Soft tissue integration to dental implants

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Abstract

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Soft tissue integration is a prerequisite for implant success. The role of the soft tissue barrier at implants is to provide an effective seal that protects the underlying bone and prevents access for microorganisms and their products.
The objectives of the present series of experimental studies were to examine the morphogenesis of the mucosal attachment to titanium implants (study 1) and healing to titanium implants coated with type I collagen (study2) and to implant abutments made of different materials (study 3). Healing around two-part implants placed in a subcrestal position (study 4) and in sites with buccal bone defects (study 5) was also studied.
The dog model was used in all experiments. Following extraction of premolars implants that represented different implant systems were placed in the edentulous premolar regions. After varying periods of healing block biopsies were collected and prepared for histological examination.

It was demonstrated that the formation of a barrier epithelium was initiated after 1-2 weeks of healing and completed at 6-8 weeks after surgery. The collagen fibers in the connective tissue became organized after 4-6 weeks of healing. The findings indicated that the overall dimension of the soft tissue interface to titanium, i.e. “biological width” was established after 6 weeks following surgery (study 1).
Similar soft tissue dimensions and composition of the connective tissue were found at collagen coated and un-coated titanium implants after 4 and 8 weeks of healing (study 2).
Abutments made of titanium and zirconia promoted proper conditions for soft tissue integration, while abutments made of gold-alloy failed to establish appropriate soft tissue integration (study 3)
Bone formation coronal of the junction between the implant and the abutment was possible when 2-part implants with sufficient surface characteristics were placed in a subcrestal position. The connective tissue interface to abutments with a TiOblast surface was comprised of a higher density of collagen and a lower fraction of fibroblasts than at abutments with a turned surface (Study 4).
Different marginal bone levels at the lingual and buccal aspects were obtained when 2-part implants with suitable surface characteristics were placed in sites with buccal bone defects (Study 5).

Key words: connective tissue, dental implants, epithelium, gold alloy, histology, peri-implant mucosa, subcrestal placement, titanium, zirconia

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To my family with love ♥

Contents

Preface

The present thesis is based on the following experimental studies, which will be referred to in the text by their numbers.

Study 1. Berglundh, T., Abrahamsson, I., Welander, M., Lang, N.P., Lindhe, J. (2007) Morphogenesis of the peri-implant mucosa: an experimental study in dogs. Clin Oral Impl Res 18:1-8.

Study 2. Welander, M., Abrahamsson, I., Linder, E., Liljenberg, B., Berglundh, T. (2007) Soft tissue healing at titanium implants coated with type I collagen. An experimental study in dogs. J Clin Periodontol 34:452-458.

Study 3. Welander, M., Abrahamsson, I., Berglundh, T. (2008) The mucosal barrier at implant abutments of different materials. Clin Oral Impl Res 19:635- 641.

Study 4. Welander, M., Abrahamsson, I., Berglundh, T. (2008) Subcrestal placement of two-part implants. Clin Oral Impl Res In press.

Study 5. Welander, M., Abrahamsson, I., Berglundh, T. (2008) Placement of two-part implants in sites with buccal bone defects. J Periodontol Submitted.

Introduction

Soft and hard tissue integration is a prerequisite for implant success. The primary function of a soft tissue barrier at implants is to effectively protect the underlying bone and prevent access for microorganisms and their products. A soft tissue seal, with structures similar to that at teeth with a true connective tissue attachment to the implant may improve this protective function. This thesis will focus on different aspects on soft tissue integration to implants.

The literature related to the peri-implant mucosa referred to in the introduction part of this thesis is also presented in Table 1 (Animal experiments), Table 2 (Human biopsy materials) and Table 3 (Clinical abutment /implant material studies).

Soft tissue at teeth

The gingiva is composed of two structurally different epithelia (junctional epithelium and oral epithelium) and the lamina propria. Stereological analysis of clinically healthy gingival units revealed that the tissue consists of 4% junctional epithelium, 27% oral epithelium and 69% connective tissue that includes a small inflammatory cell infiltrate occupying about 3-6% of the gingival volume (Schroeder et al. 1973).

The oral epithelium is a keratinized, stratified squamous epithelium. The junctional epithelium, which is structurally different, is formed from the reduced enamel epithelium during tooth eruption and from dividing basal cells of the oral epithelium. The junctional epithelium forms a collar around the tooth and is about 2 mm high and 100μm thick and is comprised of only two cell layers (a basal layer and a supra basal layer). The inner cells of the junctional epithelium form and maintain a tight seal against the tooth surface, which is called the

epithelial attachment apparatus (Schroeder & Listgarten 1997). This attachment consists of hemidesmosomes at the plasma membrane of the DAT cells (directly attached to the tooth cells) and a basal lamina-like extra cellular matrix (Salonen et al. 1989). Several protective functions with antimicrobial properties exist in the junctional epithelium: (i) the internal and external basal laminas act as barriers against infective agents, (ii) bacterial colonization on the outer epithelial surface is inhibited through rapid cell division and exfoliation, (iii) wide intercellular spaces provide a pathway for GCF (gingival crevicular fluid) and transmigrating leukocytes (Löe & Karring 1969, Schiott & Löe 1970).

The gingival lamina propria consists of about 60% collagen fibers, 5% fibroblasts and 35% vessels and nerves (Schroeder & Listgarten 1997). Most of the collagen fiber bundles are arranged in distinct directions and are classified as circular, dento-gingival, dento-periostal and trans-septal fiber groups (Feneis 1952, Page et al. 1974). This supra gingival fiber apparatus not only attaches the gingiva to the root cementum and to the alveolar bone but also provides the rigidity and resistance of the gingiva. The collagen fibers are mainly of collagen type I and III. Type I collagen is the dominating type and is found in dense fibers whereas type III collagen is detected in subepithelial and perivascular compartments. Fibroblasts are the dominating cell in the connective tissue and produce fibers and matrix. Mastcells, which are regularly present in the connective tissue, produce matrix components and vasoactive substances. Inflammatory cells, such as macrophages, polymorphonuclear cells, lymphocytes and plasma cells are also present in the connective tissue but vary in numbers depending on the need for and degree of protective activity (Schroeder & Listgarten 1997). The gingival lamina propria is highly vascularized and the terminal blood vessels form 2 networks; the subepithelial plexus under the oral epithelium and the dentogingival plexus along the junctional epithelium (Egelberg 1966).

Wound healing

A normal wound healing process is an organized and predictable process involving 3 overlapping phases: inflammation, proliferation and maturation/remodeling. The inflammatory phase allows the body to control bleeding and bacterial invasion and, additionally, to recruit the cells that are needed to restore the injured area. During the proliferative phase new tissue components are produced to fill the void caused by the tissue damage. This phase is completed when the barrier has been restored. During the maturation phase type III collagen fibers in the granulation tissue are gradually replaced by type I collagen fibers. The remodeling of the tissues continuous up to 2 years after injury but the greatest changes occur between 6 and 12 months (Myers 2004).

Wound healing at the dento-gingival junction

Repair of the gingival tissue after surgery was studied early in humans and it was observed that after the removal of the free marginal gingiva (gingivectomy) the epithelium covering the wound was very short after 6-16 days of healing. After 3 months of healing, however, the gingiva was 2.5mm wide (Bernier et al. 1947). Waerhaug (1955) reported in a study that the zero pocket depth after gingivectomy was not maintained for any length of time. It was stated “some unknown growth stimulant seems to determine that the gum must cover the tooth to a certain width coronally to the outer periodontal fibers”. Results from studies on wound healing in the dento-gingival junction area indicate that new structures with similar histologic characteristics as the pristine junctional epithelium develop from the phenotypically different oral epithelium. An intact underlying connective tissue is believed to control the migration of the cells of the newly formed junctional epithelium (Ten Cate et al. 2003). In this context it

is interesting to note that the trauma elicited during implant surgery starts a wound healing process following the adaptation of soft tissues around the implants. The aim of the **first study** of the present series was to describe the sequence in wound healing in the soft tissue after implant surgery.

Soft tissue at implants

The soft tissue that surrounds dental implants is termed peri-implant mucosa and the interface portion between the implant and the mucosa is comprised of one epithelial and one connective tissue component. The epithelial part is called barrier epithelium and resembles the junctional epithelium around teeth (James & Schultz 1974, Hansson et al. 1983, Gould et al. 1984, McKinney et al. 1985, Hashimoto et al. 1989, Arvidsson et al. 1990, Fartash et al. 1990, Mackenzi et al. 1995, Fujii et al. 1998, Kawahara et al. 1998, Marchetti et al. 2002, Glauser et al. 2005, Nevins et al. 2008, Rossie et al. 2008). It was reported that a basal lamina and hemidesmosomes occurred 2 weeks after implant placement of epoxy resin implants (Listgarten & Lai 1975) and that hemidesmosomes were formed to vitallium implants after 2-3 days of healing (Swope & James 1981). There are studies, however, that report structural and phenotype dissimilarities between the junctional epithelium at teeth and the barrier epithelium at implants (Innoue et al. 1997, Carmichael et al. 1991, Ikeda et al. 2000, Fujiseki et al. 2003).

The composition of the connective tissue interface towards implants was studied in both animal experiments and human biopsy material. Inflammatory infiltrates were frequently found in specimens prepared from human biopsies (Adell et al. 1986, Lekholm et al. 1986, Liljenberg et al. 1996), which indicated the function of an immune response (Seymour et al. 1989). Functional similarities regarding antigen presentation and density of leukocytes were found between the gingiva and peri-implant mucosa (Tonetti et al. 1993, 1995). Collagen type I was the

main constituent part of the supracrestal connective tissue of the peri-implant mucosa in human biopsies (Chavrier et al. 1999). Furthermore, gingiva and peri-implant mucosa showed similar distribution of collagen type I, III, IV, VII and fibronectin, whereas collagen type V was localized in higher amounts in peri-implant tissues. Collagen type VI was only detected in periodontal tissues (Romanos et al.1995). Dental implants lack root cementum and collagen fiber bundles in the peri-implant mucosa were mostly found to be aligned in a parallel direction with the implant surface (Hashimoto et al. 1988, van Drie et al. 1988, Berglundh et al. 1991, Listgarten et al. 1992, Chavrier et al. 1994, Comut et al. 2001, Schierano et al. 2003, Tenenbaum et al. 2003, Glauser et al. 2005, Schüpbach & Glauser 2007). In other animal experiments and studies on human biopsy material collagen fiber bundles were found to be functionally orientated and running in different directions (Schroeder et al. 1981, Arvidsson et al. 1990, Fartash et al. 1990, Nevins et al. 2008). Circular collagen fibers in the periimplant mucosa have also been demonstrated (Akagawa et al. 1989, Buser et al. 1992, Ruggeri et al. 1992, Fujii et al. 1998, Schierano et al. 2002, Schüpbach & Glauser 2007). Some studies have even suggested the presence of perpendicularly attached collagen fibers to dental implants (Buser et al. 1989, Piatelli et al. 1997, Choi et al. 2000, Schwartz et al. 2007a,b). The diameter of collagen fibrils in the peri-implant mucosa was found to be similar to that of the fibrils in the gingiva (Ruggeri et al. 1994). The connective tissue zone close to the implant surface was suggested to resemble a scar tissue that was poor in vascular structures (Buser et al. 1992, Berglundh et al. 1994, Schüpbach & Glauser 2007). In a study using stereological techniques on sections prepared for transmission electron microscopy (TEM) Moon et al. (1999) reported that the 40μm wide interface zone contained a higher density of fibroblasts and lower volume of collagen than an adjacent lateral 160μm wide zone.

Biological dimension

The dimension of the peri-implant mucosa was demonstrated to resemble that of the gingiva at teeth and included a 2 mm long epithelial portion and a connective tissue portion about 1-1.5 mm long (Berglundh et al. 1991, 1994). The entire contact length between the implant, the epithelial and the connective tissue portions is defined as "the biological width". Experimental studies have demonstrated that a minimum width of the peri-implant mucosa was required. If the thickness of the peri-implant mucosa was reduced bone resorption occurred to reestablish the mucosal dimension that was required for protection of the underlying tissues (Berglundh & Lindhe 1996). This physiological dimension was similar in loaded and unloaded conditions (Cochran et al. 1997, Hermann et al. 2000 a). Neither was the soft tissue of the peri-implant mucosa influenced of immediate functional loading or a posterior position in the mandible arch (Siar et al. 2003). In an experimental study it was reported that differently designed implants with an apically sintered porous-surface and a coronally smooth collar of varying length (0.75 or 1.8mm) demonstrated similar soft tissue dimension (Deporter et al. 2008). Furthermore, when different two-part implant systems were compared similar soft tissue dimensions were exhibited (Watzak et al. 2006). Implant systems that consisted of either one-part or two-part implants were found to exhibit similar soft tissue dimensions (Abrahamsson et al. 1996). In other studies it was suggested that the one-part implants had shorter soft tissue dimensions than the two-part implants (Hermann et al. 2001a).

