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SOME CHARACTERISTICS OF PERIIMPLANT TISSUES

EXPERIMENTAL STUDIES IN THE DOG

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DEPARTMENT OF PERIODONTOLOGY
FACULTY OF ODONTOLOGY
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II. Abrahamsson, I., Berglundh, T. & Lindhe, J. (1998) Soft tissue response to plaque-formation at different implant systems. A comparative study in the dog. *Clinical Oral Implants Research* 9: 73-79.

III. Abrahamsson, I., Berglundh, T. & Lindhe, J. (1997) The mucosal barrier following abutment dis-/re-connection. An experimental study in dogs. *Journal of Clinical Periodontology* **24**: 568-572.

IV. Abrahamsson, I., Berglundh, T., Glantz, P.- O. & Lindhe, J. (1998) The mucosal attachment at different abutments. An experimental study in dogs. *Journal of Clinical Periodontology* **25**: 721-727.

V. Abrahamsson, I., Berglundh, T., Moon, I.-S. & Lindhe, J. (1999) Peri-implant tissues at submerged and non-submerged titanium implants. *Journal of Clinical Periodontology* **26**, In press.

Abstract

Some characteristics of periimplant tissues

Ingemar Abrahamsson

Department of Periodontology, Faculty of Odontology, Göteborg University, Box 450, SE 405 30 Göteborg, Sweden.

The aim of the present experiments was to analyze aspects of the marginal tissues at implants which (i) differed with respect to design, installation technique and abutment material (ii) were challenged to repeated break-up of the "zone of connective tissue integration" and (iii) were exposed to plaque formation. The beagle dog model was used. 3 months prior to fixture installation all mandibular premolars were extracted. The implants were monitored during a six month period after abutment connection. At the end of each experiment a clinical examination, including assessment of plaque and soft tissue condition was performed, radiographs obtained and biopsies harvested. The biopsies were prepared for histometric and morphometric analyses. The peri-implant soft tissue consistently was comprised of two units, one epithelial and one connective tissue unit. A well-keratinized oral epithelium was continuous with a junctional epithelium that faced the implant. This junctional epithelium extended to a distance of about 2 mm apical of the mucosal margin. The connective tissue portion that was located between the bone crest and the junctional epithelium ("zone of connective tissue integration") was dominated by collagen. In a narrow tissue portion close to the implant, the majority of the collagen fibers were attached to the periosteum and ran a course parallel to the surface of the implant. The vertical dimension of the "zone of connective tissue integration" varied between 1.0 and 1.5 mm, when formed adjacent to a titanium or a ceramic surface. When gold or dental porcelain was used in the abutment part of the implant or when the connective tissue attachment was disrupted by repeated dis- and re-connection of the abutment, the epithelium migrated "apically", bone resorption occurred and the "zone of connective tissue integration" was formed to the titanium surface of the fixture. The structure and composition of the peri-implant tissues that formed after a submerged or nonsubmerged installation technique did not differ. 5 months of plaque formation at different implant systems, resulted in the establishment of inflammatory infiltrates in the marginal portions of the periimplant mucosae. These lesions resided in a connective tissue compartment lateral to the barrier epithelium.

Key words: abutment materials, barrier tissue, dental implants, dogs, histometry, morphometry, mucositis, non-submerged implants, peri-implant mucosa, submerged implants, titanium.

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Preface

The present thesis is based on the following publications, which will be referred to in the text by their Roman numerals.

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Introduction

The long-term function of dental implants made of c.p. titanium seems to be dependent on a sustained osseointegration providing stability, and a sufficient soft tissue barrier protecting the zone of osseointegration from factors released from the oral environment (Brånemark 1985, Ten Cate 1985, Carmichael et al. 1989, Listgarten et al. 1991).
It has been suggested that the formation of a proper union between the implant and the bone, i.e. osseointegration, requires a surgical technique where the fixture part of the implant is submerged, i.e. covered by periosteum and oral mucosa, during an initial healing period (Brånemark et al. 1977, Adell et al. 1985). The transmucosal passage is established in a subsequent surgical procedure. Some features of the peri-implant tissues formed following this installation technique have been reported in animal experiments (Sennerby 1991, Berglundh et al. 1991, 1994, Berglundh & Lindhe 1996).
In a different surgical approach the implant is not submerged but allows the establishment of the transmucosal passage to occur before the osseointegration is completed. This technique has also been demonstrated to be successful in terms of achieving osseointegration and formation of a soft tissue barrier (Buser et al. 1992, Weber et al. 1996, Cochran et al. 1997). It remains to be examined, however, if the soft and hard peri-implant tissues that will form at implants placed by the 2 techniques will differ regarding structure and composition.

The first aim of the present series of experiments was to study the marginal peri-implant tissues around implants which differed with respect to design and installation technique, i.e. non-submerged (1- stage implants) and initially submerged and subsequently exposed implants (2-stage implants).

The finding that plaque formation on implants results in the establishment of inflammatory lesions in the adjacent mucosa has been reported in experiments in the beagle dog (Berglundh et al. 1992, Ericsson et al. 1992, Lang et al. 1994). Commercially available dental implant systems differ in many aspects (e.g. design and installation technique) and it is not known whether a certain implant design or installation technique may influence the tissue response to plaque accumulation. Previous animal studies (Berglundh et al. 1992, Ericsson et al. 1992, 1995, Lang et al. 1994) demonstrated that plaque formation resulted in an inflammatory reaction (ICT) in the mucosa which in both the tooth and implant segments of the dog occupied a small portion of the connective tissue lateral to the barrier epithelium. The ICT of the peri-implant mucosa, however, showed a considerable

variation in its apical extension in the three studies. This variation may be explained either by differences in the duration of the plaque accumulation period (3 weeks to 3 months) or by the 2 different implant systems used. The Brånemark System® (2-part system, installed using a submerged technique) was used in the studies by Berglundh et al. (1992) and Ericsson et al. (1992, 1995) and the ITI Dental Implant System® (1-part system, hollow cylinder, installed using a non-submerged technique) was used in the experiment by Lang et al. (1994).

The second aim of the present thesis was to study the location and composition of plaque associated lesions (ICT) in the mucosa adjacent to implants that differed with respect to geometry, dimension and installation technique.

Studies by Berglundh et al. (1991, 1992), Buser et al. (1992), Berglundh & Lindhe, (1996) and Cochran et al. (1997), showed that the implant-mucosal barrier is comprised of (i) a junctional epithelium which is about 2 mm long and (ii) a connective tissue compartment which is about 1 - 1.5 mm high. It was suggested that during the early healing of the transmucosal passage an interaction occurs between the dioxide layer of the titanium implant and the severed connective tissue. This zone of "interaction" may not be recognized as a wound and, evidently serves the purpose of protecting the zone of osseointegration from factors released from the oral environment. Berglundh & Lindhe (1996) suggested that this soft tissue must have an adequate dimension. Hence, if the mucosa is too thin, bone resorption will occur to ensure that a "biological width" of the implant-mucosal barrier is established. When the "zone of connective tissue integration" (Berglundh et al. 1991) is severed by mechanical trauma, e.g. by removal of an abutment, a new soft tissue wound occurs, the healing of which may influence the implant-mucosal barrier.

The third objective was to study the effect of repeated abutment removal and subsequent reconnection on the implant-mucosal barrier.

