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Dimension and Structures of Biological Seal of Peri-Implant Tissues

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Abstract

Over the years, improved understanding of the nature of bone-implant interface is among the important contributors to the success of osseointegration in modern dental implantology. The focus has since shifted to the assessment of the soft tissue-implant interface to better understand the mechanism of biological seal in the transmucosal region. The importance of peri-implant mucosal region lies in the need to establish a tight seal that isolates implant and the bone from the oral environment via epithelial and connective tissue attachment, thus preventing ingrowth of bacterial plaque. Many factors may influence the soft tissue attachment at this peri-implant interface. In this chapter, the dimension of peri-implant tissues and the factors affecting the biological seal, namely surface topography and physicochemical properties, are discussed. The review also looks into the impact of the type of materials and surface modifications of dental implant, all of which may influence the formation of biological seal of soft tissue around the dental implant.

Keywords: implant-soft tissue interface, biological seal, peri-implant tissues, surface topography, three-dimensional oral mucosal model

1. Introduction

The success of dental implant in the oral cavity depends on direct bone-implant surface contact as well as the soft tissue attachment surrounding the implant abutment (and dental implant), which the latter acts as biological seal against external oral environment. Much of the attentions in the early dental implant studies were given to the bone-to-titanium interface.

These studies range from clinical [1, 2] to molecular levels [3], and from animal model [4, 5] to human biopsies [6, 7]. In all studies, the bone appears to be in direct contact with implant without the presence of any connective tissue or fibrous tissue encapsulating the implant.

The extensive and well-established researches on bone-implant interface have led to the wide acceptance of the concept of osseointegration. Presently, more focuses are placed on understanding and improving the implant-soft tissue interface. The biological seal of the soft tissue-implant interface is created by epithelium and connective tissue. The presence of keratinized mucosa surrounding an implant is thought to be one of the important factors in maintaining peri-implant soft tissue health. Moreover, materials and surface topography of implant abutment materials may also influence the biological seal formed at the implant-soft tissue interface. The available data from animal studies and emerging information from human investigations suggest that different material with different surface energy and enhanced surface topography is associated with increased soft tissue-to-implant contact [8, 9]. The nature of soft tissue-implant against normal periodontium is compared in the subsequent paragraph. By understanding the tissue around transmucosal region, the factors influencing this biological seal will be better appreciated.

2. Peri-implant tissue

The periodontium is known as a tooth-supporting structure while the peri-implant mucosa is the structure and function of the mucosa that surrounds the abutment of a dental implant. Clinically, both tooth and prosthesis of the dental implant will emerge from the gingival tissue with tight gingival cuff. **Figure 1** features the clinical pictures of healing abutment in situ and the appearance of peri-implant mucosa following removal of the healing abutment. The mucosa surrounding the dental implant formed tight gingival cuff consists of epithelium and connective tissues established during healing after the surgery. Many studies provide information on similarities and differences between peri-implant soft tissue and tissue at the dento-gingival junction. The similarities and differences of both periodontium and peri-implant mucosa are depicted in **Table 1**.

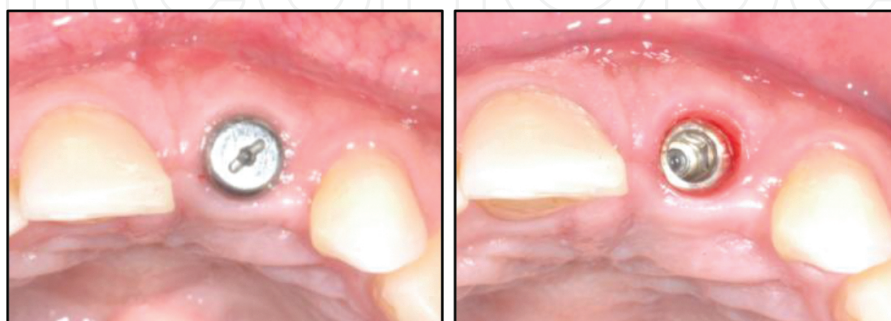


Figure 1. The clinical pictures of healing abutment in situ and mucosa at the implant neck. (Courtesy of Dr. Masfueh Razali).

