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Biological width around implants

Histological evidence: a review on animal studies

Espace biologique péri-implantaire

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ABSTRACT

Soft tissue relationship to implant surface is one of the most challenging area for implant manufacturers as it is evident by different kind of connections, implant shoulders and platforms. The presence of a quite constant dimension of soft tissue attachment to dental implants, similar for many features to dentogingival junction, has been well documented in histological studies on animal models. Based on similar histological studies, the influence of different variables, like the surgical technique, the surgical protocol, implant loading, implant structure, titanium surfaces and abutment materials on peri-implant biological width has been evaluated. The aim of this study is to produce a review on these topics.

KEY WORDS

Biologic width, implant, soft tissues healing, junctional epithelium, connective tissue.

RÉSUMÉ

Les relations entre les tissus mous et la surface implantaire représentent un défi considérable pour les fabricants d'implants si l'on considère les différents types de connexions, de cols implantaires et de plateformes qui existent. La présence assez constante d'une hauteur d'attache conjonctive sur les implants dentaires, qui est semblable sous bien des aspects à la jonction dento-gingivale, a été bien documentée dans des études histologiques réalisées sur des modèles animaux. En se fondant sur des études histologiques similaires, on a pu évaluer l'influence de différentes variables, telles que la technique chirurgicale, le protocole chirurgical, la mise en charge de l'implant, la structure de l'implant, les surfaces en titane et les matériaux composant les piliers, sur l'espace biologique péri-implantaire. Le but de cette étude est de faire le point sur ces questions.

MOTS CLÉS

Espace biologique, implant, cicatrisation des tissus mous, épithélium de jonction, tissu conjonctif.

Introduction

The large use of osteointegrated implants in modern dentistry and the increasing esthetic demands for implant rehabilitations has focused attention on soft tissue reactions to implant placement specially in the area of soft tissue relationship to implant surface. Probably this is, at the present time, the most challenging area for implant manufacturers as it is evident by different kind of connections, implant shoulders and platforms. The transmucosal area constitutes an effective barrier between the oral environment and the peri-implant bone and in many features it is similar to dentogingival junction.
The morphology of dentogingival junction has been studied since 1959 by Sicher, who found both an epithelial and a connective tissue attachment to the tooth (Sicher, 1959). In 1961, Gargiulo *et al.* measured the vertical dimension of this structure and named it "biological width" (Gargiulo *et al.*, 1961). Biological width was composed by sulcus depth (SD), junctional epithelium (JE) and connective tissue attachment (CTA). The mean value was 2.73 mm and 2.04 mm for JE + CTA.
These findings have been confirmed by Vacek in 1994: in this study, mean measurements were 1.34 ± 0.84 mm for sulcus depth; 1.14 ± 0.49 mm for epithelial attachment; 0.77 ± 0.32 mm for connective tissue attachment (Vacek *et al.*, 1994). Authors emphasized that connective tissue attachment has a variable width within a more narrow distribution and range than the epithelial attachment and sulcus depth.
Peri-implant tissues have many similarities with periodontal tissues and dentogingival junction, but there are also obvious anatomical differences like the lack of periodontal ligament and a different vascular distribution (Berglundh *et al.*, 1991). Peri-implant biological width has been studied and measured in both histological animal studies and clinical human studies.
The aim of the present study is to produce a review on the dimensions of the peri-implant biological width and to analyze the factors that may determine variations on biological width values.

Structure and biological dimensions

In the first animal studies of Berglundh *et al.* in 1991, it was demonstrated that the peri-implant mucosa established a cuff-like barrier which adhered to the surface of the titanium abutment (Berghlund *et al.*, 1991). The peri-implant tissue is a scar tissue that repairs the injury of the implant insertion: the soft tissues of the edentulous crest, once repositioned and sutured, participate to the formation of a "new" tissue that protects the exposed bone and seals the emergence of the implant.
As the gingiva, the peri-implant mucosa has a well-keratinized oral epithelium which is continuous with a junctional epithelium that faces the titanium surface.
The structure of junctional epithelium is still matter of debate: a junctional epithelium similar to the junctional epithelium on teeth has been documented in a number of studies from different authors (Abrahamsson *et al.*, 1996, 1999, 2002; Berglundh *et al.*, 1991, 2007; Moon, 1999). In a work on rats (Ikeda *et al.*, 2000), the presence of a basal lamina and hemidesmosomes in peri-implant junctional epithelium was confirmed although it was stated that the finding of a basal lamina was less evident than on the control tooth sites, and it was well detectable only in the lower part of junctional epithelium.
Results from a recent article by Shioya *et al.* differ greatly by the work of Ikeda (Shioya *et al.*, 2009): one week after implant insertion, peri-implant epithelium was observed, 8 weeks after implant insertion, the peri-implant epithelium receded, and the implant interface appeared to be sealed by aligned special cells with surrounding elongated fibroblasts and bundles of collagen fibers. No hemidesmoses and no basal lamina were found in this tissue. This finding is in contrast with the previous scientific literature and opens new phases for further research.
The most important difference between the two tissues is represented by the collagen fibers departing from the bone crest that are not inserted on the titanium surface and run parallel to implant surface, following the implant orientation **(fig. 1 and 2)**. Furthermore according to Berglundh *et al.*, experimental model, the collagen content of the peri-implant mucosa is higher, while the fibroblast density is much lower when compared to the gingival tissue (Berglundh *et al.*, 1991).
Another important observation was that all gingival and peri-implant units examined were free from infiltrates of inflammatory cells. It was suggested that under the conditions of that study, both types of soft tissues,

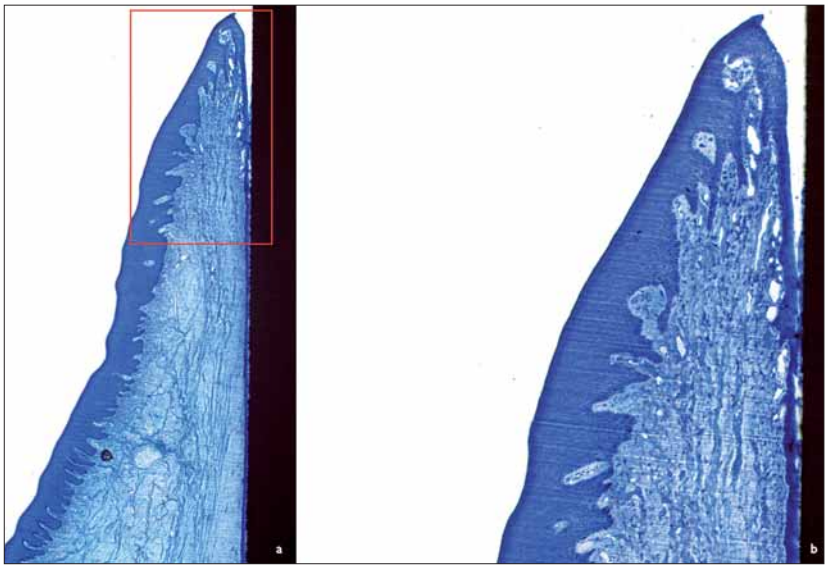

Fig. 1. a. Immediate implant (3i Osseotite Certain, Biomet 3i, USA) and surrounding tissues after 8 weeks of healing. The keratinized oral epithelium continues with the junctional epithelium which is in intimate contact with the titanium surface of the abutment. Toluidine blue staining. Original augmentation 5X. **b.** Detail of (a). The connective tissue located immediately below the epithelium is rich in collagen fibers and poor in cells.