Commercially pure (c.p.) titanium is recognized as the material of choice for oral implants (Schenk & Buser 1998, Steinemann 1998). Esthetic demands in implant dentistry, however, have called for the use of materials other than c.p. titanium, particularly in the transmucosal part of the system. Thus, gold alloys and ceramics have been used as abutment material. Data available from animal

experiments (McKinney et al. 1985, Hashimoto et al. 1988, 1989, Fartash et al. 1990, and Arvidsson et al. 1991) and clinical trials (Akagawa et al. 1989) indicated that certain ceramic materials might allow proper wound healing at the implant/mucosal passage. No data on soft tissue reactions to gold alloys used as an implant material is available.

The fourth aim of the present series of experiments was to examine the structure and composition of the mucosal barrier that formed around abutments made of different materials.

Material & Methods

The protocol of each experiment of the present series was approved by the regional Ethics Committee for Animal Research, Göteborg, Sweden.

Animals, diet and experimental design

The beagle dog model was used in all studies. The animals were at the start of the experiment about 1 year old and were during the experiment fed a soft diet. In studies I-IV only male dogs were used and in study V, 3 male and 3 female dogs were used.

Preparations of recipient implant sites

3 months prior to fixture installation, the mandibular premolars ($_4P_4$, $_3P_3$, $_2P_2$, $_1P_1$) were hemisected and the individual roots were extracted one by one in an atraumatic manner. The intention was to obtain an edentulous alveolar ridge, between the mandibular canine and the 1st mandibular molar, of optimal width and height. Therefore, in order to minimize post-extractional resorption of the alveolar ridge, small muco-periosteal flaps were elevated and mobilized to ensure a proper coverage of the extraction sockets before suturing.

Implants

The various implant components used in the present series are described in detail, regarding e.g. dimensions and surface topography in each original publication (I, II, III, IV and V).

Implant installation

A crestal incision between the 1st molar and the canine, with a buccal releasing incision in the distal part of the incision, was performed. Buccal and lingual full-thickness flaps were raised and the two mental foramina were identified in each mandibular premolar region. 3 fixtures were installed in each mandibular region.

Initially submerged implants: Following fixture installation the bone crest coincided with the fixture margin of the Astra Tech Implant System® (Studies I, II and V) and the Brånemark System® (Study I-IV). The fixtures were provided with cover screws and the flaps were adjusted and sutured, leaving the implants in a submerged position.

Abutment connection of the initially submerged implants: Following a 3 month healing period, bilateral mandibular crestal incisions were performed between the 1st molar and the canine. For proper access to the fixtures, buccal and lingual full-thickness flaps were raised, cover screws removed and abutments connected. The flaps were adjusted,

sutured and healing allowed for 2 weeks. A plaque control program was initiated immediately after suture removal.

Nonsubmerged implants: Following fixture installation the bone crest coincided with the fixture margin of the Astra Tech Implant System® (2-part system) (Study V) whereas the border between the plasma sprayed and the polished surface of the ITI Dental Implant System® (1-part system) (Studies I and II) was positioned at the level of the bone crest. The "Astra Implants" were at the same surgical session provided with abutments and the "ITI Implants" were provided with healing caps. The flaps were adjusted and sutured, allowing the "Astra Implants" and the "ITI Implants" to penetrate the mucosa. 2 weeks later, the sutures were removed and the plaque control program was started.

Plaque control

The plaque control periods of studies I - V included daily cleaning (5 days/week) of all exposed implant surfaces, using toothbrush and dentifrice.

Plaque formation

During the 5 months of plaque accumulation in study II, all oral hygiene measures were abandoned and plaque formation was allowed. The dogs were fed a soft diet to promote plaque accumulation.

Study I

Specific aim

to study the characteristics of the marginal peri-implant tissues at non-submerged (1- stage implants) and initially submerged and subsequently exposed implants (2-stage implants).

Five beagle dogs were used. Extraction of all mandibular premolars and the 1st, 2nd and 3rd maxillary premolars were performed. Three months later, fixtures of the Astra Tech Implants Dental System®, the Brånemark System® and the ITI Dental Implant System® were installed. In each mandibular quadrant one fixture of each implant system was installed in a randomized order. Following installation the bone crest coincided with the fixture margin of the Astra Tech Implant System® and the Brånemark System®, whereas the border between the titanium plasma sprayed (TPS) and the polished surface of the ITI Dental Implant System® (the 1-stage system) was positioned at the level of the bone crest. The flaps were adjusted and sutured, leaving the "Astra" and the "Brånemark implants" covered by the oral mucosa and the "ITI implants" non-submerged. Following a healing period of three months, abutment connection was carried out in the 2-stage

systems ("Astra Implants" and "Brånemark Implants"). A six month period of plaque control was initiated. At the end of the plaque control period clinical and radiographic examinations were performed and biopsies obtained.

Study II

Specific aim

to study the location and composition of plaque associated lesions in the mucosa adjacent to implants that differed with respect to both geometry and installation technique.

Five beagle dogs were included in the study. All mandibular premolars and the 1st, 2nd and 3rd maxillary premolars were extracted. After three months of healing, fixtures of the Astra Tech Implants, Dental System®; "Astra Implants", the Brånemark System®; "Brånemark Implants" and the ITI Dental Implant System®; "ITI Implants" were installed. One fixture of each implant system was installed in each mandibular quadrant in a randomized order. Following installation the bone crest coincided with the fixture margin of the Astra Tech Implant System® and the Brånemark System®, whereas the border between the plasma sprayed and the polished surface of the ITI Dental Implant System® (the 1-stage system) was positioned at the level of the bone crest. The flaps were adjusted and sutured in such a way that the "Astra" and the "Brånemark implants" became submerged while the "ITI implants" penetrated the mucosa. A plaque control program, consisting of daily cleaning with toothbrush and dentifrice, was initiated. Three months later, abutment connection was performed on the Astra Implants and the Brånemark Implants. The plaque control measures were abandoned one month later and plaque was allowed to accumulate for 5 months. At the end of this period, a clinical examination including assessment of plaque and soft tissue inflammation was performed and biopsies were collected.

Study III

Specific aim

to study the effect of repeated abutment removal and subsequent reconnection on the marginal peri-implant tissues.

Five beagle dogs were included in the study. At the start of the experiment the mandibular premolars ($_4P_4$, $_3P_3$, $_2P_2$, $_1P_1$) were extracted. After three months of healing, 2 fixtures of the Brånemark System® were installed, one in each mandibular quadrant. Three months later, abutment connection was performed. Standard abutments of commercially pure titanium were used.

A six month period of plaque control, including daily cleaning of the tooth and implant segments with toothbrush and dentifrice, was performed. Once a month during the plaque control period, the abutment of the right side (test) in each dog was disconnected, cleaned with 3% hydrogen peroxide and 70% ethanol, rinsed in saline and reconnected to the fixture. Thus, each test abutment was removed and reconnected altogether 5 times during this period. The contralateral abutment remained undisturbed for 6 months and served as control. At the end of the 6 month period and 1 month after the last reconnection of the test abutment, a clinical examination including assessment of plaque and soft tissue inflammation was performed and biopsies obtained.

Study IV

Specific aim

to examine the structure and composition of the mucosal barrier that formed around abutments of different materials.

Five beagle dogs were included in the study. The mandibular premolars ($_4P_4$, $_3P_3$, $_2P_2$, $_1P_1$) and the 1st, 2nd and 3rd maxillary premolars ($^1P^1$, $^2P^2$, $^3P^3$) were extracted. After 3 months of healing, 3 fixtures of the Brånemark System were installed in each mandibular quadrant. Abutment connection was performed after another 3 months. The abutments used and their position were the following:
-Two "control abutments" (standard) (one on each side) made of commercially pure titanium.
-Two "ceramic abutments"; CerAdapt® (one on each side).
-One "gold abutment".
-One "short titanium abutment"; EsthetiCone™. The "short titanium abutment" was provided with a super structure, made of dental porcelain fused to gold, which gave an overall geometry and dimension similar to the control-, gold- and ceramic abutments.
Following abutment connection, a six month period of plaque control, including daily cleaning with toothbrush and dentifrice was initiated. At the end of this period, a final examination was performed. At this occasion the assessment of plaque and soft tissue inflammation was followed by the harvesting of biopsies.

