Forord

Det er med glæde og ikke uden en betydelig stolthed at *Tandlægebladet*s fagredaktion i dette nummer, som et supplementum, publicerer *Søren Schous* doktordisputats.

Søren Schous arbejde med emnet periimplantitis har strakt sig over en tiårig periode. Grundlaget for disputatsen er ti internationalt publicerede artikler, som afrundes med nærværende arbejde. Disputatsen publiceres som følge heraf på engelsk. Den handler om patogenese og diagnostik af periimplantitis samt om forskellige modaliteter til kirurgisk behandling af tilstanden.

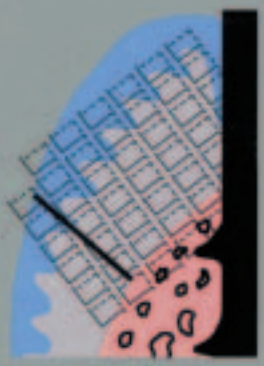
Periimplantitis defineres i den orale implantologi som en plakinduceret inflammatorisk proces i det periimplantære væv med samtidigt tab af marginal knogle. Et detaljeret kendskab til periimplantitis er afgørende for at undgå marginal knoglenedbrydning omkring implantater og dermed opnå varig behandlingssucces. Med den stigende anvendelse af implantater til behandling af tandtab er profylaktiske foranstaltninger over for udvikling af periimplantitis, diagnostik af tilstanden samt behandling af allerede opstået periimplantitis emner af den allerstørste betydning for klinisk praksis.

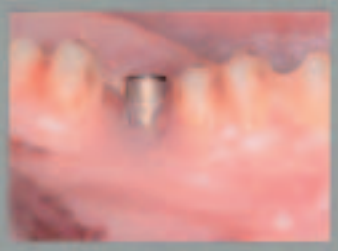
Tandlægebladets fagredaktion

Preben Hørsted Bindslev
Jens Kølsen Petersen
Palle Holmstrup
Ib Sewerin

Peri-implantitis

Pathogenesis, diagnosis, and treatment as evaluated in cynomolgus monkeys





SØREN SCHOU

Departments of Oral and Maxillofacial Surgery and Periodontology, School of Dentistry, University of Copenhagen, and Department of Oral and Maxillofacial Surgery, University Hospital (Rigshospitalet), Copenhagen, Denmark

Stereological Research Laboratory, Institute of Experimental Clinical Research, University of Aarhus, Denmark

Department of Oral Radiology, Royal Dental College, University of Aarhus, Denmark

Denne afhandling er i forbindelse med de på side 304 anførte tidligere offentliggjorte artikler af Det Sundhedsvidenskabelige Fakultet ved Københavns Universitet antaget til offentligt at forsvares for den odontologiske doktorgrad.

København, den 28. november 2003
Ralf Hemmingsen
dekan

Forsvaret finder sted onsdag den 28. april 2004, kl. 14.00 i Henrik Dam-auditoriet, Panuminstituttet, Blegdamsvej 3, 2200 København N.

Peri-implantitis

Pathogenesis, diagnosis, and treatment as evaluated in cynomolgus monkeys

(Doctoral thesis)

Søren Schou

Danish Dental Journal 2004; 108
Supplementum No. 1

Previous papers

The present thesis is based upon the following ten publications, which will be referred to by their Roman numerals (I-X):

Reviews:

I. *Schou S, Holmstrup P, Kornman KS.* Non-human primates used in studies of periodontal disease pathogenesis: A review of the literature. J Periodontol 1993; 64: 497-508.

II. *Schou S, Hansen AK.* Marburg and Ebola virus infections in laboratory non-human primates: A literature review. Comp Med 2000; 50: 108-23.

Experimental studies:

III. *Schou S, Holmstrup P, Stoltze K, Hjørting-Hansen E, Kornman KS.* Ligature-induced marginal inflammation around osseointegrated implants and ankylosed teeth. Clinical and radiographic observations in cynomolgus monkeys (*Macaca fascicularis*). Clin Oral Implants Res 1993; 4: 12-22.

IV. *Schou S, Holmstrup P, Reibel J, Juhl M, Hjørting-Hansen E, Kornman KS.* Ligature-induced marginal inflammation around osseointegrated implants and ankylosed teeth: Stereologic and histologic observations in cynomolgus monkeys (*Macaca fascicularis*). J Periodontol 1993; 64: 529-37.

V. *Schou S, Holmstrup P, Keiding N, Fiehn N-E.* Microbiology of ligature-induced marginal inflammation around osseointegrated implants and ankylosed teeth in cynomolgus monkeys (*Macaca fascicularis*). Clin Oral Implants Res 1996; 7: 190-200.

VI. *Schou S, Holmstrup P, Stoltze K, Hjørting-Hansen E, Fiehn N-E, Skovgaard LT.* Probing around implants and teeth with healthy or inflamed peri-implant mucosa/gingiva. A histologic comparison in cynomolgus monkeys (*Macaca fascicularis*). Clin Oral Implants Res 2002; 13: 113-26.

VII. *Schou S, Holmstrup P, Jørgensen T, Stoltze K, Hjørting-Hansen E, Wenzel A.* Autogenous bone graft and ePTFE membrane in the treatment of peri-implantitis. I. Clinical and radiographic observations in cynomolgus monkeys. Clin Oral Implants Res 2003; 14: 391-403.

VIII. *Schou S, Holmstrup P, Skovgaard LT, Stoltze K, Hjørting-Hansen E, Gundersen HJG.* Autogenous bone graft and ePTFE membrane in the treatment of peri-implantitis. II. Stereologic and histologic observations in cynomolgus monkeys. Clin Oral Implants Res 2003; 14: 404-11.

IX. *Schou S, Holmstrup P, Jørgensen T, Skovgaard LT, Stoltze K, Hjørting-Hansen E, Wenzel A.* Anorganic porous bovine-derived bone mineral (Bio-Oss®) and ePTFE membrane in the treatment of peri-implantitis in cynomolgus monkeys. Clin Oral Implants Res 2003; 14: 535-47.

X. *Schou S, Holmstrup P, Jørgensen T, Skovgaard LT, Stoltze K, Hjørting-Hansen E, Wenzel A.* Implant surface preparation in the surgical treatment of experimental peri-implantitis with autogenous bone graft and ePTFE membrane in cynomolgus monkeys. Clin Oral Implants Res 2003; 14: 412-22.

Acknowledgements

The present thesis is based on studies initiated in 1989 at the Departments of Oral and Maxillofacial Surgery, Periodontology, and Oral Pathology and Medicine, School of Dentistry, Faculty of Health Sciences, University of Copenhagen. The investigations were continued during employment at the Departments of Oral and Maxillofacial Surgery, School of Dentistry, Faculty of Health Sciences, University of Copenhagen, and at University Hospital (Rigshospitalet). The studies involving stereology and quantitative digital subtraction radiography were performed in collaboration with Stereological Research Laboratory, Institute of Experimental Clinical Research, and Department of Oral Radiology, Royal Dental College, University of Aarhus. Finally, the present review was completed during my employment at the Department of Oral and Maxillofacial Surgery, Aalborg University Hospital. I am deeply indebted to these departments and institutions for the excellent working conditions.

The accomplished studies were made possible by the extreme generosity of many individuals to whom I owe my deepest appreciation. My sincere and special thanks go to four persons of crucial importance, not only for the present studies, but also for my personal and professional development during the entire period. I am exceptionally thankful to Prof. E. Hjørting-Hansen, Prof. P. Holmstrup, and Dr. K. Stoltze for invaluable and outstanding discussions, tireless guidance, necessary encouragement, and always constructive criticism. I am also very thankful to Prof. H. J. G. Gundersen for introducing me to stereology. The thesis would have suffered a lot without these unbiased methods.

Also the continuous suggestions by Dr. K. S. Kornman to the experimental design of the performed studies are highly acknowledged. The investigations could not have been performed without the comprehensive experience of Dr. Kornman by using primates as laboratory animals. In addition, my true gratitude goes to the other co-authors, including Dr. N.-E. Fiehn, Prof. A. Kornerup Hansen, Dr. M. Juhl, Dr. T. Jørgensen, Prof. N. Keiding, Prof. J. Reibel, Dr. L. Theil Skovgaard, and Prof. A. Wenzel. None of the studies could have been carried out without the great support and contribution of these colleagues.

I am also extremely thankful to several persons involved in various parts of the studies over the years. The invaluable assistance of Ms. M. Anfeldt, Ms. K. Biré, Ms. K. Blicher-Nielsen, Ms. L. Füchsel, Ms. G. Glæsner, Dr. E. Gotfredsen, Dr. L. Due Hansen, Ms. H. Lykke Hansen, Ms. K. Hornshøj Jensen, Dr. H.-J. Skovgaard Jensen, Ms. S. Jensen, Ms. U. Jensen, Ms. E. Kosloff, Ms. L. Larsen, Ms. U. Larsen, Ms. T. Lemvigh, Ms. J. Lykkeaa, Ms. H. Meile, Dr. A. Nattestad, Ms. B. Schrøder Nielsen, Ms. D. Nolting, Ms. L. Olesen, Mr. J. Olsen, Dr. H. Juul Rasmussen, Mr. E. Ravn, Mr. M. Rüssel, Dr. J. Schou, Dr. I. Sewerin, Ms. A. Bøgelund Sterler, and Ms. G. Frejlev Sørensen is gratefully acknowledged. I am also indebted to Ms. L. Wermus for the invaluable rectification of grammar and style of the present review. Finally, I am deeply honoured and grateful to Dr. I. Sewerin, Scientific Editor, for the meticulous and tireless editing and for giving me the outstanding opportunity to publish the present thesis in the Danish Dental Journal.

The supply of implants by Institut Straumann and Astra Tech, a *Porphyromonas gingivalis* strain by Prof. R. C. Page, membranes by Gore and Associates, Bio-Oss by Geistlich Pharma, and Elyzol by Dumex-Alpharma is also gratefully acknowledged. Finally, a prerequisite of the experimental work is the highly appreciated grants from the ITI Foundation for the Promotion of Oral Implantology, Danish Medical Research Council, Gangstedfonden, Direktør E. Danielsen og Hustrus Fond, Familien Hede Nielsens Fond, Dansk Tandlægeforenings Forskningsfond, FUT- og CALCIN-Fondene, Astra Tech's Forskningsstipendium i Oral Implantologi och Biomaterial, Komitéen til Formidling af Forskningsstøtte, Else og Mogens Wedell-Wedellsborgs Fond, Grosserer L. F. Foghts Fond, Løvens Kemiske Fabriks Forskningsfond, and Købmand Sven Hansen og Hustru Ina Hansens Fond.

Aalborg, March 2004
Søren Schou

Contents

1. Introduction

Treatment of tooth loss with osseointegrated oral implants shows high success rates in totally and partially edentulous patients (51,52,77,88,245). However, mechanical and biological complications occasionally occur (30,77,149). The following definitions were approved at the 1st European Workshop on Periodontology in 1993 (9):

- *Peri-implant disease*: A collective term for inflammatory reactions in the tissue surrounding an implant.
- *Peri-implant mucositis*: A term for reversible inflammatory reactions in the soft tissue surrounding a functioning implant.
- *Peri-implantitis*: A term for inflammatory reactions with loss of supporting bone in the tissue surrounding a functioning implant.

It has been argued that the above definition of peri-implantitis may be too generic (78). As further discussed below, it has been shown that plaque accumulation may cause inflammatory reactions of the peri-implant mucosa and progressive loss of the occlusal part of the peri-implant bone. It has also been demonstrated in monkeys that occlusal overload can result in loss of osseointegration (120,121). Although clinical studies have indicated that occlusal overload may induce progressive loss of the occlusal portion of the peri-implant bone, this has never been demonstrated clearly in an experimental study (124). Therefore, peri-implantitis will be used exclusively as a term for plaque-induced inflammatory reactions of the peri-implant tissue with a concomitant loss of the occlusal portion of the peri-implant bone.

The precise frequency of peri-implantitis is unknown and may differ for the various implant systems. A frequency of 5-10% has been estimated (187), although a lower frequency has been claimed for the Brånemark system (77). Progressive peri-implantitis may ultimately cause implant loss. Thus, comprehensive knowledge about plaque-induced peri-implant disease is essential to avoid implant loss.

2. Purpose of studies

The present thesis is based on two reviews and eight experimental investigations performed in cynomolgus monkeys (*Macaca fascicularis*). The aim of these studies were to evaluate:

- The usefulness of primates as a model to assess plaque-induced inflammatory reactions around osseointegrated oral implants and teeth (I).
- The risk for disease transmission to humans working with primates as laboratory animals (II).
- Pathogenesis and clinical diagnosis of plaque-induced disease around osseointegrated oral implants (III-VI).
- Surgical treatments of peri-implantitis (VII-IX).
- Implant surface preparations used in the surgical treatment of peri-implantitis (X).

In the present thesis, the above-mentioned investigations will be discussed and their findings related to other studies. Results from studies involving titanium-alloy implants and implants with hydroxyapatite coatings will be included when necessary for the understanding of plaque-induced inflammatory reactions around commercially pure titanium implants.

3. Experimental model and methods of evaluation

3.1. Non-human primate model

For obvious ethical reasons, several aspects of initiation, progression, and treatment of plaque-induced peri-implant disease cannot be studied in humans, and animal models are needed. Phylogenetically lower-ranking animals in general are to be preferred. Thus, mice, rats, and rabbits are widely used due to known age, genetic background, and medical history. Moreover, high resistance to diseases, low risk of disease transmission to humans, sufficient breeding capacity, low price, ease of handling, and large amounts of data from previous studies are important aspects. However, these species may be inadequate for many studies due to significant anatomical and biological dissimilarity to humans. In addition, many intraoral procedures, including implant placement and manipulation of the peri-implant mucosa, are compromised or even impossible due to the small size of these animals. Therefore, larger animal species are necessary for most studies of the peri-implant tissue.

Primates and dogs have been used extensively as laboratory animals in studies of periodontal disease pathogenesis and treatment (I,44,204,263). The feasibility of monkeys and dogs has only been sparsely compared (86), but the close phylogenetical, anatomical, and biological similarity to humans indicate that several primate species can be used for studies of plaque-induced peri-implant disease (I,44,86,204, 263). The skeletal remodelling seems 33% more rapid in dogs as compared with humans and primates (86). It is presently unknown whether the accelerated bone remodelling in dogs may compromise them as models for studies on treat-

ment modalities of peri-implantitis. Furthermore, experimental peri-implantitis in dogs is characterised by recession of the peri-implant mucosa (160). Therefore, dogs may not be the most feasible animal model for studies of peri-implantitis.

Primates captured in their natural habitat were previously used as laboratory animals. These animals were of different age, body weight, and oral status. In addition, the unknown medical history might imply introduction of various diseases not only to the staff working with the animals, but also to established colonies of primates (I,II). The captive-born primates used today are more standardised animals with low prevalence of diseases and a known medical history. However, monkeys still include a greater risk for zoonoses than do other laboratory animals (I,II).

Although several viruses, including herpes B, Simian B, and hepatitis B and C, may cause diseases in humans and primates, special attention has been paid to Marburg and Ebola viruses causing viral hemorrhagic fever (II). Until year 2000, a total of 23 Marburg and Ebola virus outbreaks have been reported since the first outbreak occurred in Marburg, Germany, in 1967 (II). Most of the 1.100 human cases with nearly 800 deaths occurred in Africa mainly due to direct contact with infected persons. Very few human cases have been reported after contact with primates used for scientific purposes, and most of the cases occurred before the pathogenicity of these virus types was discovered. It was recently concluded that there is only a minimal risk of infection by filoviruses and other diseases for humans working with primates as laboratory animals, provided proper precautions are taken (II).

Studies of periodontal disease pathogenesis necessitate examination of sites during active tissue destruction. Placement of silk ligatures resulting in increased plaque accumulation followed by gingival inflammation, probing attachment loss, and bone loss has been used as an experimental model to study this process (I). No attachment or bone loss occur when ligature placement is combined with systemic antibiotic therapy (104,215,273). Thus, the loss appears to be due to the combined presence of the ligature and plaque rather than mechanical trauma from the ligature alone.

Cynomolgus monkeys have been used intensively to investigate periodontal disease pathogenesis (I). It has been demonstrated that gingival inflammation increased 1-3 weeks after ligature placement, while active periodontitis occurred 4-7 weeks after ligation (141). For unknown reasons, the condition stabilised between 8-17 weeks with decreased inflammation and no further bone loss. A similar experimental model appears to be applicable for investigations of plaque-induced inflammation around implants (III,IV).

The above-mentioned time sequence of periodontitis development may vary as previously discussed (I). Moreover, periodontal breakdown may not occur in all monkeys after ligature placement (63). An unchanged bone level was revealed in the present series of studies around ligated control teeth, while bone loss was seen around ligated osseointegrated implants and ankylosed teeth (III,IV). One explanation for this observation is probably that an earlier stage of plaque-induced inflammation than previously reported was assessed in these studies, because oral hygiene procedures were performed until four weeks before ligature placement in order to maintain healthy peri-implant mucosa/gingiva while osseointegration of implants and ankylosis of replanted teeth were established (III,IV).

A disadvantage of using primates as laboratory animals is that all manipulation, including oral hygiene procedures necessitates anaesthesia or sedation. The influence of anaesthesia or sedation three times weekly to enable oral hygiene procedures during an extended period of time is unknown (43). A combination of ketamine and xylazine was first used routinely, apparently without negative long-term effect on the animals (III-V). Although atropine was added, low blood pressure and heart rate were observed, and three monkeys were lost during this type of anaesthesia.

Another disadvantage of ketamine and xylazine is that the shortest period of anaesthesia is 30-45 minutes. A sedation period of 10-15 minutes was possible with minimal influence on respiration, blood pressure, and heart rate by changing the anaesthesia protocol to a combination of tiletamine, zolazepam, and atropine (158). In addition, the time necessary for the animals to recover was less than 30 minutes as compared with several hours after ketamine and xylazine. Finally, anaesthesia can be maintained safely for hours by isoflurane after orotracheal intubation provided monitoring by electrocardiogram, blood pressure apparatus, and heart rate monitor while the body temperature of the animal is maintained with a heating apparatus (VI,VII,IX,X).

Assessment of treatment modalities of peri-implantitis necessitates an animal model that closely resembles the conditions in humans. This may be accomplished by involving peri-implantitis lesions developed over an extended period of time. By combining silk ligatures and orthodontic elastics, pronounced peri-implantitis lesions can be established in cynomolgus monkeys over a reasonable period of time (VII-X). The elastics were placed to maintain submucosal retention of microorganisms, and the ligatures were either pushed into a more apical position or renewed once a month. It has

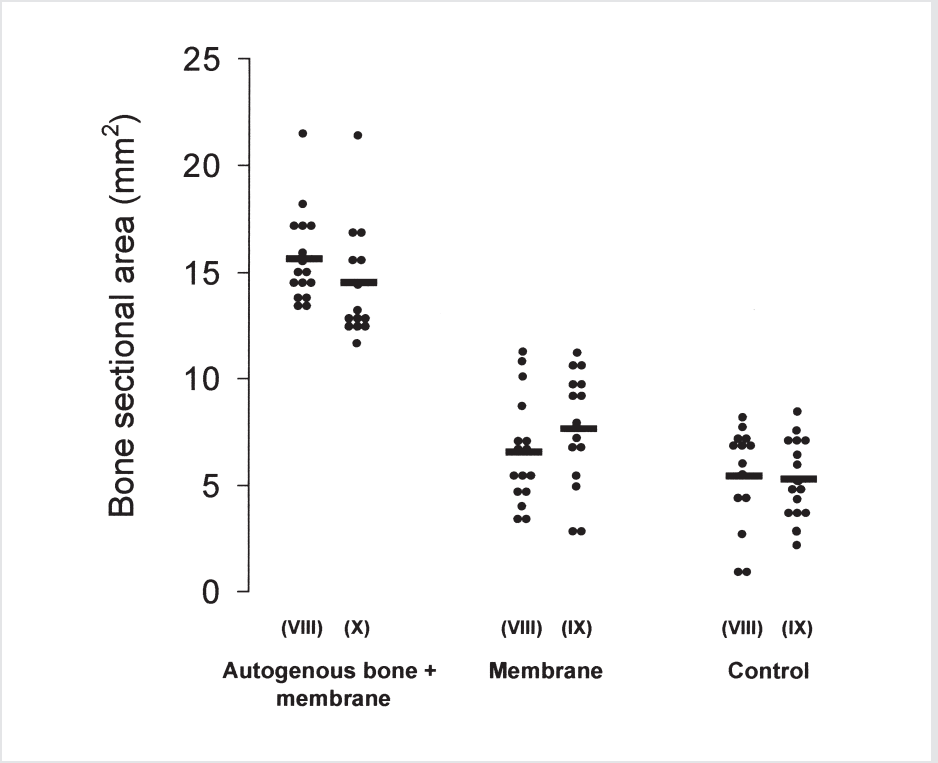


Fig. 1. Total sectional area (mm^2) of bone (autogenous bone graft particles and regenerated bone) within the former peri-implantitis defect after surgical treatment involving autogenous bone graft particles and ePTFE membrane, ePTFE membrane, or a control procedure (conventional flap procedure) in studies VIII-X. The mean value and data variation were comparable for each treatment type documenting that the reported results were reproducible (-: mean).

been demonstrated that inoculation of *Porphyromonas gingivalis* into the periodontal pocket of ligated teeth induces bone loss in cynomolgus monkeys (111). Therefore, a well-described monkey pathogenic *P. gingivalis* strain was inoculated into the peri-implant pocket to facilitate development of peri-implantitis (VII,IX,X). The effect of the inoculation procedure was not specifically evaluated, but the clinical and radiographical examination showed increased tissue inflammation and bone loss after inoculation. The susceptibility to peri-implantitis varied among the animals. Therefore, continued evaluation of the tissue destruction was mandatory to ensure comparable destruction around the implants. Individual ligation periods of 9-22 months were used to obtain a bone loss of 4-6 mm around implants with a titanium plasma-sprayed (TPS) surface (VII,IX,X).

Animals have been used to evaluate various aspects of surgical treatment of peri-implantitis (VII-X,58,76,93,114,132, 170,198,199,209-212,242,269). An advantage of the present series of studies is that the same experimental design was applied enabling comparison of the treatment outcomes (VII-X). Autogenous bone graft particles or anorganic porous bovine-derived bone mineral (Bio-Oss, Geistlich Pharma, Switzerland) with or without expanded polytetrafluoroethylene (ePTFE) membrane (Gore-Tex Regenerative Material, Gore and Associates, USA) were evaluated (VII-IX). Comparison of the treatment outcomes necessitated the inclusion of treatments involving a membrane and a control procedure in each study. Finally, autogenous bone graft particles covered with an ePTFE membrane were included in the comparison of various implant surface preparations used in the surgical treatment of peri-implantitis (X). Therefore, the treatment outcomes after using the same treatment procedure in different studies could be assessed. Comparison of the response variables including the total sectional area of bone (autogenous bone graft particles and regenerated bone) within the former peri-implantitis defects showed comparable mean value and data variation for each treatment type, thus documenting that the reported results were reproducible (Fig. 1). Moreover, the treatment outcome was in each study assessed and confirmed by different evaluation methods, i.e. clinical parameters, radiography including quantitative digital subtraction radiography, histology, and stereology (VII-X).

In conclusion, a disadvantage by using primates as lab-

oratory animals is that all manipulation, including oral hygiene procedures, necessitates anaesthesia or sedation. However, the cynomolgus monkey is a feasible animal model to investigate plaque-induced disease around osseointegrated oral implants. Also, this primate can be used with minimal risk of zoonoses, provided proper precautions are taken.

3.2. Quantitative digital subtraction radiography

Histological and stereological procedures were used for detailed examination of the peri-implant tissue at the end of the study, while clinical recordings and radiographs were used for the longitudinal evaluation. It was possible to obtain geometrically standardised radiographs throughout the studies (III,VI,VII,IX,X). The apical third of the maxillary implants and teeth was frequently not included on the radiographs due to the flat palatal vault of cynomolgus monkeys. However, the area of interest, i.e. the occlusal part of the implants and teeth was never excluded.

Quantitative digital subtraction radiography is used increasingly to detect changes of the peri-implant/-dental bone between two images (29,151,176,267). Surgical treatment modalities of peri-implantitis were evaluated with this method (Fig. 2) (VII,IX,X). The alignment of the two radiographs to be subtracted often involves manual superimposition or placement of landmarks (reference points) for the superimposition (32,33,266). A Windows-based software package for quantitative digital subtraction radiography (X-Poseit, Image Interpreter Systems, Denmark) has recently been introduced (133). The program is based on a previously reported method (266). Approximately ten reference points are placed for the superimposition on the two images to be subtracted (Fig. 3) (1,266). Furthermore, the regions of interest can be outlined with the computer mouse.

The small scaling, translation, rotation, and perspective misalignments between the two images can be reduced by including a mathematical linear transformation based on the reference points on the two images and the least squares solution (133). Another advantage by placing reference points for the superimposition is that the alignment of the two radiographs to be subtracted is probably more precise and less time-consuming than manual superimposition (266). Development of automatic or semiautomatic methods for superimposing and for outlining regions of interest will facilitate the application of quantitative digital subtraction radiography in the future.

Data variation due to differences in film, exposure, processing, digitalisation, and contrast correction must be considered when quantitative digital subtraction radiography is applied (29,151,176,223,267). Different algorithms have been used for contrast corrections (151), but the cumulative non-parametric method appears most useful (83,225). It has been stated that the region upon which the matching is based must be identical within the images to be subtracted (108,247). Moreover, the region should involve as much as possible of the radiographs to obtain the maximum number of pixels for the procedure. The contrast correction was therefore based on the entire image displayed in the two radiographs to be subtracted, excluded regions with peri-implant bone gain/loss, membrane nails, and embossed film dot or text (Fig. 3) (VII,IX,X). Contrast correction based on a region placed over the implant neck or tip was improper for several reasons. These were superimposition of plaque-induced swelling of the peri-implant tissue and bone graft particles over the implant neck, frequent absence of the tip of maxillary implants on the radiographs, and a mean grey level of the region much higher than that of the regenerated bone.

