
<tr>
<td>Paper II</td>
<td>16</td>
<td>9</td>
<td>16</td>
</tr>
<tr>
<td>Patients (n)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Plaque index</td>
<td>0.7 (0.6)</td>
<td>0.7 (0.7)</td>
<td>0.5 (0.5)</td>
</tr>
<tr>
<td>Gingival index</td>
<td>1.4 (0.6)</td>
<td>1.5 (0.4)</td>
<td>1.6 (0.5)</td>
</tr>
<tr>
<td>Pocket depth (mm)</td>
<td>2.5 (0.6)</td>
<td>2.7 (0.7)</td>
<td>2.4 (0.5)</td>
</tr>
<tr>
<td>Protein conc. (µg/µl)</td>
<td>42 (24)</td>
<td>40 (22)</td>
<td>46 (41)</td>
</tr>
</tbody>
</table>

Table 8. Mean (SD) reduction in bone height in mm over 10 years. Distance between bone crest and fixture-abutment connection mesially, distally and at surface facing teeth at the time of functional load in 1987 and 10 years later (*Paper III*).

<table>
<thead>
<tr>
<th></th>
<th>Mesial</th>
<th>Distal</th>
<th>Surface facing teeth</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>n=55</td>
<td>n=55</td>
<td>n=22</td>
</tr>
<tr>
<td>1987 (mm)</td>
<td>1.4 (0.5)</td>
<td>1.2 (0.7)</td>
<td>1.2 (0.5)</td>
</tr>
<tr>
<td>1997 (mm)</td>
<td>1.9 (0.8)</td>
<td>1.8 (0.7)</td>
<td>1.7 (0.7)</td>
</tr>
<tr>
<td>Bone reduction (mm)</td>
<td>-0.6 (0.7)</td>
<td>-0.6 (0.9)</td>
<td>-0.6 (0.7)</td>
</tr>
</tbody>
</table>
Figure 1. (A) Mean (SEM) elastase activity per µl, (B) lactoferrin concentration in Paper I and (C) elastase activity in Paper II in exudate from crevices surrounding teeth (GCF) and implants (PISF-p) in partly edentulous patients and from crevices surrounding implants (PISF-t) in totally edentulous patients. Differences assessed with the Mann-Whitney U-test.
Figure 2. Radiographic change in marginal bone level around 55 implants during 10 years of functional load (Paper III).
Figure 3. (A), Elastase activity (mAbs/µl) and (B) lactoferrin concentrations (ng/µl) in crevicular fluid from stable implants (SI), failing implants (FI) in patients with stable and failing implants and teeth in patients (TP) and controls (TC). Control implants in partly edentulous controls (CI-dent) and control implants in edentulous controls (CI-edent). P values in patients were calculated with the Wilcoxon signed-rank test. P values comparing patients and controls were calculated with the Mann-Whitney U-test (Paper V).
PERIODONTITIS

Paper IV

One hundred and forty-three consecutively treated patients were retrospectively evaluated after five years of functional load of implants. Marginal bone loss around implants and teeth was determined on intraoral and panoramic radiographs. The patients were divided into two groups: (1) 59 partly edentulous with implants and naturally remaining teeth in the same jaw, mixed jaw group (MJG) and (2) 84 edentulous in one or both jaws, full jaw group (FJG).

No correlation was found between bone loss around implants and teeth during the five years of function of implants (Figure 4). During the five years of observation, only very limited bone loss had occurred around implants and the natural teeth. After five years, mean marginal bone loss around implants of 0.75 (SD 0.85) fixture threads (0.6mm between threads) were found in the MJG and of 0.77 (SD 0.58) fixture threads in the FJG. The radiographic bone loss around implants was similar in both groups. The bone reduction, around implants was significantly greater in the maxilla than in the mandible in the MJG, but there was no difference between the maxilla and mandible in the FJG (Table 9).

Evaluation of marginal bone loss around the naturally remaining teeth was performed on panoramic radiographs taken at the time of bridge connection and at the five-year follow-up. Hardly any bone loss was noted in the natural dentition during the five years of observation (i.e. 2-7% of the root length).

Edentulous patients (FJG) lost significantly more bone at the remaining teeth than the partly edentulous (MJG). In the FJG, bone loss at teeth was more marked in those who had lost ≥ 25% of the supporting marginal bone at the beginning of the study. This was not the case in the MJG (Table 10).
Figure 4. Individual mean of bone loss at fixtures and teeth during five-years of functional load of implant-anchored prosthesis. Totally edentulous patients were excluded. *3 patients in FJG lost all remaining teeth during the five-year follow up (Paper IV).

RL = % of root length.
Table 9. Mean marginal radiographic bone loss at implants during five-years of function in patients with teeth and implants in the same jaw (MJG) and edentulous jaws (FJG). Bone loss at fixtures is expressed as the mean number of exposed fixture threads (0.6mm) above the marginal bone-crest (Paper IV).

<table>
<thead>
<tr>
<th>Jaw</th>
<th>MJG 59 patients</th>
<th>FJG 84 patients</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Maxilla</td>
<td>Mandible</td>
</tr>
<tr>
<td>No. of fixtures</td>
<td>115</td>
<td>124</td>
</tr>
<tr>
<td>Bone loss</td>
<td>1.0 (1.0)</td>
<td>0.5 (0.6)</td>
</tr>
<tr>
<td>p-value</td>
<td>0.015</td>
<td>0.423</td>
</tr>
</tbody>
</table>

p values calculated with Mann-Whitney U-test

Table 10. Mean (SD) marginal bone loss, expressed as % of total root-length, in patients classified as having no periodontitis (NP), with a mean marginal bone loss in the naturally-remaining dentition < 25% of root-length (RL) in 1992, and in patients classified as periodontitis patients (P), with a mean marginal bone loss of ≥ 25% of RL in 1992. Twenty-six patients edentulous in both jaws were excluded (Paper IV).

