The effect of material characteristics, of surface topography and of implant components and connections on soft tissue integration: a literature review

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Soft tissue interface

To be functionally useful, oral implants have to pierce the gingiva or oral mucosa and enter the oral cavity, thus establishing a transmucosal connection between the external environment and the inner parts of the body.

In order to avoid bacterial penetration that could jeopardize either initial healing or long-term behaviour of implants, the formation of an early and long-standing effective barrier capable of biologically protecting the peri-implant structures is mandatory. The establishment of this soft tissue barrier is a critical part of tissue integration and is fundamentally the result of wound healing that has to establish an effective interface between living tissues and a foreign body.

The soft tissue interface has been histologically assessed in animals and has a dimension of 3–4 mm in the apico-coronal direction called ‘biological width’. The interface consists of two zones, one of epithelium which covers about 2 mm of the surface, while the rest is devoted to connective tissue adhesion.

Both these tissues contribute to the establishment of the so-called biological width, which may prevent oral bacteria and their products from penetrating into the body (Berglundh et al. 1991; Buser et al. 1992; Berglundh & Lindhe 1996; Abrahamsson et al. 1996, 1997, 1998a, 1998b; Cochran et al. 1997).

Junctional epithelium

Owing to its capacity to proliferate and to move on surfaces, the epithelium found at the border of the incision crosses over the bridge of the fibrin clot/granulation tissue that rapidly starts forming after implant/abutment installation.

Upon reaching the surface of the implanted component, it moves in coronal-apical direction, giving rise to a junctional epithelium about 2 mm long (Listgarten 1996; Lindhe & Berglundh 1998).

It must be emphasized that the epithelium found on the border of the wound is oral epithelium; sulcular and junctional epithelium have different morphology, structure and phenotypic expressions. This means that the epithelium, during its apical proliferation along the implant surface, is subjected to many influences, undergoing major morphological and functional modifications.

Once the epithelial cells have reached the implant surface, their attachment occurs directly via a basal lamina (<200 nm)
and the formation of hemidesmosomes (James & Schultz 1974; Lístgarten & Lai 1975; Hansson et al. 1983; Gould et al. 1984; McKinney et al. 1985; Steflík et al. 1993; Kawahara et al. 1998a, 1998b). Hemidesmosomes can be formed already at 2–3 days of healing (Swope & James 1981). A study was conducted (Gould et al. 1984) to determine if the behaviour of epithelium in vitro is similar to its attachment behaviour in vivo by the use of small sections of titanium-coated implants that could be inserted in human gingiva. The size of the implants allowed their insertion in a limited region and enabled the fixation and embedding procedures that are necessary for electron microscopy to be effective.

Examination of the thin sections obtained from this material demonstrated that the epithelial cells attached to titanium in a manner similar to that observed in vitro and similar to the way that epithelium attaches to the tooth in vivo that is, there was a formation of hemidesmosomes and basal lamina.

Another possible attachment modality which has been hypothesized is an indirect epithelium/implant contact (Kawahara et al. 1998a, 1998b).

It is generally recognized that the epithelium lining the peri-implant sulcus shares many structural, ultrastructural and functional characteristics with the corresponding gingival tissue. Studies conducted in humans (Carmichael et al. 1991; Mackenzie & Tonetti 1995; Liljenberg et al. 1997) indicate that the epithelium surrounding oral implants possesses patterns of differentiation and function similar to gingival epithelium.

The presence of granulation tissue adhering to the surface of transmucosal implant components is considered the principal factor which stops the epithelium from moving further apically (Listgarten 1996). The role of the connective tissue in preventing epithelium downgrowth has been clearly demonstrated in animal models (Squier & Collins 1981; Chehroudi et al. 1992). Berglundh et al. (1991) also speculated that the reason why the epithelium stops migrating in an apical direction could be the interaction between the soft tissue and the layer of titanium oxide. It seems that mature connective tissue interferes more effectively than granulation tissue with epithelial downgrowth (Chehroudi et al. 1992). At initial phases of the healing phase, the quality and stability of the fibrin clot adhesion to the surface of the transmucosal components most probably plays a role in the formation and positioning of the junctional epithelium (Lowenguth et al. 1993).

Connective tissue adhesion

After installation of the transmucosal component, the healing of the connective tissue wound involves distinct processes: formation and (hopefully) adhesion of a fibrin clot to the implant surface, adsorption of ECM proteins and subsequently of connective tissue cells to the implant surface, transformation of the clot into granulation tissue, migration of epithelial cells on top of the fibrin clot/granulation tissue (Descouts & Aronsson 1999; Meyle 1999).

After maturation, the connective tissue portion, located between the barrier epithelium and the marginal bone, has been found to be poor in cells and in vascular structures but rich in collagen fibres.

It is now known that the connective tissue can be divided into two zones. The inner zone is in direct contact with the implant/abutment surface and is 50–100 μm thick. It is rich in fibres, with few scattered fibroblasts that appear to be in close contact with the transmucosal component. This thin fibroblasts-poor barrier next to the titanium surface probably plays a role in the maintenance of a proper seal between the oral environment and the peri-implant bone. This layer of connective tissue resembles a scar tissue (Buser et al. 1992; Berglundh et al. 1994; Abrahamsson et al. 1996; Cochran et al. 1997; Chavrier & Couble 1999; Schierano et al. 2002).

The rest of the connective tissue, the outer zone, is formed of fibres running in different directions, richer in cells and blood vessels (Buser et al. 1992).