A grey level threshold should be determined for each set-up to distinguish background noise from tissue changes. A threshold of 20 grey levels was used in the present series of studies (VII,IX,X). This value corresponded to twice the average standard deviation (95% confidence interval) of the grey level histograms of control regions not affected by the

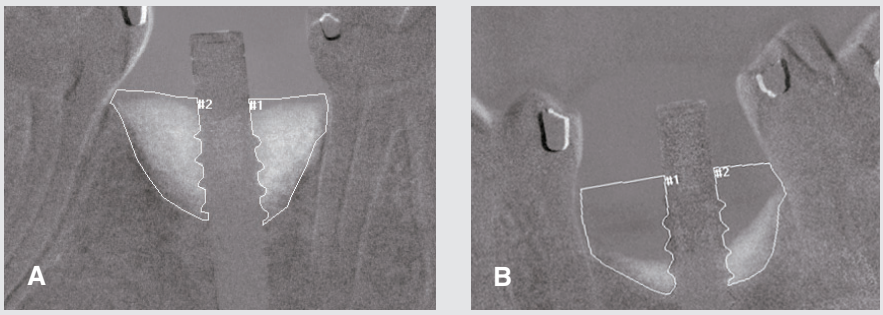


Fig. 2. Subtraction images six months after surgical treatment of peri-implantitis. A: Defect treated with autogenous bone graft particles and ePTFE membrane. B: Control defect treated with a conventional flap procedure. White lines indicate the former peri-implantitis defect border.

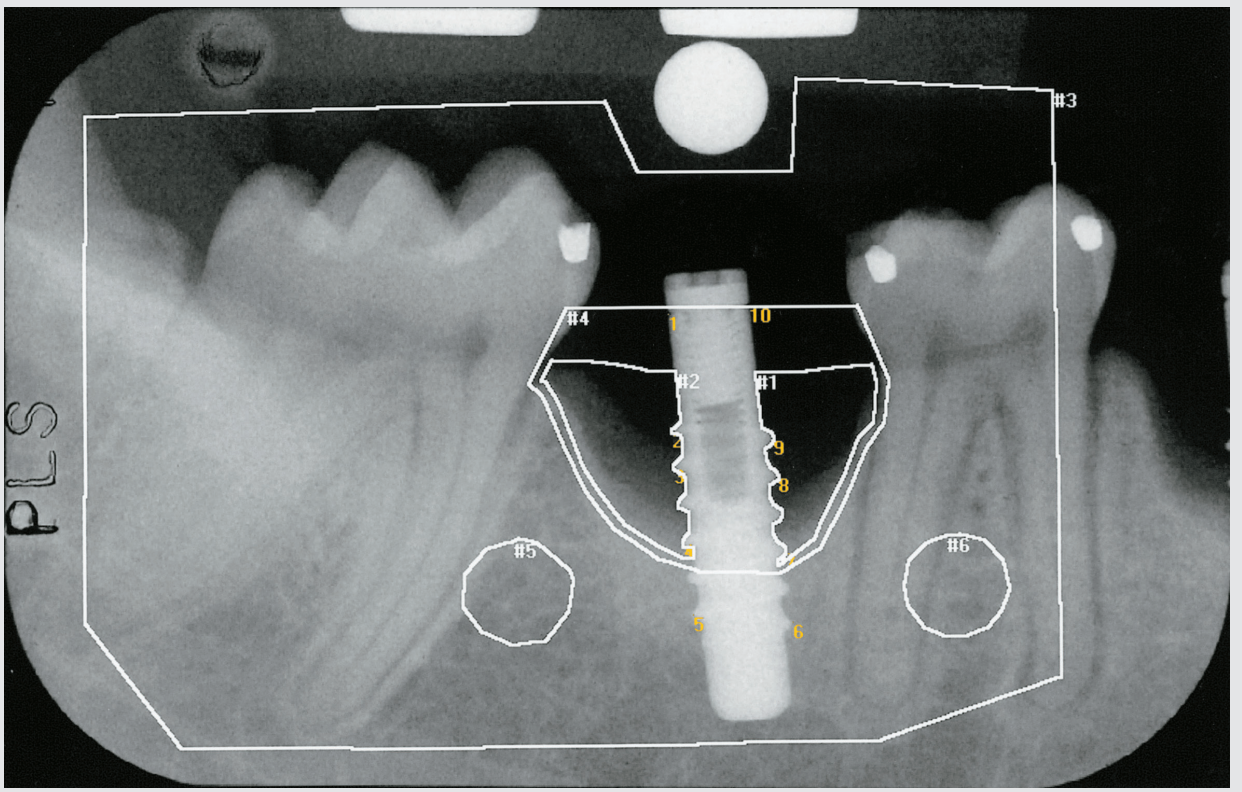


Fig. 3. Reference image at ligature removal, i.e. the time point with maximum tissue destruction. Ten reference points (1-10) were placed for the superimposition. In addition, the following regions of interest were outlined: Defect region (#1 and #2) and contrast correction region (#3) excluded regions with peri-implant bone gain/loss, membrane nails, and embossed film dot or text (#4, #5, and #6).

performed procedures (247). Previous studies have indicated that a threshold of seven grey levels was useful when performing computer-assisted densitometric image analysis (CADIA) (31,83). Moreover, a threshold of ten or thirteen grey levels has been used arbitrarily to evaluate guided bone regeneration around implants (150,202). The higher threshold used in the present investigations was due to a larger variation between the radiographs introduced during film processing as previously discussed (VII). Obviously, the background noise is reduced when the threshold is increased and the response displayed on the radiographs is concomitantly reduced. A threshold of 20 grey levels could be used due to the considerable bone regeneration.

The corrected mean grey level has been used as a parameter for the density of the regenerated bone (32,33,49,50). This parameter is estimated as the difference between the mean grey level of pixels with a grey level above a certain threshold value (representing bone gain) within the former defect and the mean grey level of a control region not affected by the performed procedure. Evaluation of control regions in the present studies revealed a mean grey level of 128 ± 4 after contrast correction. In addition, there was no systematic variation over time. Consequently, the use of this method would only have added additional variation to the recordings.

In summary, the peri-implant bone can be evaluated longitudinally on geometrically standardised radiographs in cynomolgus monkeys. Recent development has facilitated the use of quantitative digital subtraction radiography, but further development is desirable to increase the precision and reduce the time used for registration.

3.3. Histology

Undecalcified sections were prepared by the cutting-grinding technique ad modum Donath which is considered the best documented method (59-61). However, modifications of the technique were necessary to obtain sections with a thickness of 30 µm and an adequate technical quality for the histological and stereological evaluation. Pilot studies fo-

cused mainly upon different time schedules for dehydration, infiltration by a one-component light-curing methacrylate-based resin (Technovit 7200 VLC, Kulzer, Germany), and polymerisation. Optimal dehydration, resin infiltration, and polymerisation of specimens containing one implant with surrounding tissue could be obtained by following the procedure described in Table 1. Acetone and vacuum have not previously been used in conjunction with Technovit 7200, but the accomplished pilot studies showed that both steps were essential for optimal resin infiltration. Moreover, no negative effects on the preservation of cell and tissue structures were identified after including these additional procedures.

Evaluation of the peri-implant tissue necessitated division of the implant and the surrounding tissue into 2-4 separate specimens to obtain sections from different locations. The precise cutting direction of the band saw (300 CP Band Saw System, EXAKT Apparatebau, Germany) in relation to the implant axis was identified by an x-ray-guided technique (Fig. 4). The embedded tissue block was fixed with the band saw clamp. A dental radiograph and a dental x-ray machine were used to precisely demonstrate the band saw cutting direction. By repeating the cutting procedure after turning the specimen 90°, four separate specimens of each implant and surrounding tissue were obtained. Thus, sections could be prepared parallel with the implant axis mesially, distally, buccally, and lingually. This x-ray-guided method solved previously observed difficulties in obtaining sections parallel with the implant axis (28,115,268). A switch attached to the band saw ensured that x-ray exposure could only be performed with the tubus in one position. The required lead protection necessary for the installation of the dental x-ray machine in the laboratory was thereby reduced. The described method was also used for maximum reduction of the size of each tissue specimen before dehydration and resin infiltration without accidental removal of or damage to important tissue.

Numerous surface and block-staining techniques have been described to evaluate the tissue around oral implants (60,89,92,110,138,174,232,248). Further, methods have been described to identify various enzymes and antigens (109,127). Different staining procedures, including Toluidine blue and Stevenel's blue combined with alizarin red S, was first evaluated, but the staining result was unsatisfactory for the present applications. Toluidine blue previously recommended for routine use was frequently pale with inadequate differentiation between the various tissue components (59,60). Moreover, excessive staining occurred when previous recommendations for staining with Stevenel's blue combined with alizarin red S were followed (45,174). In contrast, staining with Stevenel's blue for 15 minutes followed by 0.5% alizarin red S for five minutes was a useful method for the evaluation of the peri-implant mucosa as well as bone regeneration after surgical treatment of peri-implantitis (Figs. 5,6) (VI,VIII-X). Accordingly, re-osseointegration within the

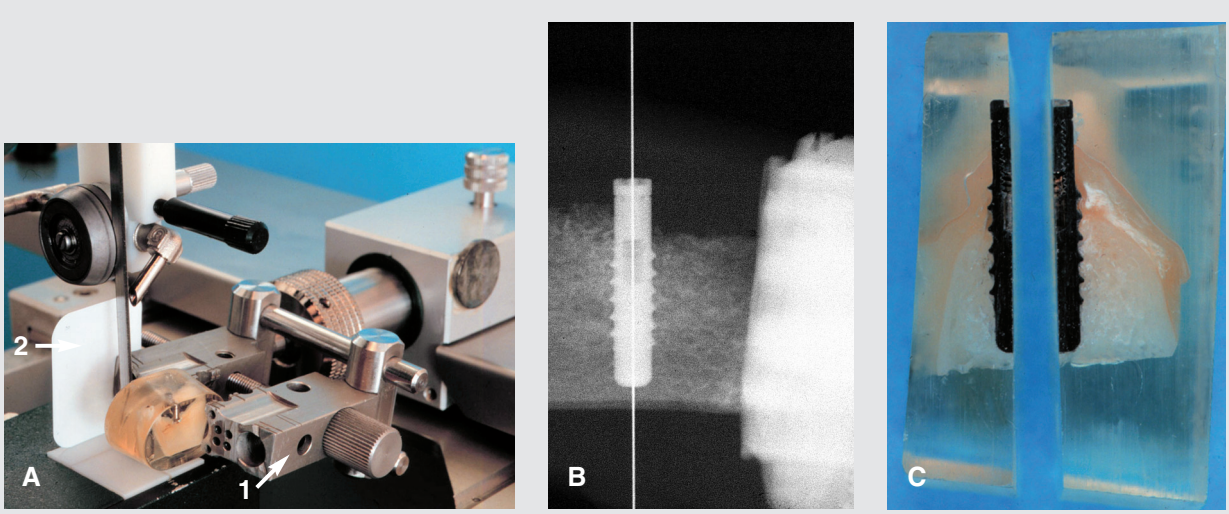


Fig. 4. A: The embedded specimen was fixed with the band saw clamp (1). The dental x-ray tubus was placed in front of the specimen and a dental radiograph (2) behind the cutting band. B: Individual localisation and adjustment of the cutting direction was possible. C: Each implant and surrounding tissue was divided into 2-4 separate specimens.

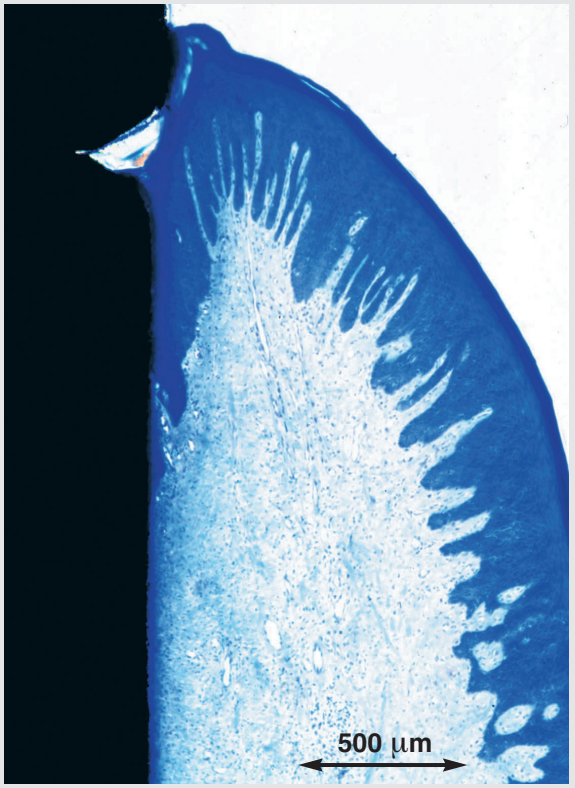


Fig. 5. Histological section of an implant surrounded by clinically healthy tissue. Evaluation of the peri-implant mucosa was possible, including easy differentiation between epithelium and connective tissue. Detailed analysis at the cellular level was difficult. Staining: Stevenel's blue and alizarin red S.

former peri-implantitis defect could be assessed. The use of 35% hydrogen peroxide for 15 minutes before Toluidine blue staining ensured a considerably improved staining result, but epithelium, connective tissue, and bone were frequently indistinctly separated (Fig. 7).

A disadvantage of the described histological method was the frequently indistinct separation between regenerated bone and native bone after surgical treatment of peri-implantitis, irrespective of the staining method used (VIII-X). The background of this observation was the extensive remodelling, normally with reformation of a normal trabecular structure six months after treatment. Therefore, an additional method was necessary to demarcate the former peri-implantitis defect on the histological sections as discussed in the following section on stereology. Moreover, the technique is usually inadequate for studies at the cellular level due to a section thickness of 30 µm (Figs. 5,6). A conventional histological technique, including demineralisation in EDTA, embedding in paraffin after gentle removal of the implant, and staining with hematoxylin-eosin, can be applied to study inflammatory reactions within the peri-implant mucosa, including identification of lymphocytes, plasma cells, and neutrophils (Fig. 8) (IV).

Removal of the implant from the tissue specimen before sectioning has been used to evaluate inflammatory reactions of the peri-implant mucosa in dogs (4,24,73,74,160). The present study included titanium-coated polycarbonate implants (IV). Previous studies have confirmed that similar implants osseointegrated in dogs and rabbits (159,166). It has been mentioned that tooth brushing and chewing abraded the titanium coating (166), but the titanium coating below the margin of the peri-implant mucosa was maintained when plaque was gently removed (IV). It was originally intended to prepare the sections without removing the implant from the specimen, but implant removal was necessary to obtain adequate technical quality of the sections for the histological and stereological evaluation.

Precise identification of the various inflammatory cells and mediators necessitates the use of antibodies, but antibodies against various relevant monkey epitopes were commercially unavailable. It was our hypothesis that antibodies against different human inflammatory cells and mediators would react with similar epitopes within the peri-implant mucosa and gingiva of cynomolgus monkeys. Previous studies involving serum, peripheral lymphocytes, and gingival crevicular fluid indicate that antibodies against various human lymphocyte subtypes, immunoglobulins, and inflammatory mediators reacted with similar epitopes in cynomolgus monkeys (62,64,65,194). Pilot studies were carried out to identify various subtypes of inflammatory cells and cytokines by antibodies and immuno-histochemistry, but the staining of the peri-implant mucosa and gingiva with the selected antibodies showed insufficient reproducibility or lack of reaction.

Therefore, the cutting-grinding procedure ad modum Donath can be utilised to assess the peri-implant tissue, provided the described modifications to improve the technical quality of the sections are used. Staining with Stevenel's blue combined with alizarin red S separates the peri-implant epithelium, connective tissue, and bone. Conventional histological technique can be applied to identify lymphocytes, plasma cells, and neutrophils. Pilot studies showed that evaluation of plaque-induced inflammatory reactions around osseointegrated oral implants in cynomolgus monkeys with the selected antibodies demonstrated insufficient reproducibility or lack of reaction.

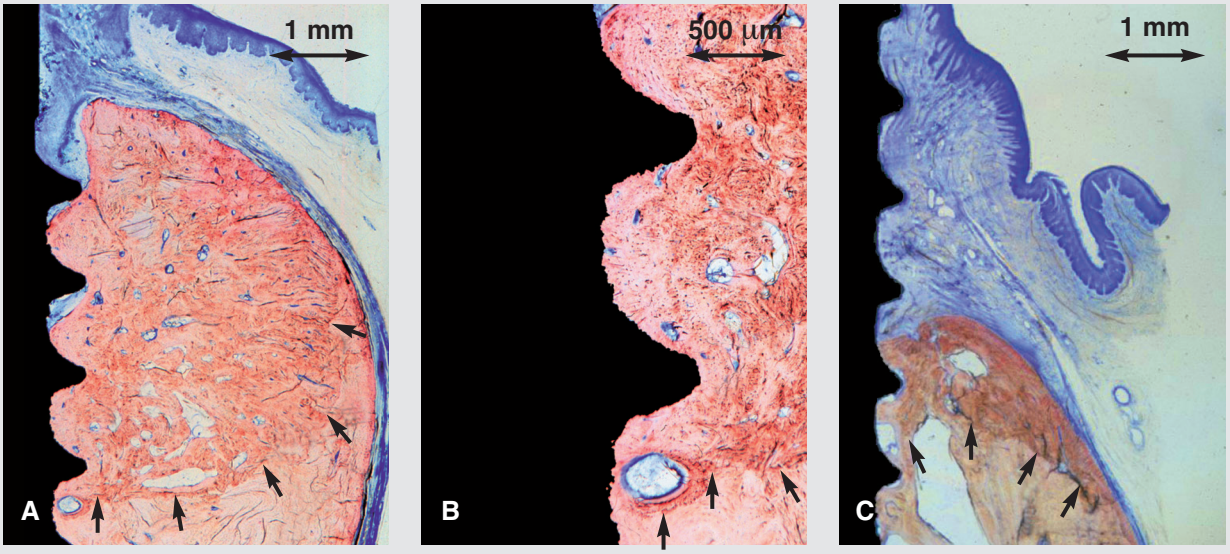


Fig. 6. A: Considerable bone regeneration after surgical treatment of peri-implantitis involving autogenous bone graft particles and ePTFE membrane. B: Higher magnification. Re-osseointegration was demonstrated due to the distinct separation of bone and soft tissue. C: Limited bone regeneration after treatment of peri-implantitis with a conventional flap procedure alone. Arrows indicate the former peri-implantitis defect border. Staining: Stevenel's blue and alizarin red S.

3.4. Stereology

Stereological methods are precise tools for acquiring quantitative information about three-dimensional microscopic structures, based mainly on observations made on sections (99,100). These methods have never previously been used to examine the tissue reactions around osseointegrated oral implants. This section will therefore describe the stereological methods used to obtain unbiased estimates of the

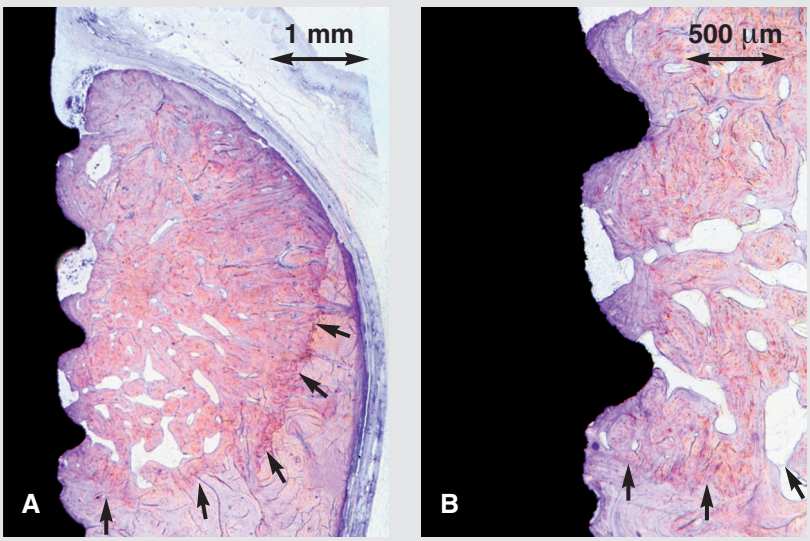


Fig. 7. A: The adjacent histological section of the section shown in Figs. 6A, 6B treated with hydrogen peroxide before staining with Toluidine blue. Extensive bone regeneration could be demonstrated also with this staining method. B: Higher magnification. Re-osseointegration was frequently difficult to evaluate by this staining method due to indistinct separation of connective tissue and bone. Arrows indicate the former peri-implantitis defect border.

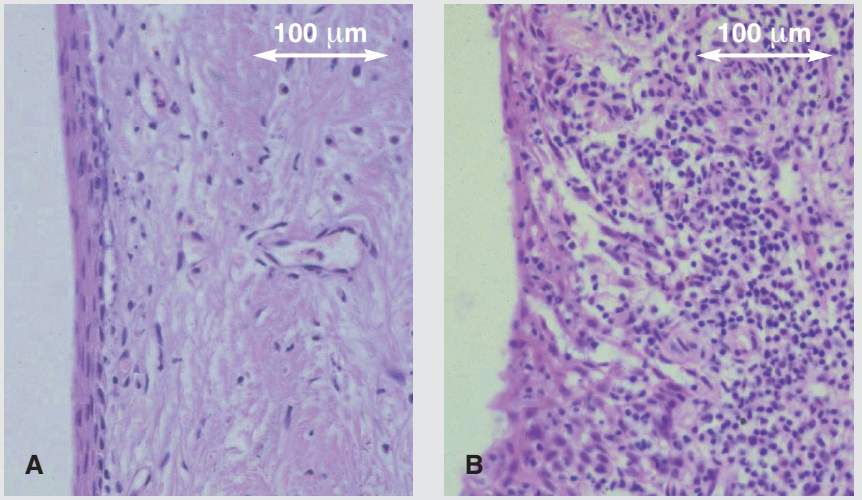


Fig. 8. Detailed evaluation of the peri-implant mucosa was possible after staining with hematoxylin-eosin. A: Section from an implant surrounded by clinically healthy tissue. B: Section from an implant surrounded by clinically inflamed tissue.

number of various inflammatory cells within the peri-implant mucosa, as well as the amount of bone regeneration and re-osseointegration after surgical treatment of peri-implantitis.

Two »concepts« are important for the understanding of stereology, i.e. »unbiased« and »precise«. Unbiased in this content means without a systematic variation from the »true value«, while precise means with a low variability (Fig. 9). In other words, a biased estimate means that the estimate on average is different from the »true value«. The »true value« is normally not known, i.e. the observations in Fig. 9 are seen without the shooting target. A biased method may have a low or high degree of precision, but bias is irreversible and cannot be detected or even estimated from the data. Thus,

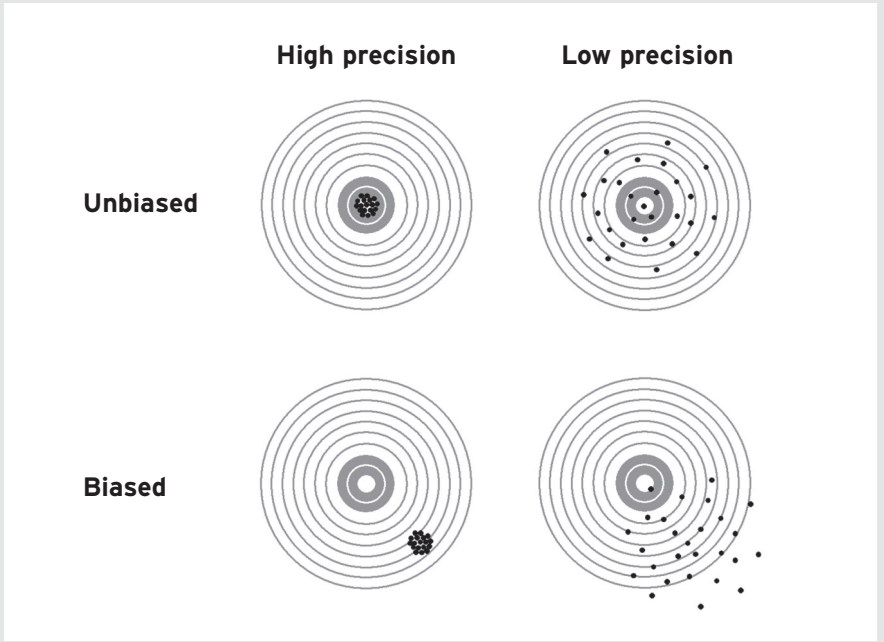


Fig. 9. Illustration modified after Gundersen (96) showing the difference between »unbiased« and »biased« as well as »high precision« and »low precision«. Centre of the shooting target is considered the »true value«.

biased methods may result in false conclusions and should obviously not be used.

Unbiased, very precise, and truly three-dimensional estimates, including total volume in e.g. mm^3 and total number of cells within an organ can be obtained by stereological methods (99,100). However, these precise three-dimensional quantitation procedures are not recommendable in all cases. The requirement of precision is dependent on the actual difference between the groups to be compared and the variation within each group (Fig. 10). Statistical identification of a small difference between two groups necessitates a precise, unbiased stereological technique, while an unbiased stereological method with a low degree of precision can be used to statistically identify a large difference between two groups.

The precision can, in contrast to bias, be evaluated from the original estimates by calculating coefficient of variation (CV) (SD/mean) and coefficient of error (CE) (SEM/mean) (IV). In addition, CE based on the differences between a first and second set of registrations can be estimated (IV,VIII-X). The total observed variance of stereological estimates is a combination of the real difference between the specimens (i.e. »biological variation«) and the variance added by the stereological estimation procedure (i.e. »methodological variation«). In the present series of studies the CVs were always much larger than the corresponding CEs, so »biological variation« was the major source of the observed variation, and not the stereological methods (IV,VIII-X). Plaque accumulation on implants and teeth is associated with pronounced infiltration of inflammatory cells and an extensive inter-individual variation of the response (IV). Therefore, estimation

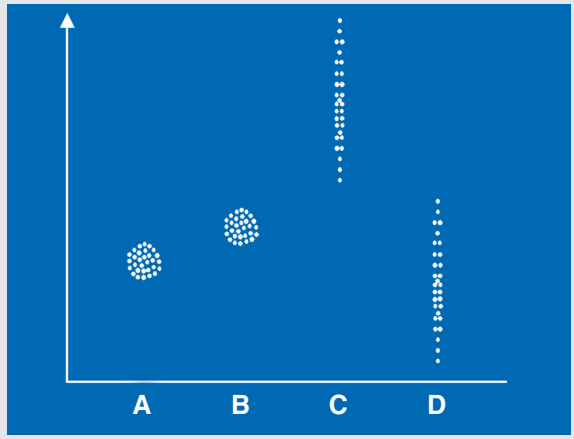


Fig. 10. An unbiased and precise stereological method is necessary to identify the small difference between group A and group B. In contrast, a stereological technique with a low degree of precision (but still unbiased) can be used to identify the large difference between group C and group D.