Fig. 1. a. Implant à mise en charge immédiate (3i Osseotite Certain, Biomet 3i, États-Unis) et tissus environnants après 8 semaines de cicatrisation. L'épithélium oral kératinisé est en continuité avec l'épithélium de jonction qui est en contact intime avec la surface en titane du pilier. Coloration au bleu de toluidine. (grossissement × 5) **b.** Détail. Le tissu conjonctif situé immédiatement en dessous de l'épithélium est riche en fibres de collagène et pauvre en cellules.

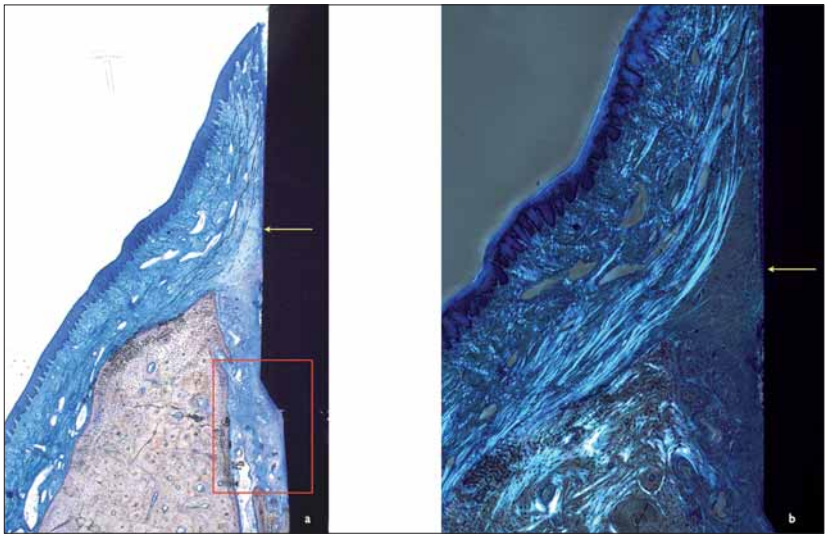

Fig. 2. Immediate implant (3i Osseotite Certain, Biomet 3i, USA) and surrounding tissues after 8 weeks of healing. The supracrestal connective tissue is cell rich in the area located close to the implant. Collagen fibers extend from periosteum of the bone crest and run lateral to this area with a direction that is parallel to the implant-abutment surface. Toluidine blue staining. Original augmentation 2.5X.

Fig. 2. Implant à mise en charge immédiate (3i Osseotite Certain, Biomet 3i, États-Unis) et tissus environnants après 8 semaines de cicatrisation. Le tissu conjonctif supracrestal est riche en cellules dans la zone située à proximité de l'implant. Les fibres de collagène s'étendent du périoste de la crête osseuse et se dirigent latéralement par rapport à cette zone dans une direction qui est parallèle à la surface implant/pilier. Coloration au bleu de toluidine. Grossissement × 2,5.

the gingiva and the peri-implant mucosa, present a proper potential to prevent subgingival plaque formation.

Buser *et al.* investigated the soft tissue dimensions around three different titanium surfaces, a rough surface, a sandplasted surface and a polished surface (Buser *et al.*, 1992). No significant difference concerning soft tissue reactions were found between the three implant surfaces. The soft tissue barrier was composed by a sulcus with a non keratinized sulcular epithelium, a junctional epithelium, and a supracrestal connective tissue with an area of dense circular fibers near to the implant surface.

Circular fibers were found in the inner zone of connective tissue, next to the titanium surface; in the outer layer, horizontal and vertical fibers were found: these fibers were

running from the periosteum and the alveolar crest towards the oral epithelium. Authors reported that the orientation of the fibers differs in rough and smooth surfaces: smooth surfaces revealed an orientation of fibers parallel to the implant surface, while porous-coated surfaces promoted the formation of perpendicular fibers. The presence of this fibers has been confirmed in a recent study of Shioya *et al.* (Shioya *et al.*, 2009). The overall tissue was described as "an inflammation free scar tissue". The zone of dense collagen fibers was surrounded by a looser connective tissue with a 3-dimensional network of collagen fibers running in different directions. Berghlund *et al.* compared the vascular system of the periodontal and peri-implant tissues in the beagle dog (Berglundh *et al.*, 1994). In the tooth, blood vessels of the sub-epithelial oral plexus appeared as they were terminal branches of the larger supraperiosteal blood vessels. The density of the vascular units in this plexus was increased in the marginal portion of the free gingival tissue unit; there were capillary loops projected into the papillae with a diameter of 7-10 m. The vasculature of tissue portion lateral to the enamel surface was located a few microns from the basal cell layer of the junctional epithelium and formed a mesh of vascular units called crevicular plexus. The vessels of the supracrestal connective tissue, lateral to the root cementum, were found to originate mainly from the vasculature of the periodontal ligament with a minor contribution from the larger supraperiosteal vessels. The two systems showed an anastomotic system. In the peri-implant mucosa thin capillary loops were found, they were terminal branches of supraperiosteal vessels with a diameter of 7-10 m, similar to those observed in the periodontal tissues. Lateral to the junctional epithelium at a distance of 50 m, a crevicular plexus could be observed. In the area of connective tissue two different portions could be found: a central part 300-500 m wide in intimate contact with the implant-abutment interface that was poor of blood vessels with only few capillaries present and a lateral part with larger vessels originating from the supraperiosteal arterioles.

The existence of a central area poor of vessels have been well documented by Moon *et al.* in an histological animal experiment (Moon *et al.*, 1999). Authors concluded that the junctional epithelium was 2 mm long and 40 m wide. Apical to the junctional epithelium the connective tissue portion (200 m wide) presented a 40 m wide zone in close contact to the titanium surface. This zone was characterized by the absence of blood vessels, abundance of fibroblasts oriented with their long axis parallel to the implant surface and thin collagen fibers that extended from the periosteum of the bone crest parallel to the implant surface (Astra Tech Implants) **(fig. 3)**.

Laterally, an area with less fibroblasts, greater collagen fibers and vascular structures could be evidenced. Connective attachment was composed by 80,61% collagen fibers, 12,98% fibroblasts, 3,42% vascular structures and 3,0% residual tissues. Authors concluded that due to the presence of a fibroblast rich layer next to the implant surface the peri-implant connective tissue was a tissue with a high turnover. These data were in contrast with previous studies that described the peri-implant tissue as scar-like tissue (Buser *et al.*, 1992).

Some authors have stressed the hypothesis of a connective tissue attachment to the implant surface. Few experiments and human biopsies have demonstrated collagen fiber bundles functionally orientated and running in different directions (Schwarz *et al.*, 2007; Nevins *et al.*, 2008). The precise tridimensional orientation of the collagen fibres in the periimplant mucosa have been described by Schierano *et al.* (Schierano *et al.*, 2002) contrasting this hypothesis.

Fig. 3. Densely packed connective tissue with absence of blood vessels, abundance of fibroblasts with thin collagen fibers. Fibroblast are oriented with their long axis parallel to implant surface. Toluidine blue staining. Original augmentation 10X.

Fig. 3. Tissu conjonctif tassé de façon dense avec absence de vaisseaux sanguins, abondance de fibroblastes avec de fines fibres de collagène. Les fibroblastes sont orientés selon leur grand axe parallèlement à la surface de l'implant. Coloration au bleu de toluidine. Grossissement × 10.