Healing after different surgical procedures was also evaluated. It was reported that similar soft tissue dimensions were established using a submerged or a non-submerged installation technique (Ericsson et al. 1996, Weber et al. 1996, Abrahamsson et al. 1999, Kohal et al. 1999) but a longer epithelial attachment was reported for the submerged installation technique (Weber et al. 1996).

Surface modification of titanium implants

Polishing, particle blasting, etching, and anodization represent different surface modifications of titanium implants. In an experimental study it was reported that the soft tissue dimensions were similar at implant abutments with either a polished smooth surface or a thermal dual acid etched surface (Abrahamsson et al. 2002), furthermore, different surface roughness failed to influence plaque accumulation in both experimental and clinical studies (Bollen et al. 1995, Zitzmann et al. 2002 and Wennerberg et al. 2003). It was reported in a study with human biopsies that the soft tissue formed to oxidized and acid etched mini implants exhibited shorter epithelial and longer connective tissue dimensions compared to the tissues around turned mini implants (Glauser et al. 2005).
Soft tissue healing to Calcium Phosphate coatings was also analyzed. In a study in dogs it was observed that epithelium and supra alveolar collagen fibers formed around dense calcium hydroxyapatite titanium implants (Kurashina et al. 1984). Parallel collagen fiber bundles were demonstrated around hydroxyapatite coated implants (Comut et al. 2001). No difference in soft tissue dimensions was found for submerged and non-submerged hydroxyapatite implants (Kohal et al. 1999). Analysis of autopsy materials showed parallel and perpendicular collagen fiber bundles to plasma sprayed titanium implants (Piatelli et al. 1997).
Titanium implants with a sol-gel derived nanoporous TiO_2 film was compared to turned titanium implants. The soft tissue of the surface treated implants was analyzed in a transmission electron microscope (TEM) and hemidesmosomes of the cells in the junctional epithelium facing the surface were observed. A shorter distance between the implant margin and the bone crest was demonstrated for the surface treated implants compared to the turned implants (Rossie et al. 2008). The use of hydroxyapatite and other coatings on titanium implants was intended to promote soft tissue formation with structures resembling the soft tissue attachment to teeth. The aim of the **second study** was to analyze the soft tissue healing at titanium implants coated with type I collagen.

Abutment materials

The traditional abutment material of dental implants was commercially pure titanium due to its well-documented biocompatibility and mechanical properties (Adell et al. 1981). Esthetic awareness in implant dentistry, however, demands the development and use of other materials than titanium in the abutment part of the implant. Soft tissue formed to implants made of alumina (Al_2O_3) and single-crystal sapphire demonstrated structures such as basal lamina, hemidesmosomes and a connective tissue with collagen fibers that were mainly oriented parallel to the implant surface (McKinney et al. 1985, Hashimoto et al. 1988, 1989, Fartash et al. 1990). Soft tissue biopsy analysis in light microscope and transmission electron microscope revealed no differences between single-crystal sapphire implants and titanium implants regarding the organization of the epithelium, the arrangement of collagen fibers, nerves and vessels and different connective tissue cells (Arvidsson et al. 1996). Cast metal alloys have extensively been used in prosthetic dentistry due to mechanical and biocompatible properties. A cast metal is easy to handle and may consequently be considered as an abutment material (Tan & Dunne 2004). In an animal study Abrahamsson et al. (1998 a) analyzed soft tissue healing to abutments made of titanium, gold-alloy, dental porcelain and Al_2O_3 ceramic. It was demonstrated that gold alloy and dental porcelain failed to establish a soft tissue attachment while abutments made of titanium and ceramic formed an attachment with similar dimensions and tissue structures. In a subsequent animal experiment, however, it was reported that the peri-implant soft tissue dimensions were not influenced if titanium or gold alloy was used in the marginal zone of the implant (Abrahamsson & Cardaropoli 2007). Different types of ceramic were also evaluated. Abutments made of zirconia (ZrO_2) showed better mechanical properties than ceramic abutments made of alumina (Al_2O_3) (Yildirim et al. 2003) and results from microbial sampling studies revealed less bacteria and plaque accumulation on zirconia

discs than on titanium discs (Rimondini et al. 2002, Scarano et al. 2004). In an animal model loaded custom-made zirconia and titanium implants demonstrated similar soft tissue dimensions (Kohal et al. 2004). Soft tissue biopsies that surrounded titanium and zirconia healing caps were analyzed and it was demonstrated that the zirconia healing caps presented a lower inflammatory level in the tissues than that at titanium healing caps (Degidi et al. 2006).
Studies utilizing clinical parameters and radiographs to compare different abutment materials were also performed. Transmucosal collars of titanium and dental ceramics were compared in a clinical study and no differences were found in soft tissue response (Barclay et al. 1996). In clinical studies titanium and ceramic (Al_2O_3) abutments were compared regarding microbial sampling (Rasperini et al. 1998) and soft tissue conditions (Andersson et al. 2003) and no differences between the materials were observed. Vigolo et al. (2006) assessed the peri-implant mucosa around abutments made of gold-alloy and titanium and no evidence of different response to the materials were found. Favorable soft tissue conditions to zirconia abutments were found in a clinical study (Glauser et al. 2004) and also abutments made of alumina-zirconia demonstrated healthy soft and hard tissue conditions (Bae et al. 2008).
Information obtained from animal experiments and clinical studies appears incomplete regarding soft tissue healing to different types of implant materials. The aim of the **third study** was to analyze the soft tissue barrier formed to implant abutments made of titanium, gold-alloy and zirconia (ZrO_2).

Microgap at two-part implants

In one-part implant systems the transmucosal part is continuous with the osseous part. The two-part implant systems, however, are provided with one intraosseous and one transmucosal part that result in a "microgap" between the components. The traditional Brånemark implant was provided with a "flat to flat" surface between the two components and the abutment was connected to the fixture with a central screw; an "open system". An experimental animal study reported that an inflammatory cell infiltrate (abutment ICT) was consistently present at the level of the interface between the two components, furthermore, the bone crest was consistently located 1-1.5 mm apical of the microgap (Ericsson et al. 1995). Persson et al. (1996) suggested that this was a result of a bacterial contamination of the inner components of the implants. In animal studies one-part implants and experimental two-part implants were placed at different levels to the bone crest. It was suggested that the most coronal bone to implant contact at two part implants was consistently located approximately 2 mm below the junction of the components (Hermann et al. 1997). In addition, placement of two-part implants at different levels in relation to the bone crest resulted in different amounts of bone loss (Hermann et al. 2000 b, Piatelli et al. 2003, Alomrani et al. 2005). Hermann et al. (2001b) and King et al. (2002) also suggested that micro-movements influenced the location of the marginal bone to implant contact. Leukocytes were analyzed in the tissue facing one- and two-part implants in an experimental animal study. Clusters of inflammatory cells were found approximately 0.5mm from the micro-gap around two-part implants, while in tissues surrounding one-part implants only scattered inflammatory cells were found (Broggini et al. 2003). The number of inflammatory cells was found to increase with the depth of the implant-abutment interface (Broggini et al. 2006). Two-part implants with non-matching implant-abutment diameters and a conical internal implant-abutment connection were used in an animal study (Jung et al.

2008). It was reported that the amount of crestal bone loss that occurred was much smaller than that observed by Hermann et al. (1997). Subcrestally placed implants in animal experiments were reported to have a wider soft tissue dimension with longer epithelium and connective tissue compartments than that at implants placed in level or coronally to the bone crest (Todescan et al. 2002, Pontes et al. 2008 a,b). The aim of the **fourth study** was to challenge the earlier results of a subcrestal placement of two-part implants by placing two-part implants in a subcrestal position. In **study five** the aim was to examine the healing adjacent to two-part implants placed in sites with buccal bone defects.

Table 1. Animal experiments

Authors	Model	Techniques	Results
James & Schultz -74	2 monkeys vitallium implants	Freeze fractured preparations TEM analysis	Structures resembling basal lamina and hemidesmosomes
Listgarten & Lai -75	3 monkeys epoxy resin implants	TEM analysis	Basal lamina and hemidesmosomes after 2 weeks
Swope & James -81	2 monkeys vitallium implants	TEM analysis	Hemidesmosomes formed after 2-3 days
Schroeder et al. -81	Monkeys Titanium implants	Light microscopy SEM/TEM analysis	Collagen fibers functionally oriented
Kurashina et al. -84	5 dogs Hydroxyapatite implants	Light microscopy Decalcified sections	Epithelial attachment and connection of supra-alveolar collagen fibers formed a biological seal around the implants.
McKinney et al. -85	18 dogs ceramic implants (aluminumoxide)	SEM/TEM analysis	Basal lamina and hemidesmosomes
Hashimoto et al. -88	10 monkeys single-crystal sapphire implants	Light microscopy Paraffin sections	Collagen fibers running parallel to the implant surface
van Drie et al. -88	4 dogs titanium implants	Light microscopy/ SEM Decalcified sections	Collagen fibers aligned parallel to the implant surface. In some specimens epithelial attachment to abutments in others epithelium separated by a layer of inflammatory cells.
Buser et al. -89	3 dogs titanium implants	Light microscopy Ground sections	Perpendicularly arranged horizontal fibers at keratinized mucosa and vertical structures parallel at non-keratinized mucosa.
Hashimoto et al. –89	10 monkeys single-crystal sapphire implants	Light microscopy TEM analysis	Basal lamina and hemidesmosomes

Arvidsson et al. -90	4 dogs titanium implants (Astra)	Light microscopy/ TEM Paraffin sections	Basal lamina and hemidesmosomes. Collagen fiber bundles in connective tissue running in different directions
Fartash et al. -90	2 dogs single crystal sapphire implants	LM, SEM,TEM Paraffin sections	Basal lamina and hemidesmosomes. Dense collagen fibers in different directions
Berglundh et al. -91	5 dogs titanium implants (Brånemark)	Light microscopy Decalcified sections	Dimensions of junctional epithelium (JE) and connective tissue (CT) resembled teeth. Collagen fibers aligned parallel to the implant surface. Collagen density higher in periimplant mucosa than teeth
Buser et al. –92	6 dogs titanium implants	Light microscopy Undecalcified sections	50-100μm zone of dense circular collagen fibers close to the implant surface.
Listgarten et al. -92	4 dogs titanium coated Epoxy resin implants	Light microscopy TEM analysis	Collagen fibers oriented parallel to implant surface
Ruggeri et al. -92	4 monkeys titanium implants	Light microscopy/ SEM ground sections Paraffin sections	Circular collagen fibers in the periimplant mucosa
Berglundh et al. -94	2 dogs titanium implants Brånemark system	Light microscopy	Connective tissue integration zone at implants poor in vessels
Ruggeri et al. -94	4 monkeys titanium implants	Light microscopy/ SEM ground sections Paraffin sections	Collagen fibril diameter in periimplant mucosa corresponds to that of gingival fibrils
Abrahamsson et al. -96	5 dogs titanium implants Brånemark, Astra, ITI system	Light microscope Decalcified sections	Similar dimension and composition of soft tissue for the 3 systems

Berglundh & Lindhe -96	5 dogs titanium implants Brånemark system	Light microscope Decalcified sections	A minimun width of the periimplant mucosa (epithelium and connective tissue zone) is required
Ericsson et al. -96	5 dogs titanium implants Brånemark system	Light microscopy	Similar soft tissue dimensions at submerged and non-submerged installation techniques
Weber et al. -96	6 dogs submerged and nonsubmerged titanium implants	Light microscopy Ground sections	Apical extension of JE greater and attachment level lower in submerged than in non submerged implants
Cochran et al. -97	6 dogs titanium implants loaded and unloaded	Light microscopy Ground sections	Similar soft tissue dimensions as around teeth. A physiologically formed and stable biological width.
Hermann et al. -97	5 dogs Ti implants 1-part non-submerged 2-part non-submerged and submerged placed at different levels to bone crest	X-rays	In 2-part implants, submerged and non-submerged, the most coronal bone to implant contact was constantly located approximately 2mm below the microgap.
Inoue et al. -97	2 dogs titanium implants	Light microscopy Immunohistochemstry	PCNA (proliferating cell nuclear antigen) score significantly lower for periimplant epithelium than for JE at teeth. Suggestion: the periimplant epithelium maintains lower capacity to act as a proliferative defense mechanism
Abrahamsson et al. -98	5 dogs ti, ceramic, Au-alloy, dental porcelain abutments	Light microscopy Decalcified sections	Abutment materials made of Au-alloy or dental porcelain failed to establish soft tissue attachment and bone resorption occurred. Ti and ceramic (Al_2O_3) allowed soft tissue formation.