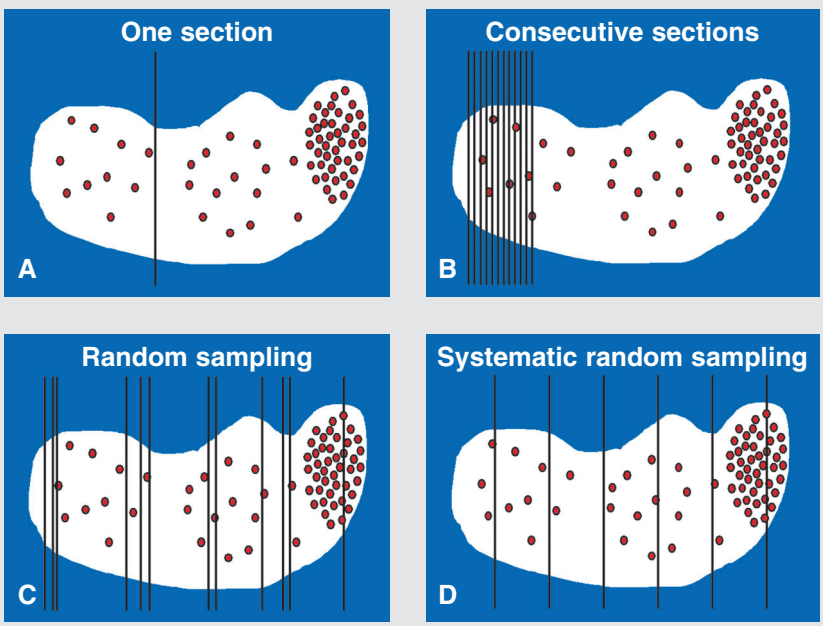


Fig. 11. Different sampling designs. A: One arbitrary section. B: Consecutive sections from a small part of the specimen. C: Uniformly randomly sampled sections. D: Systematic uniformly randomly sampled sections. Unbiased estimates can be obtained by sampling design C and D, but the most efficient method is sampling design D.

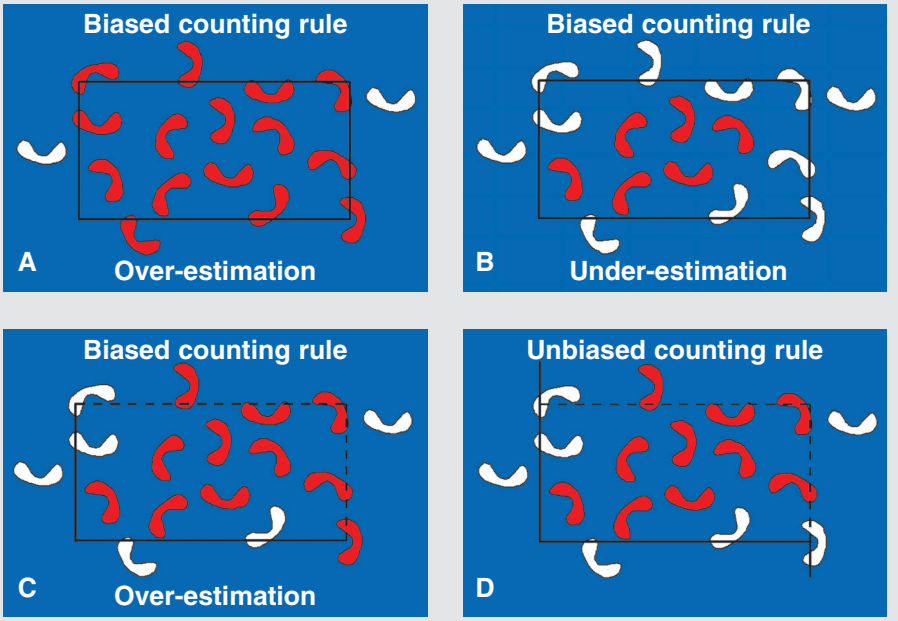


Fig. 12. A: The number of cells per test frame is over-estimated if all »edging« cells are counted. B: The number of cells is under-estimated if all »edging« cells are excluded. C: The number of cells is also over-estimated if the most frequently used counting rule is used, i.e. the counting rule used to estimate the number of erythrocytes in blood samples. D: Unbiased estimates can be obtained when all cells completely within the square and cells intersected only by the upper and right borders (inclusion-edges) are counted, while all cells intersected by the left and lower borders (exclusion-edges) or their extensions are excluded.

of the number of various inflammatory cells by simple, fast, and reliable two-dimensional quantitation is preferable, provided systematic uniform random sampling procedures are included in all steps of the quantitation procedure and unbiased counting rules are used (IV,97).

Systematic uniform random sampling at all levels of the stereological procedure is mandatory in order to efficiently obtain unbiased estimates (97). Sampling at the section level can outline the principle of systematic uniform random sampling. One arbitrary section, consecutive sections from a small part of the specimen, uniformly randomly sampled sections, or systematic uniformly randomly sampled sections may be used (Fig. 11). There may not necessarily be a direct relation between the total number of cells within an entire organ or tissue, and the number of cells observed in one arbitrary section or consecutive sections from a small part of the specimen. Although unbiased estimates can be obtained from uniformly randomly sampled sections, such a sampling design is inefficient since a considerable number of sections are necessary to obtain an acceptable precision of the estimate.

An efficient method includes sampling of sections by a systematic uniform random sampling procedure (Fig. 11D). Systematic means that the distance between the sampled sections is identical (equidistant sections), while uniform random means that the position of the sections is random by sampling the first section at a uniform random position within the distance between two consecutively sampled sections. This sampling design was used to estimate the number of inflammatory cells within the peri-implant mucosa and gingiva (IV). By using systematic uniform random sampling at all levels of the quantitation procedure, a CE below 5-10% can be obtained without known exceptions by counting less than 100-200 points/cells per specimen (97). So, in general, stereological methods are not exceptionally time-consuming.

Counting frames is an important tool to estimate the number of cells or profiles by dividing the region of interest into several small rectangular test areas. Biased counting rules have been used in all previous studies involving oral implants, because cells intersecting the delineation of the test area may cause the so-called edge-effect (94,95) (Fig. 12). To obtain unbiased estimates, each cell should be counted once only in a complete tessellation of the region of interest with test frames. Therefore, the number of cells per test frame is over-estimated if all »edging« cells are counted. Similarly, the number is under-estimated if all »edging« cells are excluded. A biased estimate is also obtained if the most frequently used counting rule is used, i.e. the counting rule used to estimate the number of erythrocytes in blood samples. However, an unbiased estimate is obtained by the unbiased two-dimensional counting frame (94,95). All cells completely within the frame are counted. In addition, all cells intersected only by the upper and right borders (inclusion-edges) are

counted, while all those intersected by the left and lower borders (exclusion-edges) or their extensions are excluded.

The unbiased two-dimensional numerical density of the various inflammatory cells was estimated by superimposing the counting frame in meander-like systematic uniform random sampling positions over the connective tissue around implants and teeth (Fig. 13) (IV). The mucogingival junction was used as a fixed anatomical structure to demarcate a reference sectional area which include all inflammatory cells. The number of cells observed on a section is a function of the size of the cell and its numerical density within the tissue or organ. Thus, large cells have a greater chance for being observed on a section than small cells. When two-dimensional quantitation procedures are used, the influence of this factor can be reduced by replacing counting of cells with counting of cell nuclei due to the smaller variation in the size of the nuclei (IV).

The number of inflammatory cells within the peri-implant mucosa has been reported previously as numerical density. Numerical density estimates may be useful to understand many biological reactions, including plaque-induced inflammation around implants. However, total cell number is probably a more relevant parameter, because the total number of cells is unaffected by shrinkage or other deformations introduced during the histological procedure. Whereas brain tissue shrinks with great variation during fixation and histological preparation (56), the dimensional changes of the alveolar bone are probably of minor significance. In contrast, considerable changes of the peri-implant mucosa and gingiva may occur and there is no reason to assume identical shrinkage of healthy and pathological tissue. Finally, in contrast to numerical density, total number is independent of reference area changes due to enlargement of inflamed tissue (IV). Therefore, total cell number is an unbiased parameter for the evaluation of plaque-induced inflammatory reactions around implants and teeth (IV).

Precise demarcation of the former peri-implantitis defect border on the histological sections is mandatory to document bone regeneration and re-osseointegration after treatment. The border between the original and the regenerated bone was often difficult or impossible to determine by conventional staining methods six months after treatment (VIII-X). Flourochromes have previously been used to demarcate the apical defect border (210-212,242,269), but a new method was introduced to demarcate the entire defect by involving transfer of the defect border from the geometrically standardised radiographical images to the histological images (Fig. 14) (VIII-X).

Point counting is an efficient method to obtain unbiased estimates of sectional areas (97,98,262). Total reference sectional areas and total sectional areas of bone graft and regenerated bone in the former peri-implantitis defects were estimated using this method (Fig. 14C) (IV,VIII-X). Efficient and unbiased estimates of surface areas can be acquired by the vertical section technique and a systematic test system of cycloids (15). The vertical section technique involves sectioning of the specimen at a uniform random orientation parallel to a vertical axis. This method was therefore improper to evaluate re-osseointegration after surgical treatment of peri-implantitis (VIII-X). The above-described transfer of defect border from radiographical to histological images dictated that all implants were sectioned in

Fig. 13. The unbiased two-dimensional numerical density and total number of the various inflammatory cells per section were estimated by moving the unbiased counting frame in meander-like systematic uniform random positions over the connective tissue around implants and teeth. The mucogingival junction was used as a fixed anatomical structure to demarcate a reference sectional area which include all inflammatory cells.

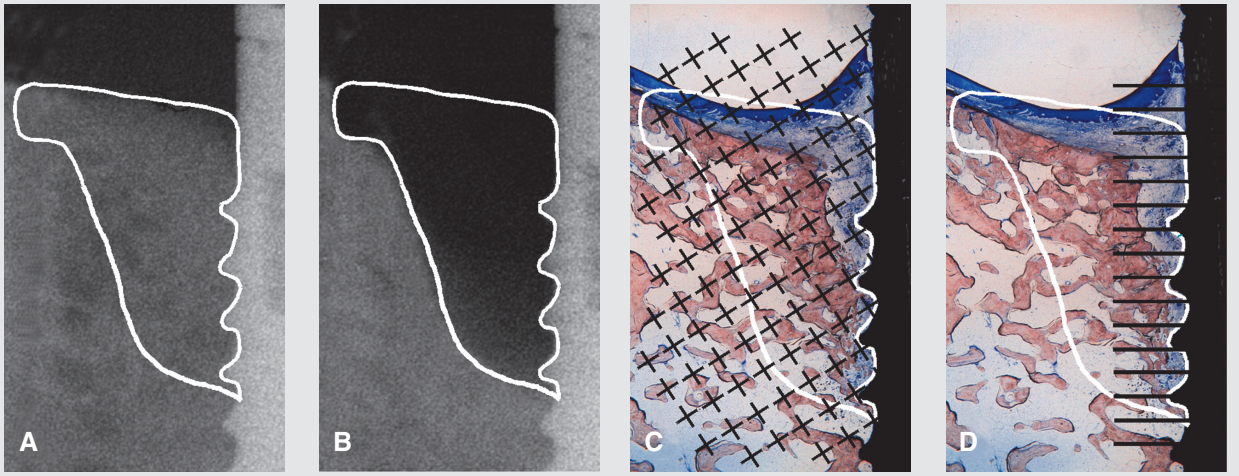


Fig. 14. A,B,C: The geometrically standardised radiographs taken before ligature placement (A) and at ligature removal (B) were used to outline the former peri-implantitis defect border on the corresponding histological sections (C). C: A fixed, systematic point set placed over the defect was applied to estimate the total sectional area of bone graft and regenerated bone within the defect. D: The proportion (%) of the implant »surface« within the defect covered by regenerated bone was estimated by placing a fixed, systematic set of straight parallel lines over the defect perpendicularly to the implant axis.

the same direction to obtain sections mesially, distally, buccally, and lingually. A systematic set of straight parallel lines placed perpendicularly to the implant axis was used to estimate the two-dimensional proportion (%) of the implant »surface« within the defect covered by regenerated bone (Fig. 14).

In conclusion, the unbiased two-dimensional stereological procedures used are effective methods to evaluate plaque-induced inflammatory reactions around osseointegrated oral implants and to assess the amount of bone regeneration and re-osseointegration after surgical treatment of peri-implantitis.

3.5. Conclusions

Although all manipulation of primates used as laboratory animals necessitates anaesthesia or sedation, the cynomolgus monkey is a feasible laboratory animal to investigate plaque-induced peri-implant disease. The risk for zoonoses is minimal, provided proper precautions are taken. Longitudinal evaluation of the peri-implant bone can be performed on geometrically standardised radiographs by bone level measurements and quantitative digital subtraction radiography. The used histological and stereological methods enable detailed examination and recordings of important characteristics of the peri-implant tissue. Unbiased two-dimensional stereological methods are effective techniques to estimate the total number of the various inflammatory cells within the peri-implant mucosa, as well as the amount of regenerated bone and re-osseointegration after surgical treatment of peri-implantitis.

4. Plaque-induced peri-implant disease

Various pathological changes have been observed around oral implants, including fistula formation and peri-implant mucosal enlargement (6,70,125,128,208). It was early reported that presence of plaque on the implant surface and inflammatory reactions of the peri-implant mucosa did not correlate (7). However, the significance of plaque accumulation on the peri-implant tissue has been comprehensively elucidated during the past decade.

4.1. Clinical and radiographical characteristics

The correlation between plaque accumulation and peri-implant bone loss has been assessed in four long-term studies of humans involving Brånemark implants with a machined surface (Table 2) (161-163,246). Three of these studies involved the same patient group (161-163). Significantly increased peri-implant bone loss was revealed in patients with poor oral hygiene as compared with patients with good oral hygiene in three of these studies (161-163). In contrast, no

correlation was recently reported (246). Surprisingly, a detailed statistical analysis based on ten-year data showed a correlation between plaque accumulation and peri-implant bone loss in smokers, while no difference in bone loss could be demonstrated among non-smokers with good and poor oral hygiene, respectively (163).

Exclusively mandibular implants in edentulous individuals were assessed in these investigations and the amount of plaque accumulation was not reported (161-163,246). A huge number of studies in humans and animals have demonstrated that plaque accumulation is associated with inflammatory changes of the peri-implant mucosa, bleeding/suppuration on probing, increased probing depth, probing »attachment« loss, and loss of the occlusal portion of the peri-implant bone (Fig. 15) (III,VI,VII,IX,X,4,24,35,53,73,74,107,120,139,143,146,152,160,175,200,216,238,249,258,274). However, long-term studies in humans investigating the influence of pronounced plaque accumulation on the peri-implant tissue are needed.

The influence of plaque accumulation on the peri-implant tissue has been evaluated in experimental studies of humans and animals (4,24,35,73,74,216,274). Cessation of oral hygiene procedures caused inflammatory reactions of the peri-implant mucosa. The peri-implant bone was not resorbed in these studies, probably because the plaque accumulation and/or the observation period were limited. Studies in animals have confirmed that pronounced plaque accumulation due to ligature placement causes destruction of the peri-implant tissue (III,53,84,107,120,139,146,160,175,177,200,238,258). An experimental study in dogs involving histological evaluation showed for unknown reasons continuous bone loss in some cases, in spite of ligature removal (175). Further studies are needed to evaluate the clinical significance of this finding. Moreover, a recently published study indicated that the presence of failing implants may influence the bone level of the remaining implants, since implant failure was associated with increased bone loss around the remaining implants as compared with implants in patients without implant failures (112). The etiological background of this observation is unknown.

Ligature-enhanced plaque accumulation on implants and teeth has been compared in monkeys and dogs (Table 3) (III,139,146,160,200). The number of animals included in each study varied between two and eight. These studies showed that ligature-enhanced plaque accumulation causes inflammatory reactions of the peri-implant mucosa/gingiva and destruction of the supporting tissue. Comparable tissue destruction was revealed around implants and teeth in two of these studies (146,200), while more pronounced destruction was demonstrated around implants as compared with

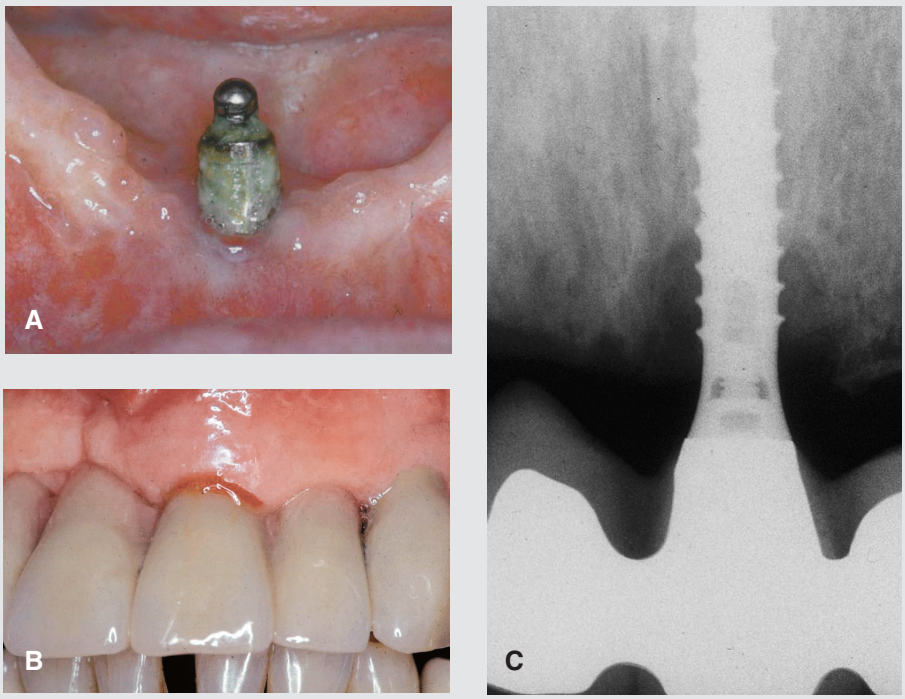


Fig. 15. A: Peri-implant mucositis characterised by inflammatory reactions of the peri-implant mucosa, including bleeding on probing. B,C: Peri-implantitis characterised by inflammatory reactions of the peri-implant mucosa, including suppuration/bleeding on probing, probing »attachment« loss, and loss of the occlusal portion of the peri-implant bone. Peri-implantitis defects normally assume the shape of a saucer around the entire implant.

teeth in two other investigations (III,160). However, the initial phase of plaque-induced inflammation seems to be associated with more pronounced tissue destruction around implants as compared with teeth (III,160), while long-term presence of inflammation appears to be characterised by comparable tissue destruction (146).

Ligature-enhanced plaque accumulation has been compared around implants, ankylosed teeth, and control teeth by focusing upon the initial breakdown phase of the supporting tissue (III). Ankylosed teeth were included in the study to evaluate the significance of the lacking periodontal ligament of implants for the breakdown of the supporting tissue. It was concluded that plaque accumulation seems, at least in the initial phase, to have more serious implications around implants and ankylosed teeth without a periodontal ligament than around teeth with a periodontal ligament. This observation can be related to several factors, including anatomical differences between the peri-implant mucosa and gingiva as discussed below.

The significance of other factors than plaque accumulation, including keratinisation of the peri-implant mucosa, smoking, implant surface characteristics, high susceptibility to periodontitis, and genetic background on the maintenance of the peri-implant tissue has only been sparsely evaluated.

The peri-implant mucosa may not always be keratinised. Actually, the frequency of buccal and lingual implant surfaces surrounded by keratinised mucosa has been reported to be between 30 and 100% (233). Implants are more frequently surrounded by keratinised mucosa in partially edentulous as compared with totally edentulous individuals (11). Moreover, long-term edentulousness seems to be associated with a decreased keratinised mucosa width (182). However, lack of keratinisation seems not to jeopardise the maintenance of healthy peri-implant tissue when plaque control is adequate (6,7,12,143,152,218,264). A study in monkeys has indicated that ligature-enhanced plaque accumulation may cause extended tissue destruction around implants surrounded by an unkeratinised mucosa as compared with implants surrounded by a keratinised mucosa (258), but the clinical relevance of this observation is presently unknown.

Smoking appears to be a considerable risk factor for peri-implant bone loss around implants inserted in the mandible (162,163). It has been reported that smoking seems to affect the development of peri-implantitis around maxillary and mandibular implants differently (101). Although no differences in plaque accumulation could be demonstrated between smokers and non-smokers, significantly higher gingival score, probing depth, and bone loss were observed in smokers as compared with non-smokers. However, these observations were for unknown reasons only characteristic for maxillary implants. Therefore, further studies are needed to elucidate the influence of smoking on the peri-implant tissue.

During the past decade, various modifications of the implant surface have been introduced to enhance the establishment of osseointegration (39). In an extensive review it was concluded that implants with a rough surface seem to have an increased failure rate due to peri-implantitis as compared with implants with a machined surface (77). However, different patient populations and/or evaluation methods may compromise the comparison of the different studies. Actually, clinical, radiographical, and histological evaluation of ligature-enhanced plaque accumulation around TPS, hydroxyapatite-coated, and machined titanium-alloy implants indicated that the evaluated implant types were equally susceptible to peri-implantitis (252,253).

Failing implants seem to concentrate in patients with inadequate oral hygiene (243,244). Although no association seems to exist between the amount of bone loss around implants and teeth in partially edentulous patients (112,221), it is presently unknown whether patients with high susceptibility to periodontitis also have high susceptibility to peri-implantitis. It has been reported that periodontitis-susceptible individuals can be treated successfully with implants, provided periodontal treatment is performed before implant placement (Table 4) (37,68,69,72,79,103,140,155,173,180,181, 195,271,272). In addition, a clinical study of patients with moderate to advanced periodontitis demonstrated stable peri-implant tissue and gingiva during a period of three years (230). Moreover, no significant correlation could be demonstrated between the bone loss around implants and teeth in a ten-year study involving patients with advanced periodontitis (155). However, recently published studies have reported slightly increased implant failure rates and peri-implant bone loss in patients susceptible to periodontitis (26,103).

The above-mentioned studies appear mainly to include elderly patients and/or patients with slowly progressive periodontitis. In addition, the implants were often followed for a limited period of time. From a clinical point of view, it is essential to obtain increased knowledge about the long-term success rate of implants inserted in younger patients with rapidly progressive periodontitis.

Non-smokers with a specific genotype of the polymorphic interleukin-1 (IL-1) gene cluster characterised by a high level of IL-1 production were associated with more severe periodontitis (142). In addition, individuals with this specific genetical modification have almost a three-times increased risk of tooth loss as compared with individuals without this

genetical modification (178). There seems to be no relation between the IL-1 positive genotype and implant failure (270). However, in this study most of the failures occurred within the first year of function. It is therefore unknown whether development of peri-implantitis around implants in function for a long period of time is related to the IL-1 positive genotype.

In conclusion, plaque accumulation can cause inflammatory reactions of the peri-implant mucosa and destruction of the supporting tissue. A study in animals involving ligature-enhanced plaque accumulation has shown that the initial phase of plaque-induced inflammation is characterised by more pronounced tissue destruction around implants and ankylosed teeth without a periodontal ligament as compared with teeth with a periodontal ligament. In contrast, long-term ligature-enhanced plaque accumulation is characterised by almost identical tissue destruction around implants and teeth. However, long-term studies in humans assessing the influence of pronounced plaque accumulation on the peri-implant tissue are needed. The influence of factors other than plaque accumulation on the peri-implant tissue, including smoking, implant surface characteristics, high susceptibility to periodontitis, and genetical background has only been sparsely investigated.

4.2. Histology

Clinically healthy peri-implant mucosa has been studied in a huge number of investigations (Table 5) (IV,3-5,7,23-25,35,36,41,42,46,53,74,75,91,106,135,144,152,153,156,157,166,171,192,224,234,237,255-257,261,274,275). Although peri-implant mucosa and gingiva have several features in common, considerable differences have been shown, as previously reviewed in detail (22,165,233,259). Mainly differences in the composition and structure of the connective tissue have been demonstrated (Table 5).

Histological evaluation of biopsies from humans has revealed that clinically inflamed peri-implant mucosa contains an increased number of inflammatory cells (36,213,229,237,274). An experimental study in dogs has demonstrated that cessation of oral hygiene procedures is characterised initially by nearly identical inflammatory reactions around implants and teeth (24). In contrast, long-term plaque accumulation seems to be associated with an increased apical extension of the inflammatory infiltrate around implants as compared with teeth (73). Plasma cells were the dominating inflammatory cell type around both implants and teeth. Further, it has been reported that the inflammatory reactions after an extended period of plaque accumulation are independent of the implant system used (4).

The inflammatory reactions due to ligature-enhanced plaque accumulation have been assessed around implants, ankylosed teeth, and control teeth by histological and stereological methods (Table 3) (IV). The junctional epithelium was changed into a pocket epithelium with rete ridges (Fig. 8). Whereas this pocket epithelium was frequently ulcerated around implants, scattered ulcerations were observed around teeth. Unbiased stereological estimates showed that the pronounced plaque accumulation was associated with significantly increased total number of lymphocytes, plasma cells, and neutrophils around implants and teeth. No significant differences could be demonstrated in the total number of plasma cells and neutrophils around implants and teeth. In contrast, the total number of lymphocytes was significantly higher around implants as compared with ankylosed teeth and control teeth. Consequently, ligature-enhanced plaque accumulation was characterised by more pronounced inflammatory reactions around implants as compared with teeth.

It has been shown in monkeys and dogs that placement of peri-implant ligatures causes bone resorption and extensive inflammatory reactions within the peri-implant mucosa (53,116,121,260). Histological assessment of the peri-implant mucosa and gingiva one month after ligature removal in dogs revealed the presence of a larger inflammatory infiltrate within the peri-implant mucosa as compared with gingiva (160). However, the assessment was hampered by the fact that the evaluation was performed one month after ligature removal. It is interesting to note that the inflammatory infiltrate within the peri-implant mucosa frequently reached the bone crest and even extended into the bone marrow (160,175). This observation has not been confirmed in other animal models.

The previously referred radiographical observation of increased tissue destruction around implants and ankylosed teeth as compared with control teeth during the initial phase of ligature-enhanced plaque accumulation was substantiated by the histological evaluation, because osteoclasts were only observed around implants and ankylosed teeth (IV). Implants are lacking cervical cementum with inserting gingival fibers as well as a periodontal ligament. The background of the increased tissue destruction around implants could be the lack of one or both of these tissue components. Cervical cementum with inserting gingival fibers was absent on approximately half of the sections from ankylosed teeth. The presence of osteoclasts was not related to the absence of cervical cementum with inserting gingival fibers around ankylosed teeth. Therefore, absence of cervical cementum with inserting gingival fibers does not seem to be the background of the increased tissue destruction around implants during

the initial breakdown phase of ligature-enhanced plaque accumulation. In contrast, the histological evaluation support that the increased tissue destruction seems for unknown reasons to be related to the absence of a periodontal ligament.