Study V

Specific aim

to study the peri-implant tissues formed around an original 2-part implant system using either a submerged or a non-submerged installation technique.

Six beagle dogs were used in the experiment. All mandibular premolars ($_4P_4$, $_3P_3$, $_2P_2$, $_1P_1$) and the bilateral 1st, 2nd and 3rd maxillary premolars ($^1P^1$, $^2P^2$, $^3P^3$) were extracted. After three months of healing, 3 fixtures of the Astra Tech Implants® Dental System were installed in the right (or the left), premolar region in each of the six dogs. The implants were placed in such a way that the fixture margin coincided with the bone crest. The mucoperiosteal flaps were resutured to cover the fixtures. Radiographs were obtained immediately after fixture installation. Three months later, abutments were connected to the initially submerged fixtures (submerged side; control side). During the same session another 3 fixtures (Astra Tech Implants® Dental System) were installed in the contralateral, edentulous premolar region and in the manner described above. Abutments were, however, immediately connected to the newly installed fixtures (non-submerged side; test side). The mucosal flaps were replaced, adjusted and sutured. A new set of radiographs were obtained from all 6 implant sites in each animal using a custom made film holder device (modified from an Eggen holder; Eggen, 1969), connected to the posterior implant. A period of plaque control was initiated, including daily cleaning of all teeth and exposed implant surfaces using toothbrush and dentifrice. Clinical examinations were performed and radiographs obtained from all implant sites after 3 months and 6 months. After 6 months of plaque control, i.e. 9 months after the first fixture installation procedure (control side), biopsies were obtained.

Clinical examinations

The clinical examinations of studies I, II, III and IV included registrations of plaque (presence or absence), bleeding on gentle probing and visible signs of inflammation. In study V the Pl.I. (Silness & Löe, 1964) was used for the plaque assessments and the MGI (Lobene et al. 1986) for the assessment of soft tissue inflammation.

Radiographic analysis

Radiographic analyses were performed in studies I-V. Data from radiographic examinations, however were presented only in study V. The radiographs of study V were obtained at the following time-points:

Day 0	Fixture installation of initially submerged implants
Day 90	Abutment connection at initially submerged implants and fixture installation and abutment connection of nonsubmerged implants
Day 180	3 months of plaque control
Day 270	6 months of plaque control and termination of the experiment

The radiographs from day 0 were obtained using a modified Eggen technique (Eggen, 1969) and the radiographs from day 90, 180 and 270 were obtained using a custom made film holder device (modified from an Eggen holder; Eggen, 1969) which was connected to the posterior implant.
In the radiographs, the distance between the most "coronal" part of the fixture (**A/F**) and the most "coronal" bone judged to be in contact with the implant surface (**B**) was determined at the mesial and distal aspect of each implant. The measurements were carried out in a Leica DM-RBE® microscope (Leica, Germany) equipped with an image system (Q-500 MC®, Leica Germany).

Biopsy procedure

In the present series of experiments (Studies I-V) the dogs were killed by an overdose of sodium Pentothal and perfused through the carotid arteries with a fixative consisting of a mixture of glutaraldehyde (5%) and formaldehyde (4%) buffered to pH 7.2 (Karnovsky, 1965). The mandibles were removed and placed in the fixative. Each implant site was further dissected using a diamond saw (Exakt®, Kulzer, Germany).

Histological processing

EPON® sections

All tissue samples, comprising the implant and the surrounding soft and hard peri-implant tissues, in studies I-IV and at implants $_2P_2$ and $_3P_3$ in study V, were decalcified in EDTA and processed using a modification of the "fracture technique" (Thomsen & Ericson, 1985) as described by Berglundh et al. 1994. Thus, before the tissue samples were fully decalcified, incisions, parallel with the long axis of the implants, were made through the entire peri-implant tissue at the mesial and distal aspect of the implants. The buccal and lingual portions of the peri-implant tissues were carefully dissected and further divided in order to obtain one mesio-lingual, one disto-lingual,

one mesio-buccal and one disto-buccal unit from each implant site. Decalcification was completed in EDTA and dehydration performed in serial steps of ethanol concentrations. Secondary fixation in OSO_4 of the tissue samples was carried out and the units were finally embedded in EPON® (Schroeder, 1969). Sections were produced from each tissue unit with the microtome set at 3µm. The sections were stained in PAS and toluidine blue (Schroeder, 1969).

Ground sections

In study V the biopsy from the most anterior implant site in each mandibular premolar region was processed for ground sectioning (Donath & Breuner 1982, Donath 1988). The tissue samples were dehydrated in serial steps of ethanol concentrations and subsequently embedded in methyl methacrylate (Technovit® 7200 VLC, Exakt®, Kulzer, Germany). The blocks were cut in a mesio-distal plane using a cutting-grinding unit (Exakt®, Apparatebau, Norderstedt, Germany). From each implant site, 2 central sections were prepared and further reduced to a final thickness of approximately 20µm using a micro - grinding unit (Exakt®, Apparatebau, Norderstedt, Germany). The sections were stained in toluidine blue (Donath, 1993) or Masson - trichrome (Donath, 1993).

Histometric and morphometric analyses

Histometric measurements (EPON sections)

In the present series of experiments 5 sections (studies I, II, III and V) and 3 sections (study IV) from each of the 4 units of every implant were used for the histological examination. Hence, 20 (5 x 4) sections from each implant site were selected to represent the major part of the implant/tissue interface. The histometric examination was performed to assess some vertical dimensions of the peri-implant tissues. The data obtained from each section were collapsed and mean values were calculated for each variable and implant. The following landmarks were identified and used for the linear measurements (Fig. 1):

PM the marginal portion of the peri-implant mucosa.

aJE the level of the apical termination of the junctional epithelium.

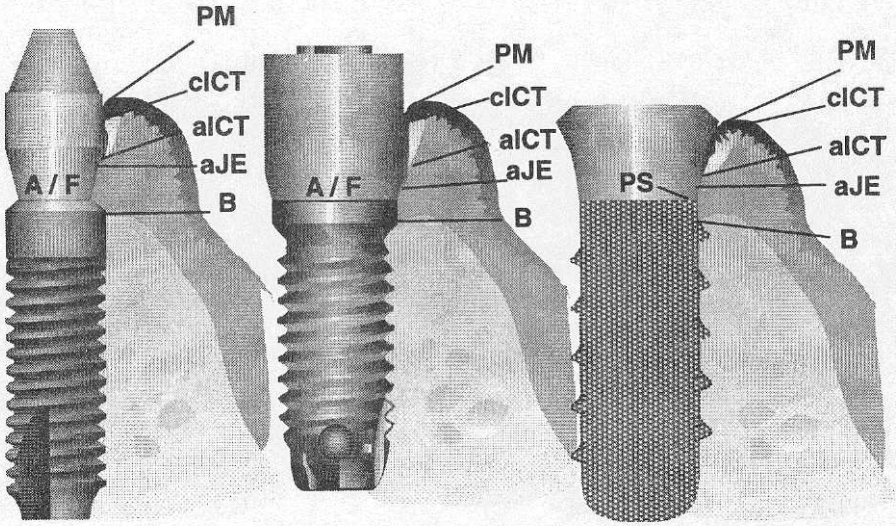
A/F the abutment/fixture borderline (studies I-V) or the interface between the titanium plasma sprayed surface and the polished surface (PS) of the fixture of the "ITI Implants" (studies I and II).