<table>
<thead>
<tr>
<th></th>
<th>MJG</th>
<th>FJG</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>NP</td>
<td>P</td>
</tr>
<tr>
<td>No. of patients</td>
<td>40</td>
<td>19</td>
</tr>
<tr>
<td>Bone loss in 1992</td>
<td>14 (7)</td>
<td>31 (6)</td>
</tr>
<tr>
<td>p-value</td>
<td>&lt;0.001</td>
<td>0.028</td>
</tr>
<tr>
<td>Bone loss during 5 years</td>
<td>2 (3)</td>
<td>3 (3)</td>
</tr>
<tr>
<td>p-value</td>
<td>0.511</td>
<td>0.004</td>
</tr>
</tbody>
</table>

p values calculated with Mann-Whitney U-test
Patient selection was based on radiographic evaluation of the marginal bone level, clinical signs of inflammation and/or the presence of pus. Compared to the one-year intra-oral radiographic examination, patients had at least one implant showing radiographic marginal bone loss of \( \geq \) three fixture threads (1.8mm) mesially or distally. 17 partly edentulous patients with 45 implants showing a marginal bone loss of \( \geq \) three fixture threads after the first year of loading and 19 controls with stable marginal tissue conditions were included.

A venous blood sample was taken from patients and controls to assess their general health and systemic markers of inflammation. Crevicular fluid samples were collected from teeth and implants in patients and controls. Since elastase activity and lactoferrin concentration are regarded as markers of inflammation, the level of IL-1\( \beta \) was chosen as a possible marker of host-response.

Clinical characteristics of the five types of sites sampled are presented in Table 5. Acute-phase reactants in plasma and blood counts were within normal range in both groups. Patients had higher white blood cells counts and CRP levels than controls, but the difference was not significant. Clinical inflammation and probing pocket depth were significantly greater at failing implants than at stable implants in patients and controls (Table 5). Neutrophil activity was also higher at failing than at control implants in healthy controls. Stable implants in patients and controls showed similar levels of elastase and lactoferrin concentrations (Table 11). The concentrations of IL-1\( \beta \) were about the same in the various sites (Fig. 5).
Table 11. Mean (SD) concentrations of lactoferrin, IL-1β and the elastase activity in crevicular fluid from five types of sites. Stable implants (SI), failing implants (FI), i.e implants with loss of surrounding bone, in patients with both stable and failing implants, control implants (CI) in subjects with only stable implants and teeth in both patients (TP) and controls (TC). Control subjects were divided into dentate and edentulous subjects. Control implants in partly edentulous controls (CI-dent) and control implants in edentulous controls (CI-edent) (Paper V).

<table>
<thead>
<tr>
<th></th>
<th>Partly edentulous patients n=17</th>
<th>Partly edentulous controls n=13</th>
<th>Edentulous controls n=6</th>
</tr>
</thead>
<tbody>
<tr>
<td>Lactoferrin (ng/µl)</td>
<td>SI 192 (180) FI 307 (207) TP 111 (99)</td>
<td>CI-dent 199 (197) TC 188 (168)</td>
<td>CI-edent 133 (243)</td>
</tr>
<tr>
<td>Elastase activity (mAbs/µl)</td>
<td>SI 250 (562) FI 474 (437) TP 142 (128)</td>
<td>CI-dent 110 (160) TC 155 (182)</td>
<td>CI-edent 85 (84)</td>
</tr>
<tr>
<td>IL-1β (pg /µl)</td>
<td>SI 27 (16) FI 42 (43) TP 32 (20)</td>
<td>CI-dent 28 (23) TC 26 (11)</td>
<td>CI-edent 13 (5)</td>
</tr>
</tbody>
</table>
Figure 5. Interleukin-1β concentrations (pg/µl) in crevicular fluid from: stable implants (SI), failing implants (FI), in patients with stable and failing implants and teeth in patients (TP) and controls (TC). Controls were divided into two groups according to their dental status-i.e., implants in partly edentulous controls (CI-dent) and implants in edentulous controls (CI-edent) (Paper V).
SMOKING

*Paper IV*

In 143 consecutively treated patients we evaluated retrospectively the effects of smoking on the occurrence of late fixture losses and marginal bone loss around teeth and implants after five years of function of implants. Patients were divided into two groups: (1) 59 partly edentulous patients with implants and teeth in the same jaw- i.e., the mixed jaw group (MJG) and (2) 84 patients edentulous in one or both jaws- i.e., the full jaw group (FJG). The distributions of age and of non-smokers and smokers in the MJG and FJG are shown in Figure 6. Categorisation of smokers and non-smokers was based on the self-reported medical history of the patient. In both groups, the smokers were younger than the non-smokers. No correlation was found between smoking and a more marked bone loss at implants or teeth (Table 12).

At the time of bridge connection, we found no difference in the number of remaining teeth between smokers and non-smokers in MJG or FJG. However after five years, smokers in FJG had fewer remaining teeth than non-smokers. The total number of teeth and of extracted teeth are shown in Table 13.

Seven of the 9 patients who lost implants after loading were smokers; these accounted for 13 of the failing fixtures. Only 15 of 744 (2%) were lost during the five years of function. Thirteen fixtures failed in the edentulous patients (FJG) and two among the partly edentulous (MJG). Four times more implants failed in the maxilla than in the mandible. Twelve fixtures were lost in the maxilla and three in the edentulous mandible. Seven of the 15 lost fixtures were lost during the first year of function.