The combined influence of plaque-induced inflammation and occlusal overload on the peri-implant tissue has only been briefly studied. An experimental study in monkeys indicated that the combination of peri-implantitis and repetitive mechanical implant loading did not resulted in increased destruction of the peri-implant tissue as compared with peri-implantitis without repetitive mechanical implant loading (116). Also static loading seems not to jeopardise the bone level around implants with established peri-implantitis lesions (90). However, the relevance of the experimental loading procedures used is unknown. Therefore, further studies are needed to clarify the significance of simultaneous plaque-induced inflammation and occlusal overload on the peri-implant tissue.

In summary, the histological and stereological evaluation revealed that the initial phase of inflammation after ligature-enhanced plaque accumulation is characterised by more pronounced inflammatory reactions around implants than around teeth. In addition, more severe tissue destruction occurs around implants and ankylosed teeth without a periodontal ligament as compared with teeth with a periodontal ligament. The background of this observation seems for unknown reasons to be the lacking periodontal ligament and not the absence of cervical cementum with inserting collagen fibers of implants. Histological examination of active peri-implantitis has been limited to animal studies. Therefore, the histopathological characteristics of active peri-implantitis in humans remain to be described. Moreover, simultaneous presence of plaque-induced inflammation and occlusal overload should be further assessed.

4.3. Microbiology

Placement of peri-implant/-dental ligatures has enabled longitudinal studies of the microbiological changes associated with initiation and progression of peri-implantitis and periodontitis in cynomolgus monkeys (V,137,141). The evaluation included phase-contrast microscopy and cultivation on selective and non-selective media (V). DNA probes are increasingly used to identify the different bacterial species in plaque samples from humans, but this method has only been validated for *P. gingivalis* in cynomolgus monkeys (191). It is therefore presently unknown whether DNA probes for human plaque samples can be used to identify other microorganisms than *P. gingivalis* in samples from monkeys with comparable sensitivity and specificity as in humans.

The submucosal microbiota of implants surrounded by healthy and inflamed tissue has been assessed in a large number of cross-sectional studies (185,186,222,233). It has been demonstrated that implants surrounded by clinically healthy mucosa are associated with a scattered submucosal flora dominated by facultative Gram-positive cocci and rods. The submucosal/-gingival microflora was evaluated before ligature placement around implants, ankylosed teeth, and control teeth (V). The total number and distribution of the evaluated microbial species and categories was almost identical around implants and teeth. Previous studies, including comparison of the total number and distribution of the different microbial species and categories, have revealed significant differences in the submucosal flora around implants surrounded by clinically healthy tissue in partially edentulous as compared with totally edentulous patients (185,186, 222,233). Most likely, this difference is accounted for by teeth and their pockets acting as bacterial reservoirs for the colonisation of the implants (189,207,217). In accordance with the present observations (V), the total number and distribution of the various microbial species and categories in the submucosal/-gingival microflora is comparable around implants and teeth surrounded by healthy tissue in partially edentulous patients and animals as previously discussed in detail (185,186,222,233). Consequently, absence of a periodontal ligament does not influence the composition of the microbiota associated with clinically healthy or slightly inflamed tissue.

Considerable changes occurred in the submucosal/-gingival microflora after establishment of inflammation associated with ligature-enhanced plaque accumulation (V). The total number of cultivable bacteria and the proportion of motile rods, anaerobic Gram-negative rods, black-pigmented rods, *P. gingivalis*, and *Prevotella intermedia* increased significantly. However, no significant differences were observed between implants, ankylosed teeth, and control teeth. Studies in dogs and pigs have shown an analogous shift in the microbiota around implants after ligature placement (107,154,200). In addition, studies in monkeys and dogs have confirmed an almost identical microflora associated with inflammation due to ligature-enhanced plaque accumulation around implants and teeth (66,154,200). Therefore, the observed difference in the initial phase of plaque-induced inflammation around implants and teeth seems to be related to factors other than those evaluated in the present microbiological study (V). Microbial species possessing the capacity of direct and indirect tissue destruction have been identified around implants with inflammatory reactions of the peri-implant mucosa, pocket formation, and bone loss in cross-sectional studies of humans (185,186,222,233). The list of peri-implan-

titis-associated microorganisms seems to increase with recognition of new species and with reclassification of others. It is presently unknown whether all changes in the submucosal microflora are the cause of the disease process rather than a consequence. Also, it is important to emphasise that only a part of the microflora is cultivable. Microorganisms other than those revealed may play a significant role in the disease progression.

4.4. Conclusions

Plaque accumulation can induce inflammatory reactions and destruction of the peri-implant tissue. Experimental studies involving ligature-enhanced plaque accumulation have demonstrated that the initial breakdown phase of plaque-induced inflammation is characterised by more pronounced inflammatory reactions around implants than around teeth. In addition, more severe tissue destruction occurs around implants and ankylosed teeth without a periodontal ligament as compared with teeth with a periodontal ligament. The background of this observation seems for unknown reasons to be the lacking periodontal ligament, and not the absence of cervical cementum with inserting collagen fibers of implants. However, long-term ligature-enhanced plaque accumulation is characterised by almost identical tissue destruction around implants and teeth. Plaque-induced inflammation is associated with a comparable composition of the microbiota around implants and teeth, i.e. a complex flora with a dominance of Gram-negative anaerobic rods. The performed microbiological evaluation could not explain the observed difference in the initial phase of plaque-induced inflammation around implants and teeth. Long-term studies in humans assessing the influence of pronounced plaque accumulation on the peri-implant tissue are needed. Also, the histopathological characteristics of active peri-implantitis in humans remain to be described. Moreover, further studies are needed to clarify the influence of factors other than plaque accumulation on the peri-implant tissue, including smoking, implant surface characteristics, high susceptibility to periodontitis, genetical background, and the combined influence of plaque-induced inflammation and occlusal overload.

5. Diagnosis of plaque-induced peri-implant disease

The applicability and significance of conventional periodontal registration methods to evaluate the peri-implant tissue have been unclear. Failed implants may be overlooked when only a clinical evaluation of the peri-implant mucosa is performed (77). Furthermore, clinical examination of the peri-implant mucosa was previously considered of no or only minor clinical relevance (7,12,47,54,152,218,220). However, limited plaque accumulation and associated mild inflammatory reactions were recorded in these studies. Moreover, the patients were in general followed for only a limited period of time. It has been confirmed that plaque accumulation may be associated with inflammatory reactions of the peri-implant mucosa, increased probing depth, probing »attachment« loss, and loss of the occlusal portion of the peri-implant bone as discussed above. Therefore, examination of the peri-implant tissue normally involves both clinical and radiographical methods, enabling diagnosis of not only implant failure, but also plaque-induced peri-implant disease (29,77,82,130,148,149,187,196,203,228,233).

5.1. Clinical methods

Clinical evaluation of implants may involve registration of percussion sound, implant stability, keratinised mucosa width, plaque accumulation, visual assessment of the peri-implant mucosa, bleeding on probing, probing depth, and probing »attachment« level. Furthermore, peri-implant crevicular fluid analyses and various microbial tests may be used to detect early stages of peri-implant disease.

Registration of percussion sound and implant stability is usually incorporated in the clinical examination of implants. A dull sound upon percussion and implant mobility are indicative of total lack of osseointegration. Implants affected by severe peri-implantitis are still characterised by a clear metallic sound upon percussion and no mobility, if osseointegration of the apical part of the implant is maintained. Therefore, these registrations cannot be used to evaluate the severity of plaque-induced disease. Only total lack of osseointegration can be diagnosed by these methods. Also Periotest recordings (Periotest, Siemens, Germany) seem so far too insensitive to evaluate the severity of plaque-induced disease (55,123).

The evaluation may also involve registration of the width of keratinised mucosa. As earlier discussed, lack of keratinisation does not prevent the maintenance of healthy peri-implant tissue, provided adequate oral hygiene is carried out. Therefore, further studies are needed to clarify the relevance of this recording.

As previously described, plaque is an important etiological factor of peri-implant disease. Assessment of plaque accumulation is therefore mandatory. Different methods have been used to evaluate plaque accumulation. Recording of presence/absence of plaque as a dichotomous variable has

been applied (233). Also the Plaque Index (239) has been used (233). Finally, a modified index has been described specifically for implants (188). However, it is presently unknown which method is preferable.

The Gingival Index (169) has been applied to evaluate inflammatory changes of the peri-implant mucosa (233), but registration of texture and colour of the peri-implant mucosa may be inappropriate parameters for the evaluation. The titanium surface and scar formation may change the colour of the peri-implant mucosa jeopardising the applicability of the index (12). Moreover, an unkeratinised peri-implant mucosa may be reddish in spite of no inflammation. Therefore, the index may be more useful when bleeding on probing to the bottom of the peri-implant sulcus/pocket is used as the parameter of inflammation (III,VI,VII,IX,X). Accordingly, a modified Sulcus Bleeding Index has been suggested for the evaluation of inflammatory reactions in the superficial part of the peri-implant mucosa (188). It has been stated that bleeding on probing is a questionable risk predictor of periodontitis progression, while absence of bleeding is a reliable indicator of periodontal health (13,145). Similar considerations appear to be valid for implants (126,168).

Increased probing depth and probing »attachment« loss are pathognomonical for peri-implantitis. A light probing force of 0.25 N (25 g) has been suggested for implants (149,184,187). Concern has been expressed about probing the peri-implant sulcus/pocket due to possible tissue damage, inoculation of bacteria, and potential damage of the implant surface by metallic probes. The epithelial damage after mechanical separation of healthy gingiva from the tooth surface is rapidly repaired (250). Although putative pathogenical bacteria can be transferred to healthy gingival sites by contaminated probes after probing periodontitis lesions, permanent colonisation does not appear to be a characteristic consequence (16,48,206). There are no data available to suggest that implants may differ from teeth in this regard.

Numerous studies have documented that probing measurements around teeth often fail to identify the occlusal connective tissue level/apical border of the junctional/pocket epithelium (13,105,164). It has been demonstrated in most studies that the probe position is influenced by the severity of gingival inflammation, probing force, and probe type/shape. The effect of inflammation and probing force on probing measurements around implants has been documented (VI,147,190). In a recently published series of experimental studies, various surgical treatment modalities of peri-implantitis were assessed (VII-IX). Although limited bone regeneration was found after treatment with a conventional flap procedure alone, a considerable probing »attachment« gain was observed. Consequently, probing »attachment« gain after treatment of peri-implantitis may be related to the elimination of the inflammatory reactions within the peri-implant mucosa rather than to peri-implant bone gain.

The significance of probing measurements around implants has been assessed in animals (VI,71,122,147). Especially the relation between the probe tip and the adjacent tissue components around implants and teeth has been studied (Table 6) (VI,71,147). Comparison of the relation between the probe tip and the apical extension of the junctional epithelium revealed advanced probe penetration around implants as compared with teeth when healthy peri-implant mucosa/gingiva was present (71). Actually, the probes penetrated through the junctional epithelium around implants. In contrast to these results, the apical epithelial border was identified by probes around implants with healthy mucosa and mucositis, while the probes penetrated up to 1.6 mm into the connective tissue of implants with peri-implantitis in another study (147). Since peri-implantitis is characterised by extensive ulceration of the pocket epithelium (IV), it may be irrelevant to assess the relation between the probe tip and the epithelium in the presence of inflammation.

Also the relation between the probe tip and the alveolar bone has been evaluated in animals (Table 6) (VI,71,147). It was concluded that even mild plaque-induced inflammation was associated with deeper probe penetration around im-

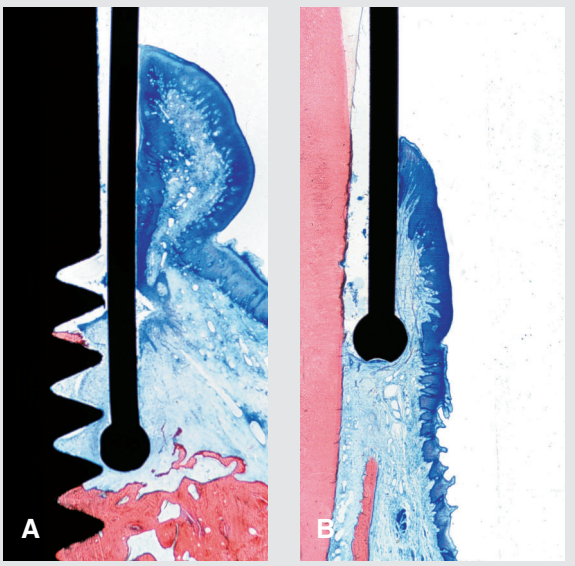


Fig. 16. Different relation between probe tip and bone around an implant with peri-implantitis (A) and a tooth with periodontitis (B).

plants as compared with teeth in eight monkeys (VI). For implants and teeth with healthy peri-implant mucosa/gingiva, a comparable distance from the probe tip to the alveolar bone of 0.5-1.5 mm was found. In contrast, the probe tip was significantly closer to bone around implants than around teeth in conditions of mild mucositis/gingivitis, severe mucositis/gingivitis, and peri-implantitis/periodontitis. A distance of less than 0.5 mm was frequently found around implants with severe mucositis and peri-implantitis, while most distances ranged between 0.5 and 1.5 mm around teeth with severe gingivitis and periodontitis. The different relation between probe tip and bone around an implant and tooth with peri-implantitis/periodontitis is presented in Fig. 16. The background of this difference in probe penetration around implants and teeth may be the different connective tissue fibre configuration of the peri-implant mucosa and gingiva (22,165,233,259). The absence of cervical cementum with inserting connective tissue fibres may facilitate probe penetration around implants surrounded with even mildly inflamed tissue.

In accordance with the above-described observations in monkeys (VI), a shorter distance was found between the probe tip and the bone around implants with peri-implantitis as compared with implants surrounded by healthy tissue in a study involving five dogs (147). Systematic comparison with teeth was not included in this study. In contrast to the two studies mentioned above (VI,147), a closer relation between probe tip and bone has been demonstrated around implants in comparison with teeth with healthy peri-implant mucosa/gingiva (71). The background of this discrepancy is probably variations in the inflammatory reactions of the peri-implant mucosa/gingiva, or the applied technique including differences in animal models, implants, probes, and probing forces. It is interesting to note that studies in humans did not disclose an intimate relation between the probe tip and bone around implants with healthy peri-implant tissue (40,190,219). However, it is still unknown why probing around implants seems more painful than around teeth (126,218,219).

Crevicular fluid around implants was demonstrated early (11,144). Analyses of the peri-implant crevicular fluid may be used as a non-invasive method to detect early stages of plaque-induced peri-implant disease. A positive correlation has been demonstrated between the peri-implant crevicular fluid volume and most clinical parameters of inflammation as well as bone loss (18,197). Also, analyses of the crevicular fluid from implants with inflammatory changes of the peri-implant mucosa and/or bone loss in most studies have shown elevated levels of several mediators of inflammation and tissue destruction, including interleukin-1β (IL-1β), prostaglandin E_2 (PGE_2), platelet-derived growth factor (PDGF), acute-phase proteins, IgG against *P. gingivalis*, alkaline phosphatase, neutral proteolytic enzyme, dipeptidyl peptidase, cathepsin B/L, aspartate aminotransferase, trypsin, matrix metalloproteinase-8 (MMP-8), lactoferrin, and elastase (2, 8,67,81,113,126,134,205,214,227,251). However, analyses of neutral proteolytic enzyme and aspartate aminotransferase activity appear to be of limited value for the prediction of peri-implantitis progression (126,226). Accordingly, it was recently concluded that further longitudinal studies are needed before peri-implant crevicular fluid analyses can be used routinely to differentiate between destructive and non-destructive inflammatory reactions (77). The same conclusion seems valid for various microbiological tests (77,184,185,187), although a recent study has indicated that the combination of microbiological and clinical parameters may offer an advantage in predicting peri-implant bone loss (168).

In summary, probing measurements around implants and teeth are different. Even mild plaque-induced inflammation is associated with a deeper probe penetration around implants as compared with teeth. It seems recommendable to evaluate probing depth and probing »attachment« level around osseointegrated oral implants, provided the described influence of inflammation on probe penetration is considered.

5.2. Radiographical methods

Preservation of the peri-implant bone is crucial for implant maintenance, and an unchanged peri-implant bone level is one of the main parameters of implant success (10). In the absence of clinical signs of inflammation, it has been recommended to take radiographs after one year of function and thereafter not more than every second year (187). Geometrically standardised intra-oral radiographs using the paralleling technique have been advocated for the detection of minor changes in the peri-implant bone (29,82,149). Mainly the peri-implant bone level and the interface between the implant and the bone are evaluated. Further development is necessary before quantitative digital subtraction radiography can be used routinely in the clinical setting.

Usually, probing measurements are able to identify bone loss due to periodontitis (13). As previously described, studies of experimental peri-implantitis have demonstrated that increased probing depth and probing »attachment« loss were associated with peri-implant bone loss. A significant positive correlation has likewise been documented between probing »attachment« level and bone level in humans (219). However, an experimental study involving implants with bone loss due to plaque accumulation or occlusal overload

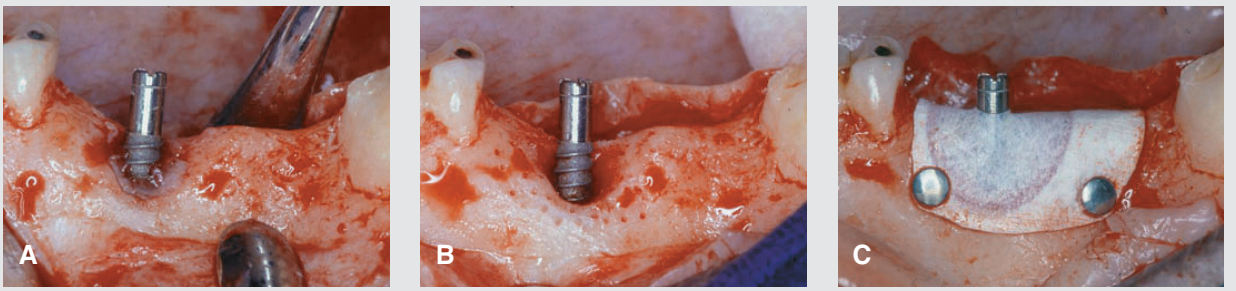


Fig. 17. Clinical features during surgical treatment of peri-implantitis. A: After elevation of full mucoperiosteal flaps. B: After removal of granulation tissue and multiple perforations of the bone surface. C: After membrane placement with or without a bone graft.

has revealed that the bone level was more reliably detected by radiographs as compared with probing measurements (122). In addition, presence of implant shoulder, threads, surface coating, and suprastructure may compromise or even render probing impossible. Actually, many implant sites are inaccessible to probing without suprastructure removal (244). Finally, deep pockets do not always indicate bone loss, but may be due to a thick peri-implant mucosa (6). Therefore, probing measurements cannot substitute the radiographical assessment of the peri-implant bone.

5.3. Conclusions

Diagnosis of implant failure and plaque-induced peri-implant disease necessitates both clinical and radiographical recordings. Probing measurements around implants and teeth are different. Although even mild plaque-induced inflammation results in a deeper probe penetration around implants as compared with teeth, it seems recommendable to evaluate probing depth and probing »attachment« level, provided the described influence of inflammation on probe penetration is considered. A risk of adverse effects by introducing periodontal probes into the peri-implant sulcus/pocket has never been demonstrated. Further development is needed before quantitative digital subtraction radiography, peri-implant crevicular fluid analyses, and various microbial tests can be used routinely in the clinical setting for early diagnosis of destructive plaque-induced inflammatory reactions of implants.

6. Treatment of peri-implantitis

Various treatment options of plaque-induced peri-implant disease have previously been meticulously reviewed (14,17, 78,129,130,184,185,187). Mechanical debridement, antiseptics, antibiotics, surgical procedures, and explantation have been recommended as a sequence of possible therapeutic procedures, depending upon the clinical and radiographical manifestations (129,130,149,184,185,187). The primary goal of peri-implantitis treatment is cessation of the disease progression to maintain the implant in function with healthy peri-implant tissue. Moreover, procedures resulting in regeneration of the lost peri-implant tissue are desired. However, it was recently concluded that no treatment procedure of peri-implantitis has so far been documented in a long-term study (17,78,185). In fact, most recommendations are lacking scientific documentation.

6.1. Surgical procedures

Surgical treatment procedures of peri-implantitis have been assessed in humans (Table 7) (14,19,85,87,102,119,136,150,167, 172,179,193,254). Most of the defects occurred several years after implant placement. Although three studies involving a total of 59 patients have been published (19,102,136), most reports involved only one patient. Predominantly, the use of membranes and/or various bone substitutes has been evaluated. Supportive systemic antibiotic therapy for 7-10 days has been included in most studies, and the membranes were used around submerged implants as well as around implants penetrating the oral mucosa during the healing period. Peri-implant health and bone regeneration have been documented after treatment, but in most studies the observation period was short. Detailed results three years after treatment have been presented in only two studies (19,136).

The assessment has also involved animals (Table 8) (VII-X, 58,76,93,114,132,170,198,199,209-212,242,269). Except for the present series of studies in monkeys and a study involving one pig (VII-X,242), the investigations have been carried out in dogs. Several of the accomplished studies have unfortunately included few animals, i.e. 1-4 animals (132,170,210-

212,242). While both maxillary and mandibular implants were included in the present series of studies (VII-X), the treatment in the other studies has been assessed around mandibular implants alone. As in the human studies, the evaluation has predominantly involved the use of membranes and/or various bone substitutes combined with systemic antibiotic therapy for 10-21 days. Although healthy peri-implant tissue was obtained in most studies, the amount of bone regeneration and re-osseointegration varied considerably.

Surgical treatment of peri-implantitis with autogenous bone graft particles and ePTFE membrane has been investigated in one animal study involving implants with a TPS surface (Fig. 17, Table 8) (VII,VIII). Healthy peri-implant tissue was obtained irrespective of the surgical procedure applied. However, quantitative digital subtraction radiography revealed a bone gain almost equivalent to the level before the peri-implantitis defects were established after using membrane-covered autogenous bone, while minimal bone regeneration occurred in defects treated with autogenous bone, membrane, or a conventional flap procedure alone (control procedure) (Fig. 2). Moreover, unbiased stereological estimates demonstrated significantly increased bone regeneration and re-osseointegration in defects treated with membrane-covered autogenous bone as compared with the other three treatment procedures (Fig. 6). A mean bone-to-implant contact of 45% was recorded in the former peri-implantitis defects when the treatment involved membrane-covered autogenous bone, while the corresponding values for the autogenous bone, membrane, and control groups were 22, 21, and 14%, respectively.

Different results have been reported in humans after the use of autogenous bone with or without membrane coverage in the surgical treatment of peri-implantitis (Table 7) (19,102, 136,254). A block or particles of autogenous bone without membrane coverage resulted in a mean bone gain of 4.2 mm corresponding to total defect regeneration (19). In contrast, a mean bone gain of 2 mm corresponding to 36% of the previous defect height was demonstrated after treatment with membrane-covered autogenous bone graft particles (102). Finally, it was recently described that additional application of a membrane did not improve the treatment outcome as compared with autogenous bone alone (136). However, the treatment procedure was apparently not randomised. Comparison of the treatment outcome obtained within these studies of humans and the present series of animal studies is complicated by differences in the examined implants and grafts (Tables 7,8). In addition, different implant surface preparations were applied.

In 1983 it was concluded that autogenous bone is the preferred bone grafting material (38). Although autogenous bone is still preferred, bone substitutes are increasingly used to simplify the surgical procedure by eliminating the need for bone harvesting and the risk of donor site morbidity (117,118). Particular attention has been paid to Bio-Oss, a protein-free porous bovine-derived bone mineral with practical no risk for transmission of bovine spongiform encephalopathy (BSE) prions (20,265).

Bio-Oss alone or combined with a membrane has only been sparsely evaluated in the treatment of peri-implantitis in dogs (Table 8) (170,198,199). No significant differences could be demonstrated between the evaluated procedures, but unfortunately only 4-5 implants were included in each group. The present evaluation showed that the combined use of Bio-Oss and ePTFE membrane should be preferred as compared with treatments involving Bio-Oss, membrane, or a conventional flap procedure alone (Table 8) (IX). However, the amount of bone regeneration and re-osseointegration was significantly higher in defects treated with membrane-covered autogenous bone graft particles as compared with membrane-covered Bio-Oss (Table 9).

Membranes are applied to prevent growth of connective tissue and epithelium into peri-implant bone defects at the time of implant placement (117,118). Various grafting materials, including autogenous bone, are increasingly being combined with membranes to maintain the created space under the membrane and to serve as an osteoconductive scaffold accelerating bone regeneration (117,118). The present studies showed that combining graft material with membrane is preferable in the surgical treatment of peri-implantitis (VII-IX). In accordance with previous studies of membranes in the treatment of peri-implantitis, membrane exposure occurred frequently. Actually, 13-38% of the membranes were exposed (VII,IX,X). Usually, the dehiscences were small and occurred shortly after membrane placement.

Exposure of ePTFE membranes results in a maintenance problem (118), because bacteria can penetrate such porous membranes (201,240,241). Although topical application of chlorhexidine seems to reduce plaque formation on the exposed membrane, bacterial penetration cannot be prevented (241). Satisfactory results may be obtained in spite of membrane exposure, but the treatment outcome is usually jeopardised (117,118). To minimise the risk of acute infection, rinsing with chlorhexidine and careful debridement of the exposed membrane have been recommended (131), and by following these guidelines, exposed membranes may be left in place for a period of time, provided no signs of acute infection occur (131).

Statistical analysis of the influence of membrane exposure

on the bone regeneration was meaningless due to the limited number of exposed membranes in each study (VII-X). Bone regeneration occurred in spite of membrane exposure, which was probably due to the extensive plaque control regime, and exclusion of the defects with exposed membranes had no impact on the conclusions. It has been recommended to remove exposed membranes immediately in order to avoid impeding bone regeneration (102), but it is unknown whether removal of the exposed membranes in the present series of studies would have influenced the amount of bone regeneration observed.