In 1996, Berglundh and Lindhe evaluated in an animal study the biologic width around implants (Brånemark Implant System) at sites showing a surgically created thin mucosa compared to control sites demonstrating a normal mucosa (Berglundh and Lindhe, 1996). Results after 6 months of healing demonstrated that the biological width dimensions were rather similar: the junctional epithelium measured approximately 2.1 mm and 2.0 mm in the control and test groups, respectively; the corresponding values for the connective tissue were 1.8 and 1.3 mm. Interestingly the wound healing processes at the test sites included bone resorption and thus the formation of an angular bone defects. The authors indicated the need of a minimum dimension of the biological width in order to accommodate the soft tissue healing process. Recently, Berglundh *et al.* described in detail the morphogenesis of the peri-implant mucosa around titanium implants in dogs (ITI Implant System) (Berglundh *et al.*, 2007). Immediately after surgery (day 0) a coagulum occupied the space between the mucosa and implant surface and between the mucosa and the alveolar process **(fig. 4)**. At 4 days of healing, the blood clot was infiltrated by numerous granulocytes and an initial mucosal seal was established by the clustering of leukocytes in a dense fibrin network. At 1 week of healing, an area of leukocyte-infiltrated fibrin tissue was still present, but it was smaller and localized only close to the soft tissue margin. The central part of the tissue was occupied by fibroblasts and collagen fibers. At 2 weeks, the peri-implant mucosa adhered to the implant surface through a connective tissue that was rich in cells and vascular structures. First signs of proliferation of junctional epithelium could be observed. At 4 weeks, the junctional epithelium was completely formed and in a more apical position a mature connective tissue could be observed. In later specimens (6-8 weeks), maturation of the connective tissue with a dense layer of elongated fibroblasts at titanium interface was evident. Fibroblasts were situated between the collagen fibers which were oriented mainly parallel to the titanium surface. From a dimensional point of view, the biologic width increased during the healing period (mainly between 1 and 2 weeks) from 3.1 to 3.5 mm. Barrier epithelium extended to a position about 0.5 mm apical to the mucosal margin, while at 4 weeks the distance was 1.42 mm. At the end of the study (6-12 weeks), the barrier epithelium varied between 1.7 and 2.1 mm.

These findings corroborated previous clinical results from a longitudinal study (Bengazi *et al.*, 1996). Authors evaluated the alterations in the position of the peri-implant soft

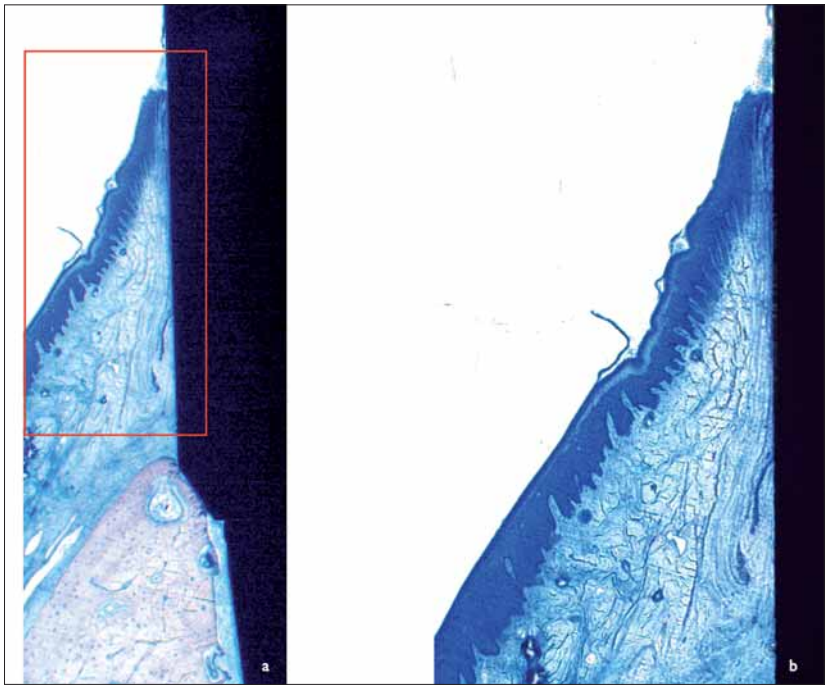

Fig. 4. a. Immediate implant (3i Osseotite Certain, Biomet 3i, USA) and surrounding tissues after 4 hours of healing. A coagulum occupies the void between the implant surface and the socket wall. Toluidine blue staining. Original augmentation 2.5X. **b.** Detail of (a). Remnants of a junctional epithelium can be recognized. Toluidine blue staining. Original augmentation 5X.

Fig. 4. a. Implant à mise en charge immédiate (3i Osseotite Certain, Biomet 3i, États-Unis) et tissus environnants après 4 heures de cicatrisation. Un coagulum occupe l'espace entre la surface implantaire et la paroi de l'alvéole. Coloration au bleu de toluidine. Grossissement × 2,5. **b.** Détail. On peut identifier les restes d'un épithélium de jonction. Coloration au bleu de toluidine. Grossissement × 5.

tissue margin, during a 2-year period follow-up. One hundred and sixty-three Brånemark implants were inserted into 41 patients that were periodonatlly evaluated and re-examined after 6 months, 1 and 2 years. The results indicated an apical displacement of the soft tissue margin that mainly occurred during the first 6 months of observation. Lingual sites in the mandible showed the most pronounced soft tissue recession, decrease of probing depth, and decrease of width of masticatory mucosa. Authors suggested that the recession of the periimplant soft tissue margin could be the result of the re-modelling of the soft tissue in order to establish the "appropriate biological dimensions" of the peri-implant soft tissue barrier.

Factors that may influence peri-implant biologic width

Surgical technique

Abrahamsson *et al.* evaluated the influence of the surgical protocol (i.e. one stage *versus* two stage) on the soft tissue healing around 3 different implant systems (Astra Tech Implants, Brånemark and Bonefit-ITI) (Abrahamsson *et al.*, 1996). The histological results demonstrated similar dimension and composition of the epithelial and connective tissue components.
Ericsson *et al.* found similar soft tissue adaptation and proper osseointegration in Brånemark implants installed according to a 1-stage or to a 2-stage surgical procedure (Ericsson *et al.*, 1996). These findings were further confirmed by Hermann *et al.* who compared non-loaded implants with loaded implants (ITI Implant System) at different time intervals (3-12 months) and according to a submerged or non-submerged healing (Hermann *et al.*, 2000). The study demonstrated that the dimension of the biological width around non-submerged, one-piece titanium dental implants was not different whether the implants were unloaded or loaded for a period of 1 year. Nevertheless differences were observed in the dimensions of the components of the biological width (sulcus depth, epithelial junction and connective tissue) at the three different healing intervals. Histometric measurements demonstrated that although the biologic width dimension remained constant over the 15-month healing period, a decrease of the sulcus depth and connective tissue contact were observed whereas an increase of the junctional epithelium occurred.
Thereafter data from different authors support the conclusion that a similar soft tissue dimensions is established regardless the use of a submerged or a nonsubmerged installation technique.

Loading

The influence of loading on soft tissue healing around implants was one of the topics most frequently investigated.
Cochran *et al.* evaluated the dimension of the implanto-gingival junction around non submerged loaded and not loaded implants testing two different surfaces (SLA and TPS) at 3 and 12 months after implant placement (Cochran *et al.*, 1997). At 3 months, the dimension of the constituents of the biological width in the unloaded group were 0.49 mm for the sulcus depth (SD), 1.16 mm for the junctional epithelium (JE), and 1.36 mm for the connective tissue component (CTC). The corresponding measurements in the loaded group were 0.50 mm for SD, 1.44 mm for JE, and 1.01 mm for CTC for the loaded group. Results were similar after 12 months of loading, confirming that the biological width around implants resembles the one present around teeth and that the dimension of its constituents are independent from the loading variable.
Siar found similar results comparing immediate *versus* delayed implant loading (Siar *et al.*, 2003). The overall mean value of the biologic width was 3.9 mm in the immediate group and 3.8 mm in the delayed group. They concluded that no statistical differences were observed in the dimensions and compositions between the two groups.
In a review of Glauser, it was concluded that, on the basis of the few available data, "once immediately loaded or loaded and restored implants integrate successfully, they appear to show a soft-tissue reaction with regard to periodontal as well as morphologic aspects comparable with those of conventionally loaded implants" (Glauser *et al.*, 2006).