Bone reduction around remaining implants was significantly greater in patients who had lost implants after loading (mean 2.0 ± 1.7 fixture threads) than in those who had not experienced implant loss (mean 0.7± 0.7 fixture threads), $p=0.003$. 


Figure 6. Age of partly edentulous patients with implants and teeth in the same jaw (MJG) and of patients edentulous in one or both jaws (FJG) at the time of the five-year follow-up. S=smokers and NS=non-smokers. P values were calculated with the Mann-Whitney U-test (Paper IV).
Table 12. Bone loss around fixtures in smokers (S) and non-smokers (NS). Mean (SD) marginal bone loss during 5 years of function. Bone loss expressed as mean number of exposed fixture threads (0.6mm) above the marginal bone crest (Paper IV).

<table>
<thead>
<tr>
<th></th>
<th>MJG n=59</th>
<th>FJG n=84</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>N</td>
<td>S</td>
</tr>
<tr>
<td>Patients (n)</td>
<td>40</td>
<td>19</td>
</tr>
<tr>
<td>Bone loss</td>
<td>0.76(0.87)</td>
<td>0.71(0.82)</td>
</tr>
<tr>
<td>p value</td>
<td>0.935</td>
<td></td>
</tr>
</tbody>
</table>

Table 13. Mean (SD) number of naturally-remaining teeth and number of teeth extracted during five years in partly edentulous (MJG) patients and those edentulous in one jaw (FJG). Patients totally edentulous in 1992 were excluded (Paper IV).

<table>
<thead>
<tr>
<th></th>
<th>MJG</th>
<th>FJG</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Smokers</td>
<td>Non-smokers</td>
</tr>
<tr>
<td>Patients (n)</td>
<td>19</td>
<td>40</td>
</tr>
<tr>
<td>Mean no. of teeth *</td>
<td>15.5 (6.0)</td>
<td>15.8 (3.9)</td>
</tr>
<tr>
<td>Total no. of teeth *</td>
<td>259</td>
<td>631</td>
</tr>
<tr>
<td>No teeth extracted (%)</td>
<td>20 (6.4)</td>
<td>39 (5.8)</td>
</tr>
</tbody>
</table>

*At five-year examination; † -Significant difference between smokers and non-smokers in mean number of naturally remaining teeth at the five-year examination (P=0.0014), using the Mann-Whitney U-test.
GENERAL DISCUSSION

Screw-shaped titanium implants are routinely used today as substitutes for lost teeth. In view of the chronic nature of diseases like periodontitis, our knowledge of long-term survival and maintenance of implants is still limited. This thesis concerns some of the biological factors related to the long-term survival and maintenance of dental implants.

MICROBIOTA

In Paper II facultative anaerobic Gram-positive cocci were the main cultivable bacteria around the three types of sites—i.e., implants and teeth in partly edentulous and around implants in edentulous patients. This finding is in agreement with several studies of the microbiota in stable implant conditions (Adell et al. 1986, Apse et al. 1989, Bower et al. 1989, Mombelli et al. 1988, Mombelli & Mericske-Stern 1990).

No significant difference was found in the microbiota of the three types of sites. Although the frequency of black pigmenting bacteria was lower in edentulous than in partly edentulous patients, the difference was not significant and might be due to the wide variation in microbial counts commonly found in cultivational studies. In patients with stable implants, our method of culture may not have been sensitive enough to reveal the difference between partly edentulous and edentulous patients, but it may also have been because the microbiota are similar in periodontal / peri-implant health. Probing pocket depth, shown by others to influence the occurrence of cocci, motile rods and spirochetes around implants (Papaioannou et al. 1995) were shallow and of similar depth around the three types of sites.

In contrast to our findings, several studies have demonstrated a difference in microbiota around implants placed in edentulous and partly edentulous cases (Apse et al. 1989, Sanz et al. 1990, Quirynen & Listgarten 1990). This difference may reflect not only the presence, but also the periodontal status of the naturally-remaining dentition (Papaioannou et al. 1996, Quirynen et al. 1996). In the present study, a microflora compatible with periodontal health was found around the natural teeth of the partly edentulous. The healthy periodontal conditions in these patients, with minimal or moderate bone loss at the natural teeth, may have influenced that no differences was observed in the microbiota between the edentulous and partly edentulous.

In Paper III, the microbial analysis of bacterial samples in 15 successfully-treated partly edentulous patients revealed no major differences in the microflora around teeth and implants after 10 years’ function of implants. Several studies of patients with mixed dentitions have reported similarities in the microbiota colonising implants and teeth (Lekholm et al. 1986, Sanz et al. 1990, Kohavi et al. 1994).

The periodontal pathogens associated with periodontal disease, P. gingivalis, P. intermedia, B. forsythus and T. denticola were present at both implants and teeth but A. actinomycetemcomitans was found only at implants (4 sites). These periodontal pathogens tended to be present at sites with marginal bone loss exceeding 2mm. However, since marginal bone loss around all 55 implants was very limited, with a mean (SD) marginal bone loss of 0.6 mm, the number of sites with bone loss exceeding 2mm were too few for statistical comparison.

Although the probing pocket depths on the distobuccal, lingual and distolingual surfaces of implants were significantly greater than on the same surfaces at “corresponding teeth” they
were not significantly deeper on the buccal, mesiobuccal and mesiolingual surfaces. The clinical inflammation was similar around teeth and implants. In accordance with Paper II, no major differences in the microbiological profiles were found between teeth and implants corresponding to comparable levels of clinical inflammation. The degree of inflammation and the pocket depth determine the types of bacteria that develop in the subgingival plaque in naturally occurring gingivitis. Deep inflamed pockets select increased numbers and higher proportions of spirochetes, motile rods and gram-negative species (Haffajee & Socransky 1994). The small difference in probing pocket depth between “corresponding teeth” and implants may have been responsible for the higher percentage of positive samples at implants that at teeth.