Fujii et al. -98	32 rats titanium implants	Light microscopy/ SEM	Periimplant epithelium show similar feature as JE after 15 days of healing. Collagen fibers in connective tissue arranged circumferentially around implants in horizontal sections
Kawahara et al. -98	3 monkeys titanium implants	Light microscopy/ SEM	Epithelial cell attachment/adhesion with basal lamina and hemidesmosomes
Abrahamsson et al. -99	6 dogs titanium implants (Astra tech system)	Light microscopy Decalcified sections	Submerged and nonsubmerged techniques similar soft tissue dimensions and connective tissue composition
Kohal et al. -99	3 dogs hydroxy apatite coated implants	Light microscopy Ground sections	Similar soft tissue dimensions for submerged and nonsubmerged implants
Moon et al. -99	6 dogs titanium implants	Light microscopy/ TEM Decalcified sections	A higher density of fibroblast in the inner interface zone (40μm) compared to an outer zone (160μm)
Choi et al. -00	3 dogs titanium implants in-stalled with periodontal ligament cells	Light microscopy Ground sections	Ligament like tissue attachment can form around dental implants
Hermann et al. – 00 a	6 dogs unloaded and loaded non submerged titanium implants	Light microscopy Ground sections	Changes occurred in the dimensions of the sulcus depth, JE and connective tissue but the overall dimension of the soft tissue was stable for loaded and unloaded implants.
Hermann et al. –00 b	5 dogs Ti implants 1-part non-submerged 2-part non-submerged and submerged placed at different levels to bone crest	Light microscopy Ground sections	Significant amounts of crestal bone loss occur around 2-part implant designs depending on the location of the interface. The location of the rough/smooth border determines the first bone to implant contact at 1-part implants. The surgical technique submerged/nonsubmerged has no influence on crestal bone changes

Ikeda et al. -00	40 rats titanium implants	Light microscopy /TEM	Internal basal lamina and hemidesmosomes only formed in the lower region of the periimplant JE. In control teeth the internal basal lamina and hemidesmosomes formed throughout the interface.
Comut et al. -01	4 dogs hydroxy apatite coated titanium implants	Light microscopy Ground sections	Collagen fibers parallel to the implant surface
Hermann et al. –01 a	5 dogs 1 and 2 piece implants unloaded, nonsubmerged and submerged	Light microscopy Ground sections	The biological width for 1-piece implants was significantly smaller compared to 2-piece implants.
Hermann et al. –01 b	5 dogs Ti implants 2-part non submerged welded and screw retained abutments, gaps 10,50,100μm	Light microscopy Ground sections	All implants in non-welded groups had significantly increased amount of bone loss compared to the welded groups.
Abrahamsson et al. -02	5 dogs titanium implants rough and smooth abutments	Light microscopy /TEM Decalcified sections	Similar dimensions for the 2 types of abutments. Soft tissue attachment was not influenced by surface roughness.
King et al. -02	5 dogs Ti implants 2-part non submerged welded and screw retained abutments, gaps 10,50,100μm	X-rays	The size of the microgap had no significant effect. Non welded implants showed significantly greater crestal bone-loss compared to welded implants at 1 and 2 months of healing. No difference at 3 months.

Todescan et al. -02	4 dogs Ti implants at different levels to crestal bone	Light microscopy Ground sections	Longer epithelium and connective tissue the deeper the implants were placed. Smallest bone loss for the subcrestally placed implants.
Zitzman et al. -02	5dogs titanium implants rough and smooth abutments	Light microscopy Decalcified sections	The surface characteristics of the abutments failed to influence plaque accumulation and inflammatory cell lesions. Plaque ICT was dominated by plasma cells and lymphocytes whereas abutment ICT was dominated by polymorphonuclear cells.
Abrahamsson et al. -03	6 dogs titanium implants	Light microscopy Decalcified sections	Single shift of abutments (healing to permanent) did not influence the dimension or quality of the soft tissue attachment.
Broggini et al. -03	5 dogs Ti implants 1-part non-submerged 2-part non-submerged and submerged placed at different levels to bone crest	Light microscopy Ground sections	Peak of inflammatory cells approximately 0.50 mm coronal of the microgap at 2–part implants. No peak for 1-part implants.
Fujiseki et al. -03	4 dogs titanium implants (ITI system)	Light microscopy /TEM Paraffin-,ground-and decalcified sections	Periimplant epithelium similar to the oral epithelium and structurally different from the JE
Piatelli et al. -03	Monkeys Ti implants placed at different levels to bone crest, insertion immediately postextraction, early and immediately loaded	Light microscopy Ground sections	Less bone loss occurred when microgap was moved coronally and if microgap was placed subcrestally greater amounts of bone resorption was present.

Siar et al. –03	6 monkeys titanium implants delayed and immediate loaded	Light microscopy Ground sections	Dimensions of the periimplant mucosa not influenced by the immediate functional loading or posterior location in the mandible
Tenenbaum et al. -03	6 dogs titanium implants (Ankylos system)	Light microscopy /TEM	Collagen fibers parallel to implant surface
Kohal et al. -04	6 monkeys zirconia and titanium implants	Light microscope Ground sections	The extent of the soft tissue compartments was similar for the 2 types of implants.
Alomraniet al. -05	5 dogs Ti implants Machined and SLA surface on collars, placed at different levels to bone crest	X-rays	Apically placed implants had greater bone loss than coronally placed implants. No difference between SLA and machined collars.
Broggini et al. -06	5 dogs Ti implants 1-part non-submerged 2-part non-submerged and submerged placed at different levels to bone crest	Light microscopy Ground sections	The periimplant neutrophil accrual increased progressively as the implant-abutment interface depth increased.
Watzak et al. –06	9 monkeys titanium implants (Brånemark and Frialen system)	Light microscopy Ground sections	No difference in soft tissue conditions between the systems

Abrahamsson et al. -07	4 dogs titanium and Au-alloy implants	Light microscopy Ground sections	Periimplant soft tissue dimensions were not influenced by the metal used in the marginal zone of the implant
Schwartz et al. –07 a	4 dogs titanium implants (SLA and mod SLA)	Light microscopy/ Immuno-histochemistry Ground sections/ Decalcified sections	Parallel and perpendicular collagen fibers to mod SLA surface
Schwartz et al. –07 b	15 dogs titanium implants (SLA and mod SLA)	Light microscopy/ Immuno-histochemistry Ground sections	Well vascularized subepithelial connective tissue, perpendicular collagen fibers attached to modSLA. Soft tissue integration was influenced by hydrophilicity and not topography of implant surface
Deporter et al. -08	4 dogs Ti sintered porous implants, short and long smooth collars	Light microscopy Ground sections	Posteriorely placed long collar implants had significantly greater bone loss than short collar implants. No difference in anterior placed implants. Soft tissue dimensions for the 2 implant types were similar.
Jung et al. -08	5 dogs Ti implants submerged and nonsubmerged at different bone levels, non-matching abutments.	X-rays	Implant abutments with non-matching abutments can be placed non submerged or submerged with comparable outcomes. The greatest bone loss between implant placement and loading was observed in the subcrestally placed group.
Pontes et al. –08 a	6 dogs Ti implants installed at different levels to crestal bone. Direct and delayed loading	Position of soft tissue margin and prosthesis-abutment junction, PD, RAL, MBI, BoP, X-rays	The first bone to implant contact was positioned in a more apical position when implant was installed subcrestally. The apical position of the implants did not influence the ridge loss or the soft tissue margin. Immediately restored sites had the soft tissue margin position significantly more coronal than the delayed restored group.

Pontes et al. –08 b	6 dogs Ti implants installed at different levels to crestal bone. Direct and delayed loading	Light microscopy Ground sections	Greater soft tissue dimension for more apical positioned implants among conventionally restored group. No differences in immediate restored group. Greater amount of boneloss around conventionally than immediately loaded implants.
Rossie et al. -08	6 dogs titanium implants (ITI system, sol-gel-derived nanoporous TiO_2 film	Light microscopy/ TEM/SEM Ground sections	Dense plaque of hemidesmosomes faced the coated surface. The distance between the implant margin and the alveolar bone crest was significantly shorter in surface treated implants.

Table 2. Human biopsy materials

Authors	Type/Implants	Techniques	Results
Hansson et al. –83	Block biopsies Brånemark system	LM, SEM, TEM	Hemidesmosomes in epithelial cells toward implant surface. 20nm thick proteoglycan layer separated the connective tissue and the implant.
Gould et al. –84	Biopsy Titanium coated implants	TEM Epon sections	Formation of basal lamina and hemidesmosomes
Adell et al. –86	Soft tissue biopsies Brånemark system	Light microscopy Epon sections	Connective tissue contained inflammatory cell infiltrates of varying size and location in 65% of biopsy material.
Lekholm et al. –86	Soft tissue biopsies Brånemark system	Light microscopy Epon sections	Inflammatory infiltrate in 42% of the biopsies of varying size and location
Akagawa et al. –89	Soft tissue biopsies Single-crystal sapphire implants	Light microscopy Paraffin sections	Inflammatory infiltrates adjacent to JE. Collagen fiber bundles in a circular fashion around the implant
Seymour et al. –89	Soft tissue biopsies Brånemark system	Light microscopy Frozen sections Immunohistochemistry	Inflammatory cell infiltrates in all biopsies. Larger lesion in clinically inflamed sites. 50-60% T-cells and 40-50% B-cells. CD4/CD8 ratio between 1.2 and 2.0. Well controlled immune response
Carmichael et al. -91	Soft tissue biopsies Gingival and periimplant mucosa Brånemark system	Light microscopy GMA sections Immunohistochemistry	Epithelia of gingiva and periimplant mucosa are not composed of identical cell populations.
Tonetti et al. –93	Soft tissue biopsies Gingival and periimplant mucosa ITI system	Light microscopy Frozen sections Immunohistochemistry	Functional similarities regarding antigen presentation in the 2 types of tissues

Chavrier et al. -94	Soft tissue biopsies Gingiva and periimplant mucosa IMZ implants	Light microscopy Frozen sections Immunohistochemistry	Collagen fibers parallel with implant. No difference in distribution of collagenous components between the gingiva and the periimplant mucosa
Romanos et al. -95	Soft tissue biopsies Gingival and periimplant mucosa ITI system	Light microscopy Frozen sections Immunohistochemistry	Collagen I,III,IV, VII and fibronectin showed similar distributions in the 2 types of tissues. Collagen type V was localized in higher amounts in lamina propria in periimplant tissue. Collagen VI only stained delicate fibrillar network in periodontal tissues.
Tonetti et al. –95	Soft tissue biopsies Gingival and periimplant mucosa ITI system	Light microscopy Frozen sections Immunohistochemistry	Higher densities of mononuclear cells in ICT than in JE in both tissues. PMN propotions similar in JE and ICT in both tissues. Density of leukocytes similar in both tissues. Regional differences in the relative proportions of immunocompetent cells in both tissues
Mackenzi et al. –95	Soft tissue biopsies Gingival and periimplant mucosa	Light microscopy Frozen sections Immunohistochemistry	The formation of oral-, oral sulcular- and junctional - epithelium was phenotypically indistinguishable from those of natural gingival.
Arvidsson et al. –96	Soft tissue biopsies Brånemark and Single-crystal sapphire implants	Light microscopy/ TEM Paraffin sections Immunohistochemistry	No qualitative structural differences between the two types of implants
Liljenberg et al. –96	Soft tissue biopsies Edentulous ridge mucosa and periimplant mucosa	Light microscopy Epon-, frozen-sections Immunohistochemistry	The composition of both tissues were close to identical in terms of collagen, cells and vascular structures. The periimplant mucosa harbored a JE that was found to contain significantly enhanced numbers of different inflammatory cells.
Piatelli et al. –97	Autopsy biopsies Plasma sprayed titanium implants	Light microscopy Ground sections	No inflammatory infiltrate in epithelium or connective tissue. Collagen fibers in the coronal part was parallel to implant surface while in the apical region the fibers were in a perpendicular fashion.
Chavrier et al. –99	Soft tissue biopsies Titanium implants	electronmicroscopy resin sections Immunohistochemisty	The connective tissue under the JE comprised of type I and III collagen. The supra crestal connective tissue was mainly comprised of type I collagen. Type IV collagen was located exclusively in the basement membrane of the JE.