B the marginal level of bone to implant contact.

cICT the coronal level of the infiltrated connective tissue (study II).

aICT the apical extension of the infiltrated connective tissue (study II).

Fig. 1



"Astra Implant" "Brånemark Implant" "ITI Implant"

The following linear measurements were performed:

PM - B	the total thickness of the peri-implant soft tissue (studies I - V)
PM-aJE	the length of the junctional epithelium (studies I - V)
aJE - B	the height of the "zone of connective tissue integration" (studies I - V)
A/F - B	the distance between the abutment/fixture border (or the border between the titanium plasma sprayed and the polished surface of the ITI implants) and the marginal level of bone to implant contact (studies I - V)
PM-A/F	the distance between the marginal position of the peri-implant mucosa and the abutment/fixture borderline (reflecting degree of soft tissue recession) (studies III, IV)
width OE	the width of the oral epithelium (study I)
width JE	the width of the junctional epithelium (study I)
PM - aICT	the apical extension of the plaque associated connective tissue infiltrate (ICT) (study II)
cICT - aICT	the outlined extension of the plaque associated connective tissue infiltrate (ICT) (study II)
area ICT	the area of the plaque associated connective tissue infiltrate (ICT) (study II)

In study I the vertical distance between the different landmarks was assessed in an Olympus® Research Stereo Macroscope connected to a PC (Compaq®) equipped with an image system and a mouse cursor, while in all the other studies (II-V) the linear histometric measurements were carried out in a Leica DM-RBE® microscope (Leica, Germany) equipped with an image system Q-500 MC® (Leica, Germany).

Morphometric measurements

The morphometric measurements of studies I, IV and V included the assessment of the volume fractions of collagen (Co), vessels (V), fibroblasts (Fi), leukocytes (Leu) (only study IV) and residual tissue (R) within the "zone of connective tissue integration". These measurements were in study I confined to a 300 - 600 µm wide zone lateral to the titanium surface, while in study V a zone 0 - 200 µm was used. In study IV the measurements were restricted to an 80 µm wide

zone of the connective tissue lateral to the ceramic and titanium abutments and between aJE and A/F.

In study II the morphometric measurements were used to describe the composition of the inflamed connective tissue. Thus, the volume fractions of the ICT occupied by collagen (Co), vascular structures (V), fibroblasts (Fi), macrophages (Mø), lymphocytes (Ly), plasma cells (Pc), polymorphonuclear cells (PMN) and residual tissue (R) were determined.

The morphometric assessments were performed using a lattice comprising 100 light points at a magnification of x 1000 (Schroeder & Münzel-Pedrazzoli, 1973). The lattice was superimposed over the tissue area and the various structures were identified using a mouse cursor. In study I the point counting procedure was performed in a Leitz microscope equipped with a Microvid® (Leica, Germany) unit connected to a PC (Compaq®) and in study II, IV and V the measurements were performed in a Leica DM-RBE® microscope (Leica, Germany) equipped with an image system Q-500 MC® (Leica, Germany).

Bone tissue analysis

Bone tissue analysis was performed in study I and V. Two different ways of describing the bone quality were used; "Bone density" and "bone-to-implant contact".

Bone density

In study I the bone density assessments were confined to the marginal 3 mm of the peri-implant bone tissue area between the implant threads. The method used was a modification of techniques originally described by e.g. Johansson (1991). The bone tissue area selected for the analysis was outlined and distinguished into lamellar and marrow type and the bone density was calculated and expressed as the area percentage of lamellar bone. The assessments were performed in a Leica DM-RBE® microscope (Leica, Germany) equipped with an image system (Q-500 MC®; Leica, Germany).

In study V ground sections were used for the assessments of bone density, which was defined as the proportion of mineralized bone tissue within a 300 µm wide zone lateral to (i) the coronal unthreaded and (ii) the remaining threaded part of the fixture. This analysis was carried out in a Leica DM-RBE® microscope (x 50) (Leica, Germany) equipped with an image system Q-500 MC® (Leica, Germany) and for the point-counting procedure a lattice comprising 100 light points

(Schroeder & Münzel-Pedrazzoli 1973) was superimposed over the bone tissue area.

Bone-to-implant contact

The degree of bone-to-implant contact was determined in study V. The ground sections were used for these measurements, describing the area percentage of mineralized bone in contact with the titanium surface. The results from the "bone-to-implant contact" assessments were presented in two different ways; (i) one value was given for the coronal unthreaded and (ii) one for the remaining threaded part of the fixture. This analysis was carried out in a Leica DM-RBE® microscope (x 50) (Leica, Germany) equipped with an image system Q-500 MC® (Leica, Germany).

Statistical analyses

Data obtained from each section in the histological analyses representing the mesio-buccal, disto-buccal, mesio-lingual and disto-lingual units and the mesial and distal surfaces in the radiographic examination, were collapsed and mean values were calculated for each variable and implant. The dog was used as the unit in the statistical analysis. Differences between different implant systems (study I and II) or between test and control sites (studies III, IV and V) were analyzed using the Student *t*- test for paired comparisons. The null hypothesis was rejected at $p < 0.05$.

Results

Clinical observations

Healing following fixture installation and subsequent abutment connection was uneventful in this series of investigations. Two fixtures in study V, one in each of two dogs and representing the control implants (submerged) perforated the mucosal lining during the first month after fixture installation. These two control sites as well as their contralateral test implants, were excluded from the analysis. All the remaining implant sites healed uneventfully.

During the dis-/re-connection procedures of study III the abutment part of the test implants was removed and the inner surfaces of the peri-implant mucosa became accessible for clinical examination. The exposed connective tissue exhibited minor signs of inflammation (redness and exudation) at the level of the abutment-fixture junction. No bleeding, however, was observed during any of the dis- and re-connections of the test implants.

In study IV, which included different abutment materials, a marked soft tissue recession, resulting in exposure of the abutment/fixture junction was observed at the mid-buccal and/or mid-lingual aspects at 3 out of 5 of the "gold abutment" sites.

The plaque control program performed in studies I, III, IV and V resulted in clean abutment surfaces. Thus, no clinically visible plaque could be identified and the adjacent peri-implant soft tissues were devoid of clinical signs of inflammation. In study V the overall mean Pl.I. scores at 6 months varied between 0.13 and 0.15 in the test and control groups. The corresponding mean MGI scores varied also within a narrow range (0.14 and 0.20).

The clinical examination performed at the end of the plaque formation period of study II, revealed that large amounts of plaque had accumulated on all implant surfaces. Obvious signs of inflammation, such as redness, swelling and bleeding on gentle probing, were observed in the peri-implant soft tissues adjacent to the implants of the 3 systems.

Gross histological observations

Clinically healthy peri-implant soft tissues

The morphology of the peri-implant soft tissues at implants with different design, different installation technique and with different materials in the abutment part had many features in common. The peri-implant mucosa consistently comprised two units, one epithelial and one connective tissue unit. A well-keratinized oral epithelium was continuous with a junctional epithelium facing the abutment part of the implant. This junctional epithelium, which had a thickness of 5-10 cell layers, extended an average distance of 1.64 - 2.35 mm from the mucosal margin (PM). The connective tissue lateral to the junctional epithelium was comprised of a dense collagenous tissue with few vascular structures and scattered inflammatory cells. The "zone of connective tissue integration" (Berglundh et al. 1991), i.e. the portion of the peri-implant mucosa that was located between the bone crest and the junctional epithelium was characterized as a collagen rich and cell poor, scar like connective tissue. In a narrow tissue portion close to the implant, the majority of the collagen fibers were attached to the periosteum and ran a course parallel to the surface of the implant. The "zone of connective tissue integration" was found to have a vertical dimension varying between 1.0 and 1.5 mm, when formed adjacent to a titanium or ceramic surface. When gold or dental porcelain was used in the abutment part of the implant (study IV) or when the titanium abutment was repeatedly dis- and re-connected (study III), the epithelium migrated "apically" and the "zone of connective tissue integration" was formed to the titanium surface of the fixture. In addition, the height of this connective tissue was smaller than the connective tissue formed adjacent to a control abutment.