Features	Periodontium	Peri-implant tissue
Gingival sulcus depth	Shallow	Dependent upon abutment length and restoration margin
Junctional epithelium	Hemidesmosome attachment to enamel	Hemidesmosome attachment on titanium [10, 11]
Gingival fibres	Complex array of fibres, some inserted into cementum above crestal bone, and onto periosteum	- Lack of fibres insertion on implant surface -Fibres orientated longitudinally, parallel or circumferential to the long axis of the implant [12]
Connective tissue attachment	Well-organised collagen fibre bundles, running perpendicular to root cementum	- A scar-like structure that is rich in collagen but deficient in fibroblasts and vascular systems [13, 14]
Blood supply	Numerous vasculatures from periodontal ligament space and gingival connective tissue which formed anastomoses	Fewer capillaries compared to tissue surrounding tooth. [14–16]
Biologic width	JE = 0.97 mm, CT = 1.07 mm	JE = 1.88 mm (average), CT = 1.05 mm [12]

Table 1. Comparison of periodontium and peri-implant tissue.

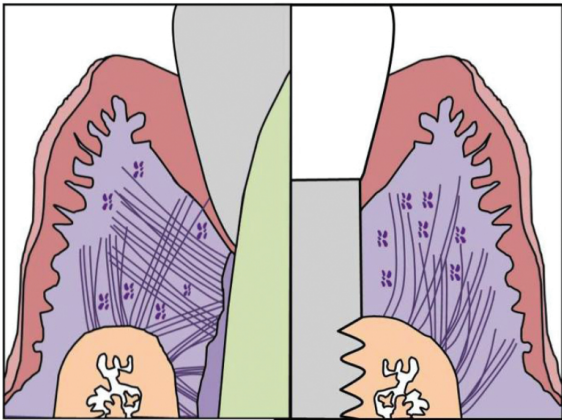


Figure 2. A schematic drawing of similarities and differences between dentogingival tissue and peri-implant mucosa (Prepared by Dr. Masfueh Razali).

Macroscopically, a tooth-supporting structure comprises the gingiva, connective tissues and periodontal ligament, which connects tooth to bone via cementum. There are three types of gingival epithelium covering the underlying connective tissue of a tooth. These are junctional epithelium, which provides the contact between the gingiva and the tooth; sulcular epithelium, which faces the tooth surfaces without any contact being made with the tooth surface; and lastly, oral epithelium, which faces the oral cavity. The oral epithelium is a keratinized, stratified squamous epithelium. The junctional epithelium, which is structurally different, is formed from the reduced enamel epithelium during tooth eruption and from dividing basal cells of the oral epithelium. The junctional epithelium forms a collar around the tooth and is about 2 mm high and 100 µm thick. It is composed of only two cell layers, namely a basal layer

and a supra basal layer. The inner cells of the junctional epithelium form and maintain a tight seal against the tooth surface. The connective tissue is composed of gingival fibres, which runs in many directions, from tooth and/or bone to gingival tissues. Similarly, the supporting structures of dental implant also consist of gingival epithelium and connective tissue attachment but without periodontal ligament. The epithelial part resembles the junctional epithelium around natural teeth [10, 12, 18, 19]. The features of both normal periodontium surrounding teeth and peri-implant tissue are illustrated in **Figure 2**.

Generally, the macroscopic and microscopic features of peri-implant mucosa are almost similar to the tooth-supporting tissue (at the dento-gingival junction) with few exceptions.

1. The junctional epithelium: the junctional epithelium faces the implant smooth surfaces or abutment of an implant is less thick, and consists of only a few cell layers especially at the apical region.
2. Biologic width: both consist of junctional epithelium and connective tissue attachment, but the junctional epithelium of an implant is longer [10, 14, 17] than that around teeth. Variation in height of these two attachments is noted between human and animal studies.
3. The gingival fibres connecting the periosteum to bone run parallel to the long axis of the implant, as compared with those around a tooth, where the gingival fibres consists of complexes arrays running from many direction including from tooth to gingival tissues, some of which perpendicular to the tooth. There were also fibres running circumferentially as shown by [15]. The arrangement of the fibres is schematically illustrated in **Figure 2**. The histological sections of transmucosal region of peri-implant soft tissue are shown in **Figure 3**. Note that there was a cell-free area adjacent to implant, and fibres appear running parallel to long axis of implant.
4. No periodontal ligament—bone is present.