Marchetti et al.- 02	Soft tissue biopsies Titanium implants	Light microscopy/ TEM Paraffin sections Immunohistochemisty	All the epithelial and connective tissue components of the mucosa are involved in the substantial regrowth of the periimplant tissue.
Schierano et al. -02	Soft tissue biopsies (en bloc) Brånemark titanium abutments	Light microscopy Ground sections	Collagen fiber bundles organized in internal longitudinal fibers and external circular fibers. No radial fibers inserted to abutment surfaces were observed.
Wennerberg et al. -03	Soft tissue biopsies Titanium abutments with different surface roughness	Light microscopy Paraffin sections	No relation was found between inflammatory response and abutment surface roughness.
Glauser et al. -05	Hard and soft tissue biopsies Mini titanium implants with different surface characteristics	Light microscopy Ground-, resin-sections	The JE established the attachment to the implant surfaces. The collagen fibers and the fibroblasts were oriented parallel to the implant surface. The oxidized and acid etched implants revealed less epithelial down growth and longer connective tissue than machined implants.
Degidi et al. –06	Soft tissue biopsies Titanium and ZrO_2 Healing caps	Light microscopy Paraffin sections Immunohisto-chemistry	Statistically significant differences were observed around the 2 types of abutments with an overall lower inflammatory level in tissues surrounding the zirconia healing caps than at the titanium healing caps.
Schüpbach et al. -07	Hard and soft tissue biopsies Mini titanium implants / different surface characteristics	Light microscopy/ TEM/SEM Ground-, resin-sections	A 100-150μm wide zone of connective tissue directly facing the implant was free from blood vessels and dominated by loosely arranged collagen fibers running parallel with the surface. An adjacent area presented circumferentially oriented fiber bundles. In oxidized surfaces the collagen fibers had become functionally oriented.

Nevins et al. -08	En bloc biopsies Lazer-Lok microchannels (BioHorizon)	Light microscopy/ μCT/SEM Ground-, resin-sections	Intimate contact of JE cells to implant surface. Connective tissue with functionally oriented collagen fibers running towards the implant surface.

Table 3. Clinical abutment/ implant material studies

Authors	Design	Evaluation parameters	Results
Bollen et al. -95	Split mouth 6 patients, titanium implants 1 standard abutment (R_a 0.2μm) 1 highly polished ceramic (R_a 0.06μm)	PPD, gingival recession, CAL, BoP, PI, Damping characteristics (Periotest) supra- and sub-gingival plaque sampling	No qualitative or quantitative differences between the 2 abutment types.
Barclay et al. -96	Split mouth 14 patients, IMZ implants Ti and ceramic transmucosal collars	PI, Peri Implant Sulcus Fluid, MBI, PPD, heigth of attached mucosa	No difference in soft tissue response to the 2 types of transmucosal collars. The plaque accumulation score for the ceramic coated collar was significantly lower than at the titanium collar.
Rasperini et al. -98	4 patients acrylic devices harboring samples of Ti and ceramic abutments	Microbial sampling at 6h, 24h, 7d and 14d	No significant differences were observed between the 2 materials
Rimondini et al.02	10 patients silicon devices with discs of fired and rectified ZrO_2 and Ti	Spectrophotometry SEM	Fewer bacteria accumulated on ZrO_2 discs than at Ti discs. No differences between the fired and rectified ZrO_2
Andersson et al. -03	Prospective 5 year 30 patients titanium implants Al_2O_3 and Ti abutments	MBI, PI	Healthy appearance of soft tissue, no diff in bleeding or plaque index.
Scarano et al. -04	10 patients removable acrylic device with discs of Ti and ZrO_2	SEM/plaque accumulation	Significantly lower plaque accumulation on ZrO_2 discs than on TI discs.
Glauser et al. -04	Prospective 4 year 24 patients titanium implants experimental ZrO_2 abutments	PI,MBI	Healthy soft tissue conditions and stable marginal bone levels were documented.

Vigolo et al. -06	Split mouth 20 patients titanium implants gold-alloy and Ti abutments	X-ray, PI, MBI, BoP, PPD, amount of keratinized gingiva	No difference between the 2 types of abutments regarding periimplant marginal bone level and soft tissue parameters.
Bae et al. -08	19 patients titanium implants alumina-zirconia abutments	X-ray	Stable and healthy soft and hard tissue conditions

Aims

The objectives of the present thesis were:

- to study the morphogenesis of the mucosal attachment to implants made of c.p. titanium

- to analyze the soft tissue healing at titanium implants coated with type I collagen

- to analyze the soft tissue barrier formed to implant abutments made of different materials

- to study the healing around two-part implants that were placed in a subcrestal position

- to study the healing adjacent to two-part implants with different surface roughness placed in sites with buccal bone defects

Material and Methods

Animals

Dogs at the age of 1-2 years were used in all experiments. The breed and number of animals, however, varied between the different studies. Twenty labrador dogs were used in study 1, while in both study 2 and 3 six labrador dogs were used. Study 4 and 5 included five mongrel dogs each. The regional Ethics Committee for Animal Research, Göteborg, Sweden, approved the experimental protocols for all studies.

Implants and components

Study 1

160 custom made solid screw implants (4.1 x 10 mm) of the ITI ®/ Straumann Dental Implant system (Straumann AG, Basel, Swizerland) were used. The implants were provided with a polished transmucosal collar that was 2.8 mm high.

Study 2

48 custom-made TG Osseotite®implants (3.75 x 10 mm) from 3i® / Biomet 3i™ (Biomet 3i, Palm Beach Gardens, Florida USA) with a 2.8 mm high transmucosal collar were used. The marginal 4.7 mm of the implant, i.e. the transmucosal collar and about 2 mm of the intraosseous portion had a turned surface, while the remaining part of the implant had a dual acid etched surface. The test implants were, in addition coated with a purified porcine Type I collagen.

Study 3
48 OsseoSpeed™ implants (4.5x 9mm) from Astra Tech implant system (Astra Tech Dental Mölndal, Sweden) were installed. Healing abutments (Zebra™ 6mm, Astra Tech Dental, Mölndal, Sweden) were used at installation and replaced with custom-made abutments made of titanium (Ti), ZrO_2 (Ceramic) and AuPt – alloy (Cast-to). The custom-made abutments had similar dimensions and geometry.

Study 4 and 5
40 OsseoSpeed™ implants (3.5mm x 8mm) from Astra Tech implant system (Astra Tech Dental, Mölndal, Sweden) were used. In the test implants the surface modification of the OsseoSpeed™ extended to the implant margin and, thus, included also the shoulder part of the implant. Two types of abutments were used: one regular abutment with a turned surface (Zebra™ 4.5 mm, Astra Tech Dental, Mölndal, Sweden) and one experimental abutment with a modified surface (TiOblast™, 4.5mm, Astra Tech Dental, Mölndal, Sweden).

Surgical procedures

General anesthesia
In all experiments the surgical procedures were performed using general anesthesia induced with propofol (10 mg/ml, 0.6 ml/kg) intravenously and sustained with N_2O:O_2 (1:1.5-2) and isoflurane employing endotracheal intubation. For suture removal and abutment shift the animals were sedated by a subcutaneous injection of Domitor Vet®(Orion Pharma AB, Animal Health, Espoo, Finland).

Study 1

All mandibular premolars were extracted. Three months later buccal and lingual muco-periosteal flaps were elevated and 4 implants were placed in each side of the mandible. The flaps were adjusted, repositioned and sutured around the transmucosal portion of the implants. When applicable, the sutures were removed 2 weeks after surgery. Biopsies were obtained at various time intervals after implant installation and represented day 0 (2 hours after implant installation) 4 days, 1, 2, 4, 6, 8 and 12 weeks of healing.

Study 2

The mandibular premolars and the 1st, 2nd and 3rd maxillary premolars were extracted. Three months later a crestal incision was made in the left or right edentulous mandibular premolar region. Buccal and lingual mucoperiosteal flaps were raised and 2 test and 2 control implants were installed in a randomized order. Cover screws were placed and flaps were adjusted and sutured around the neck of the implants. The sutures were removed two weeks after implant placement. After another 2 weeks the implant installation procedure was repeated in the contra-lateral mandibular region. The sutures were removed 2 weeks later. Biopsies were obtained 4 weeks after the second implant installation procedure.

Study 3

The mandibular premolars and the 1st, 2nd and 3rd maxillary premolars were extracted. Three months later buccal and lingual mucoperiosteal flaps were elevated and 4 implants were placed in the edentulous premolar region in one side of the mandible. Healing abutments were connected to the implants and the flaps were adjusted and sutured. One month after implant placement the sutures were removed and the healing abutments were disconnected and exchanged to

abutments made of different materials but with similar dimensions and geometry. Three months after implant surgery the implant installation procedure and the subsequent suture removal and abutment shift were repeated in the contra-lateral mandibular region. Biopsies were collected 2 months after the final abutment shift.

Study 4

The mandibular premolars and the 1st, 2nd and 3rd maxillary premolars were extracted. Three months later a crestal incision was made in the edentulous premolar region in one side of the mandible. Buccal and lingual mucoperiosteal flaps were elevated and 2 test and 2 control implants were installed in a randomized order. The implants were placed in such a way that the implant margin was located 2 mm apical to the bone crest. Regular abutments were connected to the control implants and experimental abutments were connected to the test implants. The flaps were adjusted and sutured. The sutures were removed two weeks after implant placement. Biopsies were obtained after 4 months of healing.

Study 5

The mandibular premolars and the 1st, 2nd and 3rd maxillary premolars were extracted. Immediately after the extractions in one side of the mandible, buccal and lingual mucoperiosteal flaps were elevated and a buccal defect was prepared by the resection of a 2 mm high portion of the marginal buccal bone wall of the extraction sockets. The lingual bone wall was left intact and the flaps were repositioned and sutured. The sutures were removed after 2 weeks of healing. Three months later a crestal incision was made in the premolar region with the buccal bone defect. Buccal and lingual mucoperiosteal flaps were elevated and 2 test and 2 control implants were installed. The implants were placed in a randomized order and in such a way that the implant margin at the buccal side

coincided with the buccal bone crest, while, the implant margin at the lingual side was positioned about 2mm apical of the lingual bone crest. Regular abutments were connected to the control implants and experimental abutments were connected to the test implants. The flaps were adjusted and sutured. The sutures were removed two weeks later. Four months later biopsies were harvested.

Biopsy procedure

All animals were euthanized by an overdose of Sodium Pentothal and perfused through the carotid arteries with a fixative. Access to the carotid arteries and the jugular veins was made through a 10-12 cm long incision along the external jugular vein of the shaved neck region. Using a blunt dissection technique, the jugular veins and the carotid arteries were exposed. While the arteries were cannulated for the perfusion of heparin/ saline solutions and the subsequent fixative, the jugular veins were severed to drain the solutions. The fixative consisted of a mixture of 5% glutaraldehyde and 4% formaldehyde buffered to pH 7.2 (Karnovsky, 1965). The mandibles were removed and placed in the fixative. Block biopsies containing the implant and the surrounding tissues were dissected using a diamond saw (Exakt®, Kulzer, Germany).

Histological preparation

Ground sections

The tissue blocks selected for ground sectioning in study 1,2,4 and 5 were dehydrated in serial steps of alcohol concentrations and subsequently embedded in a methyl-methacrylate resin (Technovit® 7200 VLC, Exakt®, Kulzer, Germany). Using a cutting-grinding unit and a micro-grinding system (Exakt®, Apparatebau, Norderstedt, Germany) the blocks were cut in a buccal-lingual plane and 2 central sections were obtained. The remaining mesial and distal

portions of the tissue block were remounted and 2 central sections in a mesio-distal plane were prepared. All sections were reduced to a final thickness of approximately 20 μm. Thus, from each implant block 2 mesio-distal and 2 buccal-lingual ground sections were obtained. In study 1 and 2 all ground sections were stained in toluidine blue (Donath 1993), whereas in study 4 and 5 every second ground section was stained in toluidine blue or in Ladewig (Donath 1993)

Fracture technique

The tissue samples selected for the "fracture technique" were placed in ethylene-diamine-tetra-acetic acid (EDTA). Incisions, parallel with the long axis of the implant, were made through the peri-implant tissues before the hard tissue was fully decalcified. Four different units (mesio-buccal, disto-buccal, mesio-lingual, disto-lingual) were hereby obtained. Decalcification was completed in EDTA and dehydration performed in serial steps of ethanol concentrations. Following secondary fixation in OsO_4 the specimens were embedded in epoxy resin (EPON®, Fluka Chemie GmbH, Buchs, Switzerland) (Schroeder 1969). Sections were produced with the microtome set at 3 μm and stained in PAS and toluidine blue (Schroeder 1969).