Titanium surfaces and abutment materials

The reaction of cells and tissues to implanted foreign bodies depends on the material's properties and its behavior upon contact with the body fluids. Abrahamsson *et al.*

demonstrated that surface characteristics (smooth *vs* rough titanium surfaces) do not influence the biological width dimension (3i Implant System) (Abrahamsson *et al.*, 2002). More recently, new titanium surfaces have been indicated to determine a better quality of peri-implant soft tissues relationship (Rossi *et al.*, 2008) (ITI Implant System). Glauser *et al.* demonstrated in a histological study that the dimension of the biologic width (range 4-4.5 mm) in humans are similar to values found on animal models and that the soft tissue formed to oxidized and acid etched implants showed a minor epithelial down-growth and longer connective tissue seal when compared to machined implants (Glauser *et al.*, 2005).

In two different works, it has been demonstrated that abutment materials influenced the histological outcome on biologic width dimensions and in particular, titanium and zirconia abutments seemed to produce better histological results than gold and platinum abutments (Welander *et al.*, 2008; Abrahamsson *et al.*, 1998). Nevertheless, these findings were not consistent with a later study from the same group (Abrahamsson et Cardaropoli, 2007) that reported that the peri-implant soft tissue dimensions were not influenced by the use of titanium or gold alloy in the marginal zone of the implant. Kohal *et al.* further investigated the influence of zirconia and titanium abutments on soft tissue healing and demonstrated no significant differences (Kohal *et al.*, 2004). In a review article by Rompen *et al.*, it was concluded that titanium was the only material that demonstrated soft tissue biocompatibility; zirconium and aluminum oxide showed favorable histological outcomes whereas dental porcelain and gold were less biocompatible and it was suggested to avoid them (Rompen *et al.*, 2006).

Implant structure and position

Implant structure may differ between various implant systems: one piece implants present a transmucosal part in continuity with the endosseous part, whereas two piece implants present an interface between the implant (endosseous component) and the abutment (transmucosal component), resulting in a microgap between the two components **(fig. 5 to 7)**.

Abrahamsson *et al.* evaluated the influence of three different implant systems on the biological width (Astra Tech Implants, Brånemark and Bonefit-ITI) in beagle dogs (Abrahamsson *et al.*, 1996). The authors compared a one-piece implant (Bonefit) *versus* two two-piece implants (Astra Techand Brånemark). The histological results demonstrated similar dimension and composition of the epithelial and connective tissue components at the end of the study.

Abrahamsson *et al.* further investigated the influence of the abutment dis/reconnection on the marginal peri-implant tissues (Brånemark System) (Abrahamsson *et al.*, 1997). The authors observed that abutment manipulation compromised the mucosal barrier and induced an apical migration of the connective tissue. Thus, while normal proportions and dimensions of the hard and soft tissues were observed in the control group, at test sites the abutment manipulation resulted in a mechanical injury to the soft tissue barrier that had to reestablish more apically, causing a marginal bone resorption (1.5 mm).

In contrast, a single abutment reconnection proved to induce no marginal bone remodeling (Astra Tech Implant System) resulting in a transmucosal attachment of adequate quality and dimensions (Abrahamsson *et al.*, 2003).

Hermann *et al.* further tested the hypothesis of a different biologic width between 1-piece and 2-piece implants (Hermann *et al.*, 2001). Authors reported that dimensions of the peri-implant soft tissues, as evaluated by histometric measurements, were significantly influenced by the presence/absence of a microgap (interface) between the implant and the abutment, and the location of microgap (interface) in relation to the crest of the bone.

Hermann *et al.* evaluated in an animal study (5 dogs, 59 ITI Implants) six different clinical situations: non-submerged one-piece implants with rough-smooth border placed at bone crest (group A), non-submerged one-piece implants with rough-smooth border placed 1 mm below crest (group B), non-submerged two-piece implants with a microgap placed at bone crest (group C), submerged two-piece implants with microgap placed at bone crest (group D), two-piece implants with the microgap placed above the bone crest (group E), two-piece implants with the microgap placed below the bone crest (group F) (Hermann *et al.*, 1997). Authors concluded that one-piece, non submerged implants with a rough/smooth border placed at the

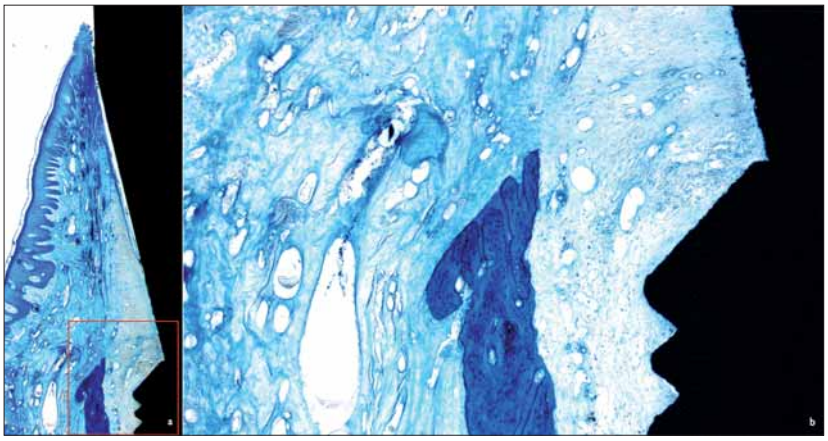

Fig. 5. Implant into fresh extraction socket. Two-piece implant (Astra Micro Thread, Osseospeed, Astra Tech, Sweden) with surrounding tissues. Biological width after 6 weeks of healing. Note the inflammation free connective tissue in intimate contact with the implant-abutment interface. Levai-Laczko staining. Original augmentation 5X.

Fig. 5. Implant dans une alvéole d'extraction fraîche. Implant en deux parties (Astra Micro Thread, Osseospeed, Astra Tech, Suède) avec les tissus environnants. Espace biologique après 6 semaines de cicatrisation. Noter l'absence d'inflammation du tissu conjonctif en contact intime avec l'interface implant/pilier. Coloration de Levai-Laczko. Grossissement × 5.

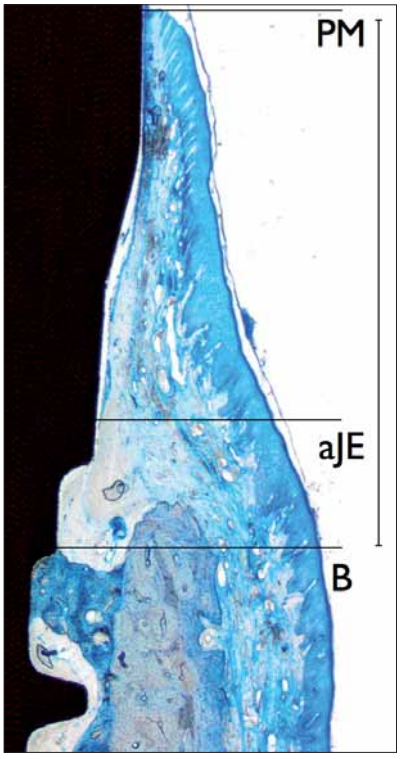

Fig. 6. Implant into fresh extraction socket. Two-piece implant (Thommen SPI Element, Thommen Medical AG, Switzerland) with surrounding tissues. Biological width after 6 weeks of healing.
PM : marginal mucosa
aJE : most apical junctional epithelium
B : most coronal bone to implant contact
Levai-Laczko staining. Original augmentation 2.5X.