Hansson et al. (1983) furthermore reported that connective tissue cells and collagen fibre bundles were consistently separated from the titanium dioxide surface by a 20 nm wide proteoglycan layer.

For most authors, these fibres run a course more or less parallel to the implant surface. This observation was made both in human subjects (Akağawa et al. 1989; Chavrier et al. 1994; Liljenberg et al. 1996, 1997) and in animal models: monkeys (Listgarten & Lai 1975; Gottfredsen et al. 1991) and dogs (Berglundh et al. 1991; Ericsson et al. 1995; Abrahamsson et al. 1996; Cochran et al. 1997; Çomut et al. 2001).

For other authors, the fibres are not parallel to the implant surface but either run in various directions (Fartash et al. 1990; Arvidson et al. 1996); a perpendicular orientation was also found, with implants harbouring a porous surface (Schroeder et al. 1981; Deporter et al. 1988), their orientation being potentially influenced by the quality of the mucosa: the fibres tend to be parallel in alveolar mucosa, while they seem to be organized more perpendicularly in keratinized mucosa.

Apart from the orientation of the fibres, the major difference between the connective tissue around teeth and around artificial abutments is related to their connection to the natural or artificial root surface.

At a natural tooth, the dento-gingival collagen fibres are firmly inserted into the cementum and the bone, and oriented perpendicular or oblique to the tooth surface, serving as a barrier to epithelial migration, and thus impeding bacterial invasion (Gargiulo et al. 1961; Stern 1981).

In contrast, implants lack cementum: the orientation of the ‘attachment’ fibres in the supracrestal soft tissue compartment is parallel to the implant surface and, more importantly, they are not inserted in the implant surface (Berglundh et al. 1991; Buser et al. 1992; Listgarten et al. 1992; Chavrier et al. 1994).

As a consequence, the connective tissue adhesion at implant has a poor mechanical resistance as compared to that of natural teeth (Herrmann et al. 2001). In other words, the gingiva at implants can hardly be qualified of ‘attached’.

As the connective tissue interface is considered of paramount importance to support the epithelium and block its apical migration, this lack of mechanical resistance can potentially endanger the prognosis of oral implants: tearing at the connective tissue/implant interface could occur due to a lack of soft tissue stability, which could induce the apical migration of the junctional epithelium, accompanied by gingival recessions or pocket formation and by bone resorption.
Soft tissue interface

Comparison between implants and teeth

From a comparative study in dogs (Berglundh et al. 1991), it is known that the soft tissue interface is slightly longer at [two-piece] implants than at teeth: if the junctional epithelium has comparable dimensions at teeth and implants (2.05 vs. 2.14 mm), the connective tissue is 1.12 mm long around teeth vs. 1.66 mm around implants. Comparable results were described by Ericsson & Lindhe (1993).

These results were obtained with transmucosal implant components made of machined titanium and cannot be extrapolated to other materials.

Clinical evaluation of the soft tissue interface

Animal studies

Despite comparable histological dimensions of the soft tissue compartments at teeth and implants, it has been shown that, when a probe pressure of 0.5 N is used in dogs, the probe tip penetrates on average 0.7 mm deeper at implant sites [Ericsson & Lindhe 1993].

The histological sections with probes in situ evidenced that, around implants, the tip of the probe ended apically to the junctional epithelium, close to the bone crest, explaining why the clinical probing depth is higher. This is in accordance with the results of Gray et al. [2005] in baboons.

Lang et al. (1994) showed that at low pressure [0.2 N], clinical probing was able to identify the connective tissue adhesion level. In contrast, the probe penetration exceeded the connective tissue level in inflamed sites.

Human studies

Some human studies have compared periodontal and peri-implant probing, and confirmed that 0.5–1.4 mm deeper measurements are generally found at implants [Quirynen et al. 1991a, 1991b; Brägger et al. 1997; Mombelli et al., 1997; Chang et al. 1999], illustrating that at implants the probe tip ends somewhere in the connective tissue and that the significance of probing at implants and that at teeth is different.

Soft tissues’ stability over time

Animal data are available to indicate a stability of the soft tissue dimensions over a 12-month period in loaded or unloaded conditions at one- or two-piece implants [Cochran et al. 1997; Hermann et al. 2000b, Assenza et al. 2003].

Using clinical indices, several studies have gathered data that strongly suggest a longstanding stability of the soft tissue interface at one- and two-piece titanium implants in human patients.

Data supporting this stability are for instance available at 12 months [Cune et al. 2004], 24 months [Bengazzi et al. 1996], 36 months [Quirynen et al. 1991a, 1991b], and even 10 years [Hultin et al. 2000; Karoussis et al. 2004a]. In the Karoussis et al. study, probing pocket depth increased from 1 to 10 years of 0.24 mm at implants vs. 0.27 mm at teeth, while the probing attachment level varied of 0.37 mm at implant vs. 0.3 mm at teeth.

Aims of the paper

To improve the quality and stability of the soft tissue/implant interface is of paramount importance for the short- and long-term prognosis of oral implants.

This goal can be reached through the combination of different approaches: the first approach is to use surgical techniques focused at preserving or re-creating a soft tissue environment made of fibrous, keratinized stable gingiva, combined with conservative prosthetic techniques in order to avoid damaging the so-called biological width.

In addition, some characteristics of the transmucosal components are also of a crucial importance to obtain an effective interface: we will here focus on the impact of material characteristics, of surface topography and of implant components and connections on the adhesion of epithelium and connective tissue.