The bacterial analyses in these patients corroborate the findings of several studies indicating bacterial transmission from teeth to implants (Apse et al. 1989, Mombelli et al. 1995). Colonisation of newly-inserted implants by periodontal pathogens occurs as early as one month after implantation. Longitudinal studies of transmucosal implants in partly edentulous patients show that the implant attracts a microbiota comparable to that colonising the remaining natural teeth in the same subject (Mombelli et al. 1995, Gouvoussis et al. 1997, van Winkelhoff et al. 2000). Although implants and teeth harboured the same periodontal pathogens, the bone loss during the 10 years of functional load was limited and these periodontal pathogens were present even when no bone loss was seen. As shown in longitudinal studies of partly edentulous patients, as long as the numbers of these potential pathogens are kept at a low level, the risk of bone loss is limited (Shordone et al. 1999).

In Paper V, the microbiological characteristics of patients with marginal bone loss and infection - i.e., peri-implantitis were studied and compared to healthy controls without signs of marginal bone loss after the first year of function. The DNA probe analysis of 12 selected microorganisms showed that the specific periodontal pathogens, *P. gingivalis, P. intermedia, B. forsythus, A. actinomycetemcomitans* and *T. denticola*, were present at all types of sites in patients and controls. These pathogens have been shown to be present at teeth and implants in longitudinal studies of patients treated for moderate-to-severe periodontal disease (Leonhardt et al. 1993, Sbordone et al. 1999). Leonhardt et al. followed the colonisation of newly-inserted implants and teeth for three years. At the end of the follow-up period, the periodontitis marker bacteria were as frequent around fixtures as around the patient’s natural teeth, which accords with the findings in patients and controls in this study.

In this study the implants had functioned > 6 years, which may have affected the high number of positive samples of periodontal pathogens in both groups. Unlike Danser et al. (1995), who suggested that the elimination of natural teeth in patients with a history of periodontitis eliminated the occurrence of *P. gingivalis and A. actinomycetemcomitans* in edentulous cases, the edentulous controls demonstrated the same bacterial species. *P. gingivalis and A. actinomycetemcomitans* were detected in 83 per cent and 100 percent of the implant sulci among the edentulous controls. Although both of these species are rarely detected in edentulous cases they can be recovered from oral mucosal surfaces, dorsum of the tongue and the tonsils (Asikainen et al. 1991, Müller et al. 1993, van Steenberghen et al. 1993). The edentulous controls in this study harboured a microflora similar that of the dentate subjects. The periodontitis-associated microflora found in both patients and controls may suggest that the frequency of peri-implantitis in patients with a history of periodontitis is not uncommon.

The only differences between healthy controls and patients were samples with bacteria exceeding ≥ 10^6 cells in a sample. Since probing pocket depths were significantly deeper at failing implants than at non-diseased sites in patients and controls, this is not a surprising finding. Deeper pockets select for an increased number of anaerobic gram-negative bacteria.
Both patients and controls harboured the selected marker bacteria which shows that the presence of periodontal pathogens do not necessarily cause tissue damage. It is generally accepted that higher levels of bacteria must be present for extended periods of time to cause tissue damage (Socransky & Haffajee 1991, Socransky & Haffajee 1992). However, colonisation of periodontal pathogens above threshold levels significantly increased the probability that the subjects for individuals to harbour deep pockets or progressing tooth sites (Papapanou et al. 1997). In patients with peri-implantitis, samples with \( \geq 10^6 \) bacterial cells were found at stable and diseased implant sites, indicating that a total increase in bacterial burden was present in patients with peri-implantitis.

In contrast to the present study, a specific microbiological profile has been found comparing healthy and diseased implant sulci. Failing sites have demonstrated an infection characterised by microbial species implicated in periodontitis (Mombelli et al. 1987, Becker et al. 1990, Rosenberg et al. 1991, Augthun et al. 1997, Sbordone et al. 1995, Listgarten et al. 1999). In these patients no distinct differences were seen between healthy and diseased sites. A periodontitis-associated microflora was found at stable and failing sites. As shown by Leonhardt et al. (1999), Staphylococcus spp, enterics and Candida spp were found in 55% of the 37 patients with peri-implantitis lesions. This study also showed a significantly higher number of samples positive for \( A. \text{actinomycteumcomitans} \) in patients with peri-implantitis than in healthy controls. The identification of other species such as Staphylococcus spp. and candida spp might have been of value in distinguishing between diseased and healthy sites in the present study.

The patients with peri-implantitis were found to have a microbiological profile of adult periodontitis at both healthy and diseased sites. The total bacterial burdens together with other factors such as loading, anatomical and local host-response may contribute to the destructive process in peri-implantitis.

**INFLAMMATION**

In *papers I and II*, significantly greater elastase activity and higher lactoferrin concentrations were found around implants in partly edentulous than around implants in edentulous. There was no difference in elastase activity and lactoferrin concentrations between teeth and implants in the partly edentulous. This accords with the findings of Adonogianaki et al. (1995), who compared acute-phase proteins (\( \alpha-2 \)-macroglobulin, \( \alpha-1 \)-antitrypsin, transferrin and lactoferrin) and IgG against *P. gingivalis*. They found no differences in absolute amounts of proteins between teeth and implants in the partly edentulous. On the other hand, Boutros et al. (1996) compared several neutrophil-derived enzymes in crevicular fluid and found no difference between implants in edentulous and partly edentulous patients. This discrepancy may be partly due to differences in the laboratory handling of the samples and elution of the strips.