Histological analysis

Linear measurements

In all studies the vertical distances between certain landmarks were determined in a direction parallel to the long axis of the implant. The analyses were performed in a Leica DM-RBE® microscope (Leica, Heidelberg, Germany) equipped with an image system Q-500 MC® (Leica Heidelberg, Germany).
Landmarks:
I-the implant margin (Study 1 and 2)

PM- the marginal portion of the peri-implant mucosa (Study 1,2,3,4,5)

B-the marginal level of bone to implant contact (Study 1,2,3,4,5)

aJE-the apical extension of the barrier epithelium (Study 1,2,3,4,5)

A/F- the abutment/ fixture borderline (Study 3,4,5)

Bc- the bone crest (Study 4,5)

Qualitative measurements

The composition of the connective tissue compartment of the peri-implant mucosa residing between aJE - B (study 1 and 2) and aJE-A/F (study 3 and 4) was analyzed in the EPON®-embedded sections. The assessments were made using a point counting procedure described previously (Schroeder & Münzel-Pedrazzoli 1973, Berglundh et al. 1989, 1991, 1992 a,b, 1993, Abrahamsson et al. 1998 a,b, 1999, 2002, Moon et al. 1999). A lattice comprising 100 cross-points was superimposed over the tissue at a magnification of x1000 and the relative proportions of the connective tissue components were determined.

Study 1

The composition of an 80μm wide area of the connective tissue facing the transmucosal portion of the implant was assessed. The measurements included 3 zones of the peri-implant mucosa: zone 1 (coronal), zone 2 (middle), zone 3 (apical). The relative proportions of the connective tissue occupied by collagen (Co), fibroblasts (Fi), vascular structures (V), mononuclear leukocytes (Mø), polymorphonuclear leukocytes (PMN) and residual tissue (R), e.g., nerves, matrix components and unidentified structures were determined.

Study 2

The assessments were confined to a 100μm wide zone of the connective tissue interposed between aJE and B. The relative proportions of the connective tissue occupied by collagen (Co), fibroblasts (Fi), vascular structures (V), macrophages (Mø), lymphocytes (Ly), plasma cells (Pc) polymorphonuclear leukocytes (PMN) and residual tissue (R), e.g. nerves, matrix components and unidentified structures, were determined.

Study 3

The composition of the connective tissue compartment of the peri-implant mucosa that was in contact with the different abutments and interposed between aJE and A/F was assessed. The analysis was confined to an 80μm wide tissue zone lateral to the abutment interface. The relative proportions of the connective tissue occupied by collagen (Co), fibroblasts (Fi), vascular structures (V), leukocytes (Leu) and residual tissue (R) (e.g. nerves, matrix components and unidentified structures) were determined.

Study 4

The composition of the connective tissue compartment of the peri-implant mucosa that was in contact with the 2 types of abutments was analyzed. This connective tissue zone was 80 μm wide and was interposed between aJE and A/F. The relative proportions of the connective tissue occupied by collagen (Co), fibroblasts (Fi), vascular structures (V), leukocytes (Leu) and residual tissue (R), e.g. nerves, matrix components and unidentified structures were determined.

Leukocytes within the barrier epithelium

In study 2 and 3 the relative volume of infiltrating leukocytes within the barrier epithelium was assessed according to methods described by

Schroeder (1973) and Berglundh et al. (1992 a,b). Thus, a lattice comprising 400 points was superimposed over the epithelium at a magnification of x 1000 and the percentage of leukocytes in relation to the volume of the barrier epithelium was determined.

Scanning electron microscope (SEM) analysis

The implants prepared according to the fracture technique (retrieved implants) in study 2 were following the separation between the implant and the surrounding tissues examined in a Scanning Electron Microscope (SEM) (Leica S420; Leica Microsystems Heidelberg, Germany, equipped with a LEO Software 15XX). The prepared implants were air-dried and sputtered with gold. In addition, 4 new (pristine) implants (2 test and 2 control implants) were also analyzed. In all implants, a 1mm high area at the level of the marginal thread was identified and analyzed at different magnifications (range: x50 – x10.000)

Data analysis

For each of the variables mean values and standard deviations were calculated for the implant group and animal (the animal was used as the statistical unit). In study 2,4 and 5 the differences were analyzed using the Student's t-test for paired observations. The null hypothesis was rejected at $p < 0.05$. In Study 3 differences were analyzed using the two-way analysis of variance (ANOVA) and the Student-Newman-Keuls test. The null hypothesis was rejected at $p < 0.05$.

Results

One implant was lost during healing in study 3. All other implant sites in all the studies healed uneventfully.

Soft tissue dimensions

Study 1

A coagulum occupied the compartments between the mucosa and the implant and between the mucosa and the alveolar process in the early healing phase. The dimensions of the mucosa were assessable from the 1st week (Table 1).

	PM-B	PM-aJE
0 d		
4 d		
1 w	2.68 (1.41)	0.48 (.20)
2 w	3.52 (.97)	0.52 (.20)
4 w	3.18 (.50)	1.42 (.32)
6 w	3.07 (.38)	1.65 (.32)
8 w	3.35 (.48)	2.06 (.21)
12 w	3.47 (.49)	1.81 (.60)

Table 1. Dimensions of the peri-implant mucosa. Mean values and standard deviations (SD).

Study 2

The dimensions of the epithelial and connective tissue components of the implant/mucosa interface at coated (test) and un-coated (control) implants were similar after both 4 and 8 weeks of healing The increase in distance from I-B between 4 and 8 weeks of healing, with a higher mucosa and a greater distance from aJE-B, was more pronounced at the control implants than at the test implants (Fig. 1).

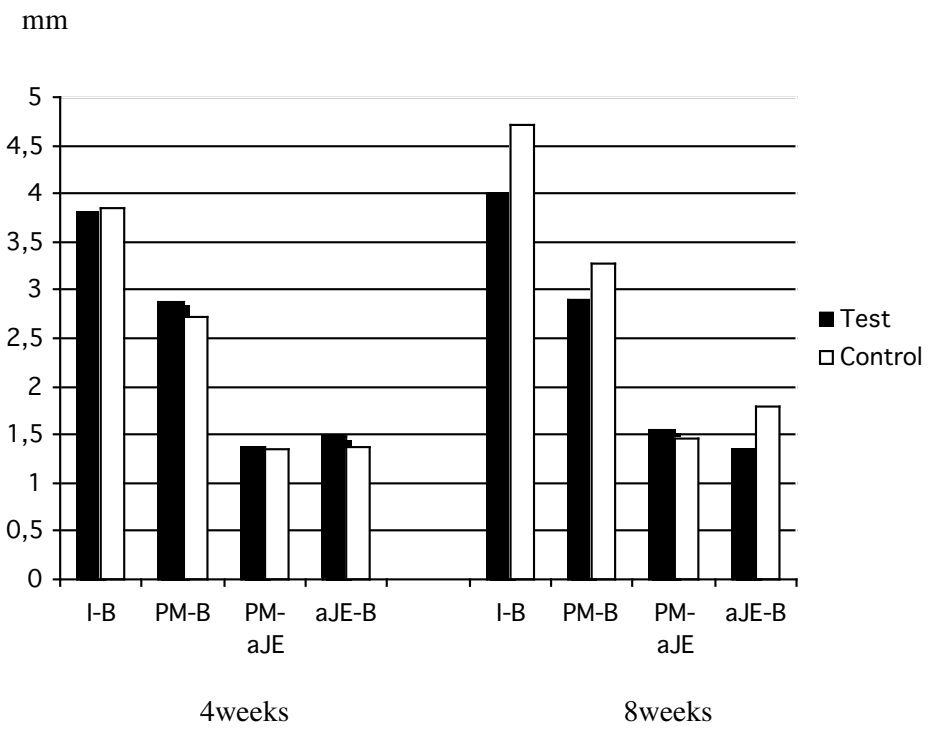

Fig. 1. Histogram illustrating the soft tissue dimensions at coated (test) and un-coated (control) implants after 4 and 8 weeks of healing.

Study 3

The soft tissue dimensions around the 3 abutment materials were similar at 2 months of healing. At 5 months of healing, however, the soft tissue height including the dimension of the barrier epithelium at cast-to (gold-alloy) abutments was larger than that at abutments made of titanium and ceramic (zirconia). Also the A/F-B distance was longer at cast-to than at titanium and ceramic abutments at 5 months (Fig. 2).

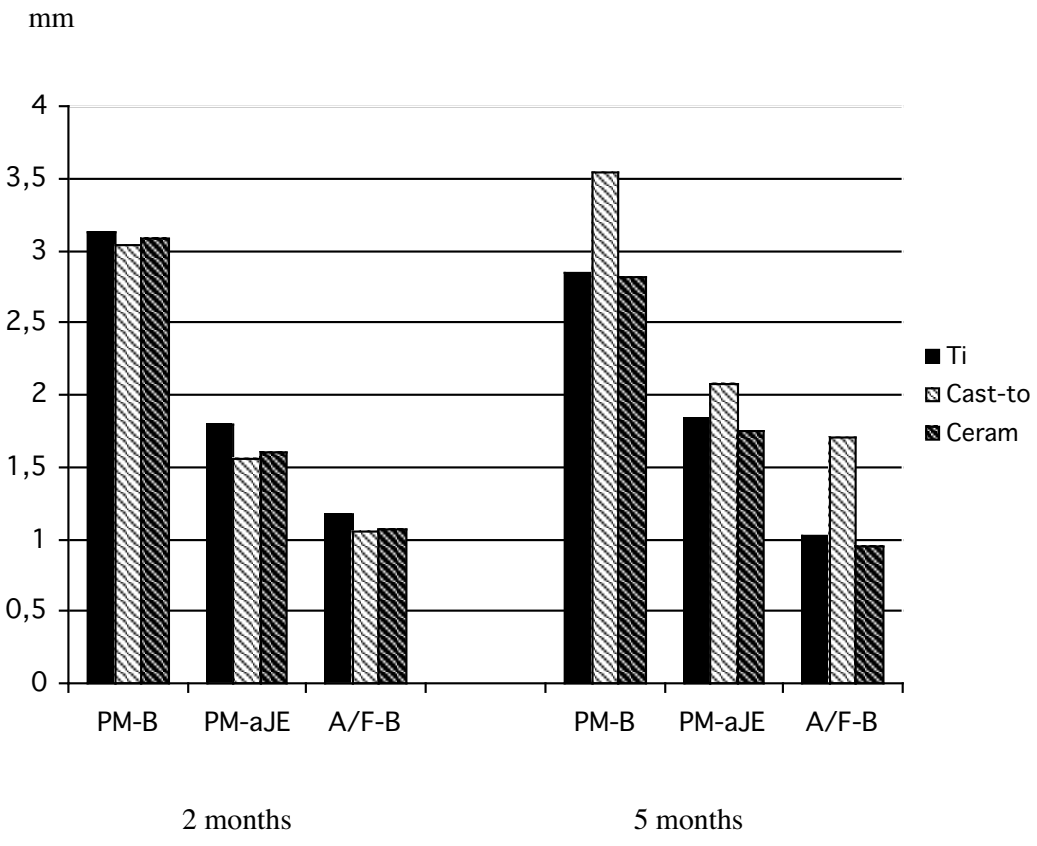

Fig. 2. Histogram illustrating the soft tissue dimensions around the 3 abutment materials (titanium, cast-to (gold-alloy), ceram (zirconia)) at 2 and 5 months of healing.

Study 4

The marginal bone level at the test implants was identified in a more coronal position than at the control implants. Thus, the linear distance from A/F to B at the test implants was shorter than at the control implants. This difference between test and control implants was statistically significant. The distance between Bc and B was also significantly shorter at test implants than at control implants (Fig. 3).