Note: A high percentage of papers looking at the implant–soft tissue interface are in vitro studies using cell cultures. The findings made in this type of study can never be fully extrapolated to the clinical situation.

Meanwhile, it must be noted that, even in vivo, the epithelial tissue is composed of cells in direct contact with each other without an extracellular matrix; they will also be in direct contact with the implant components through hemi-desmosomes and a basal lamina. This is very close from what will be reproduced in vitro.

On the other hand, connective tissue cells are dispersed in a dense extra cellular matrix. These cells do not normally get in direct touch with each other, but are rather connected to their proteic environment. They can get in direct contact with implant components, but the adhesion of the tissue is more dependent on collagen fibres.

In addition, in vivo, the formation of a fibrin network through which fibroblasts will have to secondarily migrate is the first step of connective tissue formation after implant/abutment surgery. In vitro experiments of fibroblasts adhesion to implant materials never reproduce fibrin polymerization before cell seeding, meaning that in vitro conditions are more artificial and distant from the in vivo situation for fibroblasts than for epithelial cells. The presence of 5–10% of serum in the culture medium does not allow to properly mimic the in vivo conditions.

Influence of material’s characteristics on soft tissue integration

Chemical composition

The reaction of cells and tissues to implanted foreign bodies depends on the material’s properties and its behaviour upon contact with the body fluids. It must be noted that the chemical composition of the bulk material is sometimes significantly different from that of the surface that is at the interface with the living tissues: some materials demonstrate a surface oxidation (such as titanium that exhibits a surface layer of titanium oxide), while the mode of preparation or of sterilization of others will result in chemical contamination of the surface.

As it came to be realized that the interaction of a biomaterial with its environment was governed largely by surface properties, the chemical characterization of the surfaces took on greater importance and increasingly sophisticated means of analysis have been brought into play. Currently it is not uncommon for surfaces to be characterized by their X-ray photoelectron spectroscopy (XPS) that enables specific elements and their chemical state to be assessed. For example the thickness of the
Coating titanium with a poly-vinyl-chloride polymer had a deleterious effect.

When Ti$_6$Al$_4$V was compared with c.p. titanium [Eisenbarth et al. 1996], gingival fibroblasts demonstrated a rounded cell shape and a reduced area of spreading on the alloy, presumably because of a minor toxicity to vanadium or aluminium.

Ti nitrite also proved to be suitable for fibroblasts adhesion and growth [Groessner-Schreiber et al. 2003].

Modified dental ceramics. Kokoti et al. (2001) modified chemical composition and surface morphology of dental ceramics and evaluated them, in vitro, for their ability to support fibroblasts attachment and proliferation. Four modified ceramics were constructed from body or shoulder porcelain after treatment with CaO, or CaO and P$_2$O$_5$. These oxides were selected because they had proved to improve cell attachment in bioactive ceramics (bio-glasses) [Häkkinen et al. 1988]. All modified ceramics promoted cell proliferation as compared with controls, shoulder modified ceramics proving to be the most effective.

HA surfaces. Kasten et al. [1990] found higher epithelial cell adhesion on HA compared with c.p. titanium, but the extremely low number of samples limits the significance of their results.

Human gingival fibroblasts attachment to c.p. titanium proved to be significantly higher than to non-porous and porous hydroxyapatite [Guy et al. 1993].

Metal oxides. Photolithographic techniques have been used [Scotchford et al. 2003] to apply strips of metal oxides to glass surfaces in such a manner that comparisons can be made on a side-by-side basis. Titanium, aluminium and vanadium have been produced in this way and the adsorption of cells and proteins on these surfaces studied. Titanium oxide provided the best substratum overall for cell adhesion.

Animal studies

Ti, gold, Al$_2$O$_3$, dental ceramic. Abrahamsson et al. (1998a) observed, in a dog model, that abutments made of c.p. titanium oxide layer may be determined from the intensity ratio of the metal to oxide signal. Moreover, the presence of organic and other contaminants can be determined using XPS. This information is important because some sterilization techniques, such as autoclaving, can introduce significant amount of contaminants to the surface and mask the properties of the underlying titanium. Further chemical analysis of contaminants can be obtained by such methods as mass spectroscopy. The surface, used in some of the older literature reviewed here was not characterized by such sophisticated methods, but it appears that higher standards on surface characterization are now being applied by biomaterials journals.

**In vitro** studies

Ti, gold, Al$_2$O$_3$ and dental ceramic. Räisänen et al. (2000) studied, in vitro, how epithelial cells attach to five different dental material surfaces (titanium, Ti$_6$Al$_4$V titanium alloy, dental gold alloy, dental porcelain and aluminium oxide).

The efficacy of adhesion was evaluated by SEM and immunofluorescence microscopy with antibodies to vinculin and z$\beta$4 integrin.

Epithelial cells adhered and spread more avidly on metallic surfaces (c.p. titanium, Ti$_6$Al$_4$V titanium alloy, dental gold alloy) than on ceramic surfaces (dental porcelain and aluminium oxide). Well-organized focal contacts and pre-hemidesmosomes were found on metallic surfaces, but not on porcelain and aluminium oxide.

Previously, Jansen et al. (1985) had found focal contacts, hemidesmosome-like structures and extracellular matrix contacts between epithelial cells and titanium, gold, hydroxyapatite and carbon apatite.

Simion et al. (1991) examined human gingival fibroblasts/implant materials interface in vitro using a specific but not elsewhere validated model. Their results show an effective cell growth on acid-etched titanium and titanium alloy, on gold and gold porcelain, a ‘tenacious’ cell adherence being found only on etched titanium.