Although the plaque index was higher around implants in the edentulous in *Paper I* and around teeth in partly edentulous cases in *Paper II*, there was no difference between the three types of sites in clinical inflammation or probing pocket depth. Since clinical assessment of inflammation around implants can be difficult (Mombelli & Lang 1994) the sulcular fluid volume (Niimi & Ueda 1995, Bheneke et al. 1997) and the protein concentrations (Bang & Cimasoni 1971) were measured. No difference in clinical inflammation was detected with these methods.
The low molecular weight substrate (445.5 Da) used to determine elastase activity is hydrolysed either by free elastase or elastase in complex with α-2-macroglobulin (A2MG) (Travis & Salvesen 1983). Since free elastase cannot be recovered from the paper strips used for crevicular fluid collection (Gustafsson et al. 1996), the higher elastase activity found around implants in partly edentulous cases in Paper I indicated a higher concentration of elastase-A2MG complex. This could be due to a shift from α-1-antitrypsin to A2MG inhibition of the elastase and/or a higher extracellular release of elastase (Cox 1995).

In Paper I, we found a higher lactoferrin concentration around implants in partly edentulous cases, suggesting that the latter explanation was correct. In Paper II, the concentration of elastase was assessed both functionally and immunologically to evaluate the proportions of elastase bound to the two inhibitors. Since the same standard was used in the functional and the immunological assays we could compare the concentration of elastase in complex with both A2MG and A1AT. The concentrations of elastase-A1AT complex were similar in the three types of sites, indicating that the higher elastase activity found around implants in partly edentulous cases was caused by a greater inhibition by A2MG, together with an increased extracellular release of elastase. The greater inhibition of elastase by A2MG could be due to oxidative inactivation of A1AT by reactive oxygen species generated from the neutrophils (Weiss 1989). In patients with periodontitis, peripheral neutrophils release more free oxygen radicals after Fcγ-receptor stimulation, which might explain the higher levels of elastase activity in GCF in periodontal tissue destruction (Gustafsson et al. 1996).

In Paper II, no significant differences were found in the microbiota between edentulous and partly edentulous patients which would explain the stronger neutrophilic reaction found at implants in the mixed dentition. A small but not significant difference in proportions of black pigmenting Bacteroides was seen at implants in partly edentulous and edentulous patients – i.e., two samples being positive in edentulous and six samples from implants and teeth in partly edentulous patients. A greater difference in the microbiota might have been found with a more sensitive method for bacterial detection, which would have explained the difference in inflammatory reaction between implants in partly edentulous and edentulous patients.

An alternative explanation is that the neutrophil activity was lower in samples from edentulous patients because of an altered host-response due to the complete absence of a normal periodontium. The inflammatory reaction in the gingival crevice, in contrast to the peri-implant mucosa surrounding implants, may release signal substances - for example, cytokines that cause a higher reactivity of circulating neutrophils. The process enhancing the neutrophil’s ability to respond to a second stimulus - e.g., the bacteria in the gingival crevice - has been called priming (Steinbeck & Roth 1989). Berglundh et al. (1994), in characterising the vascular topography in the peri-implant mucosa, found a connective tissue compartment between the alveolar bone and the apical portion of the junctional epithelium almost devoid of vascular structures. This morphological difference may influence the local host-response.

In Paper III, hardly any marginal bone loss occurred during 10 years of function around 55 Brånemark implants – i.e., 74% of the surfaces remaining free of radiographic bone loss exceeding 1 mm and only at 4 implants (4.5 % of the sites) showing bone loss more than 2mm. Bone reduction tended to be greater in the maxilla but the difference was not significant. This accords with the findings of Lekholm et al. (1999) who, in a prospective multicentre study of implants in partly edentulous patients found a marginal bone loss of 0.7mm in both the maxilla and mandible after 10 years of function. In 7% of the sites bone loss exceeded 2mm which is a slightly higher percentage than in the present study and may be due to the larger number of patients included in their multicentre study. Similar results have
been reported in partly edentulous patients with the same implant system after long-term function (Jemt & Lekholm 1993, Gunne et al. 1999).

In our study only a few sites around implants and teeth had a probing pocket depth of ≥ 4mm, indicating clinically healthy conditions in the mixed dentition. The marginal bone level around implants after a functional period of 10 years was similar to that reported of the same study population after five years showing that no significant change in marginal bone loss had occurred during the additional 5 years (Lekholm et al. 1994). The bone reduction found in the present study is less than that reported in periodontally compromised patients and suggests that the study population is periodontally stable, since clinical findings in the remaining teeth showed minimal signs of periodontitis (Ellegård et al. 1997).

In contrast to our findings in Papers I and II showing a greater inflammatory reaction around implants in the mixed dentition, the present study shows hardly any bone loss around implants in the partly edentulous during 10 years of function. Other factors such as overload and parafunction have been reported to affect marginal bone loss during function (Lindqvist et al. 1988, Quirynen et al. 1992). In the present study of well-maintained, partly edentulous patients with limited clinical inflammation around teeth and implants, a mean marginal bone loss around implants of 0.6mm occurred during 10 years of function. This bone loss is similar or even less than that reported in edentulous jaws (Lindqvist et al. 1997, Adell et al. 1981).

In paper V, significantly greater neutrophil activation was found around failing implant sites in patients with peri-implantitis than that around stable implants in healthy controls. This correlated to more clinical inflammation and greater probing pocket depth at peri-implantitis sites. The lactoferrin concentrations were significantly higher around failing implants than at stable implants and remaining teeth in patients with peri-implantitis. This indicates that the greater number of neutrophils at failing implant sites cause proportionally more extracellular release of elastase. Elastase activity has been shown to correlate to periodontal tissue destruction while lactoferrin, a reliable marker of the number of neutrophils reflects the degree of inflammation (Gustafsson et al. 1994, Adonogianaki et al. 1993). Lactoferrin concentrations tended to be lower around implants in the edentulous controls, supporting our previous findings of a more marked inflammatory reaction around implants in partly edentulous than that around implants in the edentulous.