Figure 3. Transmucosal region of peri-implant mucosa demonstrating fibres of gingival connective tissue, no attachment of fibres on implant surface. (Reproduced with permission from [15])

So far, the structure, dimensions and the composition of gingival and implant transmucosal regions have been investigated by many researchers. These include early animal models studies in dogs [10, 11, 14] and in human [20, 21]. From those studies, a few conclusions have been made which included:

1. On average, the attachment between mucosa and a titanium implant comprises junctional epithelium about 1.4–2.9 mm high, and a connective tissue zone approximately 0.7–2.6 mm high [10, 12]
2. The periodontium and peri-implant mucosa have common characteristics, but they differ in terms of composition of the connective tissue, the alignment of the collagen fibre bundles and the distribution of vascular structures apical to the junctional epithelium. The connective tissue-implant interface commonly consists of a non-infiltrated, densely structured, collagen-rich connective tissue. It can be divided into two zones: outer zone (located beneath the junctional epithelium) and inner zone (positioned above the bone crestal and in direct contact with implant surface) [14]

A qualitative analysis of the subepithelial connective tissue showed a cell-rich, well-vascularized outer zone with fibres running in many different directions and a poorly vascularized inner zone consisting of numerous dense collagen fibres running close to the implant surface, predominantly in a parallel direction [14, 15, 22]. The inner zone is in direct contact with the implant/abutment surface and is 50–100 mm thick. It is rich in fibres, with few scattered fibroblasts that appear to be in close contact with the transmucosal component. The peri-implant mucosa generally resembles and is recognized as a scar tissue, exhibiting an impaired resistance towards bacterial colonization [13, 16]. As a consequence, the connective tissue adhesion at implant has a poor mechanical resistance as compared to that of natural teeth.

As suggested by some studies mentioned earlier, the biological seal of the peri-implant tissue formed by both epithelial and connective tissue attachments is weak and poor in mechanical resistance [13, 16]. Hence, this area is subjected to increased risk of peri-implant diseases, as the bacterial assault begins in this area. It is important to understand the nature of both attachments as it may lead to an enhancement this biological seal. Various models been used to evaluate the implant-soft tissue interface. These models are reviewed in the next section.

3. Evaluation of implant-soft tissue response

The soft tissue interface especially the structure of collagen fibre bundles received more attention over the past 10 years with studies that include animal models such as dogs [10–12, 14] and monkeys [23, 24] and human [25, 26] used to explore the structure and dimension of soft tissue-implant interface. Recently, Chai and co-workers [18] have ventured upon the use of three-dimensional oral mucosal models by using the tissue engineering technology to investigate the nature of the peri-implant biological seal.

3.1. Implant-soft tissue interface models

The advantages and disadvantages of each implant-soft tissue interface study models are described in the next section. These models were developed in order to enhance our understanding of the soft tissue response on various materials with different surface topography and to establish best methods to evaluate the biological seal of peri-implant tissue. Generally, an *in vitro* study using monolayer cell culture model is conducted to assess the cytotoxicity of the cells and quickly observe cell activities and behaviour towards new dental implant materials. Histomorphometric analysis of *en bloc* tissue consisting of both soft tissue and implant body is the best method to demonstrate the presence of epithelial and connective tissue attachment at the soft tissue-implant interface. Yet, due to limited opportunity to obtain histological section from human, animal models were developed.