Säuberlich et al. (1999) found an effective cell adhesion to c.p. titanium, and non-significant improvement by surface treatment by sulphur dioxide plasma etching, by plasma nitration, by silane coating.

McKinney et al. (1985) had already evidenced the presence of hemidesmosomal adhesion of epithelial cells to aluminium oxide implants in dogs.

HA surfaces. Çomut et al. (2001) observed in a dog model an effective formation of a mucosal attachment on c.p. titanium and on HA-coated titanium, with a parallel fibres orientation on all samples.

Other studies indicate a favourable soft tissue response to dense HA [Kurashina et al. 1984; Jansen et al. 1991]. In an investigation of the gingival reaction to permucosal dense hydroxyapatite implants in dogs [Kurashina et al. 1984], bundles of collagen fibers are reported to terminate perpendicularly to the interface of the implants.

Single-crystal sapphire implants. Soft tissues surrounding titanium implants and single-crystal sapphire implants present no qualitative structural differences [Arvidson et al. 1996].

The epithelial cells adjacent to the sapphire implant surface have a well-ordered basal lamina with cell membrane hemidesmosomes [Hashimoto et al. 1989].

Zirconia. Kohal et al. (2004) compared bone and soft tissue integration of rough titanium vs. zirconia implants in a monkey model. They found an effective formation of a mucosal attachment at both implant materials, the mean length of connective tissue being 1.5 mm on zirconia vs. 2.4 mm...
on titanium, without evidence of perpendicular fibres. These differences did not reach the level of statistical significance.

Human studies
Zirconia. DeGidi et al. (2006) conducted a comparative immunohistochemical evaluation of peri-implant soft tissues of titanium and zirconium oxide healing caps in five patients. Statistically significant differences were observed, with an overall lower inflammatory level in tissues surrounding zirconium oxide healing caps than at titanium caps.

Otherwise, only case reports are available those show a satisfactory clinical outcome in humans of the soft tissues, but these reports are not conclusive.

Surface-free energy (wettability)
The wettability of the surface can play an important role not only regarding protein adsorption but also regarding cell attachment and spreading.

This physicochemical property of the substratum may influence cellular adhesion through:

1. effects on the adsorption of proteins on non-wettable surfaces lead to a reduced amount of proteins on the material surface, and the strength of adhesion of the molecules is reduced as well;
2. alteration of the conformation of adsorbed proteins can result from differences in the molecular sites contacting the material surface. The conformational changes can lead to differences in the expression of ligand sites interacting with cellular receptors [Colvin 1983].


Improvements in fibroblasts’ adhesion in relation with surface cleanliness and wettability were shown with germanium and Co–Cr–Mo implants [Baier et al. 1984]. No data were found concerning materials currently used for oral implants.

Surface contamination
A clean surface has a high surface free energy, while a contaminated one has a lower surface energy [Kasemo & Lausmaa 1988].

Chemical contamination by cleaning, disinfection or sterilization procedures
The ultimate goal of cleaning procedures should be to remove the contaminants and restore the elemental composition of the surface oxide without changing the surface topography, either after the fabrication process, after handling in the dental laboratory, or when transgingival components are re-used.

Although specific protocols have been developed, it proves to be rather difficult to effectively clean a contaminated titanium surface, most probably because of the strong binding of proteins and amino acids [Rowland et al. 1995; Zoller & Zentner 1996; Steinemann 1998].

Krozer et al. [1999] investigated in vitro the adsorption of amino-alcohol to machined titanium surfaces, and the possibilities to chemically remove the adsorbed alcohols in order to recover a pristine titanium surface. It was shown that rinsing in water, saline solution, or 5% H₂O₂ did not remove the amino alcohol from the surface, while exposure to ozone resulted in complete removal of the adsorbed amino-alcohol. The results show that the amino alcohol used forms a stable and dense film at the implant surface in vitro. Presence of such a film most likely prevents re-integration to occur at the implant–tissue interface in vitro.

Vezeau et al. [1996] evaluated the surface changes and effects on in vitro cell attachment and spreading brought about on prepared commercially pure titanium by multiple exposures to common sterilization methods. In vitro analysis of cell attachment and spreading using gingival fibroblasts were performed. Results indicated that steam autoclave sterilization contaminated and altered the titanium surface, resulting in decreased levels of cell attachment and spreading in vitro.

Keller et al. [1990] had also observed that sterilization of c.p. titanium surfaces by steam autoclaving caused a surface alteration and contamination, and a reduction of fibroblast cell attachment and spreading, in vitro.

Contamination by blood, saliva or plaque
Zoller & Zentner (1996) studied in vitro the influence of contaminations of titanium by saliva or serum on initial attachment of fibroblasts. Pre-treatment with serum showed consistent enhancing effect on cell adhesion. In contrast, pre-treatment with saliva diminished significantly cell adhesion. These results suggest that exposure of transgingival components to saliva at placement might inhibit adhesion of gingival fibroblasts and thus indirectly induce epithelial downgrowth.

Kawahara et al. [1998a, 1998b] investigated in vitro cell contact to titanium surfaces and adhesive strength of epithelial cells and fibroblasts under the influence of dental plaque extracts. Epithelial cells exhibited higher adhesive strength values than fibroblasts. The plaque extracts had a greater effect in decreasing the growth rate of fibroblasts than that of epithelial cells. This study suggests that the difference in growth, contact, and adhesive strength of the epithelial and fibroblastic cells to titanium surfaces may promote apical epithelialization under exposure to dental plaque.