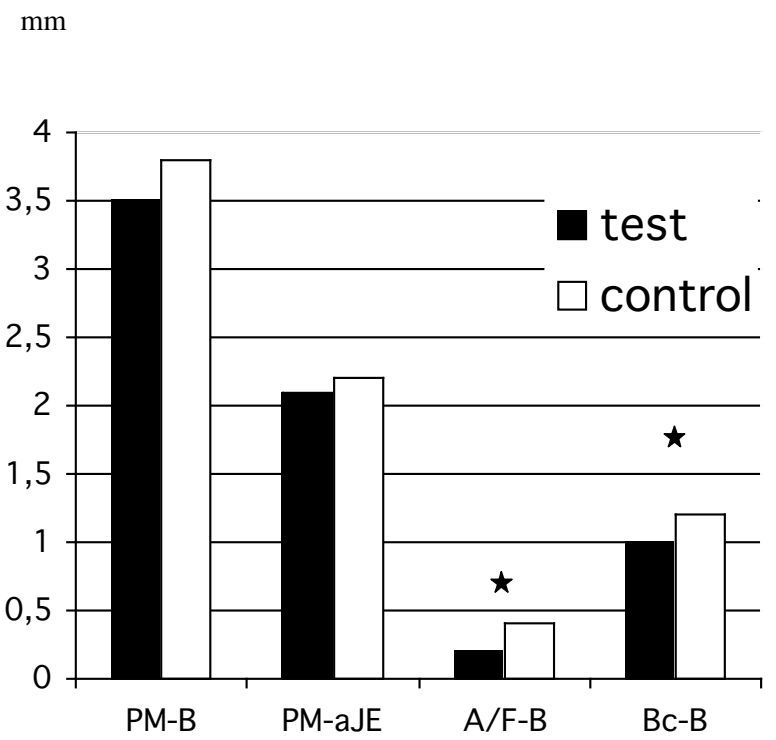

Fig. 3 Histogram illustrating the dimensions of the periimplant mucosa for test and control implants. ★ Indicates $p < 0.05$.

Study 5

A larger discrepancy was found between the buccal and lingual aspects regarding the marginal bone level (A/F-B) at the test implants than at the control implants. This difference between test and control implants was statistically significant. The distance between Bc and B of about 1 mm at the lingual aspect of test and control implants indicated the presence of an angular bony defect. At the buccal aspects, however, the vertical position of Bc in most cases coincided with that of B (Fig. 4).

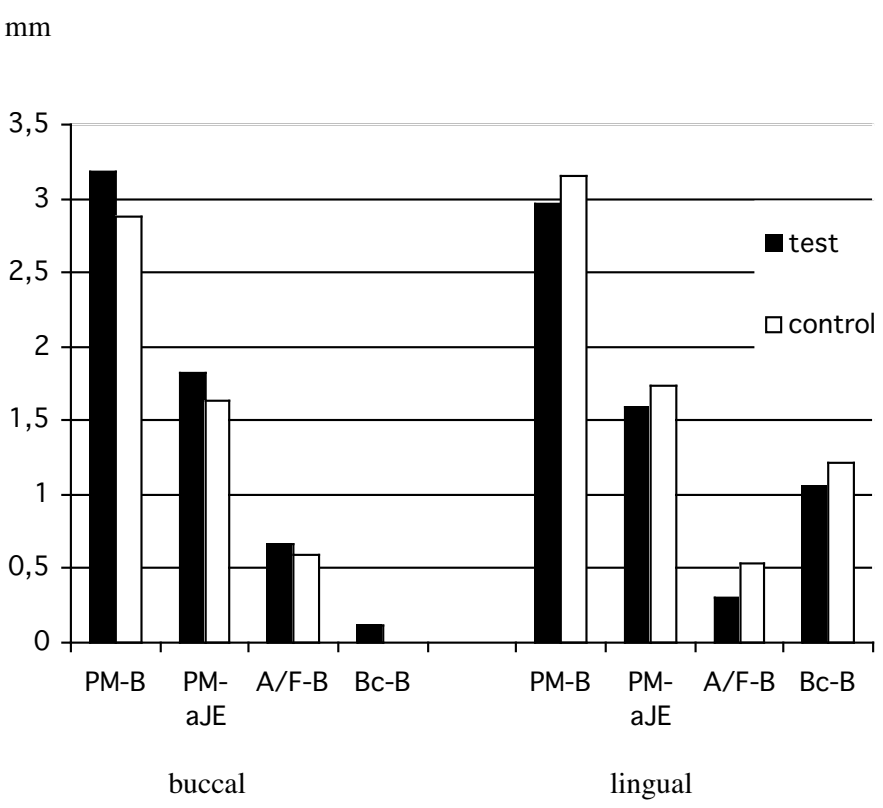

Fig. 4 Histogram illustrating the soft tissue dimensions at the buccal and lingual aspects for test and control implants.

Connective tissue composition

Study 1

The composition of the connective tissue assessed within the coronal (zone 1), middle (zone 2) and apical (zone 3) interface portion to the implant is presented in Fig. 5. The tissue in zone 1 was available for analysis at day 0, day 4, 1 week and 2 weeks, while at later healing intervals the interface portion at this level was occupied by a barrier epithelium. Day 0 included the tissue lateral to the wound surface of the mucosa secured by the sutures in the coronal and middle zones but the apical portion of the interface (zone 3) was occupied by a blood cloth containing erythrocytes. At day 4 the inflammatory phase of the wound healing process prevailed and, hence, large amounts of leukocytes were detected. Between 1 and 2 weeks there was an increase in density of fibroblasts, collagen and vessels in all 3 zones. From 4 weeks of healing, the densities of collagen and fibroblasts continued to increase and were the dominating tissue components. The connective tissue composition of fibroblasts, collagen and vessels appeared to be stable between 6 and 12 weeks.

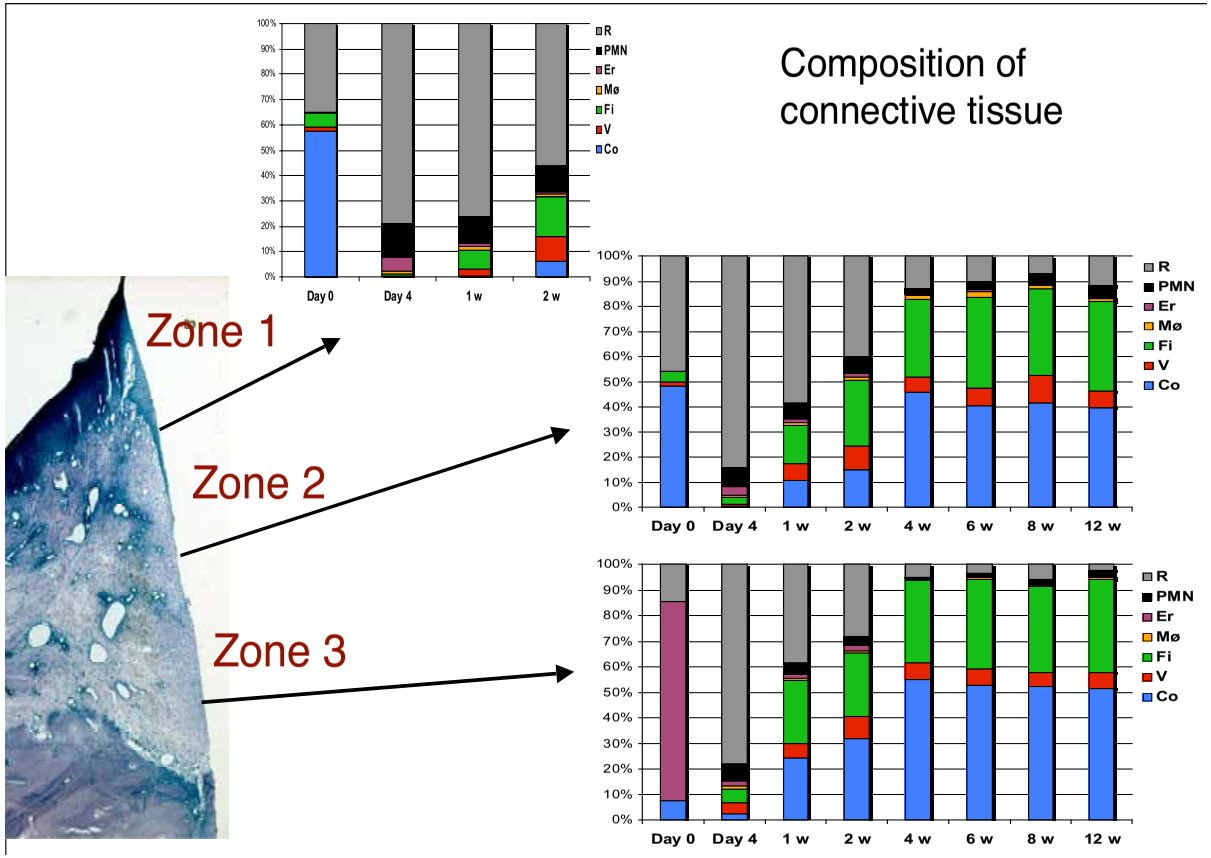

Fig. 5. Histogram illustrating the 3 zones of soft tissue composition at the interface portion of the implants from day 0 to 12 weeks.

Study 2

The overall composition of the connective tissue portion facing the implant was similar at test and control implants at both 4 and 8 weeks of healing. The percentage of collagen increased between 4 and 8 weeks, while the amount of vascular structures and leukocytes decreased. The density of fibroblasts was almost unchanged between 4 and 8 weeks of healing for both test and control implants (Fig. 6).

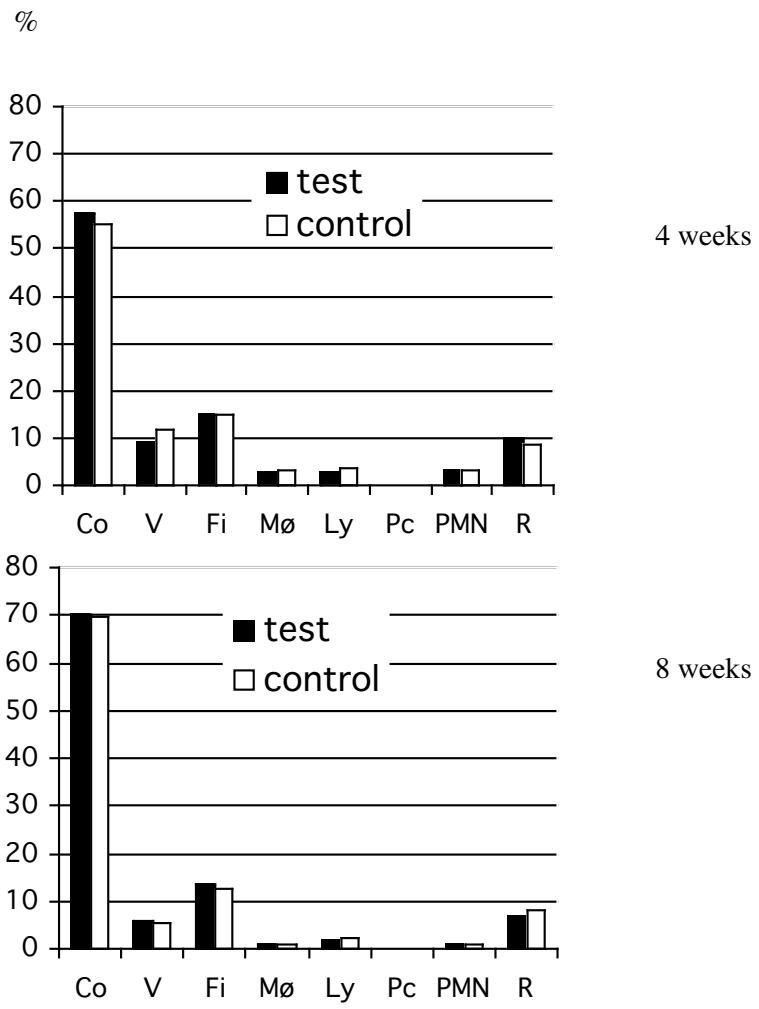

Fig. 6. Histograms illustrating the composition of the connective tissue portion facing the implants after 4 and 8 weeks of healing

Study 3

At 2 months of healing the volume fractions occupied by collagen and fibroblasts were significantly smaller at cast-to (gold-alloy) abutments than that at titanium and ceramic (zirconia) abutments. The proportions of leukocytes and residual tissue, however, were significantly larger at cast-to abutments compared to the titanium and ceramic abutments. At 5 months of healing the barrier epithelium extended to a position apical of the A/F borderline in 4 out of 6 cast-to sites and, hence, impeded the analysis of the connective tissue. The large differences in tissue composition at 2 months between sites representing cast-to abutments on the one hand and Ti and ceramic abutments on the other, persisted at 5 months of healing. Thus, in cast-to sites available for connective tissue analysis the densities of collagen and fibroblasts remained smaller, while the proportions of leukocytes and residual tissue were found to be larger than in sections representing Ti and ceramic abutments (Fig. 7).