Fig. 6. mplant dans une alvéole d'extraction fraîche. Implant en deux parties (Thommen SPI Element, Thommen Medical AG, Suisse) avec les tissus environnants. Espace biologique après 6 semaines de cicatrisation. PM : muqueuse marginale.
aJE : épithélium de jonction le plus apical.
B : os le plus coronaire au contact de l'implant.
Coloration de Levai-Laczko. Grossissement × 2,5.

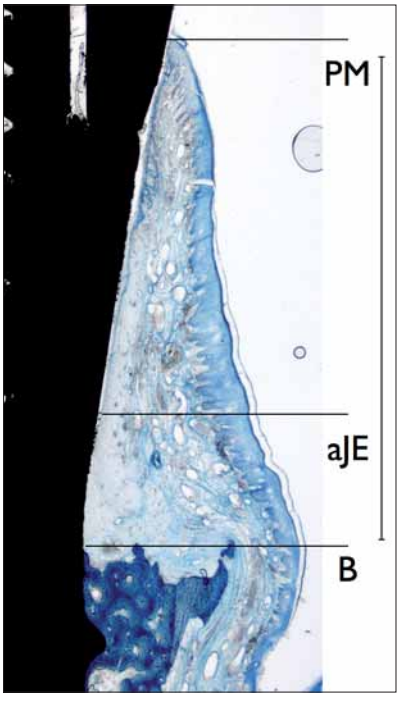

Fig. 7. Implant into fresh extraction socket. One-piece implant (Straumann Standard Plus, AG, Switzerland) with surrounding tissues. Biological width after 6 weeks of healing.
PM : marginal mucosa
a.JE : most apical junctional epithelium
B : most coronal bone to implant contact
Levai-Laczko staining. Original augmentation 2.5X.

Fig. 7. Implant dans une alvéole d'extraction fraîche. Implant monobloc (Straumann Standard Plus, AG, Suisse) avec les tissus environnants. Espace biologique après 6 semaines de cicatrisation.
PM : muqueuse marginale
aJE : épithélium de jonction le plus apical
B : os le plus coronaire au contact de l'implant
Coloration de Levai-Laczko. Grossissement × 2,5.

alveolar crest (group A) presented the smallest value of soft tissues dimension at the end of the study. The apical displacement of the rough/smooth border of the implant (type B) resulted in a wider biologic width (average increase of 0.73 mm). This change occurred through both an increase of the junctional epithelium (average 0.41 mm) and of connective tissue dimension (average 0.34 mm). Furthermore, the authors observed the negative influence of the microgap on the histological outcome evidenced by higher soft tissues dimensions as well as an increase in bone resorption. Authors speculated that this finding was probably due to a bacterical colonization as indicated by previous reports (Persson *et al.*, 1996; Quirynen *et al.*, 1993), or to abutment micromovements (King *et al.*, 2002).

Todescan *et al.* investigated the influence of the implant shoulder position on the soft tissue healing (Todescan *et al.*, 2002). The authors placed 24 implants (Brånemark System) in the mongrel dog and divided the implants into three groups: group 1, implants remained 1 mm above the bone crest, group 2, the implant shoulder was placed at the level of the bone crest and group 3, implants remained 1 mm below the bone crest. In group 2 and 3, a countersink bur was used. The junctional epithelium showed a mean value of 1.67 mm in group 1, 1.93 mm in group 2 and 2.78 mm in group 3. The corresponding values for the band of connective tissue were 1.13 mm, 0.92 mm and 1.60 mm, respectively. Differences between the groups were not statistically different, except for group 2 *versus* group 3. The authors demonstrated a tendency towards longer junctional epithelium and connective tissue component the deeper the implants were placed.

Pontes reported in a study in the mongrel dogs on 2-piece implants (Conexao System) positioned at bone level, 1 and 2 mm below the bone crest (Pontes *et al.*, 2008). Implants were further divided in two groups: immediate loading (24 hours, non occlusal contact) and conventional loading (30 days after second stage surgery). Animals were killed for histological analysis after 3 months of non-submerged healing. Findings from this study are partially consistent with Todescan *et al.* (Todescan *et al.*, 2002) demonstrating a tendency towards a longer connective tissue component the deeper the position of the implant, whereas the length of the junctional epithelium resulted to be independent of the implant position. The effect of the loading did not demonstrate any influence on the soft tissue healing.

Recently, Lazzara and Porter introduced the platform switching technique represented by a non matching implant/abutment interface (Lazzara and Porter, 2006). They elaborated the concept on the basis of a 13 years follow-up case series. Implants that presented non-matching abutments (i.e. the implant platform was wider than the abutment) showed limited marginal bone resorption when compared with normal implants covered by matching abutments. Authors speculated that the soft tissue healing around a non matching abutment might exploit a horizontal direction and utilize the space available between the implant shoulder and the abutment for the establishment of the biological width. This hypothesis would justify a less marginal bone loss apical to implant-abutment interface.

Recently, Luongo *et al.* reported on the soft tissues response to the platform switching technique (Luongo *et al.*, 2008). Authors observed in a human biopsy of one implant that the inflammatory connective tissue infiltrate measured about 0.35 mm apical and coronal to the implant-abutment interface. This finding is not consistent with data reported by Ericsson *et al.* (Ericsson *et al.*, 1996) who demonstrated in an experimental study in dogs the presence of an inflammatory connective tissue infiltrate that measured approximately 0.75 mm apical and coronal to the interface. The smaller inflammatory reaction observed with the platform switching technique (Luongo *et al.*, 2008) may in part justify the limited marginal bone loss observed by Lazzara and Porter (Lazzara and Porter, 2006).

In conclusion, the type of implant (i.e. one- or two-piece implants) and the surgical procedure (i.e. one- or two-stage surgical protocol) do no influence the dimensions and composition of the biological width. Nevertheless, limited data are available on the influence of the position of the implant shoulder in relation to the bone crest. It may be suggested that the deeper the implant shoulder position the longer the biological width. The clinical consequences of such histological findings are still unknown.

Immediate post-extractive insertion

In the past two decades, the immediate implant placement protocol has been introduced into clinical

practice, while most of the studies on biological width on animal models have been conducted on healed ridge models. It may be speculated that the surgical protocol of placing an implant immediately upon tooth extraction may influence the formation and maturation of the biological width.

Araùjo *et al*. investigated in the beagle dog the healing of implants (Straumann Implant System) placed into the distal sockets of third and fourth mandibular premolars (Araùjo *et al.*, 2005). Findings from this experiment demonstrated that the dimensions and composition of the mucosal seal around immediate implants were similar to those around standard implants (Berglundh *et al.*, 1991). The histometrical analysis only showed a different dimension of the soft tissue barrier when comparing buccal and lingual sites. The overall dimensions of the biological width after 3 months of healing was 3.9 ± 0.5 mm and 2.6 ± 0.4 mm, at the buccal and lingual aspect, respectively. The difference between buccal and lingual dimensions was due to the connective tissue component that was 1.8 ± 0.8 mm and 0.7 ± 0.2 mm on the buccal and lingual aspects, respectively. This difference could be in part explained by a greater marginal bone loss observed on the buccal aspect of the implants that corresponded to 2.6 ± 0.4 mm.