Inflamed peri-implant soft tissues

Following 5 months of plaque accumulation (study II) the peri-implant mucosa at the 3 implant systems studied (Astra Tech Implants Dental System®, the Brånemark System® and the ITI Dental Implant System®), harbored an inflammatory cell infiltrate which was located in the marginal portion of the soft tissue and was consistently separated from the implant surface by a pocket epithelium. The pocket epithelium exhibited areas of ulceration, had rete peg formations extending into the cell infiltrate, contained numerous PMN cells and some macrophages and lymphocytes and was in "apical" direction continuous with a comparatively short junctional epithelium terminating about 1-1.5 mm above the bone crest. In this suprabony region a dense connective tissue appeared to be in contact with the implant surface.

In sections representing the Astra and the Brånemark Implants (i.e. the 2-stage systems) a small inflammatory cell infiltrate was observed in the connective tissue lateral to the abutment/fixture junction. The "apico-coronal" dimension of this cell infiltrate was 0.5 mm (Brånemark Implants) and 0.4 mm (Astra Implants) and the size of the area that was occupied by the ICT was 0.08 mm^2 for the Brånemark Implants and 0.05 mm^2 for the Astra Implants. This "abutment-related ICT" was consistently separated from the plaque associated infiltrate in the marginal portion of the mucosa by a zone of normal, non inflamed connective tissue.

Histometric analysis

The marginal peri-implant tissues are schematically illustrated and the various landmarks used for the histometric assessments are described in Fig. 1. The results from the histometric measurements are reported in Table 1 - 6.

Peri-implant tissues at initially submerged implants

The findings demonstrated that the dimensions varied only slightly between the various implants systems (Table 1). The peri-implant mucosa (PM - B) formed at implants installed using a submerged technique and maintained under a meticulous plaque control program had a height which on the average was 3.0 - 3.1 mm for the "Astra Implants" (studies I and V) and varied between 3.3 and 3.4 mm for the "Brånemark Implants" (studies I, III and IV). The epithelial part of the mucosae (PM - aJE) had a length that varied between 1.6 - 1.9 mm and 2.0 - 2.1 mm for the "Astra" and the "Brånemark Implants", respectively, while the corresponding values regarding the height of the "zone of connective tissue integration" was 1.2 - 1.5 mm and 1.3 mm. The marginal level of bone-to-implant contact (B) was located 0.6 - 0.9 mm "apical" of the abutment/fixture borderline (A/F) at the "Astra Implants" (studies I and V) and 0.6 - 0.8 mm at the "Brånemark Implants" (studies I, III and IV). No statistically significant difference was observed between these two implant systems, regarding any of the variables studied (Study I).

Table 1. Results from the histometric measurements at initially submerged implants

	PM-B		PM-aJE		aJE-B		A/F-B	
(mm)	mean	SD	mean	SD	mean	SD	mean	SD
Astra Implants								
Study I	3.11	0.82	1.64	0.28	---	---	0.57	0.44
Study V	3.00	0.39	1.85	0.51	1.16	0.28	0.85	0.32
Brånemark Implants								
Study I	3.42	0.31	2.14	0.34	---	---	0.62	0.12
Studies III, IV	3.32	0.24	2.04	0.22	1.28	0.11	0.78	0.17

The landmarks are described in Fig. 1. Mean values and standard deviations (SD)

Peri-implant tissues at nonsubmerged implants

The "ITI Implants" (1-part system) and some of the "Astra Implants" (2-part system) were, in study I and V, installed using a nonsubmerged installation technique. The total thickness of the peri-implant mucosa (PM - B) at the "Astra Implants" installed with the nonsubmerged technique was 3.2 mm (study V) and for the "ITI Implants" 3.5 mm (study I). The corresponding length of the junctional epithelium was 2.0 mm and 2.4 mm, respectively. Consequently, the thickness of the "zone of connective tissue integration" was 1.2 mm for each of the two implant systems. The marginal level of bone-to-implant contact (B) at the "Astra Implants" was located 0.7 mm "apical" of the abutment/fixture borderline (A/F) and the corresponding location in relation to A/F at the "ITI Implants" was 0.5 mm. No statistically significant differences, regarding any of the variables studied, were observed regarding the "Astra Implants" comparing the outcome of a submerged or a nonsubmerged installation technique (study V) (Table 2). In addition, no differences were found when the implant system, intended for a nonsubmerged technique ("ITI Implants"), was compared with two different implant systems ("Astra Implants" and "Brånemark Implants"), placed using a technique where the fixtures were submerged during the initial phase (study I) (Table 3).

Table 2. Results from the histometric measurements at Astra and ITI implants in studies I and V

	PM-B		PM-aJE		aJE-B		A/F-B	
(mm)	mean	SD	mean	SD	mean	SD	mean	SD
ITI Implants (study I)								
Non-submerged	3.50	0.50	2.35	0.33	---	---	0.50	0.36
Astra Implants (study V)								
Non-submerged	3.15	0.34	1.97	0.52	1.18	0.31	0.68	0.33
Submerged	3.00	0.39	1.85	0.51	1.16	0.28	0.85	0.32

The landmarks are described in Fig. 1. Mean values and standard deviations (SD)

Table 3. Results from the histometric measurements at Astra Brånemark and ITI implants in study I

	PM-B		PM-aJE		A/F-B	
(mm)	mean	SD	mean	SD	mean	SD
ITI Implants	3.50	0.50	2.35	0.33	0.50	0.36
Astra Implants	3.11	0.82	1.64	0.28	0.57	0.44
Brånemark Implants	3.42	0.31	2.14	0.34	0.62	0.12

The landmarks are described in Fig. 1. Mean values and standard deviations (SD)

Peri-implant tissues after plaque accumulation

In study II the tissue response to plaque formation at 3 different implant systems was compared (Table 4). It was found that the height of the peri-implant mucosa did not differ at the Astra, Brånemark and the ITI Implants. Thus, the dimension PM-B varied between 3.03 - 3.15 mm. The length of the pocket/junctional epithelium varied between 1.6 and 2 mm, and the height of the connective tissue portion that established contact to the implant surface varied between 1 and 1.5 mm. The distance between the abutment/fixture junction (or

corresponding level at the ITI implant) and the marginal level of bone to implant contact was 0.64 mm (Astra Implants), 0.64 mm (Brånemark Implants) and 0.67 mm (ITI Implants).

The plaque associated connective tissue infiltrate extended a distance in "apical" direction (PM - aICT) of about 1.6 mm - 2.0 mm and the size of the area occupied by this infiltrate varied between 0.25 mm^2 and 0.39 mm^2. In addition, it was found that the plaque associated infiltrate adjacent to the "Astra Implants" was significantly smaller than the corresponding infiltrates at the "Brånemark Implants" and the "ITI Implants".

Table 4. Results from the histometric measurements in study II.