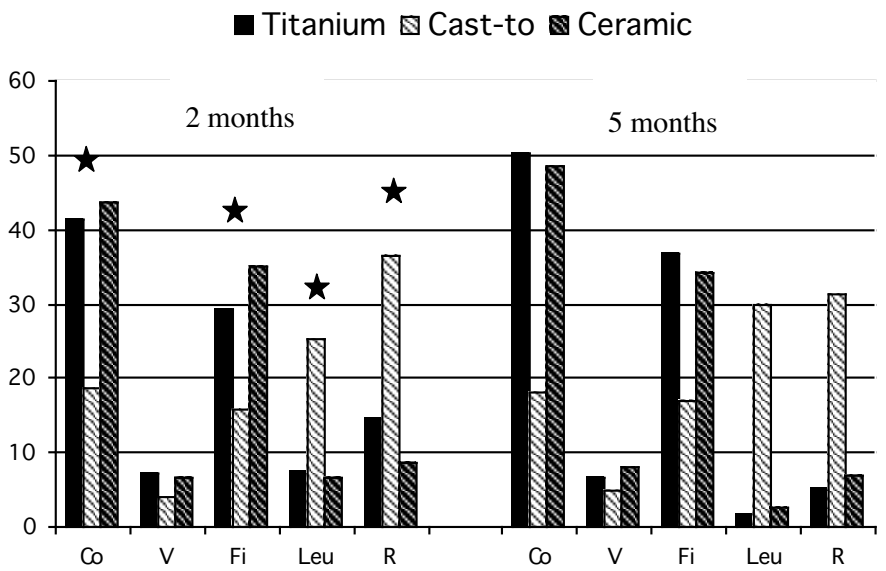

Fig. 7. Histogram illustrating the %volume of the connective tissue components in the interface zone towards the different abutment materials (titanium, cast-to (gold-alloy), ceramic (zirconia)). ★ Indicates $p < 0.05$.

Study 4

The connective tissue portion adjacent to the test abutments (TiOblast) had a higher density of collagen and a lower portion of fibroblasts than that of the control abutments. At both test and control abutments vascular units and inflammatory cells occupied small fractions of the connective tissue interface (Fig. 8).

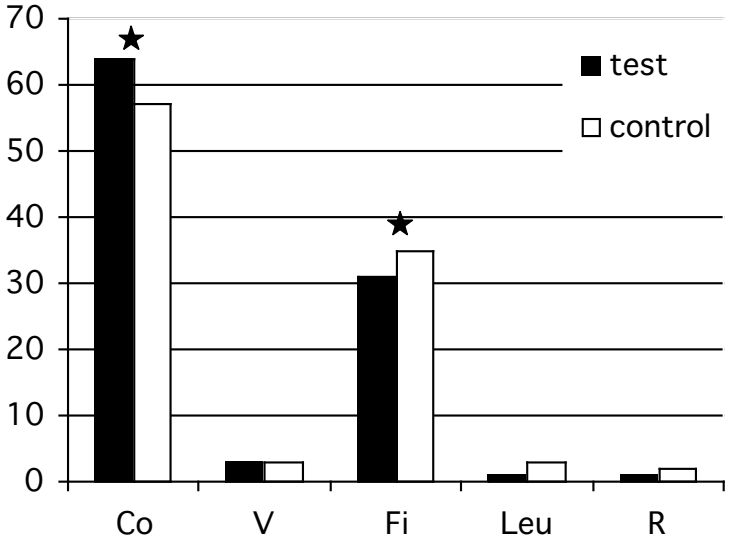

Fig. 8. Histogram illustrating the %volume of the connective tissue components in the interface zone towards the test and control implants. ★ Indicates $p < 0.05$.

Leukocytes within the barrier epithelium

Study 2

There was an increase of leukocytes residing in the barrier epithelium at test implants between 4 and 8 weeks of healing. At control implants, however, the density of leukocytes in the barrier epithelium decreased (Fig.9).

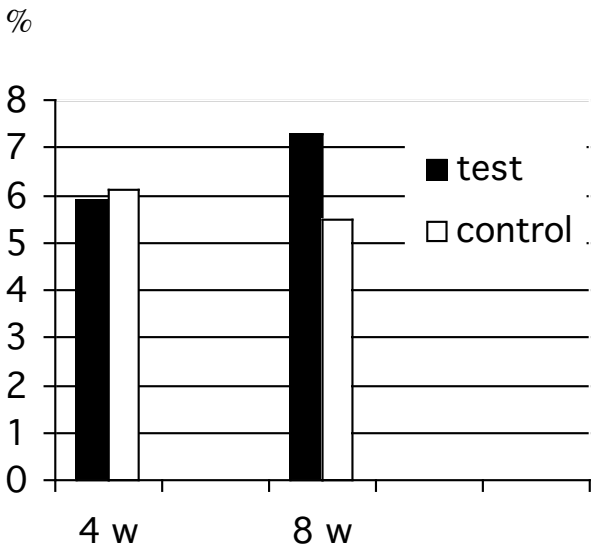

Fig. 9. Histogram illustrating the density of leukocytes residing in the barrier epithelium at test and control implants.

Study 3

At 2 months of healing there was a statistically significant difference in the density of leukocytes within the epithelium between the ceramic (zirconia) abutment sites and the abutment sites made of titanium and cast-to (gold alloy). At 5 months of healing the densities of leukocytes had decreased (Fig. 10).

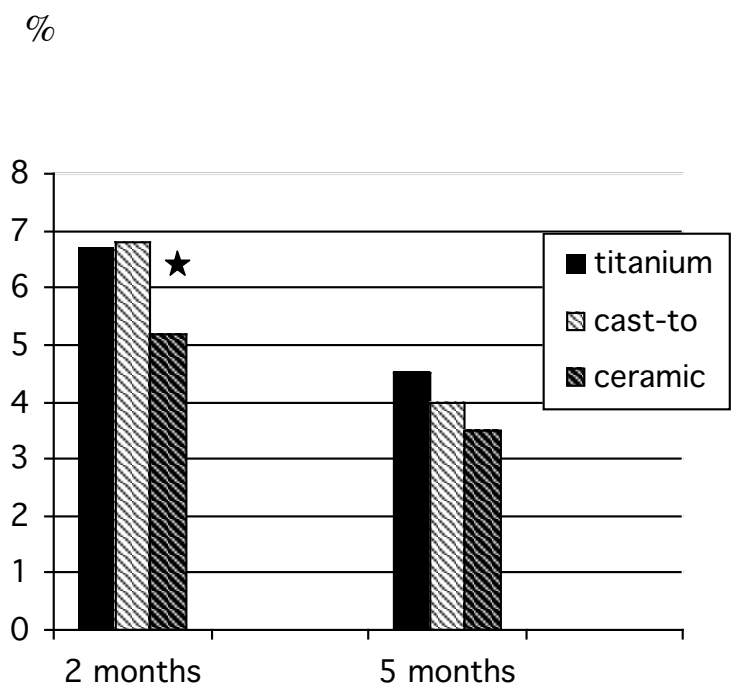

Fig. 10. Histogram illustrating the density of leukocytes residing in the barrier epithelium at the different abutment materials (titanium, gold-alloy and zirconia). ★ Indicates $p < 0.05$.

Scanning electron microscope analysis

Study 2

The SEM analysis from the retrieved test and control implants revealed similar surface characteristics. There were no signs of the dense layer of fibrils as were the case in the pristine test implants (Fig. 11).

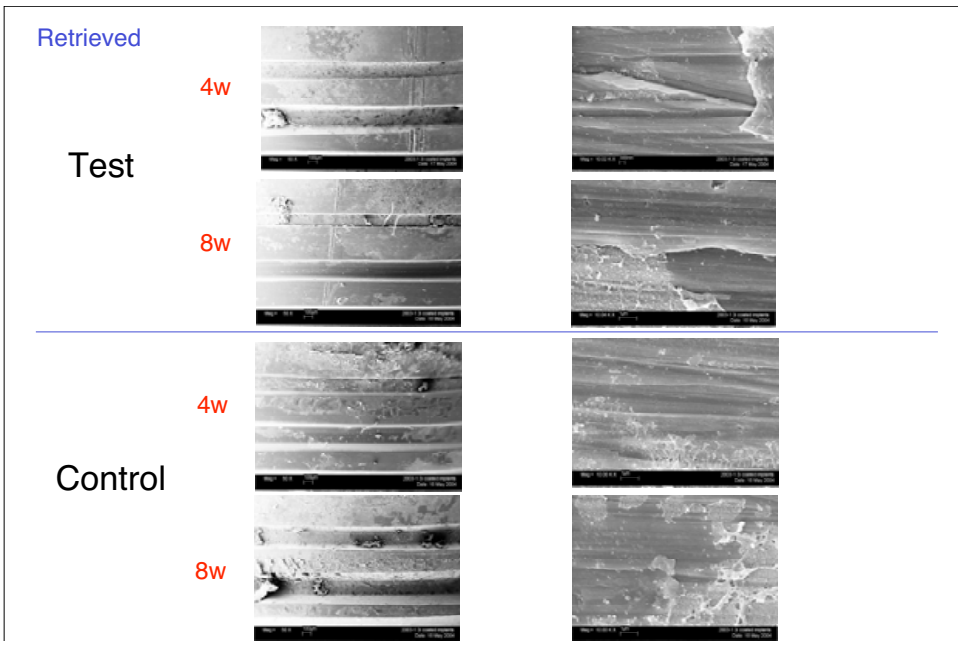

Fig. 11. Scanning electron micrographs from retrieved test and control implants after 4 and 8 weeks of healing, original magnification: x50 and x10.000.

Main Findings

- A coagulum occupied the compartment between the mucosa and the implant immediately after surgery. At day 4 the inflammatory phase of wound healing prevailed with large numbers of leukocytes. Fibroblasts substituted leukocytes in the connective tissue interface at 2 weeks after surgery. The formation of a barrier epithelium was initiated at 1 - 2 weeks. (Study 1)

- The formation of a barrier epithelium was completed at 6 - 8 weeks. Collagen fibers became organized after 4 - 6 weeks of healing. (Study 1, 2)

- The soft tissue dimension, the biologic width, at implants was established after 6 weeks following surgery. (Study 1)

- The vertical dimensions of the soft tissue, the composition of the connective tissue portion facing the implants and the proportions of leukocytes within the barrier epithelium were similar at collagen-coated and un-coated titanium implants after 4 and 8 weeks of healing. (Study 2)

- Implant abutments made of titanium, ZrO_2-based ceramic and Au/Pt-alloy had similar soft tissue dimensions after 2 months of healing. At Au/Pt-alloy abutments the connective tissue interface contained lower amounts of collagen and fibroblasts and larger fractions of leukocytes than at abutments made of Ti and ZrO_2. (Study 3)

- Bone formation coronal of the junction between the implant and the abutment was possible when two-part implants with suitable surface characteristics were placed in a subcrestal position. (Study 4)

- The connective tissue interface to abutments at test sites (TiOblast surface abutments) was comprised of a higher density of collagen and a lower fraction of fibroblasts than at control sites (turned surface abutments). (Study 4)

- Different marginal bone levels at the lingual and buccal aspects were obtained when two-part implants with suitable surface characteristics were placed in sites with buccal bone defects. (Study 5)

Concluding remarks

Animal model

In the present thesis the dog model was used in all experiments. This model has been extensively used in periodontal and implant research and is well documented. Experiments in dogs demonstrated that the dimension of the mucosal attachment to implants was similar to the gingival attachment at teeth and was comprised of an epithelial portion about 1.5-2 mm long and a cell rich connective tissue portion close to the implant that was about 1-1.5 mm high (Berglundh et al. 1991, 1992 a, b). The overall proportions of the dog mandible and the suitable anatomy in the edentulous premolar region after tooth extractions makes it possible to utilize implant components with similar dimensions as those used in humans. Furthermore, the dog is also suitable with regard to accessibility for clinical examinations and oral hygiene procedures. Ethical considerations, study design and power calculations determine the sample size of animals in experimental research. The results from experiments in a small homogeneous animal group, however, should always be interpreted with caution. Data on e.g. healing time might not always be directly transferable to the clinical situation. Thus, a given sequence of soft tissue integration to implants in a dog may not correspond exactly to an expected outcome in humans. In this context it is important to realize that differences in tissue response in healing may sometimes be more pronounced between different human subjects than between animals and humans.

Evaluation methods

Linear measurements

The vertical dimensions of the peri-implant mucosa, the position of the marginal bone level and the distance from the bone crest to bone to implant contact

(study 4 and 5) were evaluated in ground sections and decalcified Epon® embedded sections. All histological measurements were made in such a way that the examiner was blinded regarding e.g. test and control or healing time. The vertical distances that were assessed in the linear measurements may vary depending on the geometry of the particular implant analyzed. The "true" dimension of the mucosal attachment to implants, however, which is evaluated by outlining the different contours of the implant, is independent of the implant geometry.