3.1.1. *In vitro* studies

Presently, *in vitro* testing is performed as a prerequisite to *in vivo* evaluation. However, the *in vitro* techniques do not reflect the clinical situation and the progress in our understanding of extra- and intracellular processes that occur in connective tissue attachment. Thus, the data cannot be extrapolated into clinical applications. Nevertheless, the study involving monolayer cells is by far the most popular and easy-to-conduct study although more sensitive *in vitro* evaluations are now available. The cell shape, activities and response can be evaluated morphometrically via immunocytochemical staining [27], or by analysis using scanning electron [28, 29] or fluorescent [30, 31] microscopies. Additionally, the gene and protein expressions for cell adhesion and attachment can also be carried out [27, 32, 33]. Most studies used primary human gingival [29, 32, 34] and periodontal [35] fibroblasts as a cell model, which are cultured directly onto the dental implant materials surface. Keratinocytes are also frequently used [27, 36, 37]. Compared to fibroblasts, keratinocytes by far is most difficult to culture. Cochran et al. [35] compared the behaviour of periodontal and gingival fibroblast as well as keratinocytes towards the titanium with different surface textures. They found that human fibroblast and epithelial cell attachment and proliferation are significantly affected by surface characteristics of titanium. Of three cell types, gingival fibroblasts appeared to attach best, followed by periodontal ligament fibroblasts and epithelial cells. Both types of fibroblasts grow and proliferate well on both rough and smooth titanium surfaces compared to epithelial cells once they are attached to the surface [35]. Other study found a significant decrease in the number of gingival fibroblasts on rough titanium (Ti) surfaces compared with smooth polished Ti surfaces [30, 34]. On the other hand, Oates et al. [32] found that the fibroblasts adhesion and attachment are enhanced in rougher surface than smooth surface, in contrast to other findings [33] where focal adhesion kinases were immunogold labelled. In a different study using ceramic, fibroblasts attached more on the milled ceramic and appeared to follow the direction of the fine irregularities on the surface [38]. Nevertheless, most common finding of those studies is that cells were oriented in a parallel order along the grooves of the machined surface but arranged randomly when in contact with a rough surface. Hence, the *in vitro* models appear to be able to provide an insight and could be used to guide specific cell attachment or

specific material with surface characteristics for in vivo models. Animal models are the most common in vivo models carried out compared to human studies.

3.1.2. *Animal models*

Studies using animals as in vivo models for evaluation of soft tissue response around dental implant have been extensively conducted and are well documented. In animal models, the histological section of peri-implant tissue was made possible, which becomes the gold standard for the implant-soft tissue interface analysis. While dogs models such as the beagle [8, 14] being the most common animal of choice, monkeys [23, 39] and minipigs [40] were also used to demonstrate the presence of epithelial and connective tissue attachment around transmucosal region of dental implants histologically.

The experiments in animals demonstrated that the dimension of the mucosal attachment to implants was similar to the gingival attachment at teeth and was composed of an epithelial portion about 1.5–2 mm long and a cell-rich connective tissue portion close to the implant that was about 1–1.5 mm high [10]. Animal models were also used to evaluate the soft tissue response towards different abutment materials. Abrahamsson et al. [11] investigated the influence of abutment material on the location and the quality of the attachment that occurred between the peri-implant mucosa and the implant. They found no proper attachment formed at the abutment level made of gold alloy and porcelain when compared to those made of titanium and ceramic. In addition, similar finding was noted by Welander et al. [41] when titanium, zirconia and Au/Pt alloy were used. The tissue around abutment made from titanium and zirconia was stable; meanwhile, an apical migration of epithelium was noted on Au/Pt alloy. In another study, Abrahamsson et al. [42] demonstrated that the soft tissue attachment that formed at implants made of commercially pure titanium (c.p. titanium) was not influenced by the roughness of the titanium surface.

Among many, dogs have been the most common animal of choice. This is possibly due to easy access with regard to clinical examinations and oral hygiene procedures of the dogs. It must be noted that non-human primates bear more resemblance to human anatomy and histology than any other animal, thus may offer a higher degree of relevance to human. Nevertheless, the results from animal experiments should always be carefully interpreted since the healing response and immuno-reaction in animals might not be similar to human, so the data might not be comparable. A given sequence of soft tissue integration to implants in a dog may not correspond exactly to an expected outcome in humans. The differences in tissue response during healing between human-human subjects may sometimes become more pronounced between different human to human subjects than between animals and humans. Moreover, the healing response in animals is also less predictable compared to human. In the light of evidence-based dentistry, the result from animal studies should be interpreted cautiously. Additionally, animal studies are also bound to ethical considerations, where study design and calculation of sample size of animals in experiments are to be carried out with caution. Essentially, to have more clinical validity, human randomized control trials should be carried out to obtain more information on the peri-implant tissues.