In the present study, the microbiological load of periopathogenic bacteria was greater in patients with peri-implantitis than in healthy controls. However, both failing and stable implant sites harboured increased levels of periopathogenic species. The teeth of patients and controls showed similar lactoferrin concentrations and elastase activity which correlates with similar amounts of bacteria around natural teeth in patients and controls. Increases in probing pocket depth, clinical inflammation and elevated numbers of periopathogens seem to indicate that a local bacterial-driven inflammatory reaction may be responsible for the tissue destruction seen at failing implants. In long-term evaluation of edentulous mandibles, a small but significantly greater bone reduction was seen in patients with greater plaque accumulation (Lindqvist et al. 1997). Similar levels of lactoferrin concentrations and elastase activity at stable implants in patients and controls does not indicate a specific host-response in these patients with peri-implantitis as has been suggested as possible etiological factors for late implant failures (Salcetti et al. 1997).
PERIODONTITIS

In paper IV, a limited marginal bone loss was observed around implants and the naturally remaining teeth. The amount of bone loss around implants that occurred during the five years of function accords with that reported in partly edentulous and edentulous jaws treated with dental implants (Adell et al. 1990, Albrektsson et al. 1988, Jemt & Lekholm 1993, Gunne et al. 1999, Bheneke et al. 2000). The amount of bone loss differed between the maxilla and the mandible in partly edentulous, but not in edentulous jaws. This finding may indicate that the greater occluding forces on implants in the posterior regions (Book 1992) may affect implants placed in the posterior parts of the maxilla, with a poorer bone-quality.

In accordance with this view, Jemt & Lekholm (1993) showed a small but significant difference between the maxilla and mandible in 70 partly edentulous jaws with posteriorly placed implants. In clinical studies, loading has been shown to affect marginal bone loss around implants (Lindqvist et al. 1988, Quirynen et al. 1992). There was no such difference in marginal bone loss around implants between the maxilla and mandible in edentulous jaws possibly reflecting the more anterior positioning of implants in edentulous jaws.

Little marginal bone loss was occurred in the natural dentition during the five years of observation. Our findings of a marginal bone loss of 2-7% of the root-length accords with that of others of well-maintained patients treated for severe periodontal disease (Lindhe et al. 1984). Three patients edentulous in one jaw (FJG) lost all remaining teeth during the observation period. This was not due to a significant progression of periodontal disease, but rather to an already compromised periodontal situation on entry in the study. Prosthetic complications in retaining conventional bridges with a limited periodontal support resulted in the extraction of these teeth.

Although the bone loss around remaining natural teeth was very small, a significant difference in marginal bone loss at remaining teeth was observed between patients with implants and teeth in the same jaw (MJG) and patients edentulous in one jaw (FJG). The greatest bone loss at teeth occurred in patients edentulous in one jaw with a mean marginal bone loss of ≥ 25% at the beginning of the observation period. This finding accords with studies of occlusion forces applied on teeth with a reduced periodontal support (Lindhe & Svanberg 1974, Eriksson & Lindhe 1982). However, the limited bone loss that occurred around the natural teeth in the patients edentulous in one jaw (FJG) might be regarded as the response of a reduced periodontal support to the increased occlusion forces imposed by a full jaw implant anchored bridge.

No correlation was found between bone loss around implants and bone loss around teeth. When periodontitis was evaluated in a “historical perspective”, it was not found to affect the marginal bone loss that occurred around implants. The greatest marginal bone loss at teeth was seen in patients edentulous in one jaw with a mean marginal bone loss at the beginning of the study of 25% or more of the root length. This group did not show a more marked bone loss around implants.

Although host-response factors may play a role in implant maintenance (Consensus report, Proceedings of the 1996 World Workshop in Periodontology), treatment of periodontally-compromised patients with osseointegrated dental implants have proved to be successful (Ellegård et al. 1997). The present findings corroborate with those in periodontally-compromised patients treated with dental implants and show that a history of periodontitis does not affect the marginal bone loss at implants in periodontally-stable implant patients (Nevins & Langer 1995, Mengel et al. 1996, Buchman et al. 1999, Sbordone et al. 1999).
PERI-IMPLANTITIS

In Paper V, greater neutrophil activation was found in crevicular fluid around implants with loss of marginal bone and clinical signs of infection than that around stable implants in healthy controls. The levels of IL-1β were similar around stable and diseased sites. Stable implants in patients with peri-implantitis and healthy controls showed about the same levels of lactoferrin and elastase activity in crevicular fluid, indicating a site-specific inflammatory reaction around implants with marginal bone loss. Contradicting to the present findings of a site-specific bacterial-driven inflammatory reaction around failing implants, Salcetti et al. (1997) reported an aberrant host-response in patients with peri-implantitis, compared to patients with only successful and clinically healthy implants. The crevicular fluid levels of IL-1β were higher at stable and diseased implant sites in patients with peri-implantitis than at non-diseased implant sites in healthy controls. This finding accords with the generally-accepted concept of a hyperinflammatory phenotype in patients with periodontal disease. Hyperreactive neutrophils have shown to distinguish between patients with chronic inflammation and patients with periodontal tissue destruction. (Gustafsson et al. 1994). Higher levels of IL-1β in crevicular fluid have been reported in patients with periodontitis regardless of the severity of tissue destruction, than in those with chronic gingivitis alone (Figuredo et al. 1999).

Although the exact reasons for tooth extraction are difficult to evaluate retrospectively, it seems likely that most patients and controls included in this study had a history of periodontal disease. A microbiota similar to that of chronic adult periodontitis was found in both patients and controls.