Connective tissue composition

The measurements of the connective tissue composition were performed in the decalcified Epon® embedded sections. These histological sections are about 3μm thin, which makes it possible to perform analysis using a high magnification (x1000) in a light microscope and hereby distinguish between different cells and connective tissue components. The composition of the connective tissue in the interface zone towards the implant reflects the integration potential of the biomaterial used. The quality of the connective tissue is in contrast to the linear measurements not depending on the geometry or dimensions of different types of implants. Berglundh et al. (1991) in a dog study analyzed the quality of a100μm wide connective tissue zone lateral of the root cementum at teeth and the titanium surface at implants. It was found that the peri-implant mucosa contained a significantly larger volume of collagen and smaller volume of fibroblasts than the corresponding compartment of the gingiva at teeth. In a later study by Moon et al. (1999) it was shown that the inner zone (40 μm) of the connective tissue immediately lateral to the titanium implant surface differed significantly in composition compared to an outer zone (160 μm). The inner zone contained less amounts of collagen and vessels but a higher amount of fibroblasts compared to the outer zone. In study 2 of the present thesis a 100 μm wide zone immediately lateral to the implant surface

was measured whereas in study 3 and 4 the measured zone was 80 μm wide. The difference in width of the connective tissue zones may explain the different results of collagen and fibroblast density found in study 2 as opposed to the other two studies (3 and 4). Thus in study 2 the findings on 70% collagen and 13% fibroblasts corresponded to the data representing the outer zone measured by Moon et al. (1999). In studies 3 (the tissue around Au/Pt alloy excluded) and 4 the amounts of collagen and fibroblasts were 50-64% and 31-37%, respectively, which corresponded to the results from the "inner zone" measurements by Moon and coworkers (1999).

Leukocytes within the barrier epithelium

The measurements of residing leukocytes in the barrier epithelium were performed in the decalcified Epon® embedded sections that were about 3 μm in thickness. As discussed above such thin sections allows analysis using a high magnification in the light microscope (x1000). Berglundh et al. (1992) investigated the volume fraction of leukocytes within the barrier epithelium in normal healthy gingival and peri-implant tissues. It was found that the volume fraction of these cells was 0.9% in the peri-implant tissue and 0.6% in the gingival tissue. After 21 days of plaque accumulation the values had increased for both the gingival and peri-implant tissues and were significantly higher than those in healthy tissues. This demonstrated that plaque accumulation at implants and teeth resulted in the same host response. Different materials and surfaces of implants might challenge the immune response differently and high amounts of migrating leukocytes through the barrier epithelium may reflect an impaired epithelial attachment to the implant surface. Thus, in study 2 and 3 in the present thesis this evaluation method was applied to determine the host response and the epithelial attachment to the collagen coating and the different abutment materials. The results of the measurements of the volume percentage of

infiltrating leukocytes in the barrier epithelium in both study 2 and 3 corresponded to the data representing inflamed gingival tissues in the studies by Berglundh et al. (1989, 1992 a, b). This finding is difficult to interpret and it should be realized that a meticulous oral hygiene program was performed in studies of the present thesis and that similar conditions was provided for test and control implants.

Data analysis

The study design of applying intra-individual evaluations prompted the use of paired analysis with the animal as the statistical unit. Considering that the variables used, e.g. biological length units of tissue components, represent a normal distribution in a background population parametric tests were used. Thus, the Student t-test for paired observations and the two-way analysis of variance (ANOVA) together with the Student-Newman-Keuls test were used for statistical analysis in the present thesis.

Study 1

In study 1 the wound healing process in the mucosa surrounding implants was evaluated. Similar wound healing processes as after mucogingival surgery at teeth were investigated and described previously (Bernier et al. 1947, Waerhaug 1955, Wilderman et al. 1960, 1963). Wilderman et al. (1960) in an experimental study in 10 dogs excised gingival tissues after flap reflection, which resulted in 5 x 32 mm large wounds. Biopsies were collected after 0 hrs, 2, 4, 6, 10, 14, 21, 28, 93, 185 days. It was reported that immediately after surgery (0 hrs) the wound was covered by a blood clot and at 2- 4 days after injury a proliferaton of “young” connective tissue was observed beneath the clot. The “young” connective tissue extended over the entire wound. At day 14 the “young” connective tissue exhibited a definite collagen fiber formation. The wound was

fully covered by epithelium after 21 days. Wilderman et al. (1960) further reported that the process of complete maturation and functional orientation of the involved tissues extended over a six-month period.

The sequence in soft tissue healing around implants was not as extensively investigated. In one experimental study by van Drie et al. (1988) that involved 4 dogs, biopsies were obtained at 4 occasions (1, 3, 7, 15 weeks). It was reported that no distinct time-related changes occurred regarding the volume density of collagen and the position of the epithelium during the first 15 weeks of tissue healing. In study 1 of the present thesis a large number of animals (20) were used. It was thereby possible to study the sequential wound healing process that took place after implant surgery. The study showed that the wound healing process after implant installation followed the phases of inflammation, proliferation and maturation/ remodeling as described by Martin (1997), Ten Cate (2003) and Myers (2004). In study 1 it was shown that the biological width (the epithelial and the connective tissue dimensions) was established after 6 weeks following a surgical procedure with buccal and lingual mucoperiosteal flap techniques. The fact that the biological healing process requires a considerable amount of time before tissues become stable provides guidelines to clinicians in the planning of implant therapy and also to future research projects. Whether the healing is influenced by installation techniques such as flapless surgery or “punching” techniques remains to be evaluated.

Study 2

At teeth, a mechanical attachment is established between collagen fibers in the connective tissue (dentogingival, dentoperiosteal and transseptal fibers) and the root cementum. These collagen fibers are formed concomitant with cementum formation during root development of the tooth and become embedded in the newly formed cementum (Lindhe et al. 2008). In study 2 the test titanium implants were coated with a purified porcine type 1

collagen. The hypothesis was that the organic coating should mimic the organic component of the root cementum in the periodontium and, thus, during the wound healing process the collagen fibers in the mucosa would attach to the coating. The analysis of the soft tissue attachment to implants in study 2 showed no differences in dimensions or connective tissue composition between the coated and the un-coated titanium implants. Although some collagen fibers in the peri-implant mucosa were aligned in oblique directions towards the implant surface, the direction of the fibers were parallel with the implant in areas close to the surface. There were no signs of mechanical attachment between the collagen coated implants and the surrounding peri-implant mucosa. One explanation to this finding was that the ultra thin (40nm) collagen coating was probably degraded during the inflammatory phase of the wound healing process. The task of this phase in wound healing is to "clean the wound of debris and set the stage for further healing by calling cells necessary for repair to the injured area" (Myers 2004). The porcine-derived collagen did not generate any adverse reactions in the connective tissue, which indicated that the dog apparently "tolerated" collagen xenograft material. The tissue response to organic materials from other species was previously analyzed in studies on regenerative procedures at teeth (Owens & Yukna 2001, Rothamel et al. 2005). It may be speculated that the achievement of a mechanical attachment between the implant surface and the peri-implant mucosa provides an improved seal and, thus, more effectively protects the underlying peri-implant bone against products in the oral cavity. Further research is needed to achieve a mechanical attachment between collagen fibers in the mucosa and implants provided with organic surfaces.

Study 3

The mucosal appearance to titanium is well investigated but the esthetical awareness in implant dentistry sometimes demands the use of other materials than titanium in the abutment part of the implant. In an experiment in dogs Abrahamsson et al. (1998 a) analyzed the mucosal attachment at abutment materials made of titanium, gold alloy (Au, Pt, Pd, Ir), ceramic (highly sintered Al_2O_3) and dental porcelain fused to gold. It was reported that the titanium and the ceramic abutments formed proper mucosal attachment, while at the abutments made of gold and dental porcelain bone resorption and recession of the mucosal margin occurred. Later, the zirconia material used in the orthopedic field was introduced in implant dentistry. Although mechanical properties for zirconia (ZrO_2) were suggested to be superior to ceramics made of alumina (Al_2O_3) (Piconi et al. 1999) there were no reports on soft tissue integration to the zirconia material. Thus, the 3rd study in the present thesis was designed to analyze the soft tissue formed at different abutment materials. The abutments used were custom-made from (i) commercially pure titanium, (ii) ceramic (ZrO_2) and (iii) a traditional casting alloy (Au/Pt- alloy). Healing periods of 2 and 5 months were studied. The shorter healing period (2 months) was chosen with the information gained from the first study in the present thesis, which demonstrated that the biological width was established after 6-8 weeks following surgery. The longer healing period of 5 months was suggested to provide information on the matured soft tissue. During the experiment the initially placed healing abutments were exchanged to the custom-made abutments of different materials 1 month after implant installation. This procedure was applied to mimic a frequently used clinical protocol where healing abutments are used prior to the placement of standard or custom-made abutments. Abrahamsson et al. (2003) studied the tissue response to a single shift of abutments (from healing abutment to permanent abutment). It was found that a shift from a

healing abutment to a permanent abutment resulted in similar dimensions and quality of the transmucosal attachment as that surrounding permanent abutments placed directly after surgery. The results from the third study in the present thesis demonstrated that abutments made of titanium and ZrO_2 promoted proper conditions for soft tissue integration, while abutments made of Au/Pt alloy failed to establish appropriate soft tissue integration. These results verified that abutments made of ZrO_2 are preferable to abutments made of Au/Pt- alloy.

Study 4

A natural appearance of the peri-implant mucosa includes a soft tissue integration that is established in a supracrestal compartment. If an angular bony defect is present (difference in BC-B) a substantial portion of this integration (the biological width) occurs subcrestally, which may effect the possibility to maintain or reform a papilla between implants (Tarnow et al. 2003). Thus, it is of great importance to sustain the bone to implant contact at the implant/abutment level. In the literature advantages and disadvantages related to one-part and two-part implant systems were discussed. The interface between the components (abutment/implant) at two-part implants was suggested to be a "weak point". As discussed in study 4 the microbial leakage and the possible movement between the two implant components were suggested to cause tissue reactions resulting in a more apical position of the peri-implant bone at two-part implants (see discussion section in study 4). The fourth study in the present thesis challenged the conclusions presented previously about two-part implants placed in a subcrestal position (Hermann, 1997, 2000 b, 2001 a, b, King et al. 2002, Broggini et al. 2003). Thus, in study 4 implants provided with a conical implant/abutment interface design and a solid abutment (closed system) were placed in a subcrestal position. The abutment parts of the test implants were provided with a

rougher surface than that at the control implants. It was demonstrated that the marginal bone level at the test implants was identified in a more coronal position than that at the control implants. In 40% of the test implants the bone to implant contact extended coronal of the junction of the two components, i.e. in contact with the abutment part of the implant. It may be suggested that the rougher surface of the test implants provided an enhanced ability to retain the coagulum that during the wound-healing process was replaced by bone and soft tissue as discussed in study 1. In an experimental study from Broggini et al. (2006) two-part implants with an "open system" was used and the density of inflammatory cells surrounding implants with a supra crestal (1mm above the crest), crestal or subcrestal (1mm below the crest) implant-abutment interface was descriebed. It was reported that the density of neutrophils increased progressively when the implant abutment interface depth increased. In study 4 the fractions of leukocytes in the connective tissue at both test and control implants were small (1.3% and 2.9%) and similar to the values representing normal conditions around teeth (2.5% leukocytes) in the study by Berglundh et al. (1992). The model to test the solid abutment with the conical seal design provided with different surface characteristics in study 4 provided evidence of osseointegration coronal to the abutment/fixture interface of two-part implants.

Study 5

There is no clinical relevance in placing the entire circumferential part of an implant in a subcrestal position. The bone crest in implant patients, however, often exhibits insufficient horizontal dimensions as discussed in study 5. Instead of using resective therapy of the ridge at implant installation a preservation of a sloped bone crest would be preferable to support the soft tissue and thereby possibly achieving esthetic advantages. Carmangnola et al. (1999) performed a study with a design that had many features in

common with that in study 5 of the present thesis. Implants were placed in bone with large buccal defects (6mm of the buccal bone wall was resected) and implants were placed in such a way that the lingual part of the implant was invested in bone, whereas the buccal marginal portion of the implant was exposed. It was reported that despite a discrepancy of about 1 mm in marginal bone levels between the buccal and lingual aspects, the soft tissue margin was located at similar levels bucally and lingually. The hypothesis of study 5 was to test if the lingual bone could be preserved using the concept with a roughened solid abutment with a conical implant/abutment interface. The study demonstrated that different lingual and buccal bone levels were obtainable when two-part implants with suitable surface characteristics were placed in sites with buccal bone defects. These results promote prerequisites for future implant development.

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