3.1.3. Human studies

The composition of the connective tissue interface towards implants was studied in both animal experiments and human biopsy materials. While human studies are very limited due to ethical issues, the evidence of epithelial and connective tissue attachment around peri-implant regions are obtained mostly from failed implant [43], autopsy [44, 45] or clinical studies [1, 46], where the presence of connective tissue attachment on these studies is still difficult to demonstrate. Most of the human studies that have been carried out were clinical studies in which the traditional periodontal parameters were used for monitoring the soft tissue responses around dental implants intra-orally. According to clinical studies that involve the marginal bone levels, we can conclude that bone level is stable as it implies that the soft tissue integration has not migrated apically [1, 46, 47]. Liljenberg et al. [48] in their study of soft tissue biopsies of edentulous ridge mucosa and peri-implant mucosa revealed that the composition of both tissues were nearly identical in terms of collagen, cells and vascular structures. The peri-implant mucosa harboured a junctional epithelium that contained significantly enhanced numbers of different inflammatory cells infiltration. On the other hand, Piatelli et al. [44] found that there was no inflammatory infiltrate in epithelium or connective tissue in human autopsy biopsies of titanium dental implants. It is also interesting to note that the collagen fibres in the coronal part were parallel to implant surface while in the apical region the fibres were in a perpendicular fashion was found. Additionally, Glauser et al. [49] used both hard and soft tissue biopsies of mini titanium implants with different surface characteristics to demonstrate the establishment of junctional epithelium attachment to the implant surfaces. They noted that collagen fibres and the fibroblasts were oriented parallel to the implant surface. The oxidized and acid-etched implants revealed less epithelial downgrowth and longer connective tissue than machined implants [49]. As for different types of materials, Vigolo et al. [46] assessed the peri-implant mucosa around abutments made of gold alloy and titanium and found no difference between the two types of abutments with regard to peri-implant marginal bone level and soft tissue parameters. Meanwhile, Nevins et al. [26] using en bloc biopsy demonstrated intimate contact of junctional epithelium cells to implant surface and connective tissue with functionally oriented collagen fibres running towards the implant surface designed with Laser-Lok microchannels. Nonetheless, it is unethical to remove implant in order to attain en bloc tissue for histological analyses in human, and data from autopsy did not necessarily represent the ultrastructural nature of the peri-implant interface. In addition, not all animal experiments can be replicated in human samples due to cost and ethical considerations. For this reason, the investigation of peri-implant interface for improvement of connective tissue attachment is rather difficult to conduct in human. Thus, the need of development of different models for histological analyses may be essential.

3.1.4. Three-dimensional oral tissue engineering

As the opportunity to undertake human studies is limited, many studies that evaluated the peri-implant interface were carried out using animal models. With advances in knowledge on tissue regeneration, tissue-engineered oral mucosal equivalents (three-dimensional oral mucosal model, 3D OMM) have been developed for clinical applications and also for con-

ducting in vitro studies on biocompatibility, mucosal irritation, disease and other basic oral biological phenomena such as for grafting of oral mucosal defects [50, 51]. The 3D OMM consists of both epithelium and connective tissue layers, grown in the laboratory using collagen membrane as the scaffold. Therefore, evaluation of cell-cell interaction between epithelium, connective tissue and implant surface using 3D OMM is possible and could become an alternative method to study the nature of peri-implant interface. The use of 3D OMM will permit histological preparation and histomorphometric analysis of the interface. With the modification of culture technique, Chai et al. [18] have constructed 3D OMM and have demonstrated the presence of peri-implant tissue with features that mimicked those seen in vivo when tested with titanium. Chai and co-workers [19] further developed the 3D OMM and succeeded in obtaining formed peri-implant-like-epithelium (PILE) on the polished, machined, sand-blasted and TiUnite titanium surfaces. Using the 3D OMM, ultrastructural investigation of the soft tissue-implant interface with transmission electron microscopy (TEM) is also possible. It is also interesting to note that the presence of hemidesmosome-like structure as an epithelial attachment to the material surface is shown using this model (**Figure 4**). Moreover, the biological seal of peri-implant tissue can also be demonstrated quantitatively with 3D OMM [52, 53]. This can be carried out via assessment of penetrative behaviour of radioisotope material through the 3D OMM model [52]. Alternatively, the biological seal of peri-implant can also be assessed through the measurement of degree formed by pocket or non-pocket epithelial attachment at the oral mucosal model-material interface [18, 53]. Although only limited study is available on the use of 3D OMM for evaluating the peri-implant interface, this model appears to have a more promising prospect than the monolayer cell culture model. This model is a useful method to evaluate the soft tissue response prior to investigation with an animal model.