These differences were confirmed by another study from the same laboratory (Araùjo *et al.*, 2006) that utilized a similar experimental model with different time intervals. At 1 month of healing the distance from the mucosal margin to the first bone-implant contact varied from 3.3 mm at the buccal sites and 3.5 mm at the lingual sites whereas at 3 months the corresponding values were 4.2 ± 0.8 mm and 2.7 ± 0.2 mm respectively. As previously reported, the difference was due to the dimensions of the connective tissue that reached 1.9 ± 0.6 mm and 0.6 ± 0.2 mm at the buccal and lingual sites after 3 months of healing.

Nevertheless, other experimental studies that have compared healing at implants placed in a healed ridge and implants immediately placed in fresh extraction sockets have reported, at 8 months, a larger dimension of the soft tissue barrier in implants placed immediately (Schultes and Gaggl, 2001). Similar results were reported in a recent experimental study in the minipig model (Rimondini *et al.*, 2005). They evaluated the epithelial dimensions after placing implants in fresh extraction sockets in minipigs and reported that the epithelial length was 3.02 mm 30 days after implant installation and then remained stable up to 60 days **(fig. 8 to 10)**.

In a recent study, Vignoletti described the differences in the healing of the soft tissue barrier when placing four different implant systems immediately in fresh extraction sockets (3i Implant System, Astra Tech

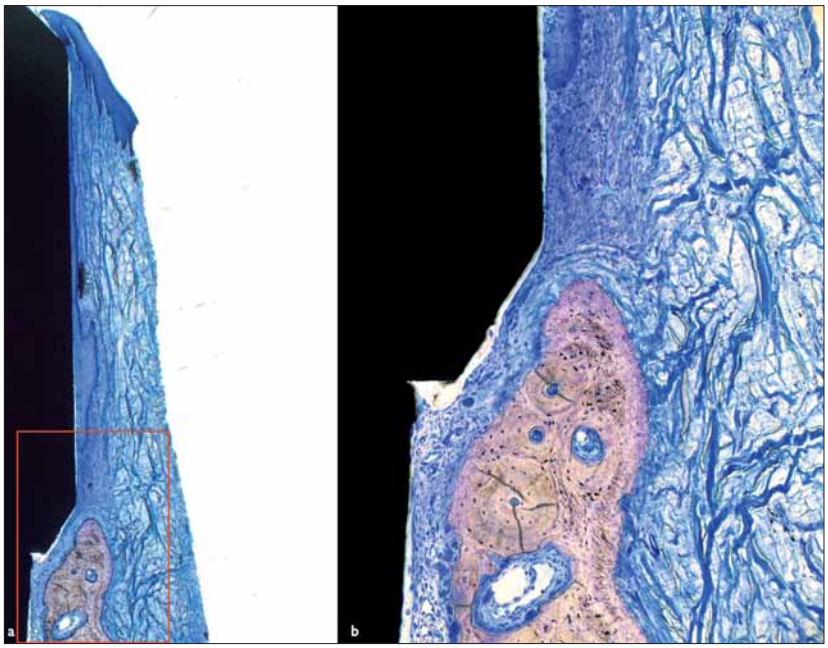

Fig. 8. a. Implant into fresh extraction sockets. Two-piece implant (3i Osseotite Certain, Biomet 3i, USA). Biological width formation at 1 week of healing. A junctional epithelium can be clearly detected. Toluidine blue staining. Original augmentation 2.5X. **b.** Detail of (a). The connective tissue immediately apical of the junctional epithelium is rich in inflammatory cells. Toluidine blue staining. Original augmentation 5X.

Fig. 8. a. Implant dans une alvéole d'extraction fraîche. Implant en deux parties (3i Osseotite Certain, Biomet 3i, États-Unis). Formation d'un espace biologique au bout de 1 semaine de cicatrisation. Un épithélium de jonction peut être clairement détecté. Coloration au bleu de toluidine. Grossissement × 2,5. **b.** Détail. Le tissu conjonctif situé immédiatement apicalement à l'épithélium de jonction est riche en cellules inflammatoires. Coloration au bleu de toluidine. Grossissement × 5.

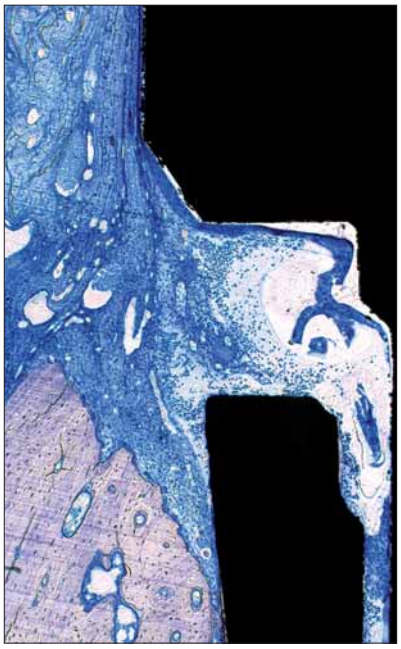

Fig. 9. Implant into fresh extraction sockets. Two-piece implant (3i Osseotite Certain, Biomet 3i, USA). Biological width formation at 2 weeks of healing. A gap is present between the implant shoulder and the abutment. Proliferation of the junctional epithelium can be detected at the implant-abutment interface. Toluidine blue staining. Original augmentation 2.5X.

Fig. 9. Implant dans une alvéole d'extraction fraîche. Implant en deux parties (3i Osseotite Certain, Biomet 3i, États-Unis). Formation d'un espace biologique au bout de 2 semaines de cicatrisation. Il existe un hiatus entre l'épaulement de l'implant et le pilier. La prolifération de l'épithélium de jonction peut être détectée à l'interface implant/pilier. Coloration au bleu de toluidine. Grossissement × 2,5.

Implants, Thommen Implant System, ITI Implant System) (Vignoletti *et al.*, 2009). The biological width 6 weeks after implant placement averaged between 3.5-4.1 mm and 2.8-3.2 mm at the buccal and lingual aspects, respectively. On the buccal aspect the soft tissues barrier was comprised of a junctional epithelium that measured between 2-2.7 mm and a connective tissue that ranged between 1-1.8 mm. The corresponding values at the lingual side were 1.6-2 mm of epithelium and 0.9-1.4 mm of connective tissue. The study failed to demonstrate differences in the healing pattern when placing four different implant systems in fresh extraction sockets. Nevertheless, the obtained length of the epithelium with the four implant systems is longer than what has been reported when placing implants in healed-ridge experimental models.