In conclusion, autogenous bone graft particles covered by an ePTFE membrane is a useful surgical treatment modality of experimental peri-implantitis around implants with a TPS surface in monkeys. The bone gain is almost to the pre-peri-implantitis level, and unbiased stereological estimates demonstrated a mean bone-to-implant contact of 45%. The treatment outcome after using membrane-covered Bio-Oss seems less encouraging than after the use of membrane-covered autogenous bone. Although evaluation was undertaken in a primate model with many anatomical and biological similarities to humans, further long-term human studies are needed to draw firm conclusions about surgical treatment of peri-implantitis. From a theoretical point of view, treatment procedures ensuring pronounced bone regeneration and re-osseointegration is preferable to treatments resulting in limited or no bone regeneration and re-osseointegration. This aspect has never been scientifically elucidated. In this respect, it is important to underline that a statistically significant difference in treatment outcome may be clinically insignificant, although it is difficult to determine the »border« between clinical significance and insignificance. This general statement is also true for surgical treatment of peri-implantitis. However, the present animal studies indicate that future research of surgical treatment of peri-implantitis in humans should focus on the use of membrane-covered autogenous bone graft particles, and not on membrane-covered Bio-Oss.

6.2. Implant surface preparations

It has been mentioned that creation of an implant surface conducive to bone formation is mandatory for a successful treatment outcome of peri-implantitis (235). Contaminants such as bacteria, bacterial products, calculus, and soft tissue cells should be removed without modifying the implant surface or compromising the healing potential of the peri-implant tissue. However, it is still unknown to what extent these contaminants have to be removed for a successful treatment outcome, as previously discussed (185).

Numerous implant surface preparation methods have been used, either alone or in various combinations as part of the surgical treatment of peri-implantitis in humans and animals (Tables 7,8). It was recently concluded that no consensus has been reached about the most adequate cleaning method (78). *In vitro* studies focusing on various methods to clean the implant surface have currently been reviewed and discussed in detail (X). It was concluded that several methods are inappropriate for implant surface cleaning. Especially metal curettes for hand scaling, conventional sonic/ultrasonic scalers, and some laser types may severely damage the implant surface. Although implant surface damage can be almost prevented by using ultrasonic scalers with a non-metallic tip or resin curettes, the presence of implant threads and/or implant surface roughness may compromise their effect.

A useful model to evaluate various implant surface preparation methods in the surgical treatment of peri-implantitis is treatment of established peri-implantitis defects in animals, followed by histological evaluation of the treatment outcome. Few studies have been carried out according to these guidelines (X,58,210). No difference could be demonstrated when cotton pellet soaked in saline or rotating brush with pumice was combined with conventional flap surgery (210). Finally, no difference could be revealed when decontamination by a carbon dioxide (CO_2) laser and/or an air-powder abrasive unit was combined with flap surgery, with or without coverage of the defect by an ePTFE membrane (58).

Four implant surface preparation methods have recently been compared in monkeys: 1) air-powder abrasive unit followed by citric acid application, 2) air-powder abrasive unit, 3) gauze soaked in saline followed by citric acid application, and 4) gauze soaked alternately in chlorhexidine and saline (X). The experimental peri-implantitis defects were surgically exposed, and each implant was prepared according to one of the above-mentioned procedures. All defects were then filled with autogenous bone graft particles and covered by an ePTFE membrane. Clinical parameters, radiography, including quantitative digital subtraction radiography, histology, and stereology did not reveal significant differences between these methods. Almost total bone regeneration and considerable re-osseointegration were obtained irrespective of the method applied.

The influence of various air-powder abrasive systems on the titanium surface has been evaluated *in vitro* as previously discussed (X). Although increased implant surface roughness and retained powder particles have been observed, no or only minor surface changes were identified in most studies. Air-powder abrasive units have been used in the surgical treatment of peri-implantitis in several studies of humans

and animals without adverse effects (Tables 7,8). Although the mixture of water and abrasive powder is driven by compressed air, usually with a higher pressure than needed for other dental instruments, the number of emphysema and pneumoparotitis cases induced by air-powder abrasive units is very low (21,27,80). Only one case report on implants has been presented including submucosal emphysema, acute infection, and bone loss after removal of calculus in the maintenance care of titanium abutments (21). Consequently, there appears to be minimal risk associated with the use of air-powder abrasive units in the surgical treatment of peri-implantitis. However, a simpler method, i.e. gauze soaked alternately in chlorhexidine and saline, resulted in a comparable treatment outcome around implants with a TPS surface when membrane-covered autogenous bone graft particles were used in cynomolgus monkeys (X).

It has been questioned in many of the above-mentioned animal studies whether osseointegration can be established on an implant surface previously exposed to plaque and the oral environment. Osseointegration was early defined as a direct structural and functional connection between ordered living bone and the surface of a load-bearing implant (34). It has been stated that a successful treatment outcome of peri-implantitis depends on prevention of acute infection, mechanical wound stability with establishment and maintenance of a blood clot-filled defect, prevention of connective tissue in-growth, and creation of an implant surface conducive to bone formation (235). However, re-osseointegration seems dependent on implant surface characteristics. The amount of re-osseointegration in former peri-implantitis defects was higher on a rough implant surface (84%) than on a machined surface (22%) after treatment with a conventional flap procedure in dogs (211).

The mechanisms of bone healing after implant placement have been discussed previously (57). Osseointegration can be established by contact osteogenesis where new bone is formed directly on the implant surface, or by distance osteogenesis where new bone is formed on the surrounding bone surface, thereby creating contact with the implant (57). A series of coordinated events, including protein adsorption, cellular adhesion, cellular differentiation, cellular proliferation, and bone formation, occur at the interface between the implant and the bone after implant placement (57,183,231, 236). *In vitro* studies have indicated that several of these events are influenced by implant surface roughness (57,183,235,236). Implants with a rough surface generally exhibit a higher bone-to-implant contact as compared with implants with a machined surface, probably due to an increased osteoconductivity of the rough implant surface (39). The mechanisms of bone healing after treatment of peri-implantitis have not been described, but a difference in osteoconductivity may explain the apparently more favourable treatment outcome of peri-implantitis around implants with a rough surface.

6.3. Conclusions

Autogenous bone graft particles covered by an ePTFE membrane is a useful surgical treatment procedure of experimental peri-implantitis around implants with a TPS surface in cynomolgus monkeys. Healthy peri-implant tissue is obtained and the bone gain is almost to the pre-peri-implantitis level. Moreover, the mean bone-to-implant contact is 45% in the former peri-implantitis defects. The outcome after treatment with membrane-covered Bio-Oss seems less encouraging than after the use of membrane-covered autogenous bone. Therefore, surgical treatment of peri-implantitis involving autogenous bone graft particles and an ePTFE membrane should be evaluated in a long-term study in humans. Gauze soaked alternately in chlorhexidine and saline seems to be the preferred implant surface preparation method of implants with a TPS surface during surgical treatment of peri-implantitis. It seems reasonable to assume that the regenerated bone within the former peri-implantitis defects is able to withstand functional loading and plaque accumulation, as bone regenerated within defects adjacent to a sterile implant surface present at the time of implant placement. This aspect has neither been assessed in humans nor in animals. The use of resorbable membranes and growth factors, including bone morphogenetic proteins, should also be investigated in the surgical treatment of peri-implantitis.

7. Conclusions and suggestions for further research

The accomplished reviews and experimental studies warrant the following main conclusions and suggestions for future research:

- Although all manipulation of primates used as laboratory animals necessitates anaesthesia or sedation, the cynomolgus monkey is a feasible laboratory animal for studies of plaque-induced disease around osseointegrated oral implants. The risk of zoonoses is minimal, provided proper precautions are taken.
- The peri-implant bone can be assessed longitudinally on geometrically standardised radiographs in cynomolgus monkeys by bone level measurements and quantitative digital subtraction radiography. Further development of

quantitative digital subtraction radiography is desired to increase the precision of the method and to reduce the time used for registration.

- The cutting-grinding procedure ad modum Donath can be used to study the peri-implant tissue, provided addition of the described modifications to improve the technical quality of the sections.
- Unbiased two-dimensional stereological methods are effective tools to evaluate plaque-induced inflammatory reactions around osseointegrated oral implants and to assess the amount of bone regeneration and re-osseointegration after treatment of peri-implantitis.
- The initial phase of ligature-enhanced plaque accumulation is characterised by more pronounced inflammatory reactions around implants than around teeth. In addition, more pronounced tissue destruction occurs around implants and ankylosed teeth without a periodontal ligament than around teeth with a periodontal ligament. The background of this observation seems for unknown reasons to be the lacking periodontal ligament, and not the absence of cervical cementum with inserting collagen fibers of implants.
- Plaque-induced inflammation is associated with a comparable shift in the submucosal/-gingival microbiota around implants and teeth, i.e. from a scattered flora dominated by facultative Gram-positive cocci and rods, to a more complex flora where Gram-negative anaerobic rods constitute a major proportion.
- The histopathological characteristics of active peri-implantitis in humans remain to be described. Further studies are also needed to clarify the significance of simultaneous plaque-induced inflammation and occlusal overload on the peri-implant tissue.
- Probing measurements around implants and teeth are different. Even mild plaque-induced inflammation results in deeper probe penetration around implants as compared with teeth. It seems recommendable to evaluate probing depth and probing »attachment« level around osseointegrated oral implants, provided the influence of inflammation on probe penetration is considered.
- Autogenous bone graft particles covered by an ePTFE membrane is a useful surgical treatment modality of experimental peri-implantitis around implants with a TPS surface. The bone gain is almost to the pre-peri-implantitis level. Unbiased stereological estimates demonstrated considerable re-osseointegration, i.e. a mean bone-to-implant contact of 45%.
- Surgical treatment of peri-implantitis with Bio-Oss covered by an ePTFE membrane seems less encouraging than after the use of membrane-covered autogenous bone.
- Surgical treatment of peri-implantitis was evaluated in a primate model with many anatomical and biological similarities to humans, but long-term studies in humans are needed to draw firm conclusions about surgical treatment of peri-implantitis. The performed animal studies indicate that future research in humans should focus on the use of membrane-covered autogenous bone graft particles, and not on membrane-covered Bio-Oss.
- Gauze soaked alternately in chlorhexidine and saline seems to be the preferred implant surface preparation method of implants with a TPS surface during surgical treatment of peri-implantitis.
- The mechanisms of bone healing after treatment of peri-implantitis have not been described. Also the ability of the regenerated bone to withstand functional loading and plaque-induced inflammation has neither been assessed in humans nor in animals. The use of resorbable membranes and growth factors, including bone morphogenetic proteins, should be investigated in the surgical treatment of peri-implantitis.

8. Dansk resumé

Behandling af tandtab med osseointegrerede orale implantater resulterer normalt i langtidsholdbare resultater. Mekaniske og biologiske komplikationer forekommer imidlertid. Plakinducerede komplikationer ses som periimplantær mucositis og periimplantitis. Periimplantitis kan defineres som en plakinduceret inflammatorisk proces i det periimplantære væv med samtidigt tab af marginal knogle. Et detaljeret kendskab til periimplantitis er afgørende for at undgå implantattab. Formålet med nærværende oversigtsartikler og eksperimentelle undersøgelser på Java-aber (*Macaca fascicularis*) har på denne baggrund været at vurdere:

- Anvendeligheden af primater som forsøgsdyr til belysning af plakinducerede inflammatoriske reaktioner omkring osseointegrerede orale implantater og tænder (I).
- Risikoen for overførsel af sygdomme til personer der arbejder med primater som forsøgsdyr (II).
- Patogenese og klinisk diagnostik af plakinducerede patologiske tilstande omkring osseointegrerede orale implantater (III-VI).
- Kirurgisk behandling af periimplantitis (VII-IX).
- Konditionering af implantatoverfladen ved kirurgisk behandling af periimplantitis (X).

På baggrund af de udarbejdede oversigtsartikler og de gen-

nemførte eksperimentelle undersøgelser kan følgende konkluderes:

- Eksperimentelle procedurer på primater nødvendiggør anvendelse af sedering eller anæstesi. På trods heraf er Java-aber brugbare forsøgsdyr til studiet af plakinducerede inflammatoriske reaktioner omkring osseointegrerede orale implantater. Aber kan anvendes med minimal risiko for overførsel af sygdomme til mennesker, såfremt der tages visse forholdsregler.
- Evaluering af det periimplantære knoglevæv kan foretages på geometrisk standardiserede intraorale røntgenoptagelser på Java-aber. Bedømmelsen involverer måling af det marginale knogleniveau og kvantitativ digital subtraktionsradiografi.
- Den såkaldte »*cutting-grinding*-teknik *ad modum* Donath« kan anvendes til undersøgelse af det periimplantære væv efter visse modifikationer af teknikken, således at der opnås vævssnit af en tilfredsstillende teknisk kvalitet.
- Det er muligt ved hjælp af *non-biased* stereologiske todimensionelle teknikker at vurdere plakinducerede inflammatoriske reaktioner omkring osseointegrerede orale implantater samt mængden af nydannet knoglevæv og genetableret osseointegration efter behandling af periimplantitis.
- Den initiale fase af ligaturfremmet plakakkumulering er karakteriseret ved kraftigere inflammation omkring implantater end omkring tænder. Endvidere ses kraftigere vævsdestruktion omkring implantater og ankyloserede tænder uden et parodontalligament end omkring kontroltænder med et parodontalligament. Baggrunden herfor synes af endnu ukendte årsager at være det manglende parodontalligament og ikke mangel på cervikal cement med insererende dentogingivale fibre.
- Plakinduceret inflammation er karakteriseret ved en sammenlignelig ændring af den submukosale/-gingivale plak omkring implantater og tænder, nemlig fra en sparsom flora bestående overvejende af fakultative grampositive kokker og stave til en mere kompleks flora med dominans af gramnegative anaerobe stave.
- Pochedybdemåling er forskellig omkring implantater og tænder. Der ses selv ved let plakinduceret inflammation dybere vævspenetration af pochedybdemåleren omkring implantater end omkring tænder. Pochedybdemåling omkring implantater er relevant, såfremt der tages højde for inflammationens påvirkning af pochedybdemålerens vævspenetration.
- Kirurgisk behandling af periimplantitis med partikulært autologt knogletransplantat og ePTFE-membran er en anvendelig behandling af periimplantitis omkring implantater med en titanplasmabelagt overflade. Det periimplantære knoglevæv kan genopbygges næsten til samme niveau som før udvikling af periimplantitis. Den stereologiske evaluering viste at 45% af implantatoverfladen i de tidligere periimplantitis-defekter i gennemsnit er dækket af nydannet knoglevæv.
- Kirurgisk behandling af periimplantitis med Bio-Oss og ePTFE-membran medfører et signifikant dårligere behandlingsresultat end med membrandækket autologt knogletransplantat. Fremtidige undersøgelser på mennesker bør derfor fokusere på anvendelsen af membrandækket autologt knogletransplantat og ikke membrandækket Bio-Oss ved behandling af periimplantitis.
- Gaze vædet skiftevis med klorheksidin og saltvand bør foretrækkes til konditionering af implantatoverfladen ved kirurgisk behandling af periimplantitis.

9. References

1. Aagaard E, Donslund C, Wenzel A, Sewerin I. Performance for obtaining maximal gain from a program for digital subtraction radiography. Scand J Dent Res 1991; 99: 166-72.
2. Aboyoussef H, Carter C, Jandinski JJ, Panagakos FS. Detection of prostaglandin E_2 and matrix metalloproteinases in implant crevicular fluid. Int J Oral Maxillofac Implants 1998; 13: 689-96.
3. Abrahamsson I, Berglundh T, Wennström J, Lindhe J. The peri-implant hard and soft tissues at different implant systems. A comparative study in the dog. Clin Oral Implants Res 1996; 7: 212-9.
4. Abrahamsson I, Berglundh T, Lindhe J. Soft tissue response to plaque formation at different implant systems. A comparative study in the dog. Clin Oral Implants Res 1998; 9: 73-9.
5. Abrahamsson I, Berglundh T, Moon I-S, Lindhe J. Peri-implant tissues at submerged and non-submerged titanium implants. J Clin Periodontol 1999; 26: 600-7.
6. Adell R, Lekholm U, Rockler B, Brånemark P-I. A 15-year study of osseointegrated implants in the treatment of the edentulous jaw. Int J Oral Surg 1981; 10: 387-416.
7. Adell R, Lekholm U, Rockler B, Brånemark P-I, Lindhe J, Eriksson B, et al. Marginal tissue reactions at osseointegrated titanium fixtures. (I). A 3-year longitudinal prospective study. Int J Oral Maxillofac Surg 1986; 15: 39-52.
8. Adonogianaki E, Mooney J, Wennström JL, Lekholm U, Kinane DF. Acute-phase proteins and immunoglobulin G against *Porphyromonas gingivalis* in peri-implant crevicular fluid: A comparison with gingival crevicular fluid. Clin Oral Implants Res 1995; 6: 14-23.
9. Albrektsson T, Isidor F. Consensus report of session IV. In: Lang NP, Karring T, editors. Proceedings of the 1st European workshop on periodontology. London: Quintessence; 1994. p. 365-9.
10. Albrektsson T, Zarb G, Worthington P, Eriksson AR. The long-term efficacy of currently used dental implants: A review and

proposed criteria of success. Int J Oral Maxillofac Implants 1986; 1: 11-25.
11. Apse P, Ellen RP, Overall CM, Zarb GA. Microbiota and crevicular fluid collagenase activity in the osseointegrated dental implant sulcus: A comparison of sites in edentulous and partially edentulous patients. J Periodontal Res 1989; 24: 96-105.
12. Apse P, Zarb GA, Schmitt A, Lewis DW. The longitudinal effectiveness of osseointegrated dental implants. The Toronto study: Peri-implant mucosal response. Int J Periodontics Restorative Dent 1991; 11: 95-111.
13. Armitage GC. Periodontal diseases: Diagnosis. Ann Periodontol 1996; 1: 37-215.
14. von Arx T, Kurt B, Hardt N. Treatment of severe peri-implant bone loss using autogenous bone and a resorbable membrane. Case report and literature review. Clin Oral Implants Res 1997; 8: 517-26.
15. Baddeley AJ, Gundersen HJG, Cruz-Orive LM. Estimation of surface area from vertical sections. J Microsc 1986; 142: 259-76.
16. Barnett ML, Baker RL, Olson JW. Material adherent to probes during a periodontal examination. Light and electron microscopic observations. J Periodontol 1982; 53: 446-8.
17. Baron M, Haas R, Dörtbudak O, Watzek G. Experimentally induced peri-implantitis: A review of different treatment methods described in the literature. Int J Oral Maxillofac Implants 2000; 15: 533-44.
18. Behneke A, Behneke N, d'Hoedt B, Wagner W. Hard and soft tissue reactions to ITI screw implants: 3-year longitudinal results of a prospective study. Int J Oral Maxillofac Implants 1997; 12: 749-57.
19. Behneke A, Behneke N, d'Hoedt B. Treatment of peri-implantitis defects with autogenous bone grafts: Six-month to 3-year results of a prospective study in 17 patients. Int J Oral Maxillofac Implants 2000; 15: 125-38.
20. Benke D, Olah A, Möhler H. Protein-chemical analysis of Bio-Oss bone substitute and evidence on its carbonate content. Biomaterials 2001; 22: 1005-12.
21. Bergendal T, Forsgren L, Kvint S, Löwstedt E. The effect of an airbrasive instrument on soft and hard tissues around osseointegrated implants. A case report. Swed Dent J 1990; 14: 219-23.
22. Berglundh T. Soft tissue interface and response to microbial challenge. In: Lang NP, Karring T, Lindhe J, editors. Proceedings of the 3rd European workshop on periodontology. Implant dentistry. Berlin: Quintessence; 1999. p. 153-74.
23. Berglundh T, Lindhe J, Ericsson I, Marinello CP, Liljenberg B, Thomsen P. The soft tissue barrier at implants and teeth. Clin Oral Implants Res 1991; 2: 81-90.
24. Berglundh T, Lindhe J, Marinello C, Ericsson I, Liljenberg B. Soft tissue reaction to de novo plaque formation on implants and teeth. An experimental study in the dog. Clin Oral Implants Res 1992; 3: 1-8.
25. Berglundh T, Lindhe J, Jonsson K, Ericsson I. The topography of the vascular systems in the periodontal and peri-implant tissues in the dog. J Clin Periodontol 1994; 21: 189-93.
26. Brocard D, Barthet P, Baysse E, Duffort JF, Eller P, Justumus P, et al. A multicenter report on 1,022 consecutively placed ITI implants: A 7-year longitudinal study. Int J Oral Maxillofac Implants 2000; 15: 691-700.
27. Brown FH, Ogletree RC, Houston GD. Pheumoparotitis associated with the use of an air-powder prophylaxis unit. J Periodontol 1992; 63: 642-4.
28. Brunel G, Benqué E, Elharar F, Sansac C, Duffort JF, Barthet P, et al. Guided bone regeneration for immediate non-submerged implant placement using bioabsorbable materials in beagle dogs. Clin Oral Implants Res 1998; 9: 303-12.
29. Brägger U. Radiographic parameters for the evaluation of peri-implant tissues. Periodontol 2000 1994; 4: 87-97.
30. Brägger U. Technical failures and complications related to prosthetic components of implant systems and different types of suprastructures. In: Lang NP, Karring T, Lindhe J, editors. Proceedings of the 3rd European workshop of periodontology. Implant dentistry. Berlin: Quintessence; 1999. p. 304-32.
31. Brägger U, Pasquali L, Rylander H, Carnes D, Kornman KS. Computer-assisted densitometric image analysis in periodontal radiography. A methodological study. J Clin Periodontol 1988; 15: 27-37.
32. Brägger U, Bürgin W, Lang NP, Buser D. Digital subtraction radiography for the assessment of changes in peri-implant bone density. Int J Oral Maxillofac Implants 1991; 6: 160-6.
33. Brägger U, Hämmerle CHF, Mombelli A, Bürgin W, Lang NP. Remodelling of periodontal tissues adjacent to sites treated according to the principles of guided tissue regeneration (GTR). J Clin Periodontol 1992; 19: 615-24.
34. Brånemark P-I. Introduction to osseointegration. In: Brånemark P-I, Zarb GA, Albrektsson T, editors. Tissue-integrated prostheses. Osseointegration in clinical dentistry. Chicago: Quintessence; 1985. p. 11-76.
35. Brånemark P-I, Breine U, Adell R, Hansson BO, Lindström J, Ohlsson Å. Intra-osseous anchorage of dental prostheses. I. Experimental studies. Scand J Plast Reconstr Surg 1969; 3: 81-100.
36. Brånemark P-I, Hansson BO, Adell R, Breine U, Lindström J, Hallen O, et al. Osseointegrated implants in the treatment of the edentulous jaw. Experience from a 10-year period. Scand J Plast Reconstr Surg 1977; 11 (Suppl): 1-132.
37. Buchmann R, Khoury F, Faust C, Lange DE. Peri-implant conditions in periodontally compromised patients following maxillary sinus augmentation. A long-term post-therapy trial. Clin Oral Implants Res 1999; 10: 103-10.
38. Burchardt H. The biology of bone graft repair. Clin Orthop 1983; 174: 28-42.
39. Buser D. Effects of various titanium surface configurations on osseointegration and clinical implant stability. In: Lang NP, Karring T, Lindhe J, editors. Proceedings of the 3rd European workshop on periodontology. Implant dentistry. Berlin: Quintessence; 1999. p. 88-101.
40. Buser D, Weber H-P, Lang NP. Tissue integration of non-submerged implants. 1-year results of a prospective study with 100 ITI hollow-cylinder and hollow-screw implants. Clin Oral Implants Res 1990; 1: 33-40.
41. Buser D, Weber HP, Donath K, Fiorellini JP, Paquette DW, Williams RC. Soft tissue reactions to non-submerged unloaded titanium implants in beagle dogs. J Periodontol 1992; 63: 226-36.