Mouhyi et al. [1998] tested the surface composition of failed and retrieved machined titanium implants after various cleaning and disinfection techniques. Cleaning in citric acid followed by rinsing with deionized water for 5 min followed by cleaning in ultrasonic baths with trichloroethylene and absolute ethanol gave the best results with regard to macroscopic appearance and surface composition.

Sennerby et al. [1989] retrieved titanium cover screws and either rinsed them in saline or subjected them to ultrasonic cleaning and sterilization. After implantation in the abdominal wall of rats, cover screws induced the formation of a thick fibrous capsule, when unused screws did not. None of the decontamination procedures was effective.

Sennerby & Lekholm [1993] implanted titanium abutments in rats, after intra-oral contamination in humans for 1 min or 2 weeks and either rinsing in saline or ultrasonic treatment in amino-alcohols. All pre-contaminated abutments induced an altered tissue response as compared with pristine abutments, irrespective of the cleaning procedure.

In contrast, Ericsson et al. [1996a] failed to show differences in soft tissue reaction between pristine titanium abutments with various surface roughness and corresponding contaminated abutments.
Mouhyi et al. [2000] evaluated the soft tissue response to clinically retrieved and decontaminated cover screws. The cover screws were cleaned by using citric acid, sterile water, hydrogen peroxide or CO2 laser alone or combined. After cleaning the cover screws were implanted in the abdominal wall of the rat for 6 weeks. It was concluded that only CO2 laser used alone or in combination with hydrogen peroxide may be used clinically for sufficient decontamination of titanium surfaces.

Coating with bioactive molecules

Epithelial cells and fibroblasts have different affinities for adhesive proteins of the extracellular matrix.

Dean et al. [1995] observed in vitro that a fibronectin coating enhanced gingival fibroblast attachment to smooth [machined], plasma-sprayed, and hydroxyapatite-coated titanium surfaces two- to threefold, but it was less effective on epithelial cell attachment. In contrast, coating surfaces with laminin-1, a component of epithelial cell basement membranes, resulted in three- to fourfold enhancement of gingival epithelial cell binding but has less effect on fibroblast attachment.

Tamura et al. [1997] and El-Ghannam et al. [1998] observed in vitro the enhancement of epithelial cell attachment, spreading and hemidesmosomes assembly on laminin-5-coated titanium alloy.

Type IV collagen has also been shown to provide an excellent substratum for epithelial cell attachment on titanium surfaces whereas vitronectin restrains attachment of epithelial cells, compared with noncoated titanium surfaces [Park et al. 1998].

Influence of surfaces’ topography on soft tissue integration

Definitions

A large number of surface treatment processes are available to alter surface topography of titanium implants, including machining/micromachining, particle blasting, Ti plasma spraying, HA plasma spraying, chemical/electrochemical etching, anodization.

The topographic features that are obtained on the implant surface can range from nanometers to millimetres, that is from below the cell-size scale to the tissue scale.

One approach to characterizing the topography of implant surfaces is that of Wannerberg & Albrektsson [2000] who use a confocal laser scanning profilometer. The topography of the surface is defined in terms of form, waviness and roughness [Fig. 1, with the waviness and roughness often presented together under the term texture [Thomas 1999]. The form relates to the largest structure [profile] while the roughness describes the smallest irregularities in the surface. Typical surface roughness is described by three parameters: Sa, Sx and Sdr.

A problem with the use of parameters based on averages, such as those listed above, is that surfaces with markedly different distributions of feature size may yield similar Ra values. Moreover fine roughness features that may be important for performance in a given application may not contribute significantly to the calculated overall roughness value if much larger features are also present. This discrepancy can occur when complex surfaces are prepared using different processes. For example the Ra value of sandblasted and etched surface will be largely determined by the contribution of the large surface features produced by grit blasting. A more sophisticated, albeit computationally intensive approach to this problem is the use of Fourier transforms to fit observed profiles of surfaces and enable roughness in different size ranges, termed windows, to be determined and correlated with biological responses [Wieland et al. 2001].

Surface roughness can occur in two principal planes: one perpendicular to the surface and one in the plane of the surface [Thomas 1999]. The orientation of the irregularities may be either isotropic or anisotropic. Surface structures without a dominating direction are called isotropic. Techniques to produce such surfaces include abrasive blasting, plasma-spraying, etching and oxidizing.

Other processes such as turning or milling result in a surface that has a distinct and regular pattern. Such a surface structure is denoted anisotropic.

Surface texture

Impact on protein adsorption

The composition of the protein film and the orientation of the molecules that are adsorbed on the implant surface may be affected by the surface roughness. Di Iorio et al. (2005) evaluated the fibrin clot extension in vitro on three different textures of c.p. titanium, and found that the surface microtexture complexity determines the formation of a more extensive and three-dimensionally complex fibrin scaffold.

This could be of crucial importance both for osseointegration and for the early formation of an effective connective tissue seal that would impair epithelial cells downgrowth.

Walivaara et al. [1994] also showed that if the wettability of smooth titanium surfaces is correlated to fibrin adsorption, this correlation no longer exists on rough titanium.

François et al. [1997] showed a 50% decrease of fibronectin adsorption on acid-etched and on sandblasted and acid-etched (SLA) surfaces as compared to polished titanium.

Fig. 1. From Wannerberg & Albrektsson [2000].