	Astra implants		**Brånemark implants**		**ITI implants**	
(mm)	mean	SD	mean	SD	mean	SD
PM-B	3.10	0.32	3.15	0.58	3.03	0.42
PM-aJE	1.61	0.51	1.94	0.79	2.02	0.19
PM-aICT	1.56	0.32	1.64	0.29	1.98	0.31
outline ext.ICT	1.58	0.31	1.66	0.34	2.16	0.32
area ICT	0.25	0.11	0.32	0.10	0.39	0.13
A/F-B	0.64	0.44	0.64	0.72	0.67	0.25
aICT / aJE %	99	11	91	22	98	15

The landmarks are described in Fig. 1. Mean values and standard deviations (SD)
* Indicates a statistically significant difference with a P-value < 0.05

Peri-implant tissues at implants with different abutment materials

In study IV the peri-implant tissues adjacent to control abutments (c.p. titanium) were compared with the corresponding tissues adjacent to abutments made of ceramic (highly sintered Al_2O_3), gold or abutments with a short titanium sleeve fitted with a crown made of dental porcelain (Table 5).

Table 5. Results from the histometric measurements in study IV.

	PM-B	PM-aJE	aJE-B	PM-A/F	A/F-B
(mm)	mean SD	mean SD	mean SD	mean SD	mean SD
Control	3.32 0.24	2.04 0.22	1.28 0.11	2.54 0.35	0.78 0.17
Ceramic	3.36 0.26	2.01 0.44	1.34 0.33	2.56 0.22	0.80 0.16
Gold	2.55* 0.28	1.75 0.20	0.79* 0.31	0.75* 0.44	1.80* 0.21
Short titanium	2.99 0.31	1.81 0.36	1.19 0.38	1.73* 0.43	1.26* 0.31

Mean values and standard deviations (SD)
* Indicates statistically significant difference versus Control (titanium). P-value < 0.05.

Control abutment (Titanium) and ceramic abutment

The peri-implant mucosa at the "ceramic abutments" had many features in common with the mucosa of the "control abutment" sites. Thus, the mucosa surrounding the "ceramic abutments" and the "control abutments" had a 2 mm long junctional epithelium and a zone of connective tissue that was about 1.3 mm high. The distance between A/F and B was for both abutment types about 0.8 mm.

Gold abutment

The peri-implant mucosa surrounding the "gold abutments" differed in several aspects from the mucosa at the control sites. Thus, the dimension PM - B at the "gold abutment" sites was significantly smaller than the corresponding dimension at the control sites; 2.6 mm vs. 3.3 mm. The length of the junctional epithelium and the height of the zone of connective tissue were consistently smaller at the "gold abutment" than at the "control abutment" (1.8 mm vs. 2.0 mm and 0.8 mm vs. 1.3 mm respectively). The marginal bone tissue was located a distance of 1.8 mm "apical" of A/F, i.e. about 1 mm further "apical" compared to its location at the "control abutment". This difference was statistically significant. The distance PM - A/F was also significantly smaller at the "gold abutment" (0.8 mm) than at the control sites (2.5 mm).

Short titanium abutment

The "short titanium abutment", which was provided with a crown, made of dental porcelain (fused to gold), was surrounded by a mucosa the height of which was smaller than the corresponding soft tissue at the control sites; 3.0 mm vs. 3.3 mm. The height of the junctional epithelium and the connective tissue interface was similar to the corresponding dimensions at the control sites; 1.8 mm vs. 2.0 mm and 1.2 mm vs. 1.3 mm, respectively. The marginal bone level, was located at a distance of 1.3 mm "apical" of A/F, i.e. about 0.5 mm more "apical" than at the controls. This difference was statistically significant. The distance PM - A/F was at the "short titanium abutment" sites significantly smaller than at the control sites (1.7 mm and 2.5 mm, respectively).

Peri-implant tissues after repeated dis- and re- connection of the abutment

In study III the mucosal barrier that was allowed to heal undisturbed for 6 months at implants (control) were compared with the peri-implant tissues at implants where the abutment were dis- and re-connected once a month during 6 months (test) (Table 6).

Control sites:

The dimensions of the epithelial and connective tissue components were 2.0 mm and 1.3 mm, respectively. The most marginal position of bone to implant contact was located a distance of 0.8 mm "apical" of the abutment-fixture junction.

Test sites:

The height of the peri-implant mucosa of the test sites was smaller than the corresponding dimension of the control sites; 2.5 mm vs. 3.3 mm. Also the length of the junctional epithelium and the connective tissue was consistently smaller at the test sites than at the controls, i.e. 1.7 mm and 0.9 vs. 2.0 mm and 1.3 mm, respectively.
The marginal level of the bone was located about 1.5 mm from the abutment/fixture-junction, i.e. about 0.7 mm further "apically" than at the control sites. This difference between test and control sites was statistically significant. The distance between the mucosal margin and the abutment/fixture junction was for the control sites and the test sites 2.5 mm and 1.0 mm respectively. This difference was statistically significant and indicated that a soft tissue recession of about 1.5 mm had occurred at the test sites. Hence, the junctional epithelium of the test sites consistently terminated at a position "apical" to the abutment / fixture junction and the "zone of connective tissue integration" was established to the marginal portion of the fixture.

Table 6. Results from the histometric measurements in study III.

	PM-B	PM-aJE	aJE-B	A/F-B	PM-A/F
(mm)	mean S.D.	mean S.D.	mean S.D.	mean S.D.	mean S.D.
Test	2.50 ± 0.42	1.65 ± 0.24	0.85 ± 0.26	1.49 ± 0.19 *	1.02 ± 0.54 *
Control	3.32 ± 0.24	2.04 ± 0.22	1.28 ± 0.11	0.78 ± 0.17	2.54 ± 0.35

Mean values and standard deviation (S.D.). * indicates $p < 0.05$.

Morphometric analysis

Zone of connective tissue integration

The composition of the connective tissue lateral to the implant surface in a zone between aJE and B was found to be similar irrespective of installation technique used and/or design of the implant system. Thus, in study I, IV and V, the collagen fraction of the connective tissue was consistently found to be high and varied between 80.0 and 86.8 %. The volume fraction of vessels and residual tissue was found to be small, 2.4 - 4.3 % and 2.2 - 3.9 %, respectively, while the percentage of fibroblasts varied between 7.2 % and 13.4 %.

Infiltrated connective tissue

In study II, the morphometric measurements were used to describe the composition of the inflamed connective tissue. The overall composition of the ICT did not differ between the 3 implant systems. The volume fraction of the ICT occupied by collagen and vascular structures varied between 22.3 - 26.6 % and 23.2 - 30.6 % respectively. The density of fibroblasts was between 10.0 - 13.7 % of the ICT and the corresponding proportion for the inflammatory cells was 25.0 - 29.4 %, amongst which plasma cells and lymphocytes dominated.

Bone tissue analysis

Bone density

In study I, the bone density between the threads of the implants within the marginal 3 mm of the peri-implant bone was 85.4% at the Astra Implants, 87.4% at the Brånemark Implants and 88.4% at the ITI Implants. No statistically significant differences were found between the 3 implant systems.

In study V, the proportion of mineralized bone (bone density) in a 300 µm wide zone adjacent to the fixture was in the marginal unthreaded portion 84.6 % in the test and 81.9 % in the control group. The corresponding values for the remaining threaded part of the fixtures were 45.8 % (test group) and 48.9 % (control group).

Bone-to-implant contact

The percentage of bone to implant contact in study V was within the unthreaded marginal portion of the fixtures 75.0 % for the test (nonsubmerged) implants and 72.6 % for the controls (initially submerged). The corresponding figures for the threaded part the fixtures were 61.4 % (test) and 66.7 % (control).