42. Carmichael RP, McCulloch CAG, Zarb GA. Quantitative immunohistochemical analysis of keratins and desmoplakins in human gingiva and peri-implant mucosa. J Dent Res 1991; 70: 899-905.
43. Caton J. Establishing and maintaining clinically healthy gingivae in rhesus monkeys. J Clin Periodontol 1979; 6: 260-3.
44. Caton J, Mota L, Gandini L, Laskaris B. Non-human primate models for testing the efficacy and safety of periodontal regeneration procedures. J Periodontol 1994; 65: 1143-50.
45. del Cerro M, Cogen J, del Cerro C. Stevenel's blue, an excellent stain for optical microscopical study of plastic embedded tissues. Microsc Acta 1980; 83: 117-21.
46. Chavrier C, Couble ML, Hartmann DJ. Qualitative study of collagenous and noncollagenous glycoproteins of the human healthy keratinized mucosa surrounding implants. Clin Oral Implants Res 1994; 5: 117-24.
47. Chaytor DV, Zarb GA, Schmitt A, Lewis DW. The longitudinal effectiveness of osseointegrated dental implants. The Toronto study: Bone level changes. Int J Periodontics Restorative Dent 1991; 11: 113-25.
48. Christersson LA, Slots J, Zambon JJ, Genco RJ. Transmission and colonization of *Actinobacillus actinomycetemcomitans* in localized juvenile periodontitis patients. J Periodontol 1985; 56: 127-31.
49. Christgau M, Wenzel A, Hiller K-A, Schmalz G. Quantitative digital subtraction radiography for assessment of bone density changes following periodontal guided tissue regeneration. Dentomaxillofac Radiol 1996; 25: 25-33.
50. Christgau M, Bader N, Schmalz G, Hiller K-A, Wenzel A. GTR therapy of intrabony defects using 2 different bioresorbable membranes: 12-month results. J Clin Periodontol 1998; 25: 499-509.
51. Cochran DL. A comparison of endosseous dental implant surfaces. J Periodontol 1999; 70: 1523-39.
52. Cochran DL. The scientific basis for and clinical experiences with Straumann implants including the ITI® dental implant system: A consensus report. Clin Oral Implants Res 2000; 11 (Suppl): 33-58.
53. Çomut AA, Weber HP, Shortkroff S, Cui F, Spector M. Connective tissue orientation around dental implants in a canine model. Clin Oral Implants Res 2001; 12: 433-40.
54. Cox JF, Zarb GA. The longitudinal clinical efficacy of osseointegrated dental implants: A 3-year report. Int J Oral Maxillofac Implants 1987; 2: 91-100.
55. Cranin AN, DeGrado J, Kaufman M, Baraoidan M, DiGregorio R, Batgitis G, et al. Evaluation of the Periotest as a diagnostic tool for dental implants. J Oral Implantol 1998; 24: 139-46.
56. Dam AM. Shrinkage of the brain during histological procedures with fixation in formaldehyde solutions of different concentrations. J Hirnforsch 1979; 20: 115-9.
57. Davies JE. Mechanisms of endosseous integration. Int J Prosthodont 1998; 11: 391-401.
58. Deppe H, Horch H-H, Henke J, Donath K. Peri-implant care of ailing implants with the carbon dioxide laser. Int J Oral Maxillofac Implants 2001; 16: 659-67.
59. Donath K. Die Trenn-Dünnschliff-Technik zur Herstellung histologischer Präparate von nicht schneidbaren Geweben und Materialien. Präparator 1988; 34: 197-206.
60. Donath K. Preparation of histologic sections by the cutting-grinding technique for hard tissue and other material not suitable to be sectioned by routine methods. Norderstedt: EXAKT-Kulzer-Publication; 1993.
61. Donath K, Breuner G. A method for the study of undecalcified bones and teeth with attached soft tissues. The säge-schliff (sawing and grinding) technique. J Oral Pathol 1982; 11: 318-26.
62. Ebersole JL, Kornman KS. Systemic antibody responses to oral microorganisms in the cynomolgus monkey: Development of methodology and longitudinal responses during ligature-induced disease. Res Immunol 1991; 142: 829-39.
63. Ebersole JL, Brunsvold M, Steffensen B, Wood R, Holt SC. Effects of immunization with *Porphyromonas gingivalis* and *Prevotella intermedia* on progression of ligature-induced periodontitis in the nonhuman primate *Macaca fascicularis*. Infect Immun 1991; 59: 3351-9.
64. Ebersole JL, Bauman GR, O'Dell SEC, Giardino A. Evidence for serum immunoglobulin G (IgG) antibody responses in *Macaca fascicularis* identified by monoclonal antibodies to human IgG subclasses. Oral Microbiol Immunol 1997; 12: 193-203.
65. Ebersole JL, Cappelli D, Holt SC, Singer RE, Filloon T. Gingival crevicular fluid inflammatory mediators and bacteriology of gingivitis in nonhuman primates related to susceptibility to periodontitis. Oral Microbiol Immunol 2000; 15: 19-26.
66. Eke PI, Braswell LD, Fritz ME. Microbiota associated with experimental peri-implantitis and periodontitis in adult *Macaca mulatta* monkeys. J Periodontol 1998; 69: 190-4.
67. Eley BM, Cox SW, Watson RM. Protease activities in peri-implant sulcus fluid from patients with permucosal osseointegrated dental implants. Correlation with clinical parameters. Clin Oral Implants Res 1991; 2: 62-70.
68. Ellegaard B, Baelum V, Karring T. Implant therapy in periodontally compromised patients. Clin Oral Implants Res 1997; 8: 180-8.
69. Ellegaard B, Kølsen-Petersen J, Baelum V. Implant therapy involving maxillary sinus lift in periodontally compromised patients. Clin Oral Implants Res 1997; 8: 303-15.
70. Engquist B, Bergendal T, Kallus T, Linden U. A retrospective multicenter evaluation of osseointegrated implants supporting overdentures. Int J Oral Maxillofac Implants 1988; 3: 129-34.
71. Ericsson I, Lindhe J. Probing depth at implants and teeth. An experimental study in the dog. J Clin Periodontol 1993; 20: 623-7.
72. Ericsson I, Lekholm U, Brånemark P-I, Lindhe J, Glantz P-O, Nyman S. A clinical evaluation of fixed-bridge restorations supported by the combination of teeth and osseointegrated titanium implants. J Clin Periodontol 1986; 13: 307-12.
73. Ericsson I, Berglundh T, Marinello C, Liljenberg B, Lindhe J. Long-standing plaque and gingivitis at implants and teeth in the dog. Clin Oral Implants Res 1992; 3: 99-103.
74. Ericsson I, Persson LG, Berglundh T, Marinello CP, Lindhe J, Klinge B. Different types of inflammatory reactions in peri-implant soft tissues. J Clin Periodontol 1995; 22: 255-61.
75. Ericsson I, Nilner K, Klinge B, Glantz P-O. Radiographical and histological characteristics of submerged and nonsubmerged titanium implants. An experimental study in the labrador dog. Clin Oral Implants Res 1996; 7: 20-6.

76. Ericsson I, Persson LG, Berglundh T, Edlund T, Lindhe J. The effect of antimicrobial therapy on periimplantitis lesions. An experimental study in the dog. Clin Oral Implants Res 1996; 7: 320-8.
77. Esposito M, Hirsch J-M, Lekholm U, Thomsen P. Biological factors contributing to failures of osseointegrated oral implants. (I). Success criteria and epidemiology. Eur J Oral Sci 1998; 106: 527-51.
78. Esposito M, Hirsch J, Lekholm U, Thomsen P. Differential diagnosis and treatment strategies for biologic complications and failing oral implants: A review of the literature. Int J Oral Maxillofac Implants 1999; 14: 473-90.
79. Fardal Ø, Johannessen AC, Olsen I. Severe, rapidly progressing peri-implantitis. J Clin Periodontol 1999; 26: 313-7.
80. Finlayson RS, Stevens FD. Subcutaneous facial emphysema secondary to use of the Cavi-Jet. J Periodontol 1988; 59: 315-7.
81. Fiorellini JP, Nevins ML, Sekler J, Chung A, Oringer RJ. Correlation of peri-implant health and aspartate aminotransferase levels: A cross-sectional clinical study. Int J Oral Maxillofac Implants 2000; 15: 500-4.
82. Fourmousis I, Brägger U. Radiologic interpretation of peri-implant structures. In: Lang NP, Karring T, Lindhe J, editors. Proceedings of the 3rd European workshop on periodontology. Implant dentistry. Berlin: Quintessence; 1999. p. 228-41.
83. Fourmousis I, Brägger U, Bürgin W, Tonetti M, Lang NP. Digital image processing. I. Evaluation of gray level correction methods *in vitro*. Clin Oral Implants Res 1994; 5: 37-47.
84. Fritz ME, Braswell LD, Koth D, Jeffcoat M, Reddy M, Cotsonis G. Experimental peri-implantitis in consecutively placed, loaded root-form and plate-form implants in adult *Macaca mulatta* monkeys. J Periodontol 1997; 68: 1131-5.
85. Gammage DD, Bowman AE, Meffert RM. Clinical management of failing dental implants: Four case reports. J Oral Implantol 1989; 15: 124-31.
86. Giannobile WV, Finkelman RD, Lynch SE. Comparison of canine and non-human primate animal models for periodontal regenerative therapy: Results following a single administration of PDGF/IGF-I. J Periodontol 1994; 65: 1158-68.
87. Goldman MJ. Bone regeneration around a failing implant using guided tissue regeneration. A case report. J Periodontol 1992; 63: 473-6.
88. Goodacre CJ, Kan JYK, Rungcharassaeng K. Clinical complications of osseointegrated implants. J Prosthet Dent 1999; 81: 537-52.
89. Gotfredsen K, Budtz-Jörgensen E, Jensen LN. A method for preparing and staining histological sections containing titanium implants for light microscopy. Stain Technol 1989; 64: 121-7.
90. Gotfredsen K, Berglundh T, Lindhe J. Bone reactions at implants subjected to experimental peri-implantitis and static load. A study in the dog. J Clin Periodontol 2002; 29: 144-51.
91. Gould TRL, Westbury L, Brunette DM. Ultrastructural study of the attachment of human gingiva to titanium *in vivo*. J Prosthet Dent 1984; 52: 418-20.
92. Gross UM, Strunz V. Surface staining of sawed sections of undecalcified bone containing alloplastic implants. Stain Technol 1977; 52: 217-9.
93. Grunder U, Hürzeler MB, Schüpbach P, Strub JR. Treatment of ligature-induced peri-implantitis using guided tissue regeneration: A clinical and histologic study in the beagle dog. Int J Oral Maxillofac Implants 1993; 8: 282-93.
94. Gundersen HJG. Notes on the estimation of the numerical density of arbitrary profiles: The edge effect. J Microsc 1977; 111: 219-23.
95. Gundersen HJG. Estimators of the number of objects per area unbiased by edge effects. Microsc Acta 1978; 81: 107-17.
96. Gundersen HJG. Stereology: The fast lane between neuroanatomy and brain function – or still only a tightrope? Acta Neurol Scand 1992; 137 (Suppl): 8-13.
97. Gundersen HJG, Jensen EB. The efficiency of systematic sampling in stereology and its prediction. J Microsc 1987; 147: 229-63.
98. Gundersen HJG, Boysen M, Reith A. Comparison of semiautomatic digitizer-tablet and simple point counting performance in morphometry. Virchows Arch B Cell Pathol 1981; 37: 317-25.
99. Gundersen HJG, Bagger P, Bendtsen TF, Evans SM, Korbo L, Marcussen N, et al. The new stereological tools: Disector, fractionator, nucleator and point sampled intercepts and their use in pathological research and diagnosis. APMIS 1988; 96: 857-81.
100. Gundersen HJG, Bendtsen TF, Korbo L, Marcussen N, Møller A, Nielsen K, et al. Some new, simple and efficient stereological methods and their use in pathological research and diagnosis. APMIS 1988; 96: 379-94.
101. Haas R, Haimböck W, Mailath G, Watzek G. The relationship of smoking on peri-implant tissue: A retrospective study. J Prosthet Dent 1996; 76: 592-6.
102. Haas R, Baron M, Dörtbudak O, Watzek G. Lethal photosensitization, autogenous bone, and e-PTFE membrane for the treatment of peri-implantitis: Preliminary results. Int J Oral Maxillofac Implants 2000; 15: 374-82.
103. Hardt CRE, Gröndahl K, Lekholm U, Wennström JL. Outcome of implant therapy in relation to experienced loss of periodontal bone support. A retrospective 5-year study. Clin Oral Implants Res 2002; 13: 488-94.
104. Hausmann E, Ortman LF, Sedransk N. Experimental alveolar bone loss in the monkey evaluated by ^{125}I absorptiometry. Calcif Tissue Int 1979; 29: 133-9.
105. Hefti AF. Periodontal probing. Crit Rev Oral Biol Med 1997; 8: 336-56.
106. Hermann JS, Buser D, Schenk RK, Schoolfield JD, Cochran DL. Biologic width around one- and two-piece titanium implants. A histometric evaluation of unloaded nonsubmerged and submerged implants in the canine mandible. Clin Oral Implants Res 2001; 12: 559-71.
107. Hickey JS, O'Neal RB, Scheidt MJ, Strong SL, Turgeon D, van Dyke TE. Microbiologic characterization of ligature-induced peri-implantitis in the microswine model. J Periodontol 1991; 62: 548-53.
108. Hildebolt CF, Brunsden B, Yokoyama-Crothers N, Pilgram TK, Townsend KE, Vannier MW, et al. Comparison of reliability of manual and computer-intensive methods for radiodensity measures of alveolar bone loss. Dentomaxillofac Radiol 1998; 27: 245-50.
109. Hillmann G, Hillman B, Donath K. Enzyme, lectin and immu-

nohistochemistry of plastic embedded undecalcified bone and other hard tissues for light microscopic investigations. Biotech Histochem 1991; 66: 185-93.
110. Hipp JA, Brunski JB, Cochran GVB. Method for histological preparation of bone sections containing titanium implants. Stain Technol 1987; 62: 247-52.
111. Holt SC, Ebersole J, Felton J, Brunsvold M, Kornman KS. Implantation of *Bacteroides gingivalis* in nonhuman primates initiates progression of periodontitis. Science 1988; 239: 55-7.
112. Hultin M, Fischer J, Gustafsson A, Kallus T, Klinge B. Factors affecting late fixture loss and marginal bone loss around teeth and dental implants. Clin Implant Dent Rel Res 2000; 2: 203-8.
113. Hultin M, Gustafsson A, Hallström H, Johansson L-Å, Ekfeldt A, Klinge B. Microbiological findings and host response in patients with peri-implantitis. Clin Oral Implants Res 2002; 13: 349-58.
114. Hürzeler MB, Quiñones CR, Schüpback P, Morrison EC, Caffesse RG. Treatment of peri-implantitis using guided bone regeneration and bone grafts, alone or in combination, in beagle dogs. Part 2: Histologic findings. Int J Oral Maxillofac Implants 1997; 12: 168-75.
115. Hürzeler MB, Kohal RJ, Naghshbandi J, Mota LF, Conradt J, Hutmacher D, et al. Evaluation of a new bioresorbable barrier to facilitate guided bone regeneration around exposed implant threads. An experimental study in the monkey. Int J Oral Maxillofac Surg 1998; 27: 315-20.
116. Hürzeler MB, Quiñones CR, Kohal RJ, Rohde M, Strub JR, Teuscher U, et al. Changes in peri-implant tissues subjected to orthodontic forces and ligature breakdown in monkeys. J Periodontol 1998; 69: 396-404.
117. Hämmerle CHF. Membranes and bone substitutes in guided bone regeneration. In: Lang NP, Karring T, Lindhe J, editors. Proceedings of the 3rd European workshop on periodontology. Implant dentistry. Berlin: Quintessence; 1999. p. 468-99.
118. Hämmerle CHF, Karring T. Guided bone regeneration at oral implant sites. Periodontol 2000 1998; 17: 151-75.
119. Hämmerle CHF, Fourmousis I, Winkler JR, Weigel C, Brägger U, Lang NP. Successful bone fill in late peri-implant defects using guided tissue regeneration. A short communication. J Periodontol 1995; 66: 303-8.
120. Isidor F. Loss of osseointegration caused by occlusal load of oral implants. A clinical and radiographic study in monkeys. Clin Oral Implants Res 1996; 7: 143-52.
121. Isidor F. Histological evaluation of peri-implant bone at implants subjected to occlusal overload or plaque accumulation. Clin Oral Implants Res 1997; 8: 1-9.
122. Isidor F. Clinical probing and radiographic assessment in relation to the histologic bone level at oral implants in monkeys. Clin Oral Implants Res 1997; 8: 255-64.
123. Isidor F. Mobility assessment with the Periotest system in relation to histologic findings of oral implants. Int J Oral Maxillofac Implants 1998; 13: 377-83.
124. Isidor F. Occlusal loading in implant dentistry. In: Lang NP, Karring T, Lindhe J, editors. Proceedings of the 3rd European workshop on periodontology. Implant dentistry. Berlin: Quintessence; 1999. p. 358-75.
125. Jemt T, Lekholm U. Implant treatment in edentulous maxillae: A 5-year follow-up report on patients with different degrees of jaw resorption. Int J Oral Maxillofac Implants 1995; 10: 303-11.
126. Jepsen S, Rühling A, Jepsen K, Ohlenbusch B, Albers H-K. Progressive peri-implantitis. Incidence and prediction of peri-implant attachment loss. Clin Oral Implants Res 1996; 7: 133-42.
127. Johansson CB, Röser K, Bolind P, Donath K, Albrektsson T. Bone-tissue formation and integration of titanium implants: An evaluation with newly developed enzyme and immunohistochemical techniques. Clin Implant Dent Rel Res 1999; 1: 33-40.
128. Johns RB, Jemt T, Heath MR, Hutton JE, McKenna S, McNamara DC, et al. A multicenter study of overdentures supported by Brånemark implants. Int J Oral Maxillofac Implants 1992; 7: 513-22.
129. Jovanovic SA. The management of peri-implant breakdown around functioning osseointegrated dental implants. J Periodontol 1993; 64: 1176-83.
130. Jovanovic SA. Diagnosis and treatment of peri-implant disease. Curr Opin Periodontol 1994; 2: 194-204.
131. Jovanovic SA, Buser D. Guided bone regeneration in dehiscence defects and delayed extraction sockets. In: Buser D, Dahlin C, Schenk RK, editors. Guided bone regeneration in implant dentistry. Chicago: Quintessence; 1994. p. 155-88.
132. Jovanovic SA, Kenney EB, Carranza FA, Donath K. The regenerative potential of plaque-induced peri-implant bone defects treated by a submerged membrane technique: An experimental study. Int J Oral Maxillofac Implants 1993; 8: 13-8.
133. Jørgensen T. X-Poseit reference manual. Lystrup: Image Interpreter Systems; 2000.
134. Kao RT, Curtis DA, Richards DW, Preble J. Increased interleukin-1β in the crevicular fluid of diseased implants. Int J Oral Maxillofac Implants 1995; 10: 696-701.
135. Kavanagh P, Gould TRL, Brunette DM, Weston L. A rodent model for the investigation of dental implants. J Prosthet Dent 1985; 54: 252-7.
136. Khoury F, Buchmann R. Surgical therapy of peri-implant disease: A 3-year follow-up study of cases treated with 3 different techniques of bone regeneration. J Periodontol 2001; 72: 1498-508.
137. Kiel RA, Kornman KS, Robertson PB. Clinical and microbiological effects of localized ligature-induced periodontitis on non-ligated sites in the cynomolgus monkey. J Periodontal Res 1983; 18: 200-11.
138. Kihara A, Morimoto K, Suetsugu T. An improved method using a bubble-free adhesion technique for the preparation of semiserial undecalcified histologic sections containing dental implants. J Oral Implantol 1989; 15: 87-94.
139. Klinge B. Implants in relation to natural teeth. J Clin Periodontol 1991; 18: 482-7.
140. Knabe C, Schendel KU. The use of implant-supported titanium prostheses for treatment of periodontally compromised patients including functional and orthodontic therapy. Report of 2 cases. Clin Oral Implants Res 1997; 8: 332-8.
141. Kornman KS, Holt SC, Robertson PB. The microbiology of ligature-induced periodontitis in the cynomolgus monkey. J Periodontal Res 1981; 16: 363-71.
142. Kornman KS, Crane A, Wang H-Y, di Giovine FS, Newman MG,

Pirk FW, et al. The interleukin-1 genotype as a severity factor in adult periodontal disease. J Clin Periodontol 1997; 24: 72-7.
143. Krekeler G, Schilli W, Diemer J. Should the exit of the artificial abutment tooth be positioned in the region of the attached gingiva? Int J Oral Surg 1985; 14: 504-8.
144. Köndell PÅ, Söder P-Ö, Landt H, Frithiof L, Anneroth G, Engström P-E, et al. Gingival fluid and tissues around successful titanium and ceramic implants. A comparative clinical, laboratory, and morphologic study. Acta Odontol Scand 1991; 49: 169-73.
145. Lang NP, Tonetti MS. Periodontal diagnosis in treated periodontitis. Why, when and how to use clinical parameters. J Clin Periodontol 1996; 23: 240-50.
146. Lang NP, Brägger U, Walther D, Beamer B, Kornman KS. Ligature-induced peri-implant infection in cynomolgus monkeys. I. Clinical and radiographic findings. Clin Oral Implants Res 1993; 4: 2-11.
147. Lang NP, Wetzel AC, Stich H, Caffesse RG. Histologic probe penetration in healthy and inflamed peri-implant tissues. Clin Oral Implants Res 1994; 5: 191-201.
148. Lang NP, Mombelli A, Brägger U, Hämmerle CHF. Monitoring disease around dental implants during supportive periodontal treatment. Periodontol 2000 1996; 12: 60-8.
149. Lang NP, Wilson TG, Corbet EF. Biological complications with dental implants: Their prevention, diagnosis and treatment. Clin Oral Implants Res 2000; 11 (Suppl): 146-55.
150. Lehmann B, Brägger U, Hämmerle CHF, Fourmousis I, Lang NP. Treatment of an early implant failure according to the principles of guided tissue regeneration (GTR). Clin Oral Implants Res 1992; 3: 42-8.
151. Lehmann TM, Gröndahl H-G, Benn DK. Computer-based registration for digital subtraction in dental radiology. Dentomaxillofac Radiol 2000; 29: 323-46.
152. Lekholm U, Adell R, Lindhe J, Brånemark P-I, Eriksson B, Rockler B, et al. Marginal tissue reactions at osseointegrated titanium fixtures. (II). A cross-sectional retrospective study. Int J Oral Maxillofac Surg 1986; 15: 53-61.
153. Lekholm U, Ericsson I, Adell R, Slots J. The condition of the soft tissues at tooth and fixture abutments supporting fixed bridges. A microbiological and histological study. J Clin Periodontol 1986; 13: 558-62.
154. Leonhardt Å, Berglundh T, Ericsson I, Dahlén G. Putative periodontal pathogens on titanium implants and teeth in experimental gingivitis and periodontitis in beagle dogs. Clin Oral Implants Res 1992; 3: 112-9.
155. Leonhardt Å, Gröndahl K, Bergström C, Lekholm U. Long-term follow-up of osseointegrated titanium implants using clinical, radiographic and microbiological parameters. Clin Oral Implants Res 2002; 13: 127-32.
156. Liljenberg B, Gualini F, Berglundh T, Tonetti M, Lindhe J. Some characteristics of the ridge mucosa before and after implant installation. A prospective study in humans. J Clin Periodontol 1996; 23: 1008-13.
157. Liljenberg B, Gualini F, Berglundh T, Tonetti M, Lindhe J. Composition of plaque-associated lesions in the gingiva and the peri-implant mucosa in partially edentulous subjects. J Clin Periodontol 1997; 24: 119-23.
158. Lin HC, Thurmon JC, Benson GJ, Tranquilli WJ. Telazol – a review of its pharmacology and use in veterinary medicine. J Vet Pharmacol Ther 1993; 16: 383-418.
159. Linder L, Albrektsson T, Brånemark P-I, Hansson H-A, Ivarsson B, Jönsson U, et al. Electron microscopic analysis of the bone-titanium interface. Acta Orthop Scand 1983; 54: 45-52.
160. Lindhe J, Berglundh T, Ericsson I, Liljenberg B, Marinello C. Experimental breakdown of peri-implant and periodontal tissues. A study in the beagle dog. Clin Oral Implants Res 1992; 3: 9-16.
161. Lindquist LW, Rockler B, Carlsson GE. Bone resorption around fixtures in edentulous patients treated with mandibular fixed tissue-integrated prostheses. J Prosthet Dent 1988; 59: 59-63.
162. Lindquist LW, Carlsson GE, Jemt T. A prospective 15-year follow-up study of mandibular fixed prostheses supported by osseointegrated implants. Clinical results and marginal bone loss. Clin Oral Implants Res 1996; 7: 329-36.
163. Lindquist LW, Carlsson GE, Jemt T. Association between marginal bone loss around osseointegrated mandibular implants and smoking habits: A 10-year follow-up study. J Dent Res 1997; 76: 1667-74.
164. Listgarten MA. Periodontal probing: What does it mean? J Clin Periodontol 1980; 7: 165-76.
165. Listgarten MA, Lang NP, Schroeder HE, Schroeder A. Periodontal tissues and their counterparts around endosseous implants. Clin Oral Implants Res 1991; 2: 1-19.
166. Listgarten MA, Buser D, Steinemann SG, Donath K, Lang NP, Weber HP. Light and transmission electron microscopy of the intact interfaces between non-submerged titanium-coated epoxy resin implants and bone or gingiva. J Dent Res 1992; 71: 364-71.
167. Lozada JL, James RA, Boskovic M, Cordova C, Emanuelli S. Surgical repair of peri-implant defects. J Oral Implantol 1990; 16: 42-6.
168. Luterbacher S, Mayfield L, Brägger U, Lang NP. Diagnostic characteristics of clinical and microbiological tests for monitoring periodontal and peri-implant mucosal tissue conditions during supportive periodontal therapy (SPT). Clin Oral Implants Res 2000; 11: 521-9.
169. Löe H, Silness J. Periodontal disease in pregnancy. I. Prevalence and severity. Acta Odontol Scand 1963; 21: 533-51.
170. Machado MAN, Stefani CM, Sallum EA, Sallum AW, Tramontina VA, Nociti FH Jr. Treatment of ligature-induced periimplantitis defects by regenerative procedures: A clinical study in dogs. J Oral Sci 1999; 41: 181-5.
171. Mackenzie IC, Tonetti MS. Formation of normal gingival epithelial phenotypes around osseointegrated oral implants in humans. J Periodontol 1995; 66: 933-43.
172. Malmquist JP. Management of the failing dental implant. Oral Maxillofac Surg Clin North Am 1994; 6: 647-57.
173. Malmstrom HS, Fritz ME, Timmis DP, van Dyke TE. Osseo-integrated implant treatment of a patient with rapidly progressive periodontitis. A case report. J Periodontol 1990; 61: 300-4.
174. Maniatopoulos C, Rodriguez A, Deporter DA, Melcher AH. An improved method for preparing histological sections of metallic implants. Int J Oral Maxillofac Implants 1986; 1: 31-7.
175. Marinello CP, Berglundh T, Ericsson I, Klinge B, Glantz PO,