Contradicting with our findings, higher levels of IL-1β have been found in crevicular fluid from diseased implant sites than in that from stable implant sites (Kao et al. 1995, Curtis et al. 1997). Panagakos et al. (1996) compared the crevicular fluid levels of IL-1β from implant sites divided into healthy, early peri-implantitis and advanced peri-implantitis lesions. The difference between early and advanced implant lesions was based on the amount of radiographic bone loss that had occurred. Implants that were diagnosed as having early peri-implantitis had higher levels of IL-1β than did those having advanced lesions. The authors ascribed this difference to the more acute nature of early peri-implantitis versus the more chronic nature of advanced lesions.

In the present study increases in probing pocket depth, clinical inflammation and numbers of periopathogens seem to indicate that a local bacterial-driven inflammatory reaction may be responsible for the tissue destruction seen at failing implants. Similar levels of elastase activity, IL-β and lactoferrin concentrations at stable implants in patients and controls did not indicate a specific host-response in the present group of patients with peri-implantitis. A polymorphism of the gene encoding for interleukin-1β (allele 2 of IL-1β at +3953) has been shown to correlate with an increased risk of severe periodontitis (Kornman et al. 1997, Gore et al. 1998, McGuire et al. 1999). Individuals positive for this allele produce up to four times more IL-1β (Pociot et al. 1992) Wilson et al. (1999) failed to correlate this genotype with early or late implant failures. Mombelli et al. (1987) regarded peri-implantitis as a site-specific infection having many features in common with chronic adult periodontitis.

Although this study did not show a specific microbiological profile at failing implants, a greater bacterial burden was found in patients with peri-implantitis. In a recent study of non-submerged ITI implants in the treatment of edentulous upper jaws, the results after one year of loading indicated that peri-implantitis occurred in 7.2% of the inserted fixtures (Åstrand et al. 2000). The bone loss that occurred around 12 of the 167 inserted implants before bridge connection might have been due to the negative effect of dentures worn during initial healing.
on both loading and microbiology. Several experimental studies, have shown that the
placement of submarginal ligatures and plaque accumulation can induce peri-implantitis
lesions (Lindhe et al. 1992, Lang et al. 1993). Anti-microbial therapy in conjunction with flap
elevation and debridement has shown to resolve the inflammatory lesion and create new bone-
1999). Re-osseointegration occurs where a pristine implant component is placed in the bone
defect after surgical debridement (Persson et al. 2001).

Peri-implantitis may have a multifactorial background. Although the present study
failed to show a hyperinflammatory trait in patients with peri-implantitis this may set off the
initiation of tissue destruction around implants in a few patients. Clustering of implant losses
in certain individuals can be interpreted as an indication of systemic or host-related factors of
importance for fixture losses (Weyant & Burt 1993). In some case reports aggressive forms of
periodontitis, have been associated with rapidly progressing peri-implantitis (Malmström et
al. 1990, Fardal et al. 1999). In patients treated for periodontal disease, a complex interaction
between the host-response and the periodontally-associated microflora may thus constitute a
risk of development of peri-implantitis.

SMOKING

In Paper IV, smoking was not found to associate with bone loss around implants or teeth,
which may reflect the limited bone loss that occurred during the study. Lindqvist et al. (1996,
1997) showed that marginal bone loss was significantly correlated with smoking in patients
wearing mandibular-fixed prostheses. However, the mean differences between smokers and
non-smokers was only 0.8 mm over a 10-year period. A difference between smokers and non-
smokers might have been found in the present study, if the observation period had been
longer. The additional effect of smoking and poor oral hygiene were not within the limits of
the present study, but may have influenced bone loss in smokers with poor oral hygiene.
Little marginal bone loss occurred in the natural dentition during the study period (i.e., 2-7% of RL), which is in accordance with other studies of well-maintained and periodontally stable
patients (Lindhe 1984). A reduced alveolar bone-height in smokers compared to non-smokers
has frequently been reported (Bergström & Eliasson 1987, Bolin et al. 1986, Bolin et al.
1993). A direct association between smoking and periodontal bone loss has also been found
(Haber 1993). Notwithstanding, smoking was not found to induce bone loss in the remaining
dentition of the patients in the present study. However, smoking was related to an increase in
the number of teeth extracted during the study in patients edentulous in one jaw, which
accords with several studies concerning risk factors and tooth loss (Krall et al. 1997, Axelsson
et al. 1998). Smokers in the two study groups edentulous and partly edentulous, were younger
than other subjects which indicates more tooth loss in smokers.

In this study, a significantly greater bone reduction was observed around remaining and
stable implants in patients who had lost implants after loading. Since the study design was
retrospective, it is difficult to determine the reason for a greater marginal bone loss at the
remaining implants in patients who had lost implants. However, this finding suggests that
marginal bone loss around implants could be related to patient-associated factors such as
inflammatory response and smoking. Haas et al. (1996) found, in a retrospective study of
1366 implants, more marginal bone loss and peri-implant tissue inflammation in smokers than
in non-smokers. In the present study, seven of the nine patients who lost implants after
loading were smokers with most fixture losses occurring in the edentulous maxilla.
MAIN FINDINGS

- The inflammation around implants in partly edentulous patients induced a stronger neutrophil reaction than did the inflammation around implants in edentulous patients albeit a similar degree of clinical inflammation and in essence no difference in microbiota (Paper I, II).

- Marginal bone loss around implants after long term function (10 years) in partly edentulous patients was limited and comparable to that in edentulous jaws. There was no major difference in the microbiota colonising teeth and implants in partly edentulous patients (Paper III).