Impact on cell and tissue adhesion

In vitro experiments. Hormia et al. [1991] compared the attachment and spreading of human gingival epithelial cells on three differently processed titanium surfaces (electropolished, acid-etched and sandblasted) by means of immunostaining. The results showed that epithelial cells attached and spread more readily on polished and etched titanium than on rougher surfaces [sandblasted titanium].
Kononen et al. [1992] and Hormia & Körnönen [1994] showed the same results with human gingival fibroblasts.

Based on their model, smooth or finely grooved titanium surfaces could be optimal in maintaining the adhesion and specialized phenotype of gingival epithelial cells and fibroblasts. The authors also showed that the surface roughness of the substrate can affect the expression of integrin subunits.

Cochran et al. [1994] compared in vitro attachment and proliferation of human gingival or periodontal fibroblasts and epithelial cells grown on titanium surfaces with varying roughness [electropolished vs. fine or coarse sandblasted/acid-etched]. Initial adhesion of fibroblasts was higher on smooth titanium, but their growth was good on all surfaces. Epithelial cells proliferation only happened on electropolished titanium.

Meyle [1999] showed that a sandblasted titanium surface delayed the adhesion and spreading of epithelial cells, while the corresponding features of fibroblasts and osteoblasts were enhanced.

Di Carmine et al. [2003] observed that a rough surface [sandblasted, 2.14 μm] promoted the formation of multiple filopodia at the periphery of immortalized epithelial cells, while the cells were round and in direct contact with each other on more smooth titanium [machined, 0.8 μm] and on tissue culture plastic. They assume that the presence of filopodia suggests a higher level of adhesion. This assumption is dubious. It is not a normal behaviour for epithelial cells to display filopodia: in vitro and in vivo situations, these ‘cell-cup like’ cells normally have a polygonal shape and are in close contact with each other. In addition, the SEM images in the paper clearly suggest that the epithelial cells are not in direct contact with the valleys of the roughened titanium, but rather bridge over the valleys.


Mustafa et al. [1998] observed that human gingival fibroblasts initially attach more to polished aluminium oxide abutments, but display a higher rate of proliferation on rougher Al2O3.

A recent paper from Baharloo et al. [2005] compared the adhesion, spreading and growth of epithelial cells on polished, rough grit-blasted, acid-etched and grit-blasted and acid-etched titanium (SLA). They evidenced a negative effect of titanium roughness on epithelial cells growth and spreading. As assessed by immunofluorescence staining for vinculin, they showed that epithelium formed less and smaller focal adhesions on rough titanium, suggesting that epithelial cells on rough surfaces are more susceptible to mechanical removal.

They also demonstrated that focal adhesions were primarily located on the ridges rather than the valleys on rough surfaces, with a tendency to bridge over the valleys, which confirms the images of Di Carmine et al. [2003]. TEM measurements demonstrated this phenomenon: the average cell to titanium distance increased as the surface roughness increased.

Animal experiments
In a dog model, Abrahamsson et al. [2002] compared the soft tissue integration of turned (S0 0.22 μm, S0 3.26%) vs. acid-etched (S0 0.45 μm, S0 8.57%) titanium abutments. They demonstrated that the soft tissue adhesion was not influenced by this kind of roughness of the transmucosal titanium components. The connective tissue fibres were found parallel both at smooth and at rough abutments.

In the past, a perpendicular orientation of the connective fibres had been found by some authors, particularly with implants harbouring a porous surface [Schroeder et al. 1981; Déporter et al. 1988; Buser et al. 1992].

Their orientation appeared to be influenced by the quality of the mucosa: the fibres tended to be parallel in alveolar mucosa, while they seemed to be organized more perpendicularly in keratinized mucosa.

Human studies
Glauser et al. [2005] studied histometrically, in human biopsies, the soft tissue formed around one-piece micro-implants with different surface topographies (turned, oxidized or acid etched). The overall height of the soft tissue seal was approximately the same for all surfaces. However, the length of the junctional epithelium was higher on smooth titanium (2.9 mm) than for rough surfaces (1.4–1.6 mm), with an inverse relationship for the length of the connective tissue. The limited number of samples unfortunately limits the impact of their findings.

Contact guidance
Impact on cell and tissue adhesion
An isotropic surface texture may influence growth and proliferation of cells, leading to contact guidance, which depends upon the micro-pattern and size of the different geometrical elements. Contact guidance refers to the tendency of cell locomotion to be guided or directed by the dominating direction of the surface topography of the substrate to which the cells are adhering.

Brunette et al. [1983] reported that cells outgrowing from gingival explants are guided by grooves of a titanium-coated silicon wafer. Grooved surfaces were also found to orient fibroblasts and epithelium [Brunette 1986a, 1986b, 1987]. Similar observations were made by Inoue et al. [1987], who found that circumferential grooves on Ti surfaces guide fibroblasts to form oriented capsule-like structures, whereas cells grown on porous surface showed no preferred orientation. Subsequent work demonstrated that there was a hierarchy in cell response to features, with larger features dominating smaller ones [Brunette 1986b].

The effects of grooved topography are considerable: Dunn & Brown [1986] showed the relationship between surface textural configuration and the shape that cells assume when cultured on it: they determined that 90% of cell shape, specifically elongation, was determined by the surface texture.

Moreover cells can be exquisitely sensitive to features, features as small as 0.2 μm, having been observed to produce a cell response [Clark et al. 1987]. Meyle et al. [1993] suggested that focal adhesions are mostly seen on ridges instead of contacting
the surface in the groove, depending upon the groove’s width and depth.