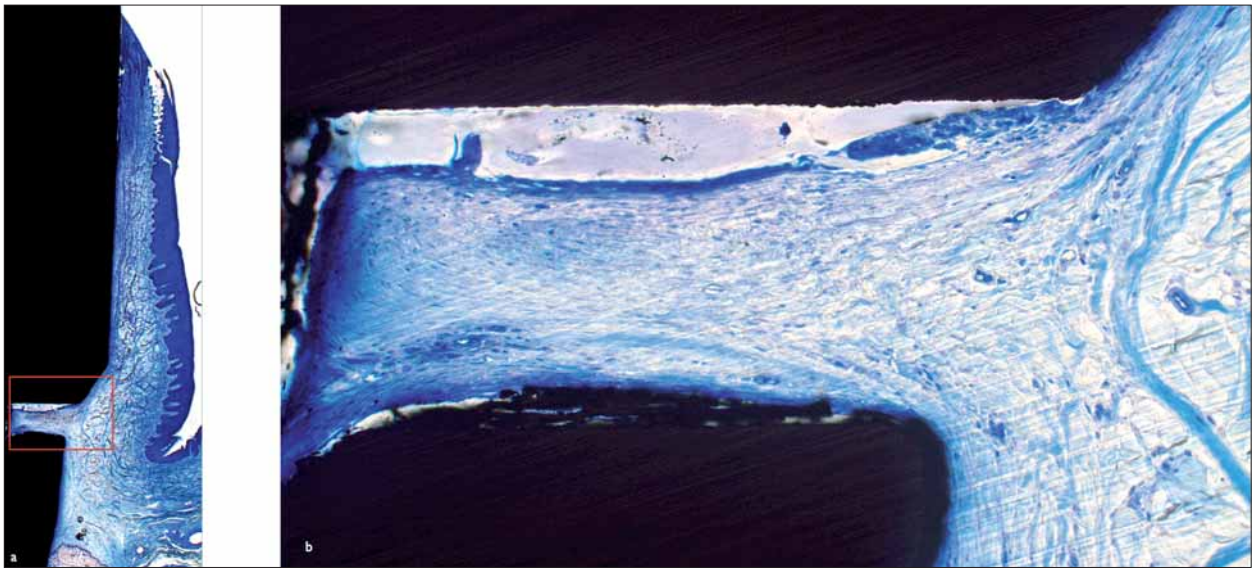

Fig. 10. Implant into fresh extraction sockets. **a.** Two-piece implant (3i Osseotite Certain, Biomet 3i, USA). Biological width formation at 4 weeks of healing. Note the mature connective tissue. **b.** Detail of (a). No inflammatory cells can be detected at the implant-abutment interface.

Fig. 10. Implant dans une alvéole d'extraction fraîche. **a.** Implant en deux parties (3i Osseotite Certain, Biomet 3i, États-Unis). Formation d'un espace biologique au bout de 4 semaines de cicatrisation. Noter le tissu conjonctif mature. **b.** Détail. Aucune cellule inflammatoire ne peut être détectée à l'interface implant/pilier.

In conclusion, contradictory data are available on the influence of placing implants immediately upon tooth extraction on the biological width. The limited experimental evidence available seem to indicate a tendency towards higher dimensions of the mucosal seal around implants placed according to this surgical protocol.

Conclusion

The structure of peri-implant mucosa has many similarities with periodontal tissues. The soft tissue barrier is composed by a sulcus with a non keratinized sulcular epithelium, a junctional epithelium, and a supracrestal connective tissue with an area of dense circular fibers near to the implant surface. The presence of a junctional epithelium facing the titanium surface, similar to the one around teeth, has been evidenced by a large number of studies.
Nevertheless recent ultrastructural studies on rats models have failed to demonstrate the presence of a "true" junctional epithelium, because of the absence of the basal lamina and of the hemidesmosomes in the samples 8 weeks after implant insertion. Further researches are needed to clarify these issues.
Connective fibers orientation represents the most important difference between periodontal and peri-implant tissues that is while in the periodontal structure, fibers run perpendicular the long axis of the tooth, in perimplant tissue, the fibers from the bone crest run parallel to the implant surface.
The dimension the soft tissue barrier around implant seems to be constant, similarly to what has been described around teeth. This dimension has been described as "peri-implant biologic width": This is composed by the dimension of the sulcus, and by the supra-crestal epithelial and connective tissue component.
Most studies report bigger values for peri-implant biologic width than the ones reported for periodontal biologic width. The difference is generally related to a bigger epithelial component at implant sites when compared to the tooth.
A minimum dimension of the biological width is needed in order to accommodate for the soft tissue healing process: when this dimension is not present, bone resorption may occur, to allow for an "appropriate biological dimension" of the peri-implant soft tissue barrier.
The influence of five different factors on peri-implant biologic width dimensions has been evaluated reviewing the available literature, these are: surgical technique, loading time, titanium surfaces and abutment materials, implant structure and position, immediate post-extractive insertion.
Surgical technique, one-stage or two-stage surgery and loading time, both immediate or delayed, do not influence the dimensions of the soft tissue barrier around the implants.
More controversial is the issue of the influence of titanium surfaces and abutment materials.
Titanium is the only material that demonstrated soft tissue biocompatibility, zirconium and aluminum oxide have showed favorable histological outcomes whereas dental porcelain and gold were less biocompatible.
There is no agreement in literature on the influence of titanium surface, in fact while there are studies indicating a smaller dimension of the biologic width in smooth surfaces nevertheless while other studies suggest that these differences are not present.
Further research is needed to allow for a conclusion.
The type of implant (i.e. one- or two-piece implants) and the surgical procedure (i.e. one- or two-stage surgical protocol) do not influence the dimensions and composition of the biological width. Nevertheless limited data are available on the influence of the position of the implant shoulder in relation to the bone crest. It may be suggested that the deeper the implant shoulder position is, the longer the biological width.
Microgap between implant and abutment when present can modify the dimension of biologic width, the longer epithelial component described may be determined by bacterial colonization or abutments micro movements.
The clinical consequences of such histological findings are still unknown.
Contradictory data are present on dimension of the biological width when an immediate postextractive approach is utilized: differences between buccal and lingual sites have been documented, demonstrating a bigger biologic width in buccal sites of immediately placed implants. This difference is related to a bigger connective component in buccal sites.
The limited experimental evidence available seems to indicate a tendency towards larger dimensions of the mucosal seal around implants placed according to this surgical protocol. □