Radiographic analysis

The marginal level of bone to implant contact (B; Fig. 2) in the radiographs obtained immediately after fixture installation in study V coincided with the most marginal portion of the cylindrical part of the fixture, in other words B was in both groups positioned at a distance about 0.3 mm below the fixture margin (A/F; Fig. 2). In the control group, the radiographic bone level (B) was found to slightly decrease (0.23 mm) between Day 0 and 3 months (abutment connection). A minor additional apical displacement of the bone level (0.19 mm) occurred in the control group in the interval between 3 months and 9 months. The corresponding (3 months - 9 months) alteration in the test group was 0.30 mm.

Fig. 2

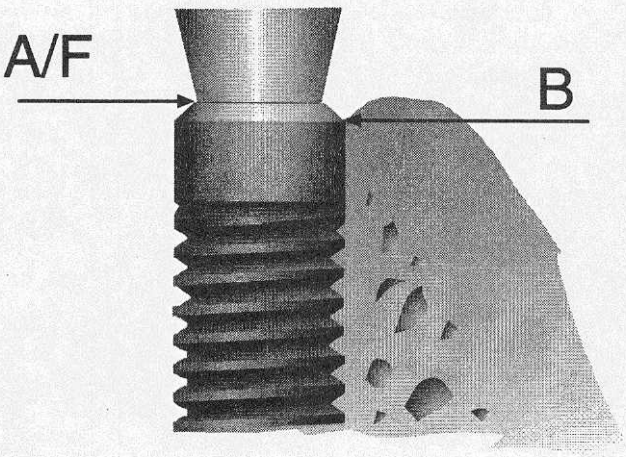


Fig. 2. Schematic drawing illustrating the marginal level of bone to implant contact (B) at fixture installation. Note that B is located 0.3 mm below the fixture margin (A/F).

Main Findings

The present series of investigations demonstrated that:

* the peri-implant tissues that formed to the titanium surface of different implant systems or with different installation techniques did not differ with respect to, structure and composition

* 5 months of undisturbed plaque formation at different implant systems, resulted in the establishment of inflammatory infiltrates (ICT) in the marginal portions of the periimplant mucosae. In all 3 systems tested, the plaque induced lesions resided in the connective tissue lateral to the barrier epithelium.

* repeated abutment removal and subsequent reconnection resulted in marginal bone resorption and soft tissue recession. The apical portion of the barrier epithelium (aJE) consistently terminated "apical" of the abutment/fixture border (A/F) and the "zone of connective tissue integration" was established to the marginal portion of the fixture.

* the material used in the abutment portion of the implant influenced the location and the quality of the attachment that occurred between the mucosa and the implant. Abutments made of c.p. titanium or aluminum based ceramic allowed the formation of a mucosal attachment which included one epithelial and one connective tissue portion that had a dimension of about 2 mm and 1- 1.5 mm, respectively. At sites where abutments made of gold alloy or dental porcelain were used, no proper attachment formed at the abutment level, but the mucosal barrier became established at the fixture portion of the implant.

Concluding remarks

The main objective of the present series of investigations was to study some characteristics of the peri-implant tissues using the beagle dog model. This model is well known, well-documented and valuable in the study of biological and pathological features of periodontal tissues (e.g. Egelberg 1967, Attström 1971, Lindhe et al. 1975, Berglundh et al. 1989, 1990, 1992 Berglundh & Lindhe 1993) as well as peri-implant tissues (Berglundh et al. 1991, 1994, Buser et al. 1992).

The findings from the current studies confirm data from previous animal experiments showing that the transmucosal barrier at implants is comprised of one epithelial and one connective tissue component, the height of which measures about 2 mm and 1 - 1.5 mm, respectively (Berglundh et al. 1991, Buser et al. 1992, Cochran et al. 1997). It has been suggested that these dimensions represent a biological width (Berglundh & Lindhe 1996, Cochran et al. 1997) of the mucosal attachment to dental implants.

In the following, some characteristics of the peri-implant tissues will be discussed in relation to different implants, installation techniques and accumulation of plaque.

Implant design

In a study in dogs, Hermann et al. (1997) evaluated, in radiographs, the influence of the presence of a microgap between implant components on the peri-implant hard tissues. The authors used six different types of one-part and two-part implants, of which the latter included a microgap between the two parts. The authors concluded that "the bone loss was not dependent on whether the implant was placed using a nonsubmerged or a submerged technique; rather, the bone loss was dependent on the microgap". They also stated that approximately 2 mm of bone loss was observed below a microgap, a loss that was "necessary to re-establish a biologic width with epithelium migrating below the microgap". The data presented by Hermann et al. (1997) also indicated that the position of the rough/smooth surface interface of one part implants influenced the marginal bone level. The above findings, are in several aspects not consistent with observations made in the present experiments. Thus, in studies I and V, it was observed that (i) installation technique, (ii) surface texture or (iii) presence of a "microgap", did not significantly influence the location of the marginal bone level. The 2 mm of bone loss that occurred below a "microgap", according to Hermann et al. (1997) was not observed in the present series of experiments. On the contrary, the marginal bone loss was small and varied only between 0.57 mm and 0.85 mm. This minor bone loss was also noticed by Weber et al

(1996) who, in an experiment in beagle dogs, found minor bone level alterations to occur around submerged (2-part) and non-submerged (1-part) implants. In this context, it should be emphasized that the size of the "microgap" in the study by Hermann et al. (1997) was about 50 µm, whereas the width of the "microgap" in commercially available implants is normally considered to be less than 10 µm (Weber et al. 1996, Jansen et al. 1997).

In the present studies the proportion of collagen and vascular structures, within the "zone of connective tissue integration" of the peri-implant mucosa, varied between 80 and 87% and between 2 and 4%, respectively. These data are consistent with findings reported by Berglundh et al. (1991), who studied the peri-implant mucosa at implants of the Brånemark System® in the Beagle dog model. They also stated that a dense network of collagen fibers extended from the alveolar bone crest to the mucosal margin and ran a course mainly parallel to the implant surface. This description was confirmed by Buser et al. (1992), who reported on the presence of (i) an inner zone (50-100 µm) of poorly vascularized connective tissue in the peri-implant mucosa and that this zone contained circularly oriented collagen fibers and (ii) an outer zone characterized by vertical fibers, running from the periosteum towards the oral epithelium.

Non-submerged and submerged installation techniques

Gotfredsen et al. (1991) in an experiment in monkeys, found that the "bone-to-implant contact length fractions" after 22 weeks of healing was similar at non-submerged and permanently submerged implants. This finding is supported by observations made in studies I (ITI implants vs. Brånemark and Astra implants) and V (1-stage vs. 2-stage Astra implants) of the present series. Levy et al. (1996), however, in a study on the initial healing (6 weeks) at non-submerged and permanently submerged porous-coated endosseous dental implants, reported on a greater "bone-to-implant contact length fraction" for the submerged implants. It should be emphasized, however that in the current studies, the non-submerged and the initially submerged implants were allowed to heal for a considerably longer time (6 months and 9 months) than the 6 weeks in the study by Levy et al. (1996).