Lindhe J. Resolution of ligature-induced peri-implantitis lesions in the dog. J Clin Periodontol 1995; 22: 475-9.
176. Matteson SR, Deahl ST, Alder ME, Nummikoski PV. Advanced imaging methods. Crit Rev Oral Biol Med 1996; 7: 346-95.
177. McCracken M, Lemons JE, Jeffcoat M, Koth DL, Fritz ME. Histomorphological evaluation of loaded plate-form and root-form implants in *Macaca mulatta* monkeys. Clin Oral Implants Res 2002; 13: 214-20.
178. McGuire MK, Nunn ME. Prognosis versus actual outcome. IV. The effectiveness of clinical parameters and IL-1 genotype in accurately predicting prognoses and tooth survival. J Periodontol 1999; 70: 49-56.
179. Mellonig JT, Griffiths G, Mathys E, Spitznagel J. Treatment of the failing implant: Case reports. Int J Periodontics Restorative Dent 1995; 15: 385-95.
180. Mengel R, Stelzel M, Hasse C, Flores-de-Jacoby L. Osseointegrated implants in patients treated for generalized severe adult periodontitis. An interim report. J Periodontol 1996; 67: 782-7.
181. Mengel R, Schröder T, Flores-de-Jacoby L. Osseointegrated implants in patients treated for generalized chronic periodontitis and generalized aggressive periodontitis: 3- and 5-year results of a prospective long-term study. J Periodontol 2001; 72: 977-89.
182. Mericske-Stern R, Schaffner TS, Marti P, Geering AH. Peri-implant mucosal aspects of ITI implants supporting overdentures. A five-year longitudinal study. Clin Oral Implants Res 1994; 5: 9-18.
183. Meyle J. Cell adhesion and spreading on different implant surfaces. In: Lang NP, Karring T, Lindhe J, editors. Proceedings of the 3rd European workshop on periodontology. Implant dentistry. Berlin: Quintessence; 1999. p. 55-72.
184. Mombelli A. Prevention and therapy of peri-implant infections. In: Lang NP, Karring T, Lindhe J, editors. Proceedings of the 3rd European workshop on periodontology. Implant dentistry. Berlin: Quintessence; 1999. p. 281-303.
185. Mombelli A. Microbiology and antimicrobial therapy of peri-implantitis. Periodontol 2000 2002; 28: 177-89.
186. Mombelli A, Lang NP. Microbial aspects of implant dentistry. Periodontol 2000 1994; 4: 74-80.
187. Mombelli A, Lang NP. The diagnosis and treatment of peri-implantitis. Periodontol 2000 1998; 17: 63-76.
188. Mombelli A, van Oosten MAC, Schürch E, Lang NP. The microbiota associated with successful or failing osseointegrated titanium implants. Oral Microbiol Immunol 1987; 2: 145-51.
189. Mombelli A, Marxer M, Gaberthüel T, Grunder U, Lang NP. The microbiota of osseointegrated implants in patients with a history of periodontal disease. J Clin Periodontol 1995; 22: 124-30.
190. Mombelli A, Mühle T, Brägger U, Lang NP, Bürgin WB. Comparison of periodontal and peri-implant probing by depth-force pattern analysis. Clin Oral Implants Res 1997; 8: 448-54.
191. Moncla BJ, Braham PH, Persson GR, Page RC, Weinberg A. Direct detection of *Porphyromonas gingivalis* in *Macaca fascicularis* dental plaque samples using an oligonucleotide probe. J Periodontol 1994; 65: 398-403.
192. Moon I-S, Berglundh T, Abrahamsson I, Linder E, Lindhe J. The barrier between the keratinized mucosa and the dental implant. An experimental study in the dog. J Clin Periodontol 1999; 26: 658-63.
193. Muller E, González YM, Andreana S. Treatment of peri-implantitis: Longitudinal clinical and microbiological findings – A case report. Implant Dent 1999; 8: 247-54.
194. Murayama Y, Fukao K, Noguchi A, Takenaka O. Epitope expression on primate lymphocyte surface antigens. J Med Primatol 1986; 15: 215-26.
195. Nevins M, Langer B. The successful use of osseointegrated implants for the treatment of the recalcitrant periodontal patient. J Periodontol 1995; 66: 150-7.
196. Newman MG, Flemmig TF. Periodontal considerations of implants and implant associated microbiota. J Dent Educ 1988; 52: 737-44.
197. Niimi A, Ueda M. Crevicular fluid in the osseointegrated implant sulcus: A pilot study. Int J Oral Maxillofac Implants 1995; 10: 434-6.
198. Nociti FH Jr, Machado MÂN, Stefani CM, Sallum EA. Absorbable versus nonabsorbable membranes and bone grafts in the treatment of ligature-induced peri-implantitis defects in dogs: A histometric investigation. Int J Oral Maxillofac Implants 2001; 16: 646-52.
199. Nociti FH Jr, Machado MÂN, Stefani CM, Sallum EA, Sallum AW. Absorbable versus nonabsorbable membranes and bone grafts in the treatment of ligature-induced peri-implantitis defects in dogs. Part I. A clinical investigation. Clin Oral Implants Res 2001; 12: 115-20.
200. Nociti FH Jr, de Toledo RC, Machado MAN, Stefani CM, Line SRP, Gonçalves RB. Clinical and microbiological evaluation of ligature-induced peri-implantitis and periodontitis in dogs. Clin Oral Implants Res 2001; 12: 295-300.
201. Nowzari H, Slots J. Microbiologic and clinical study of polytetrafluoroethylene membranes for guided bone regeneration around implants. Int J Oral Maxillofac Implants 1995; 10: 67-73.
202. Nyman S, Lang NP, Buser D, Brägger U. Bone regeneration adjacent to titanium dental implants using guided tissue regeneration: A report of two cases. Int J Oral Maxillofac Implants 1990; 5: 9-14.
203. Orton GS, Steele DL, Wolinsky LE. The dental professional's role in monitoring and maintenance of tissue-integrated prostheses. Int J Oral Maxillofac Implants 1989; 4: 305-10.
204. Page RC, Schroeder HE. Periodontitis in man and other animals. A comparative review. Basel: Karger; 1982.
205. Panagakos FS, Aboyoussef H, Dondero R, Jandinski JJ. Detection and measurement of inflammatory cytokines in implant crevicular fluid: A pilot study. Int J Oral Maxillofac Implants 1996; 11: 794-9.
206. Papaioannou W, Bollen CML, van Eldere J, Quirynen M. The adherence of periodontopathogens to periodontal probes. A possible factor in intra-oral transmission? J Periodontol 1996; 67: 1164-9.
207. Papaioannou W, Quirynen M, van Steenberghe D. The influence of periodontitis on the subgingival flora around implants in partially edentulous patients. Clin Oral Implants Res 1996; 7: 405-9.
208. Payne AGT, Solomons YF, Tawse-Smith A, Lownie JF. Inter-abutment and peri-abutment mucosal enlargement with mandibular implant overdentures. Clin Oral Implants Res 2001; 12: 179-87.

209. Persson LG, Ericsson I, Berglundh T, Lindhe J. Guided bone regeneration in the treatment of periimplantitis. Clin Oral Implants Res 1996; 7: 366-72.
210. Persson LG, Araújo MG, Berglundh T, Gröndahl K, Lindhe J. Resolution of peri-implantitis following treatment. An experimental study in the dog. Clin Oral Implants Res 1999; 10: 195-203.
211. Persson LG, Berglundh T, Sennerby L, Lindhe J. Re-osseointegration after treatment of peri-implantitis at different implant surfaces. An experimental study in the dog. Clin Oral Implants Res 2001; 12: 595-603.
212. Persson LG, Ericsson I, Berglundh T, Lindhe J. Osseintegration following treatment of peri-implantitis and replacement of implant components. An experimental study in the dog. J Clin Periodontol 2001; 28: 258-63.
213. Piattelli A, Scarano A, Piattelli M. Histologic observations on 230 retrieved dental implants: 8 years' experience (1989-1996). J Periodontol 1998; 69: 178-84.
214. Plagnat D, Giannopoulou C, Carrel A, Bernard J-P, Mombelli A, Belser UC. Elastase, α2-macroglobulin and alkaline phosphatase in crevicular fluid from implants with and without periimplantitis. Clin Oral Implants Res 2002; 13: 227-33.
215. Polson AM, Zappa UE, Espeland MA, Eisenberg AD. Effect of metronidazole on development of subgingival plaque and experimental periodontitis. J Periodontol 1986; 57: 218-24.
216. Pontoriero R, Tonelli MP, Carnevale G, Mombelli A, Nyman SR, Lang NP. Experimentally induced peri-implant mucositis. A clinical study in humans. Clin Oral Implants Res 1994; 5: 254-9.
217. Quirynen M, Listgarten MA. The distribution of bacterial morphotypes around natural teeth and titanium implants ad modum Brånemark. Clin Oral Implants Res 1990; 1: 8-12.
218. Quirynen M, Naert I, van Steenberghe D, Teerlinck J, Dekeyser C, Theuniers G. Periodontal aspects of osseointegrated fixtures supporting an overdenture. A 4-year retrospective study. J Clin Periodontol 1991; 18: 719-28.
219. Quirynen M, van Steenberghe D, Jacobs R, Schotte A, Darius P. The reliability of pocket probing around screw-type implants. Clin Oral Implants Res 1991; 2: 186-92.
220. Quirynen M, Naert I, van Steenberghe D, Nys L. A study of 589 consecutive implants supporting complete fixed prostheses. Part I: Periodontal aspects. J Prosthet Dent 1992; 68: 655-63.
221. Quirynen M, Peeters W, Naert I, Coucke W, van Steenberghe D. Peri-implant health around screw-shaped c.p. titanium machined implants in partially edentulous patients with or without ongoing periodontitis. Clin Oral Implants Res 2001; 12: 589-94.
222. Quirynen M, de Soete M, van Steenberghe D. Infectious risks for oral implants: A review of the literature. Clin Oral Implants Res 2002; 13: 1-19.
223. Reddy MS. Radiographic methods in the evaluation of periodontal therapy. J Periodontol 1992; 63: 1078-84.
224. Romanos GE, Schröter-Kermani C, Weingart D, Strub JR. Healthy human periodontal versus peri-implant gingival tissues: An immunohistochemical differentiation of the extracellular matrix. Int J Oral Maxillofac Implants 1995; 10: 750-8.
225. Ruttimann UE, Webber RL, Schmidt E. A robust digital method for film contrast correction in subtraction radiography. J Periodontal Res 1986; 21: 486-95.
226. Rühling A, Jepsen S, Kocher T, Plagmann H-C. Longitudinal evaluation of aspartate aminotransferase in the crevicular fluid of implants with bone loss and signs of progressive disease. Int J Oral Maxillofac Implants 1999; 14: 428-35.
227. Salcetti JM, Moriarty JD, Cooper LF, Smith FW, Collins JG, Socransky SS, et al. The clinical, microbial, and host response characteristics of the failing implant. Int J Oral Maxillofac Implants 1997; 12: 32-42.
228. Salvi GE, Bardet P, Lang NP. Clinical parameters in longitudinal implant studies. In: Lang NP, Karring T, Lindhe J, editors. Proceedings of the 3rd European workshop on periodontology. Implant dentistry. Berlin: Quintessence; 1999. p. 217-27.
229. Sanz M, Alandez J, Lazaro P, Calvo JL, Quirynen M, van Steenberghe D. Histo-pathologic characteristics of peri-implant soft tissues in Brånemark implants with 2 distinct clinical and radiological patterns. A histometric and ultrastructural study. Clin Oral Implants Res 1991; 2: 128-34.
230. Sbordone L, Barone A, Ciaglia RN, Ramaglia L, Iacono VJ. Longitudinal study of dental implants in a periodontally compromised population. J Periodontol 1999; 70: 1322-9.
231. Schenk RK, Buser D. Osseointegration: a reality. Periodontol 2000 1998; 17: 22-35.
232. Schenk RK, Olah AJ, Herrmann W. Preparation of calcified tissues for light microscopy. In: Dickson GR, editor. Methods of calcified tissue preparation. Amsterdam: Elsevier; 1984. p. 1-56.
233. Schou S, Holmstrup P, Hjørting-Hansen E, Lang NP. Plaque-induced marginal tissue reactions of osseointegrated oral implants: A review of the literature. Clin Oral Implants Res 1992; 3: 149-61.
234. Schroeder A, van der Zypen E, Stich H, Sutter F. The reactions of bone, connective tissue, and epithelium to endosteal implants with titanium-sprayed surfaces. J Maxillofac Surg 1981; 9: 15-25.
235. Schwartz Z, Kieswetter K, Dean DD, Boyan BD. Underlying mechanisms at the bone-surface interface during regeneration. J Periodontal Res 1997; 32: 166-71.
236. Schwartz Z, Lohmann CH, Cochran DL, Sylvia VL, Dean DD, Boyan BD. Bone regulating mechanisms on implant surfaces. In: Lang NP, Karring T, Lindhe J, editors. Proceedings of the 3rd European workshop on periodontology. Implant dentistry. Berlin: Quintessence; 1999. p. 41-54.
237. Seymour GJ, Gemmell E, Lenz LJ, Henry P, Bower R, Yamazaki K. Immunohistologic analysis of the inflammatory infiltrates associated with osseointegrated implants. Int J Oral Maxillofac Implants 1989; 4: 191-8.
238. Shibutani T, Inuduka A, Horiki I, Luan Q, Iwayama Y. Bisphosphonate inhibits alveolar bone resorption in experimentally-induced peri-implantitis in dogs. Clin Oral Implants Res 2001; 12: 109-14.
239. Silness J, Löe H. Periodontal disease in pregnancy. II. Correlation between oral hygiene and periodontal condition. Acta Odontol Scand 1964; 22: 121-35.
240. Simion M, Baldoni M, Rossi P, Zaffe D. A comparative study of the effectiveness of e-PTFE membranes with and without early

exposure during the healing period. Int J Periodontics Restorative Dent 1994; 14: 167-80.
241. Simion M, Trisi P, Maglione M, Piattelli A. Bacterial penetration *in vitro* through GTAM membrane with and without topical chlorhexidine application. A light and scanning electron microscopic study. J Clin Periodontol 1995; 22: 321-31.
242. Singh G, O'Neal RB, Brennan WA, Strong SL, Horner JA, van Dyke TE. Surgical treatment of induced peri-implantitis in the micro pig: Clinical and histological analysis. J Periodontol 1993; 64: 984-9.
243. van Steenberghe D, Lekholm U, Bolender C, Folmer T, Henry P, Herrmann I, et al. The applicability of osseointegrated oral implants in the rehabilitation of partial edentulism: A prospective multicenter study on 558 fixtures. Int J Oral Maxillofac Implants 1990; 5: 272-81.
244. van Steenberghe D, Klinge B, Lindén U, Quirynen M, Herrmann I, Garpland C. Periodontal indices around natural and titanium abutments: A longitudinal multicenter study. J Periodontol 1993; 64: 538-41.
245. van Steenberghe D, Quirynen M, Naert I. Survival and success rates with oral endosseous implants. In: Lang NP, Karring T, Lindhe J, editors. Proceedings of the 3rd European workshop on periodontology. Implant dentistry. Berlin: Quintessence; 1999. p. 242-54.
246. van Steenberghe D, Quirynen M, Naert I, Maffei G, Jacobs R. Marginal bone loss around implants retaining hinging mandibular overdentures, at 4-, 8- and 12-years follow-up. J Clin Periodontol 2001; 28: 628-33.
247. Stentz WC, Mealey BL, Nummikoski PV, Gunsolley JC, Waldrop TC. Effects of guided bone regeneration around commercially pure titanium and hydroxyapatite-coated dental implants. I. Radiographic analysis. J Periodontol 1997; 68: 199-208.
248. Stich, H. Producing the histologic specimens. In: Schroeder A, Sutter F, Buser D, Krekeler G, editors. Oral implantology. Basics, ITI hollow cylinder system. New York: Thieme; 1996. p. 104-11.
249. Strub JR, Gaberthüel TW, Grunder U. The role of attached gingiva in the health of peri-implant tissue in dogs. Part I. Clinical findings. Int J Periodontics Restorative Dent 1991; 11: 317-33.
250. Taylor AC, Campbell MM. Reattachment of gingival epithelium to the tooth. J Periodontol 1972; 43: 281-93.
251. Teronen O, Konttinen YT, Lindqvist C, Salo T, Ingman T, Lauhio A, et al. Human neutrophil collagenase MMP-8 in peri-implant sulcus fluid and its inhibition by clodronate. J Dent Res 1997; 76: 1529-37.
252. Tillmanns HWS, Hermann JS, Cagna DR, Burgess AV, Meffert RM. Evaluation of three different dental implants in ligature-induced peri-implantitis in the beagle dog. Part I. Clinical evaluation. Int J Oral Maxillofac Implants 1997; 12: 611-20.
253. Tillmanns HWS, Hermann JS, Tiffee JC, Burgess AV, Meffert RM. Evaluation of three different dental implants in ligature-induced peri-implantitis in the beagle dog. Part II. Histology and microbiology. Int J Oral Maxillofac Implants 1998; 13: 59-68.
254. Tinti C, Parma-Benfenati S. Treatment of peri-implant defects with the vertical ridge augmentation procedure: A patient report. Int J Oral Maxillofac Implants 2001; 16: 572-7.
255. Tonetti MS, Schmid J, Hämmerle CH, Lang NP. Intraepithelial antigen-presenting cells in the keratinized mucosa around teeth and osseointegrated implants. Clin Oral Implants Res 1993; 4: 177-86.
256. Tonetti MS, Gerber L, Lang NP. Vascular adhesion molecules and initial development of inflammation in clinically healthy human keratinized mucosa around teeth and osseointegrated implants. J Periodontal Res 1994; 29: 386-92.
257. Tonetti MS, Imboden M, Gerber L, Lang NP. Compartmentalization of inflammatory cell phenotypes in normal gingiva and peri-implant keratinized mucosa. J Clin Periodontol 1995; 22: 735-42.
258. Warrer K, Buser D, Lang NP, Karring T. Plaque-induced peri-implantitis in the presence or absence of keratinized mucosa. An experimental study in monkeys. Clin Oral Implants Res 1995; 6: 131-8.
259. Weber H-P, Cochran DL. The soft tissue response to osseointegrated dental implants. J Prosthet Dent 1998; 79: 79-89.
260. Weber HP, Fiorellini JP, Paquette DW, Howell TH, Williams RC. Inhibition of peri-implant bone loss with the nonsteroidal anti-inflammatory drug flurbiprofen in beagle dogs. A preliminary study. Clin Oral Implants Res 1994; 5: 148-53.
261. Weber HP, Buser D, Donath K, Fiorellini JP, Doppalapudi V, Paquette DW, et al. Comparison of healed tissues adjacent to submerged and non-submerged unloaded titanium dental implants. A histometric study in beagle dogs. Clin Oral Implants Res 1996; 7: 11-9.
262. Weibel ER. Stereological methods. Vol. 1. Practical methods for biological morphometry. London: Academic Press; 1979.
263. Weinberg MA, Bral M. Laboratory animal models in periodontology. J Clin Periodontol 1999; 26: 335-40.
264. Wennström JL, Bengazi F, Lekholm U. The influence of the masticatory mucosa on the peri-implant soft tissue condition. Clin Oral Implants Res 1994; 5: 1-8.
265. Wenz B, Oesch B, Horst M. Analysis of the risk of transmitting bovine spongiform encephalopathy through bone grafts derived from bovine bone. Biomaterials 2001; 22: 1599-606.
266. Wenzel A. Effect of manual compared with reference point superimposition on image quality in digital subtraction radiography. Dentomaxillofac Radiol 1989; 18: 145-50.
267. Wenzel A. Computer-aided image manipulation of intraoral radiographs to enhance diagnosis in dental practice: A review. Int Dent J 1993; 43: 99-108.
268. Wetzel AC, Stich H, Caffesse RG. Bone apposition onto oral implants in the sinus area filled with different grafting materials. A histological study in beagle dogs. Clin Oral Implants Res 1995: 6: 155-63.
269. Wetzel AC, Vlassis J, Caffesse RG, Hämmerle CHF, Lang NP. Attempts to obtain re-osseointegration following experimental peri-implantitis in dogs. Clin Oral Implants Res 1999; 10: 111-9.
270. Wilson TG, Nunn M. The relationship between the interleukin-1 periodontal genotype and implant loss. Initial data. J Periodontol 1999; 70: 724-9.
271. Yalçin S, Yalçin F, Günay Y, Bellaz B, Önal Ş, Firatli E. Treatment of aggressive periodontitis by osseointegrated dental implants. A case report. J Periodontol 2001; 72: 411-6.

272. Yi S-W, Ericsson I, Kim C-K, Carlsson GE, Nilner K. Implant-supported fixed prostheses for the rehabilitation of periodontally compromised dentitions: A 3-year prospective clinical study. Clin Implant Dent Rel Res 2001; 3: 125-34.
273. Zappa UE, Polson AM. Factors associated with occurrence and reversibility of connective tissue attachment loss. J Periodontol 1988; 59: 100-6.
274. Zitzmann NU, Berglundh T, Marinello CP, Lindhe J. Experimental peri-implant mucositis in man. J Clin Periodontol 2001; 28: 517-23.
275. Zitzmann NU, Berglundh T, Marinello CP, Lindhe J. Expression of endothelial adhesion molecules in the alveolar ridge mucosa, gingiva and periimplant mucosa. J Clin Periodontol 2002; 29: 490-5.

Correspondance

Søren Schou, Department of Oral and Maxillofacial Surgery, Aalborg University Hospital, Hobrovej 18-22, DK-9000 Aalborg, Denmark
E-mail: ss@aas.nja.dk

Table 1. Procedure for dehydration, resin infiltration, and polymerisation.

Procedure	Time
Dehydration:	
Ethanol 60% w/v	3 days
Ethanol 70% w/v	3 days
Ethanol 80% w/v	3 days
Ethanol 90% w/v	3 days
Ethanol 96% w/v	3 days
Ethanol 99% w/v	3 days
Ethanol 99% w/v	3 days
Ethanol 99% w/v	3 days
Acetone 100%	3 days
Resin infiltration:	
Technovit 7200 VLC 30% w/v + ethanol (99%) 70% w/v	4 days
Technovit 7200 VLC 50% w/v + ethanol (99%) 50% w/v	4 days
Technovit 7200 VLC 70% w/v + ethanol (99%) 30% w/v	4 days
Technovit 7200 VLC 100%	4 days
Technovit 7200 VLC 100%	4 days
Technovit 7200 VLC 100%	7 days
Vacuum in Technovit 7200 VLC 100%	30 minutes
Polymerisation (Histolux Photocuring Unit, Kulzer, Germany):	
White light	4 hours
Blue light	6 hours

Table 2. Long-term studies in humans on the correlation between plaque accumulation and peri-implant bone loss.

Patient	Implant	Supra-structure	Observation period	Registration method	Results and conclusions by authors	Ref.
21 edentulous patients. Female: 81%, male: 19%	276 Brånemark	Fixed mandibular prostheses	Approximately 3-4 years	Plaque accumulation evaluated at each visit according to a 3-point scale: 0: No visible plaque. 1: Local plaque accumulation (< 25% of the visible abutment area). 2: General plaque accumulation (> 25% of the visible abutment area). Oral hygiene index calculated by adding scores and dividing by number of visits. Radiographs at baseline and after 1 year and 3 years as well as at final examination	Bone loss during 1st year: < 0.5 mm. Bone loss per year after 1 year: < 0.08 mm. More bone loss in patients with poor oral hygiene as compared with patients with good oral hygiene (amount of plaque accumulation not reported)	(161)
25 edentulous patients. Female: 76%, male: 24%			5 1/2-6 years			
21 edentulous patients. Female: 67%, male: 33%	273 Brånemark	Fixed mandibular prostheses	12 years clinically and 10 years radio-graphically	Plaque accumulation evaluated at each visit according to a 3-point scale: 0: No visible plaque. 1: Local plaque accumulation (< 25% of the visible abutment area). 2: General plaque accumulation (> 25% of the visible abutment area). Oral hygiene index calculated by adding scores and dividing by number of visits. Smoking habits recorded at 10-year follow-up. Radiographs at baseline and after 1, 3, 5/6, 10, and 15 years	Bone loss during 1st year: 0.5 mm. Bone loss per year after 1 year: < 0.1 mm. More bone loss in patients with poor oral hygiene as compared with patients with good oral hygiene after 10 years: Good oral hygiene (13 patients): 0.7 mm; Poor oral hygiene (14 patients): 1.3 mm. More bone loss in smokers as compared with non-smokers. When both smoking and oral hygiene were included in the analysis, more bone loss was observed in patients with poor oral hygiene, but the influence of poor oral hygiene was greater in smokers as compared with non-smokers (amount of plaque accumulation not reported)	(162)
26 edentulous patients. Female: 73%, male: 27%			15 years clinically and radio-graphically			
45 edentulous patients. Age: 33-64 years. Female: 71%, male: 29%. Smokers: 47%, non-smokers: 53%. Median No. of cigarettes per day: 20 (8-30)	266 Brånemark	Fixed mandibular prostheses	10 years	Annual registration of plaque accumulation according to a 3-point scale: 0: No visible plaque. 1: Local plaque accumulation (< 25% of the visible abutment area). 2: General plaque accumulation (> 25% of the visible abutment area). Oral hygiene index calculated by adding scores and dividing by number of visits. Smoking habits recorded at 10-year follow-up. Radiographs at baseline and after 1, 3, 5/6, and 10 years	Evaluation of smokers and non-smokers together: More bone loss in patients with poor oral hygiene as compared with patients with good oral hygiene. Bone loss in smokers and non-smokers after 10 years: Smokers: / Non-smokers: — Good oral hygiene: 1.0 mm / 0.7 mm — Poor oral hygiene: 1.6 mm / 0.7 mm. More bone loss in smokers as compared with non-smokers. Bone loss correlated to amount of cigarette consumption. Smokers: More bone loss in patients with poor oral hygiene as compared with patients with good oral hygiene. Non-smokers: No difference in bone loss in patients with poor oral hygiene as compared with patients with good oral hygiene (amount of plaque accumulation not reported)	(163)
158 edentulous patients. Age: 59 (32-82) years. Female: 72%, male: 28%	316 Brånemark	Mandibular over-dentures	Up to 12 years. Only a minority followed for more than 4 years	Plaque score (239) and radiographs at baseline and after 4, 8, and 12 years	Bone loss after 4 years: 1.0 mm. Bone loss after 8 years: 1.4 mm. Bone loss after 12 years: 1.7 mm. Bone loss not influenced by plaque accumulation (amount of plaque accumulation not reported)	(246)

All group values referred to are expressed as mean values, if not otherwise specified.

Table 3. Studies in animals on ligature-enhanced plaque accumulation on implants and teeth.

Animal	Implant	Ligation period	Results			Conclusions by authors	Ref.
			Clinical characteristics	Radiography	Histology		
2 dogs	7 Brånemark (machined surface)	5 months	No information	More pronounced bone loss around implants as compared with teeth: Implants: 1 mm; Teeth: 5 mm	No information	The pilot study showed more rapid tissue destruction around implants as compared with teeth	(139)
5 dogs	10 Brånemark (machined surface)	6 weeks with ligatures followed by 1 week without ligatures. Ligatures renewed after 3 weeks	Peri-implant mucosa/ gingiva severely inflamed and receded several mm	More pronounced bone loss around implants as compared with teeth: Implants: 3.2 mm; Teeth: 1.1 mm	Size of inflammatory cell infiltrate considerably larger around implants than around teeth. Inflammatory cell infiltrate reached frequently the bone crest and even extended into the bone marrow around implants	Tissue destruction more pronounced around implants as compared with teeth	(160)
4 monkeys	16 ITI (TPS surface)	8 months. Supplementary ligatures placed after 3 and 6 months	Increased plaque score, gingival score, probing depth, and probing »attachment« loss around implants and teeth. Comparable features around implants and teeth	Comparable bone loss around implants and teeth: Implants: 1.0 mm; Teeth: 0.6 mm	No information	Peri-implantitis and periodontitis progressed at a similar rate	(146)
8 monkeys	32 titanium-coated poly-carbonate implants	7 weeks	Increased plaque score, gingival score, probing depth, and probing »attachment« loss around implants, ankylosed teeth, and control teeth. No differences between implants, ankylosed teeth, and control teeth	More pronounced bone loss around implants and ankylosed teeth in comparison to control teeth: Implants: 1.5 mm; Ankylosed teeth: 0.5 mm; Control teeth: 0.1 mm	Osteoclasts exclusively observed around implants and ankylosed teeth. Unbiased stereological methods demonstrated: 1: Increased total number of lymphocytes, plasma cells, and neutrophils around implants, ankylosed teeth, and control teeth. 2: Total number of lymphocytes higher around implants as compared with ankylosed teeth and control teeth. 3: No differences in total number of plasma cells and neutrophils around implants, ankylosed teeth, and control teeth	More pronounced inflammatory reactions around implants than around teeth. More pronounced tissue destruction around implants and ankylosed teeth without a periodontal ligament than around teeth with a periodontal ligament. The background of this observation seems for unknown reasons to be the lacking periodontal ligament and not the absence of cervical cementum with inserting collagen fibers of implants	(III,IV)
5 dogs	20 Napio (acid-etched surface)	30 days	Peri-implant mucosa/ gingiva inflamed. Comparable probing »attachment« loss around implants and teeth: Implants: 3.7 mm; Teeth: 3.6 mm	No information	No information	Peri-implantitis and periodontitis progressed at a similar rate	(200)

All group values referred to are expressed as mean values. Abbreviation: TPS: titanium plasma-sprayed.