A considerable body of literature has now developed and reviews are available from some of the most active laboratories in this field (Curtis & Wilkinson 1998; Brunette 2001).

Surface’s form

Impact on cell and tissue adhesion

In vitro experiments. Chehroudi (1988) and Chehroudi et al. (1989) studied in vivo and in vitro the effects of a grooved (V-shaped grooves, 10 μm deep) titanium-coated substratum on epithelial cell behaviour. More epithelial cells were found attached to the grooved titanium surfaces than to adjacent flat surfaces. Clusters of epithelial cells were markedly oriented along the long axis of the grooves. In the grooved portion of the implant, epithelial cells interdigitated into the grooves and had rounded nuclei.

Histomorphometric measurements indicated that there was a shorter length of epithelial attachment, a longer length of connective tissue attachment, and less recession in the grooved, compared to the smooth portion of implants after 7 and 10 days.

These results indicate that horizontal grooves produced by micromachining can significantly impede epithelial downgrowth on titanium-coated epoxy implants.

The same authors (1990–1991) studied the effect of varying groove parameters such as depth, spacing, and vertical/horizontal orientation on epithelial downgrowth and attachment of epithelial cells and fibroblasts to percutaneous implants in vivo.

Close attachment of epithelial cells was found on the smooth, 10 and 3 μm deep, horizontally or vertically aligned grooved titanium surfaces; in contrast, epithelial cells bridged over the 22 μm deep, horizontally oriented grooves. Although epithelium was in contact with the flat ridges between the 22 μm grooved surfaces, the cell nuclei were rarely found inside the 22 μm grooves.

Fibroblasts formed a capsule on the smooth surface as well as the 10 and 3 μm deep horizontally oriented grooves, but they inserted obliquely into the 22 μm deep, horizontally aligned grooved surface, with nuclei located within the grooves.

Epithelial downgrowth was accelerated on the vertically oriented grooved surfaces and inhibited on the horizontally oriented grooved surfaces. Moreover, the mechanism of inhibition of the epithelial downgrowth may differ among these surfaces. Epithelial cells bridged over the 22 μm deep grooves and their migration appeared to be inhibited by the fibroblasts that inserted into the implant surface. Thus, the optimal surface topography for cell attachment to implants may differ for different cell types.

However, in those studies, connective tissue and epithelium interacted with the same surface so that the effects of the surfaces on each population could not be determined separately.

In 1992, the same authors examined cell behaviour on implants in which connective tissue contacted grooved topographies and epithelium encountered only a smooth surface: at grooved surfaces, the orientation of fibroblasts changed from an oblique to a more complex pattern which included cells having round nuclei within the grooves, as well as cells oriented oblique or perpendicular to the grooves.

The apical migration of the epithelium was significantly inhibited by those micromachined surfaces due to an improved connective tissue anchorage.

Influence of implant’s components and connections on soft tissue integration

Definitions

In a one-piece implant, the transmucosal component facing the soft tissues makes part of the implant.

In a two-piece implant, the transmucosal component (the abutment) dedicated at soft tissue integration is a separate part from the implant body. The interface between the transmucosal component and the implant is generally located in the neighbourhood of the alveolar bone level.

A one-piece implant is, in general, placed according to a one-stage surgery where the implant immediately pierces the soft tissue’s barrier (non-submerged fashion), when a two-piece implant system can either be submerged under the soft tissues for a waiting period (two-stage surgery) or be placed according to a one-stage surgery like one-piece implants.

Influence of surgical procedure on soft tissue integration

Animal studies

Several studies have looked at the potential impact of a submerged or non-submerged placement of implants on the localisation, the type and the dimensions of the soft tissues.

Weber et al. (1996) found no difference neither in the global dimensions of the soft tissue interface nor in the bone level and length of connective tissue between submerged and non submerged implants, but a longer junctional epithelium with two-stage surgery. These results were obtained using experimental implants.

With Brånemark two-piece implants (Ericsson et al. 1996a, 1996b; Abrahamsen et al. 1999) the dimensions and position of the soft tissues were found similar in both types of surgical approach.

Human studies

No clinical experiment has specifically compared the soft tissue integration after one- or two-stage surgery, but a number of clinical studies have looked at the marginal bone levels, which allow us to draw some conclusions, as a stable bone level implies that the soft tissue integration has not migrated apically. It has been demonstrated that there is no difference in marginal bone resorption, even in the long-term perspective, between one- and two-step surgical approaches with two-piece Brånemark implants (Ericsson et al. 1994, 1997; Petersson et al. 2001).

Soft tissue integration at one- or two-piece implants

Animal studies

Comparative studies were performed in dogs to determine the influence of implant design on soft tissue integration. Abrahamsen et al. (1996) demonstrated that the dimensions of the junctional epithelium and of the connective tissue are similar on one-piece implants (Straumann, Basel, Switzerland) and on two-piece implants (Brånemark system, Nobel Biocare Norden AB, Göteborg, Sweden and Astra Tech, Malmö, Sweden). In addition, their position relative to the bone crest was also comparable with the soft tissue integration located on the smooth implant’s neck on one-piece implants and at the abutment level on two-piece implants.
Using the same experimental conditions, but after 6 months of undisturbed plaque accumulation, it was shown [Abrahamsson et al. 1998a] that the extent of the plaque-related inflammatory infiltrate was comparable around one- and two-piece implants.