- Marginal bone loss around remaining fixtures was greater in patients who experienced fixture loss after loading. Only 2% of the fixtures were lost during five years of functional load. No correlation was found between bone loss around implants and teeth. A history of periodontitis did not affect the marginal bone loss that occurred around implants. Smoking was not associated with bone loss around fixtures or teeth but a majority of late fixture losses occurred in smokers (Paper IV).

- A site-specific inflammatory reaction rather than a patient-associated host response was found in patients with peri-implantitis (Paper V).

CONCLUDING REMARKS

Although the inflammation around implants in partly edentulous induces a stronger neutrophil reaction, than that in edentulous patients, the marginal bone loss after long-term function in the former is limited and similar to that in edentulous jaws. In patients treated for periodontal disease stable periodontal and peri-implant conditions can be maintained during long term function.

The periodontally-associated microbiota constitute a risk for future development of peri-implantitis. In patients with a history of periodontitis i.e. individuals who previously have shown a tissue destructive inflammatory response, this risk is more pronounced.

The findings of a stronger inflammatory reaction around implants in partly edentulous than in the edentulous together with a periodontally-associated microflora in patients with a history of periodontitis may thus emphasise the importance of establishing stable periodontal conditions prior to implant treatment.
FUTURE DIRECTIONS

A multicentre approach has to be applied for identification of the rare individuals susceptible to implant failures in order to evaluate possible risk factors related to implant failures. There is also a need to further develop methods for detecting early tissue changes surrounding implants complementary to the clinical and radiographic parameters currently used for evaluation. Markers of inflammation and of tissue destruction in crevicular fluid may thus prove important in detecting early signs of tissue change. Markers of proteolytic enzymes, tissue damage and osseous breakdown have been detected in crevicular fluid and related to peri-implant tissue disease status (Oringer et al. 1998, Fiorellini et al 2000, Ma et al 2000).

Anti-microbial therapy in conjunction with flap elevation, cleaning of the bacterial contaminated portion of the fixture have shown to resolve the inflammatory lesion and create new bone fill in a previous peri-implant bone defect in animals (Persson et al. 1996, 1999). Reosseointegration fails to occur at implant surfaces exposed to bacterial contamination but has been demonstrated where a pristine implant component was placed in the bone defect following surgical debridement (Persson et al. 2001).

However, only few clinical controlled studies and several case reports have been published on the treatment of biological failures of implants (for review see Esposito et al. 1999, Lang et al 1997). Therefore a systematic approach in both treatment regiments and the regeneration of lost tissues is needed. Presently the treatment is based mainly on empirical experience (von Arx et al. 1997) and inference from animal experiments. Well-designed clinical trials and experimentally controlled investigations on early signs of tissue destruction, treatment of peri-implant breakdown and regeneration of tissues are currently important tasks for future research (Brägger 1994, Albrektsson & Isidor 1994, Lang 1997, Nevins et al 1996).
ACKNOWLEDGEMENTS

I wish to express my sincere gratitude to all of you who helped me with these studies in the Karolinska Institutet, Institute of Odontology, Huddinge.
I also would like to thank collaborating clinics: Department of Periodontology, Centre of Oral Health Sciences, Malmö University. The Specialist Centre for Dental Implants, Nacka, Stockholm. The Departments of Periodontology and Prosthetic Dentistry, Dental and Medical Health Centre, Halmstad.

In particular, I wish to thank:

Professor Björn Klinge, my tutor and supervisor, for introducing me to scientific research and sharing with me his knowledge of implantology.
Thanks to his respect in the dental community, these studies could be done in collaboration with other dental clinics. His constant support, sincere concern about my research and me made this thesis possible.

Associate professor Anders Gustafsson, my tutor, supervisor and friend, for his never-failing enthusiasm in the daily research. Without his introduction to clinical research, encouragement, optimistic approach and expertise on neutrophils and inflammation there might have been no thesis.

Associate professor Kristina Arvidson, for encouraging me to do research and as valued co-author in the preparation of my first manuscript.

Dr. Thomas Kallus for his honest concern, professional support and constructive criticisms as a co-author.

Dr Juhani Fischer, for clinical expertise and constructive ideas as my co-author.

Dr. Lennart Boström, for introducing me to the world of microbes and valuable criticisms as co-author on microbial analyses.

Dr. Hadar Hallström, for his enthusiasm giving me the opportunity to collaborate with Specialisttandvården, Länssjukhuset, Halmstad and to Dr. Lars-Ake Johansson, for sharing his clinical experience and patients with me.

The staff, and especially dental nurses Barbro Hermansson and Britt-Marie Meldert in the Specialist Clinic, Institute of Odontology, Karolinska Institutet, Huddinge for their patience and help during clinical examinations and samplings.

The staff, and especially dental nurse Helena Ahlgren, Department of Periodontology, Centre of Oral Health Sciences, Malmö University, for excellent assistance in assembling of patients and in the clinical examinations of them.

The staff of the Specialist Centre for Dental Implants, Nacka, Stockholm, colleagues and dental nurses, for their warm friendship, great interest in these studies and for outstanding memories of the Orcinus orca.
The staff, and especially Dental Hygienist Inger Persson in the Departments of Periodontology and Prosthetic Dentistry, Dental and Medical Health Centre, Halmstad for all their help and the warm atmosphere that they created during clinical examinations of patients.

Secretary Kerstin Smedberg, for always being so kind and helpful in our everyday-work at the Institute.

Dr. Zoe Walsh and Mr. Francis Walsh, for quickly and professionally revising my English text and making it more understandable.

My husband, Peter and our two sons Fredrik and Christian, for all their love, patience and endless support throughout this thesis.

My mother Kajsa, and my late father, Folke, for never-ending love and for giving me long happy summers in the archipelago of Blekinge.

This work has been supported by grants from the Karolinska Institutet and from the Swedish Dental Society.
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