Table 4. Studies on implant treatment of patients with tooth loss due to periodontitis.

Patient	Implant	Suprastructure	Observation period	Results		Conclusions by authors	Ref.
				Survival rate	Other results		
10 partially edentulous patients with advanced periodontitis. Age: 31-60 years	41 Brånemark	Fixed prostheses supported by implants and teeth	18 (6-30) months	No information	Stable peri-implant/dental tissue	No information	(72)
1 partially edentulous patient with rapidly progressive periodontitis. Smoker	Maxilla: 6 Mandible: 3	Fixed and removable prostheses	No information	4 implants (44%) removed within the first 2 months	Continuous dehiscences, abscess formation, and bone loss	Aggressive types of periodontitis should be treated before implant treatment by involving anti-microbial therapy	(173)
59 patients with partially edentulous and totally edentulous jaws. Tooth loss due to recalcitrant periodontitis (failed to respond to appropriate periodontal treatment). Age: 42-86 years	Brånemark Maxilla: 177 Mandible: 132	Fixed and removable prostheses	1 year: 23 implants 2 years: 42 implants 3-5 years: 185 implants 6-7 years: 38 implants 8 years: 21 implants	Maxilla: 98% Mandible: 97%	Bone loss to the 1st or 2nd thread around several implants. Bone loss to the 4th thread around 7 implants	Patients with strong susceptibility to periodontitis can be treated successfully with implants	(195)
5 partially edentulous females with generalised severe adult periodontitis. Age: 31-44 years. 1 patient was a smoker (5 cigarettes per day)	Brånemark Maxilla: 21 Mandible: 15	Fixed and removable prostheses	1 year	Maxilla: 85% Mandible: 93%	Minimally increased plaque and gingival scores around implants and teeth after 1 year. Bone loss around implants after 1 year: 0.6 mm. Implants with ≤ 1 mm bone loss after 1 year: 78%	Implant treatment seems to be successful in partially edentulous patients with generalised severe adult periodontitis	(180)
68 partially edentulous, periodontally compromised patients. Age: 60 (41-78) years. Female: 79%, male: 21%. Smokers: 63%	31 Astra	Predominantly fixed prostheses. 2 patients treated with removable partial prosthesis	30 (12-40) months	After 3 years: 100%	After 3 years: Implants with < 1.5 mm bone loss: 76-86%. Implants with < 3.5 mm bone loss: 93-100%	Periodontally compromised patients can be successfully treated with implants	(68)
Female: 75%, male: 25%. Smokers: 64%	93 ITI		33 (3-84) months	95%			
24 partially edentulous, periodontally compromised patients. Implant treatment with/without sinus lift procedure. No bone graft was used	51 Astra No sinus lift	Predominantly fixed prostheses. 1 patient treated with removable partial prosthesis	31 months	100%	After 3 years: Implants with < 1.5 mm bone loss: 76% / Implants with < 3.5 mm bone loss: 100%	Sinus lift procedure can be used successfully in periodontally compromised patients	(69)
	Sinus lift		30 months	95%	82% / 95%		
	29 ITI No sinus lift		29 months	91%	71% / 91%		
	Sinus lift		25 months	86%	29% / 86%		

Table 4, continued.

Patient	Implant	Suprastructure	Observation period	Results		Conclusions by authors	Ref.
				Survival rate	Other results		
1 partially edentulous, periodontally compromised female. Age: 65 years	4 ITI and 1 Frialit-2	Fixed prostheses	1-2 years	100%	Healthy and stable peri-implant tissue	Implants may be a valuable part of restorative therapy for periodontally compromised patients	(140)
1 partially edentulous, periodontally compromised female. Age: 57 years	7 ITI			100%			
50 patients with chronic adult periodontitis treated with sinus lift procedure, autogenous bone graft, and simultaneous implant placement. Age: 52 years. Female: 58%, male: 42%	167 Brånemark, IMZ, and Frialit-2	No information	5 years	No information	Healthy and stable peri-implant tissue	Comparable conditions of the peri-implant tissue	(37)
37 periodontally healthy patients treated with implants without sinus lift procedure and autogenous bone graft. Age: 44 years	60 IMZ, ITI, and Ledermann						
1 partially edentulous patient with refractory periodontitis. Age: 45 years. 10-15 cigarettes per day	8 Brånemark	Removable prosthesis	2 years	2 implants (25%) remained osseointegrated after 2 years	No information	No information	(79)
5 partially edentulous females with generalised aggressive periodontitis. Age: 31-44 years	36 Brånemark	Predominantly fixed prostheses. 1 patient treated with removable prosthesis	5 years	Maxilla: 85% Mandible: 93%	Healthy peri-implant tissue and gingiva. Limited bone loss around implants and teeth	Implants can be used successfully in partially edentulous patients treated for generalised aggressive periodontitis and generalised chronic periodontitis	(181)
5 partially edentulous females with generalised chronic periodontitis. Age: 35-42 years	12 Brånemark		3 years	Maxilla: 100% Mandible: 100%			
43 partially and totally edentulous patients treated for advanced periodontitis. Age: 26-65 years	125 Astra	Fixed prostheses	3 years	100%	Bone loss after 3 years: 0.2 mm. Implants with ≤ 0.5 mm bone loss: 81%. Implants with 0.5-2.0 mm bone loss: 19%	Implant-supported fixed prostheses are a predictable treatment option in patients with tooth loss due to periodontitis	(272)
1 partially edentulous patient with aggressive periodontitis. Age: 17 years	2 mandibular implants (TPS surface)	Removable prosthesis	3 years	100%	No information	Implant treatment may be an alternative treatment for patients suffering from aggressive periodontitis	(271)

Table 4, continued.

Patient	Implant	Suprastructure	Observation period	Results			Conclusions by authors	Ref.
				Survival rate	Other results			
25 partially edentulous patients susceptible to periodontitis. Age: 54 years. Female: 52%, male: 48%	100 Brånemark	Fixed prostheses in posterior part of the maxilla	5 years	92%	Bone loss after 5 years: 2.2 mm	Implants with ≥ 2 mm bone loss: 62%	Bone loss around implants is correlated to previous experience of periodontal bone loss. Higher implant failure rate in individuals susceptible to periodontitis	(103)
25 partially edentulous patients with minimal periodontal breakdown. Age: 57 years. Female: 64%, male: 36%	92 Brånemark			97%	1.7 mm	44%		
15 partially edentulous patients with advanced periodontitis. Age: 21-71 years. Female: 47%, male: 53%	7 Brånemark	Fixed prostheses	10 years	Maxilla: 94% Mandible: 96%	Bone loss around teeth: 0.7 mm. Bone loss around implants: 1.7 mm. Implants with ≤ 0.5 mm bone loss: 15%. Implants with 0.5-2.0 mm bone loss: 52%. Implants with > 2.0 mm bone loss: 33%. No correlation between bone loss around implants and teeth		Implants can be maintained with excellent results over a 10-year period in patients earlier treated for advanced periodontitis	(155)

All group values referred to are expressed as mean values. Abbreviation: TPS: titanium plasma sprayed.

Table 5. Main histological characteristics of clinically healthy peri-implant tissue.

Tissue component	Results and comments	Ref.
Epithelium	Comparable vertical dimension of junctional epithelium around implants and teeth irrespective of implant system and irrespective of a 1- or 2-stage installation procedure	(3,5,23,24,75,106,261)
	Junctional epithelium attached to titanium surface with hemidesmosomes and basal lamina	(91,135)
	No or only minor structural differences in epithelial differentiation around implants and teeth when evaluated by antibodies directed against keratins, desmoplakins, and intercellular cell adhesion molecule 1 (ICAM-1)	(42,171)
Connective tissue, blood vessels, and inflammatory cells	Peri-implant connective tissue contains more collagen fibers and lower density of fibroblasts as compared with gingiva	(23)
	Cervical cementum with inserting collagen fibers absent on implant surface. Region between junctional epithelium and alveolar crest where peri-implant connective tissue is in direct contact with implant surface. Not understood why junctional epithelium does not proliferate along implant surface to bone crest	(IV,3,5,23,35,41,74,75,166,234)
	Region between junctional epithelium and alveolar bone close to implant surface contains abundance of fibroblasts and no or very few blood vessels	(25,41,166,192)
	Collagen fibers adjacent to implant surface run in a direction more or less parallel with implant surface. Remaining collagen fibers run in different directions forming a 3-dimensional network	(23,41,53,74,166)
	Similar distribution of laminin, fibronectin, and collagen types I, III, IV, and VII around implants and teeth. Different distribution of collagen types V and VI around implants and teeth. The relevance of this observation is unknown	(46,224)
	Few inflammatory cells in connective tissue adjacent to junctional epithelium. Predominantly T-lymphocytes, few plasma cells	(IV,3,7,23,24,35,36,41,53,74,144,152,153,156,157, 166,234,237,255-257,274)
	No or only minor differences in presence of intraepithelial antigen-presenting cell markers, inflammatory cell subtypes, and cellular adhesion molecules in peri-implant mucosa and gingiva. Biopsies of peri-implant tissue taken rather shortly after implant placement may compromise these results	(157,255,256,275)
	An inflammatory infiltrate present in connective tissue at connection between fixture and abutment when connection between fixture and abutment is beyond the margin of peri-implant mucosa	(4,74,75)

Table 6. Studies in animals on probe tip position around implants and teeth.

Animal	Implant	Probe		Inflammatory status	Results				Conclusions by authors	Ref.
					Distance from probe tip to apical extension of junctional epithelium		Distance from probe tip to alveolar bone			
		Type	Force		Implants	Teeth	Implants	Teeth		
5 dogs	10 Brånemark in the mandible (machined surface)	20 probes (10 at implants, 10 at teeth) with a diameter of 0.5 mm	0.5 N	Healthy or only minor signs of inflammation	-1.3 mm	0.1-0.2 mm	0.2 mm	1.1-1.2 mm	Probe penetration more advanced around implants as compared with teeth with healthy peri-implant mucosa/gingiva	(71)
5 dogs	30 ITI in the mandible (TPS surface)	80 probes (60 at implants, 20 at teeth) with a diameter of 0.45 mm or with a rectangular tip (0.2x0.83 mm)	0.2 N	Healthy	0.05 mm	-0.2 mm (n=2)	0.6 mm	1.1 mm (n=2)	Probing around implants represents a relevant clinical parameter to indicate stability or pathological conditions of peri-implant tissue. Tooth crown convexity hindered proper probe insertion around teeth	(147)
				Mucositis/ gingivitis	0.02 mm	-0.1 mm (n=2)	0.8 mm	1.2 mm (n=2)		
				Peri-implantitis/ periodontitis	-0.5 mm	-0.5 mm (n=5)	0.3 mm	0.2 mm (n=5)		
8 monkeys	32 Astra in the maxilla and mandible (machined surface)	128 probes (64 at implants, 64 at teeth) with a ball-shaped tip and a diameter of 0.5 mm	0.3-0.4 N	Healthy	No information	No information	0.7-1.2 mm#	0.8-1.2 mm#	Probing around implants and teeth was different. Even mild inflammation associated with deeper probe penetration around implants as compared with teeth	(VI)
				Mild mucositis/ gingivitis	No information	No information	0.5-1.2 mm#	0.7-1.7 mm#		
				Severe mucositis/ gingivitis	No information	No information	0.2-0.4 mm#	0.7-1.2 mm#		
				Peri-implantitis/ periodontitis	No information	No information	0.1-0.2 mm#	1.2-1.8 mm#		

All group values referred to are expressed as mean values, if not otherwise specified. Abbreviations: TPS: titanium plasma-sprayed, #: median.

Table 7. Studies in humans on surgical treatment modalities of peri-implantitis.

Patient	Implant and time since placement	Antibiotic	Treatment	Implant surface preparation	Healing period	Observation period	Results		Comments	Ref.
							Inflammation	»Bone« regeneration		
1	4 Core-Vent (threaded surface). 5 years	Erythromycin (10 days)	Free gingival graft. After 12 weeks: Flap surgery and HA or DFDB	No information	Non-submerged	1 year	No	HA: Yes DFDB: No	No comments	(85)
1	2 cylindric hollow-basket implants (threaded surface). 1 year	No information	Flap surgery and DFDB	Smoothening, air-powder abrasive unit, and chloramine T	Non-submerged	17 months	No	Yes	No complications	(167)
1	1 Screw-Vent. 7 months	Amoxicillin (1 week)	Flap surgery and ePTFE	No information	Submerged	11 months	No information	Yes	Membrane removal after 6 weeks	(87)
1	1 ITI (TPS surface). 5 weeks	Amoxicillin (10 days) followed by ornidazol (10 days)	Flap surgery and ePTFE	Irrigation alternately with chlorhexidine and saline	Non-submerged	6 months	No	Yes	Membrane removal after 5 months	(150)
1	1 titanium screw implant. 2 years	No information	Flap surgery, DFDB, and ePTFE	No information	Submerged	6 months	No information	Yes	No complications	(172)
2	2 ITI (TPS surface). 4 years	Metronidazole and amoxicillin (10 days)	Flap surgery and ePTFE	Irrigation alternately with chlorhexidine and saline	Non-submerged	1 year	No. Probing depth: 3.3 mm	2.7 mm	Membrane removal after 4.5 months	(119)
		Amoxicillin (10 days)					No. Probing depth: 3.7 mm	1.9 mm	Membrane removal after 6.5 months	
3	3 titanium hollow cylinders (TPS surface). 1.5-2.5 years	No information	Flap surgery, HA or DFDB soaked in tetracycline, and ePTFE	Tetracycline irrigation	Non-submerged	8-12 months	No	Yes	Membrane removal after 6-9 weeks	(179)
1	1 ITI (TPS surface). 3 months	Amoxicillin (1 week)	Flap surgery, AB, and Guidor membrane	Chlorhexidine irrigation	Submerged	6 months	No	Yes	Membrane exposure after 2 weeks	(14)
1	2 titanium implants. 2 years	Tetracycline (10 days)	Flap surgery and Bio-Oss or ePTFE	Smoothening and tetracycline irrigation	Non-submerged	8 years	No. Probing depth: 1.5 mm	Yes	Membrane removal after 2 months	(193)

Table 7, continued.

Patient	Implant and time since placement	Antibiotic	Treatment	Implant surface preparation	Healing period	Observation period	Results		Comments	Ref.
							Inflammation	»Bone« regeneration		
17	25 ITI (TPS surface). 8 implants within 2 years of function, 17 implants after 2 years of function	Metronidazole (7 days)	Flap surgery and AB (block or particulate) fixated with screws or fibrin glue	Air-powder abrasive unit (30 seconds)	Non-submerged	6-36 months	No. Probing depth after 3 years: 1.6 mm	After 3 years: 4.2 mm (100%)	No comments	(19)
17	24 IMZ (TPS surface)	Penicillin (5 days)	Flap surgery, AB, and ePTFE	Photo-sensitisation by Toluidine blue and soft laser irradiation	Submerged	9.5 months	No	2.0 mm (36%)	All membranes exposed	(102)
25	41 IMZ and Friadent. 5.8 years of function	According to antimicrobial susceptibility test (2 weeks)	Flap surgery, AB (block or particulate), and ePTFE	Chlorhexidine irrigation, citric acid, and hydrogen peroxide	Submerged	3 years	Probing depth: 2.8 mm	2.8 mm	Membrane removal after 6 months. 45% of the membranes exposed	(136)
			Flap surgery, AB (block or particulate), and Bio-Gide membrane				Probing depth: 5.1 mm	1.9 mm	33% of the membranes exposed	
			Flap surgery and AB (block or particulate)				Probing depth: 2.9 mm	2.4 mm	No complications	
1	3 Brånemark (machined surface). 30 months of function	Amoxicillin (1 week)	Flap surgery, AB, DFDB, and ePTFE	Air-powder abrasive unit (3 minutes), tetracycline, and saline irrigation	Submerged	12 months	Probing depth: < 2 mm	Yes	Membrane removal after 12 months. No membrane exposure	(254)

All group values referred to are expressed as mean values. Abbreviations: HA: hydroxyapatite, DFDB: demineralised freeze-dried bone, AB: autogenous bone, TPS: titanium plasma-sprayed, ePTFE: expanded polytetrafluoroethylene membrane.

Table 8. Studies in animals on surgical treatment modalities of peri-implantitis.

Animal	Implant	Antibiotic	Treatment	Implant surface preparation	Healing period	Results			Comments	Ref.
						Inflam-mation	Re-osseo-integration	Bone regeneration		
10 dogs	40 Screw-Vent (acid-etched surface)	No information	Flap surgery with/without ePTFE for 1 month	Air-powder abrasive unit	Submerged and non-submerged	No	≤ 0.3 mm in all groups	Minimal	Suspension membrane sutures used in the non-submerged group. High frequency of membrane exposures	(93)
3 dogs	10 Brånemark (machined surface), 10 IMZ (TPS surface), and 10 Integral (HA-coated surface)	No information	Flap surgery and ePTFE for 2 or 4.5 months	Air-powder abrasive unit (30 seconds) and supersaturated citric acid (30 seconds)	Submerged	No information	Limited	Yes	No comments	(132)
			Flap surgery				Minimal	Minimal		
1 pig	6 Brånemark (machined surface)	No information	Flap surgery and ePTFE for 6 weeks	Air-powder abrasive unit	Submerged	No information	Reference: Previous defect. 36%	Vertical measurements during surgery: 2.1 mm	Flourochrome injected on the day of surgery	(242)
			Flap surgery		Submerged		8%	1.4 mm		
					Non-submerged		0%	0.9 mm		
5 dogs	30 Brånemark (machined surface)	Amoxicillin and metronidazole (3 weeks)	Flap surgery	1% delmopinol and abutments autoclaved	Non-submerged	No	No	No	No comments	(76)
			No	No		Yes	No	No		
5 dogs	30 Brånemark (machined surface)	Amoxicillin and metronidazole (3 weeks)	Flap surgery and ePTFE for 4 months	1% delmopinol	Submerged	No	Minimal	Minimal	Vertical measurements of defect during surgery	(209)
			No	No	Non-submerged	Yes	No	No		
7 dogs	42 Brånemark (machined surface)	Metronidazole (3 weeks)	Flap surgery, HA, and ePTFE for 4 months	Air-powder abrasive unit (30 seconds)	Submerged	No	2.3 mm	2.4 mm	No comments	(114)
			Flap surgery, DFDB, and ePTFE for 4 months				2.2 mm	3.0 mm		
			Flap surgery and HA				0.9 mm	1.3 mm		
			Flap surgery and DFDB				0.9 mm	1.6 mm		
			Flap surgery and ePTFE for 4 months				1.0 mm	2.5 mm		
			Flap surgery				0.3 mm	0.5 mm		

Table 8, continued.

Animal	Implant	Antibiotic	Treatment	Implant surface preparation	Healing period	Results			Comments	Ref.
						Inflam-mation	Re-osseo-integration	Bone regeneration		
4 dogs	16 Napio (acid-etched surface)	Metroni-dazole (3 weeks)	Flap surgery, Bio-Oss, and PTFE for 4 months	Air-powder abrasive unit (30 seconds)	Submerged	No information	No information	1.6 mm	Vertical measurements of defect during surgery	(170)
			Flap surgery and Bio-Oss					1.6 mm		
			Flap surgery and PTFE for 4 months					1.4 mm		
			Flap surgery					0.9 mm		
4 dogs	24 Brånemark (machined surface)	Amoxicillin and metroni-dazole (3 weeks)	Flap surgery	Rotating brush with pumice	Submerged	No	0.4 mm	Reference: Previous defect. 2.0 mm^2 (59%)	Flourochrome injected 2 weeks after surgery	(210)
				Cotton pellet soaked in saline			0.4 mm	1.6 mm^2 (64%)		
7 dogs	41 ITI SLA surface	Metroni-dazole (10 days)	Flap surgery and ePTFE for 5.5 months	Copious chlorhexidine irrigation	Submerged	No	Reference: Previous defect.	Reference: Previous defect.	Flourochrome injected 2 weeks before surgery. Titanium ring placed around implant at defect bottom	(269)
							20% (0.6 mm)	2.3 mm (83%)		
	TPS surface						14% (0.5 mm)	2.6 mm (73%)		
	Machined surface						2% (0.1 mm)	2.2 mm (62%)		
	SLA surface		Flap surgery				11% (0.3 mm)	0.4 mm (15%)		
	TPS surface						14% (0.3 mm)	0.3 mm (14%)		
	Machined surface						7% (0.2 mm)	0.8 mm (31%)		
6 dogs	60 Frialit-2 (TPS surface)	No information	Flap surgery	Air-powder abrasive unit (60 seconds)	Submerged	No information	0.8 mm	Reference: Previous defect. 0.5 mm (29%)	No signs of thermal damage to surrounding bone after implant surface preparation with CO_2 laser	(58)
			Flap surgery and ePTFE for 4 months				1.2 mm	1.6 mm (94%)		
			Flap surgery	Carbon dioxide (CO_2) laser (60 seconds)			1.0 mm	0.9 mm (53%)		
			Flap surgery and ePTFE for 4 months				1.2 mm	1.9 mm (112%)		
			Flap surgery	Air-powder abrasive unit (60 seconds) and carbon dioxide laser (60 seconds)			0.8 mm	0.8 mm (47%)		
			Flap surgery and ePTFE for 4 months				1.1 mm	1.5 mm (88%)		

Table 8, continued.

Animal	Implant	Antibiotic	Treatment	Implant surface preparation	Healing period	Results			Comments	Ref.
						Inflam-mation	Re-osseo-integration	Bone regeneration		
5 dogs	30 Napio (acid-etched surface)	Metroni-dazole (3 weeks)	Flap surgery alone or combined with Bio-Oss and PTFE for 4 months, Bio-Oss and Bio-Gide membrane, PTFE for 4 months, Bio-Oss, or Bio-Gide membrane	Air-powder abrasive unit (30 seconds)	Submerged	No information	Reference: Previous defect. 26-31% irrespective of treatment	Reference: Previous vertical defect height. 14-28% irrespective of treatment	Vertical measurements of defect during surgery	(198, 199)
2 dogs	16 Brånemark (machined surface) Modified fixtures	Amoxicillin and metroni-dazole (3 weeks)	Flap surgery	Replacement of the coronal contaminated implant part with a non-contaminated	Submerged	No	New osseo-integration on the replaced implant part	Yes	Flourochrome injected 2 weeks after surgery	(212)
	Standard fixtures			Cotton pellet soaked in saline			Minimal	Yes		
4 dogs	24 ITI Machined surface	Amoxicillin and metroni-dazole (17 days)	Flap surgery	Cotton pellet soaked in saline	Submerged initially, but all non-submerged after 1 month	No	Reference: Previous defect. 22% (0.4 mm)	Reference: Previous defect. 3.8 mm^2 (72%)	Flourochrome injected 2 weeks after surgery	(211)
	SLA surface						84% (1.2 mm)	3.2 mm^2 (77%)		
8 monkeys	64 ITI (TPS surface)	Ampicillin and metroni-dazole (12 days)	Flap surgery, AB, and ePTFE for 3 months	Gauze soaked alternately in chlorhexidine and saline (5 minutes)	Non-submerged	No	Reference: Previous defect. 45%	Reference: Previous defect. 16.1 mm^2 4.7 mm (94%)	Transfer of defect border from radiographs to histological sections. Unbiased stereological estimates	(VII, VIII)
			Flap surgery and AB				22%	9.2 mm^2 4.0 mm (80%)		
			Flap surgery and ePTFE for 3 months				21%	6.3 mm^2 3.0 mm (65%)		
			Flap surgery				14%	5.4 mm^2 1.9 mm (40%)		
8 monkeys	64 ITI (TPS surface)	Ampicillin and metroni-dazole (12 days)	Flap surgery, Bio-Oss, and ePTFE for 3 months	Gauze soaked alternately in chlorhexidine and saline (5 minutes)	Non-submerged	No	Reference: Previous defect. 36%	Reference: Previous defect. 11.2 mm^2 5.0 mm (111%)	Transfer of defect border from radiographs to histological sections. Unbiased stereological estimates	(IX)
			Flap surgery and Bio-Oss				16%	8.7 mm^2 4.6 mm (96%)		
			Flap surgery and ePTFE for 3 months				23%	7.9 mm^2 3.7 mm (74%)		
			Flap surgery				13%	5.3 mm^2 2.1 mm (47%)		

Table 8, continued.

Animal	Implant	Antibiotic	Treatment	Implant surface preparation	Healing period	Results			Comments	Ref.
						Inflam-mation	Re-osseo-integration	Bone regeneration		
8 monkeys	64 ITI (TPS surface)	Ampicillin and metroni-dazole (12 days)	Flap surgery, AB, and ePTFE for 3 months	Air-powder abrasive unit (5 minutes) and super-saturated citric acid (2 minutes)	Non-submerged	No	Reference: Previous defect. 46%	Reference: Previous defect. 14.1 mm^2 4.6 mm (99%)	Transfer of defect border from radiographs to histological sections. Unbiased stereological estimates	(X)
				Air-powder abrasive unit (5 minutes)			39%	14.1 mm^2 4.7 mm (105%)		
				Gauze soaked in saline (5 minutes) and super-saturated citric acid (2 minutes)			43%	14.2 mm^2 4.4 mm (94%)		
				Gauze soaked alternately in chlorhexidine and saline (5 minutes)			40%	13.9 mm^2 4.7 mm (105%)		

All group values referred to are expressed as mean values. Abbreviations: HA: hydroxyapatite, DFDB: demineralised freeze-dried bone, AB: autogenous bone, SLA: sandblasted and acid-etched, TPS: titanium plasma-sprayed, ePTFE: expanded polytetrafluoroethylene membrane, PTFE: polytetrafluoroethylene membrane.

Table 9. Treatment outcome in monkeys after using autogenous bone or Bio-Oss covered by an ePTFE membrane in the surgical treatment of peri-implantitis.

Response variable	Autogenous bone graft particles and ePTFE membrane (Study VIII)	Bio-Oss and ePTFE membrane (Study IX)	Estimated difference between the 2 treatments
Mean total sectional area of graft and regenerated bone within defect	16.1 mm^2	11.2 mm^2	4.9 mm^2 (95% confidence interval: 3.3-6.4 mm^2) ($p \leq 0.0001$)
Mean proportion (%) of implant »surface« within defect covered by regenerated bone (re-osseointegration)	45%	36%	9% (95% confidence interval: 3-15%) ($p = 0.004$)

Abbreviation: ePTFE: expanded polytetrafluoroethylene membrane.