BIBLIOGRAPHY

- **Abrahamsson I, Berglundh T, Wennström J, Lindhe J.** The peri implant hard and soft tissues at different implant systems. A comparative study in the dog. Clin Oral Implants Res 1996;7:212-219.
- **Abrahamsson I, Berglundh T, Lindhe J.** The mucosal barrier following abutment dis/reconnection. An experimental study in dogs. J Clin Periodontol 1997;24:568-572.
- **Abrahamsson I, Berglundh T, Glantz PO, Lindhe J.** The mucosal attachment at different abutments. An experimental study in dogs. J Clin Periodontol 1998;25:721-727.
- **Abrahamsson I, Berglundh T, Moon IS, Lindhe J.** Peri-implant tissues at submerged and non-submerged titanium implants. J Clin Periodontol 1999;26:600-607.
- **Abrahamsson I, Zitzmann NU, Berglundh T, Linder E, Wennerberg A, Lindhe J.** The mucosal attachment to titanium implants with different surface characteristics: an experimental study in dogs. J Clin Periodontol 2002;29:448-455.
- **Abrahamsson I, Berglundh T, Sekino S, Lindhe J.** Tissue reactions to abutment shift: an experimental study in dogs. Clin Implant Dent Related Res 2003;5:82-88.
- **Abrahamsson I, Cardaropoli G.** Peri-implant hard and soft tissue integration to dental implants made of titanium and gold. Clin Oral Implants Res 2007;18:269-274.
- **Araùjo MG, Sukekava F, Wennström JL, Lindhe J.** Ridge alterations following implant placement in fresh extraction sockets: an experimental study in the dog. J Clin Periodontol 2005;32:645-652.
- **Araùjo MG, Sukekava F, Wennström JL, Lindhe J.** Tissue modeling following implant placement in fresh extraction sockets. Clin Oral Implants Res 2006;17;615-624.
- **Bengazi F, Wennström JL, Lekholm U.** Recession of the soft tissue margin at oral implants. A 2 year longitudinal prospective study. Clin Oral Implants Res 1996;7:303-310.
- **Berglundh T, Lindhe J, Ericsson I, Marinello CP, Liljenberg B, Thornsen P.** The soft tissue barrier at implants and teeth. Clin Oral Implants Res 1991;2:81-90.
- **Berglundh T, Lindhe J, Jonsson K, Ericsson I.** The topography of the vascular systems in the periodontal and peri-implant tissues in the dog. J Clin Periodontol 1994;21:189-193.
- **Berglundh T, Lindhe J.** Dimension of the periimplant mucosa. Biological width revisited. J Clin Periodontol 1996;23:971-973.
- **Berglundh T, Abrahamsson I, Welander M, Lang NP, Lindhe J.** Morphogenesis of the peri-implant mucosa: an experimental study in dogs. Clin Oral Implants Res 2007;18:1-8.
- **Buser D, Weber HP, Donath K, Fiorellini JP, Paquette DW, Williams RC.** Soft tissue reactions to non-submerged unloaded titanium implants in beagle dogs. J Periodontol 1992;63:225-235.
- **Cochran DL, Hermann JS, Schenk RK, Higginbottom FL, Buser D.** Biologic width around titanium implants. A histometric analysis of the implantogingival junction around unloaded and loaded nonsubmerged implants in the canine mandible. J Periodontol 1997;68:186-198.
- **Ericsson I, Nilner K, Klinge B, Glantz PO.** Radiographical and histological characteristics of submerged and nonsubmerged titanium implants. An experimental study in the Labrador dog. Clin Oral Implants Res 1996;7(1):20-26.
- **Gargiulo AW, Wentz FM, Orban B.** Dimensions and relations of the dentogingival junction in humans. J Periodontol 1961;32:261-267.
- **Glauser R, Schüpbach P, Gottlow J, Hämmerle CH.** Periimplant soft tissue barrier at experimental one-piece mini-implants with different surface topography in humans: a light-microscopic overview and histometric analysis. Clin Implant Dent Related Res 2005;7(suppl. 1):S44-S51.
- **Glauser R, Zembic A, Hammerle CHF.** A systematic review of marginal soft tissue at implants subjected to immediate loading or immediate restoration. Clin Oral Implants Res 2006;17(suppl. 2);82-92.
- **Hermann JS, Cochran DL, Nummikoski PV, Buser D.** Crestal bone changes around titanium implants. A radiographic evaluation of unloaded nonsubmerged and submerged implants in the canine mandible. J Periodontol 1997;68:1117-1130.
- **Hermann JS, Buser D, Schenk RK, Higginbottom FL, Cochran DL.** Biologic width around titanium implants. A physiologically formed and stable dimension over time. Clin Oral Implants Res 2000;11:1-11.
- **Hermann JS, Buser D, Schenk RK, Schoolfield JD, Cochran DL.** Biologic width around one- and two-piece titanium implants. A histometric evaluation of unloaded nonsubmerged and submerged implants in the canine mandible. Clin Oral Implants Res 2001;12;559-571.
- **Ikeda H, Yamaza T, Yoshinari M, Ohsaki Y, Ayukawa Y, Kido MA *et al.*** Ultrastructural and immunoelectron microscopic studies of the peri-implant epithelium-implant (Ti-6Al-4V) interface of rat maxilla. J Periodontol 2000;71:961-973.
- **King GN, Hermann JS, Schoolfield JD, Buser D, Cochran DL.** Influence of the size of the microgap on crestal bone levels in non-submerged dental implants: a radiographic study in the canine mandible. J Periodontol 2002;73:1111-1117.
- **Kohal RJ, Weng D, Bachle M, Strub JR.** Loaded custom-made zirconia and titanium implants show similar osseointegration: an animal experiment. J Periodontol 2004;75:1262-1268.
- **Lazzara J, Porter S.** Platform switching: a new concept in implant dentistry for controlling posterestorative crestal bone levels. Int J Periodontics Restorative Dent 2006;26:9-17.
- **Luongo R, Traini T, Guidone PC, Bianco G, Cocchetto R, Celletti R.** Hard and soft tissue responses to the platform-switching technique. Int J Periodontics Restorative Dent 2008;28:551-557
- **Moon IS, Berglundh T, Abrahamsson I, Linder E, Lindhe J.** The barrier between the keratinized mucosa and the dental implant. An experimental study in the dog. J Clin Periodontol 1999;26:658-663.
- **Nevins M, Nevins ML, Camelo M, Boyesen JL, Kim DM.** Human histologic evidence of a connective tissue attachment to a dental implant. Int J Periodontics Restorative Dent 2008;28:111-121.
- **Persson LG, Lekholm U, Leonhardt A, Dahlin G, Lindhe J.** Bacterial colonization on internal surfaces of Brånemark system implant components. Clin Oral Implants Res 1996;7:90-95.
- **Pontes AEF, Ribeiro FS, Iezzi G, Piattelli A, Cirelli JA, Marcantonio E Jr.** Biologic width changes around loaded implants inserted in different levels in relation to crestal bone: histometric evaluation in canine mandible. Clin Oral Implants Res 2008;19;483-490.
- **Quirynen M, van Steenberghe D.** Bacterial colonization of the internal part of two-stage implants. An *in vivo* study. Clin Oral Implants Res 1993;4:158-161.
- **Rimondini L, Bruschi GB, Scipioni A, Carrassi A, Nicoli-Aldini N, Giavaresi G *et al.*** Tissue healing in implants immediately placed into postextraction sockets: a pilot study in a mini-pig model. Oral Surg Oral Med Oral Pathol Oral Radiol Endod 2005;100:43-50.
- **Rompen E, Domken O, Degidi M, Pontes AEF, Piattelli A.** The effect of material characteristics, of surface topography and of implant components and connections on soft tissue integration: a literature review. Clin Oral Implants Res 2006;17 (suppl. 2);55-67.
- **Rossi S, Tirri T, Paldan H, Kuntsi-Vaattovaara H, Tulamo R, Närhi T.** Periimplant tissue response to TiO2 surface modified implants. Clin Oral Implants Res 2008;19:348-55.

- **Schierano G, Ramieri G, Cortese M, Aimetti M, Preti G.** Organization of the connective tissue barrier around long-term loaded implant abutments in man. Clin Oral Implants Res 2002;13:460-464.
- **Schultes G, Gaggl A.** Histologic evaluation of immediate *versus* delayed placement of implants after tooth extraction. Oral Surg Oral Med Oral Pathol Oral Radiol Endod 2001;92(1):17-22.
- **Schwarz F, Ferrari D, Herten M, Mihatovic I, Wieland M, Sager M *et al.*** Effects of surface hydrophilicity and microtopography on early stages of soft and hard tissue integration at non-submerged titanium implants: an immunohistochemical study in dogs. J Periodontol 2007;78:2171-2184.
- **Shioya K, Sawada T, Miake Y, Inoue S, Yanagisawa T.** Ultrastructural study of tissues surrounding replanted teeth and dental implants. Clin Oral Implants Res 2009;20:299-305.
- **Siar CH, Toh CG, Romanos G, Swaminathan D, Ong AH, Yaacob H *et al.*** Peri-implant soft tissue integration of immediately loaded implants in the posterior macaque mandible: a histomorphometric study. J Periodontol 2003;74:571-578.
- **Sicher H.** Changing concepts of the supporting dental structures. Oral Surg Oral Med Oral Pathol Endod 1959;12:31-35.
- **Todescan FF, Pustiglioni FE, Imbronito AV, Albrektsson T, Gioso M.** Influence of the microgap in the peri-implant hard and soft tissues: a histomorphometric study in dogs. Int J Oral Maxillofac Implants 2002;17:467-472.
- **Vacek JS, Gher ME, Assad DA, Richardson AC, Giambarresi LI.** The dimensions of the human dentogingival junction. Int J Periodontics Restorative Dent 1994;14:155-165.
- **Vignoletti F, de Sanctis M, Berglundh T, Abrahamsson I, Sanz M.** Early healing of implants placed into fresh extraction sockets: an experimental study in the beagle dog. III. Soft tissue findings. J Clin Periodontol 2009;36:1059-1066.
- **Welander M, Abrahamsson I, Berglundh T.** The mucosal barrier at implant abutments of different materials. Clin Oral Implants Res 2008;19:635-641.

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