In Study V, the hard and soft tissues that formed around an original 2-part implant system (Astra Tech Implants Dental System®) installed using either a non-submerged or an initially submerged procedure were analyzed. The findings disclosed that (i) the length of the junctional epithelium, (ii) the height and quality of the "zone of connective tissue integration", (iii) the percentage of bone to implant contact and (iv) the density of the peri-implant bone were similar in the non-submerged

and the submerged groups. These observations are, to some extent, in agreement with findings presented by Weber et al. (1996). They examined the peri-implant tissues around non-submerged and initially submerged implants in 6 beagle dogs and stated that the two techniques yielded similar results both with respect to the overall dimension of the peri-implant mucosa and the marginal level of bone to implant contact. It was also reported, however, that the junctional epithelium extended more apically in the initially submerged (1.71 ± 0.13 mm) than in the non-submerged (1.18 ± 0.27 mm) implant group. This observation is not in agreement with data from study V in which the junctional epithelium in both the non-submerged and submerged groups was about 2 mm long. This difference between the two studies may simply be related to design of the experiments. Thus, in the study by Weber et al. (1996) soft tissue healing was allowed for 4.5 months for the nonsubmerged and 6 weeks for the submerged implants. In study V, both groups of implants healed for 6 months. Additionally, while the carefully performed plaque control program in study V maintained a healthy mucosa in both groups of implants, Weber et al. (1996) reported on the presence of mucositis at the initially submerged implants. On the other hand, the observations regarding the peri-implant mucosa, made in study V, corroborate findings reported from a dog experiment by Ericsson et al. (1996), who concluded that implants installed according to a "1-step or 2-step surgical procedure" obtained matching soft tissue adaptation, i.e. an epithelial length of 2.4 mm and connective tissue dimension of 1.5 mm for the non-submerged and 2.1 mm and 1.4 mm for the submerged implants.

Ericsson et al. (1996) also performed measurements in radiographs and reported that the bone loss amounted to 2.6 mm at the non-submerged implants and 2.1 mm at the initially submerged implants. The corresponding bone alteration in study V was considerably smaller; 0.40 and 0.34 mm for the 2 groups. The reason for this difference between the radiographic data reported by Ericsson et al. (1996) and the results in study V is presently not understood.

Plaque accumulation

In study II the soft tissue response to plaque formation was studied at 3 different implant systems. 5 months of plaque formation resulted in clinically marked signs of mucositis. In the histological analysis, it was observed that the epithelium facing the implant had the character of a pocket epithelium with rete peg formations extending into the underlying connective tissue. The pocket epithelium was in its "apical" part continuous with a short junctional epithelium, which terminated about 1-1.5 mm above the bone crest. Lateral to this pocket epithelium a cell rich, collagen poor infiltrate was consistently found. This infiltrated connective tissue (ICT) had a vertical extension which for the 3 implant systems varied between 1.6 mm (Astra and Brånemark

Implants) and about 2.0 mm (ITI Implants). The plaque associated ICT in the periimplant mucosae in all 3 systems did not involve the connective tissue portion of the mucosal/titanium barrier. Thus, the vertical extension of the ICT was in all systems within 91 - 99 % of the dimension of the barrier epithelium. These observations corroborate findings reported in previous animal studies (Berglundh et al. 1992, Ericsson et al. 1992, 1995).

In study II the size of the area of the ICT, differed somewhat between the 3 implant systems. Thus, the ICT in the peri-implant mucosa at the Astra Implants was significantly smaller than those in the mucosae at the Brånemark and ITI Implants (0.25 mm^2, 0.32 mm^2 and 0.39 mm^2, respectively). The significance of this difference in ICT size is presently not understood but may simply be related to the design of the transmucosal part of the implants.

Break-up of "the zone of connective tissue integration"

During the formation of the implant-mucosal barrier, an interaction probably occurs between the connective tissue and the titanium dioxide of the implant surface. A "zone of connective tissue integration" is established which (i) is not recognized as a wound and may therefore prevent further epithelial migration (Berglundh et al. 1991), (ii) may serve as an important biological barrier and (iii) if severed may allow undue change of the marginal portion of the peri-implant bone (Berglundh et al. 1991, Berglundh & Lindhe, 1996). In order to further test this hypothesis, the effect of repeated mechanical disruption of the mucosal barrier was analyzed in study III. The findings indicated that the repeated dis- and subsequent reconnections of the abutment component compromised the mucosal barrier. This resulted in an apical shift of the epithelium. In order to reestablish a connective tissue barrier of proper dimensions, bone resorption (0.7 mm) obviously occurred and the "zone of connective tissue integration" formed at the fixture part of the implants.

Abutment material

The results of study V demonstrated that abutments made of c.p. titanium (control) or a ceramic material allowed the formation of a mucosal attachment which included one epithelial and one connective tissue portion that were about 2 mm and 1- 1.5 mm high. The observation that the "ceramic" (Al_2O_3) abutment established healing conditions similar to those at the titanium abutment corroborates data reported from animal experiments and clinical trials using so called single crystal sapphire implants (McKinney et al. 1985, Hashimoto et al. 1988, 1989, Akagawa et al. 1989, Fartash et al. 1990, Arvidsson et al. 1991, 1996). Furthermore, results from histological analyses of

human and animal biopsy material (McKinney et al. 1985, Hashimoto et al. 1989, Fartash et al. 1990, Arvidsson et al. 1996) demonstrated the presence of ultrastructural features of epithelial attachment to the Al_2O_3 surface.

In this context it must be realized that the bio-adhesive properties of ceramics and ceramic like materials (e.g. titanium) are good. It is commonly accepted that ceramics and titanium dioxide have a high corrosion resistance (Handbook of Chemistry and Physics). Thus, the surface layers of titanium and ceramics are regarded to be chemically stable and may therefore allow cells to grow in contact with the surface.

The finding that the gold alloy abutments failed to properly interact with the connective tissue is in agreement with results by Thomsen et al. (1997). They studied, in rabbits, the interface between cortical bone and implants made of gold, zirconium and titanium and found that the amount of bone formed within the threads of the fixtures as well as the degree of bone to implant contact was smaller at gold than at titanium and zirconium implants. Thomsen et al. (1997) further reported that areas of connective tissue including multinuclear cells and macrophages were observed more frequently at gold implants than at implants made of titanium or zirconium. A prerequisite for cells to grow in contact with a surface is that the surface is chemically stable, i.e. has a high resistance to corrosion. It is commonly accepted that gold alloys are less resistant to corrosion than ceramics and titanium dioxide (Handbook of Chemistry and Physics). Gold resists oxidation in air but resists oxidation much less in sea water and biological fluids and has a resistance to corrosion that is about 100 times lower than titanium (Steinemann 1998).

The validity of the biological width concept (Berglundh & Lindhe 1996) was supported by findings made at the "short titanium abutment" sites in study IV. At such abutments, the available height of titanium was only 1 mm, and hence, insufficient for a proper mucosal attachment. During healing following connection of the "short titanium abutments" (i) marginal bone resorption (about 1.3 mm compared to 0.8 mm at the control sites) occurred, (ii) a corresponding portion of the fixture was exposed and (iii) the mucosal attachment became established to a surface of titanium that included the abutment and a marginal part of the fixture.

The concept that a minimum amount of soft tissue attachment must exist to prevent a compensatory bone resorption was argued by Kastenbaum et al. (1998). They evaluated radiographically the alterations of the marginal bone level at 200 Brånemark implants provided with Estheti-Cone™ abutments. The authors reported a marginal bone resorption of about 0.9 mm, calculated as the difference

of the bone level between baseline (i.e. abutment connection) and the 3-year follow up examination. Kastenbaum et al (1998) stated that "the 1 mm transgingival abutment height did not cause any increased marginal bone resorption" as compared with other studies of the Brånemark System® components. Despite the fact that the study by Kastenbaum et al (1998) did not include any controls, an additional explanation regarding bone resorption was given by the authors themselves by stating that "the fixtures are not always countersunk which leaves the fixture collar height of 0.8 mm in the soft tissue, giving a total of at least 1.8 mm". In other words, the 1.8 mm of mucosal attachment to the fixture surface reported by Kastenbaum et al (1998) will match the 1.3 mm presented at the short titanium abutments of study IV.

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