Using experimental implants with either a one-piece or a two-piece design, Hermann et al. (2000a, 2001) showed significantly higher apical migration of the soft tissues and marginal bone resorption with two-piece implants, suggesting a role of the sub-gingival position of the abutment/implant interface (so-called microgap) on tissue remodelling. It must be noted that in this experiment, all two-piece implants were clinically and histologically surrounded by an intense inflammatory process. This is in strong opposition with several animal studies (Berglundh et al. 1991, 1994, 1996; Ericsson et al. 1995; Abrahamsson et al. 1996, 1997, 1998a, 1998b, 1999, 2001, 2002; Lindhe & Berglundh 1998; Hermann et al. 2001) in which a soft tissue integration occurs at the abutment level.

In another experiment of the same group [Hermann et al. 2001], it was demonstrated that the size of the microgap between implants and abutments has little influence on marginal bone remodelling, whereas micromovements of the abutments induce a significant bone loss, independent of the microgap’s size. This strongly suggests that the mechanical disruption of the soft tissue interface is of importance.

An inflammatory cell infiltrate has been demonstrated at two-piece implants, in the close vicinity of the abutment/implant interface [Ericsson et al. 1995]. This infiltrate does not impair the formation of effective soft tissue integration, and seems to be present at implants systems with an external implant/abutment connection as well as at systems with an internal morse taper connection, but not at one-piece implants [Abrahamsson et al. 1996, 1998a, 1998b].

In some experiments using commercially available implants, the infiltrate proved to be very limited in size (<0.5 mm) and was not linked to a higher bone loss as compared with one-piece implants [Abrahamsson et al. 1996, 1998a, 1998b], while Broggini et al. (2003), with experimental implants, linked the 0.5 mm inflammatory infiltrate seen in their samples to a higher bone loss than at one-piece implants.

It has been shown that the seal provided by a locking taper connection at the implant/abutment interface effectively impairs bacterial leakage [Dibart et al. 2005]. But it has not been clearly evidenced if the bacterial contamination of the internal components of some two-piece implant systems [Persson et al. 1996] is responsible for the inflammatory cell infiltrate seen at the abutment/implant interface.

Clinical studies
Several studies have demonstrated long standing stability of the soft tissue interface and comparable marginal bone remodelling at both one-piece and two-piece implant systems [Quirynen et al. 1991a, 1991b; Bengazi et al. 1996; Hultin et al. 2000; Cune et al. 2004; Karoussis et al. 2004a].

Influence of abutment disconnection
The presence of a transmucosal component at two-piece implant systems can lead to intentional or unintentional disconnections of this abutment. Based on Hermann et al. (2001) results, an unintentional abutment loosening will lead to a disruption of the soft tissue integration and to increased bone remodelling.

It has also been shown that repeated intentional abutment disconnections and reconnections after alcoholic disinfection induces an apical repositioning of the soft tissues and marginal bone resorption [Abrahamsson et al. 1997]. In contrast, a single shift of a healing abutment and replacement by a final abutment proved to induce no marginal bone remodelling [Abrahamsson et al. 2003].

Conclusions
To be functionally useful, oral implants have to pierce the oral mucosa and enter the oral cavity, thus establishing a transmucosal connection between the external environment and the inner parts of the body.

In order to avoid bacterial penetration through this transmucosal piercing, the early formation of a long-standing effective barrier capable of biologically protecting the peri-implant structures is of paramount importance. It is a critical part of tissue integration, and may in part depend on:

Material chemistry
It is mandatory to place at the transmucosal level a material tissues can adhere to:

- c.p. titanium is the only material that has proven his biocompatibility towards the soft tissues in long-term clinical studies;
- some favourable clinical data become available for zirconium and aluminium oxide;
- animal studies have shown that dental porcelain or gold are less biocompatible and should be avoided. Materials such as resins and composites should not be recommended up to now;
- the surface of the core material can be contaminated, altering the composition of the interface. Saliva has shown deleterious and hardly reversible effects in vivo. Other contaminations, such as handling in the dental laboratory, could also be detrimental.

Surface topography
No clinical studies are currently available on the effect of altered surface topographies on implant prognosis. Results from in vitro and in vivo studies indicate that surface roughness and surface texture in the micrometer range may have an impact on the early events of healing by influencing attachment, orientation, proliferation and metabolism of epithelial and connective tissue cells.

- Some roughened titanium surfaces seem to improve the formation of a superficial fibrin network, which could hypothetically be positive for the initial stability of the interface and impair epithelial cells downgrowth.
- In vitro and in vivo studies tend to indicate that epithelial cells adhesion is lower on rough titanium surfaces than on machined titanium.
- Animal studies show that micromachined grooved surfaces of appropriate
dimensions can improve connective-tissue ingrowth and inhibit epithelial downgrowth.

Implant components and connections

Comparative animal studies have shown equivalent soft tissue integration at one-piece implants and at abutments of two-piece implant systems.

These data are confirmed by long-term clinical studies demonstrating the stability of soft tissue integration and comparable marginal bone remodelling at both concepts. It is meanwhile noteworthy that:

- At two-piece implants systems, animal studies have noticed a discrete inflammatory cell infiltrate at the abutment/implant interface, the effect of which on marginal bone level being limited and controversial.
- Unintentional or repeated intentional disconnections of the abutment at two-piece implant systems has been shown to disrupt the soft tissue integration and to induce an increased marginal bone remodelling.

As it is also more likely to place transmucosal components with an altered biocompatibility on two-piece implant systems (c.f. supra), effective soft tissue integration at one-piece implants seems easier to reproduceably obtain.

References


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