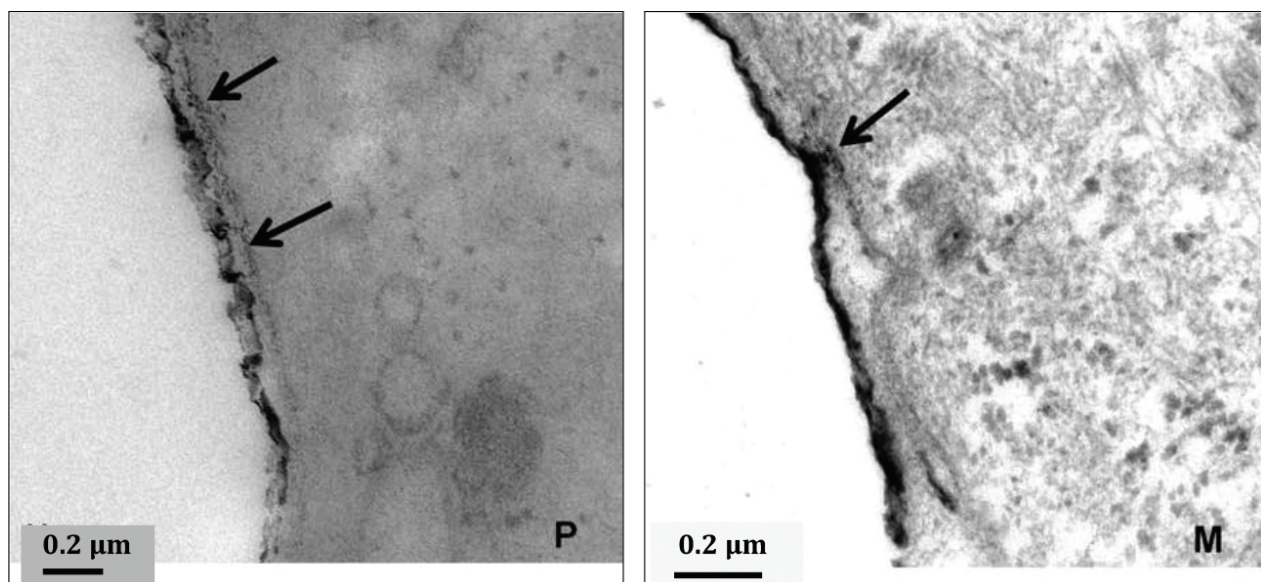


Figure 4. Hemidesmosome-like structures (black arrows) formed from 3D OMM and specimens (Ti). P = polished and M = machined surfaces. (Reproduced with permission from [19]).

3.2. Analyses of the soft tissue-implant interface

The soft-tissue implant interface can be investigated through histomorphometric and histologic analyses. Of both, the preparation for latter analysis is very difficult to carry out especially if the implant is attached to the tissue. The histological studies also allow identification of specific protein markers expressed by any of the tissue or cells in response to dental implants. The histological sections can then be analysed under different types of microscopies. Among the known microscopic analyses for assessing the peri-implant interface are light microscopy (LM), scanning electron microscopy (SEM), confocal laser scanning microscopy (CLSM), focus ion beam (FIB) and transmission electron microscopy (TEM). Similar to SEM, CLSM allows assessment of peri-implant interface and cell-cell interaction without the need of histological processing as for light microscopy. With these two, direct visualization of implant-soft tissue interface is possible with appropriate preparation for each microscopy. The tissue or specimens can also be fluorescently labelled for the identification of adhesion molecules or cells and examined under CLSM [43]. While the use of 3D oral mucosal model with implant material intact may allow direct examination of the connective tissue attachment, the method to prepare the specimen still remains challenging and technically demanding. More studies in term of optimization of certain promising technique such as FIB for TEM analysis must be explored in order to obtain the ultrastructural nature of the implant-soft tissue interface.

4. Factors influencing biological seal of implant-soft tissue interface

The existence and function of biologic width around dental implant are well documented in animal and human histological studies. Any factors affecting soft tissue reaction around dental implant might also affect the biologic width, thus the biologic seal of the peri-implant region. As mentioned earlier in the text, the nature and health of soft tissue surrounding an implant may be influenced by many factors. The presence of keratinized mucosa surrounding an implant is thought to influence the dimension of biological seal [54]. Moreover, the attachment of epithelial and connective tissues may also be influenced by material properties and surface modifications of implant abutment materials. Within the context of this chapter, how soft tissue responds to material and surface modification of implant/implant abutment is only discussed briefly.

4.1. Bulk of materials

Material properties appear to affect the attachment formed by epithelial tissue. Most often, titanium is the material used for dental implants and abutments, and is therefore the most extensive and widely studied material. Commercially pure titanium (Grade 2 and Grade 4) is commonly used in the fabrication of dental implants and implants abutments. Recently, zirconia is gaining more popular and seems to be a suitable implant material because of its excellence aesthetics, mechanical properties and biocompatibility. The presence of zirconia in dentistry is now being embraced, with the manufacturers promoting the esthetic, biomechanical and biological qualities of the material. Despite the extensive literature in the field of

osseointegration of zirconia [39, 55], the response of soft tissue towards zirconia is starting to gain attention from many researchers [9, 11, 40, 41]. In animal experiments, Abrahamsson et al. [11] showed that an epithelial downgrowth occurred and migrated towards the implant neck and associated bone loss which was noted around the abutments of gold and gold alloys fused with dental ceramics, as compared to abutments made of pure titanium and aluminium oxide (Al_2O_3) ceramics where peri-implant cuff of about 3.5 mm width was noted to be present. Kohal et al. [39] also reported a satisfactory soft-tissue formation on both titanium and zirconium oxide (ZrO_2) surfaces, without evidence of perpendicular fibres on the monkey model. Likewise, another study showed that the soft-tissue dimension at Ti and ZrO_2 abutments remained stable after 5 months of healing, meanwhile at gold/platinum alloys abutment sites, an apical shift of the barrier epithelium and marginal bone loss occurred [41]. In contrast, a human clinical study conducted by Vigolo et al. [46] revealed no significant differences regarding peri-implant bone loss and soft-tissue level when abutments of titanium and gold alloy were used with cemented single implant crown. Similarly, Linkevicius and Apse [56] in their systematic review concluded that available data failed to give evidence that titanium abutments are better at maintaining stable peri-implant tissues as compared to gold, aluminium oxide and zirconium oxide abutments. The performance of zirconia vs titanium abutments over long term is yet to be available. Recently, Zembic et al. [57] has published a 5-year comparison of the clinical performance of both titanium and zirconia abutments, and they found no statistically and clinically relevant difference between the survival rates, and technical and biological complication of these two abutment types.

4.2. Surface modifications

Surface modifications of titanium dental implants or implant abutment are performed to improve the biological, chemical and mechanical properties of implants. Over the years, specific surface properties such as topography, structure, chemistry, surface charge and wettability have been investigated to help enhance the soft tissue attachment. Commonly, the surface modification can be broadly classified into modification of physical properties of the surface or chemical properties of the surface. In the subsequent paragraphs, the surface modifications of titanium dental implant/abutment are divided into surface topography and surface/chemical composition of the material. The surface topography of the implant can be altered in many ways. However, the methods of surface modifications of dental implant are not discussed since they are not within the scope of this chapter.

4.2.1. Surface topography

Different materials exhibit different surface energy. The differences in surface free energy may reflect their wettability characteristics. The higher the hydrophilicity of the material, the better adhesion of the cells thus enhancing the attachment formed by these cells [58]. Improving the surface texture with various techniques, thus altering the surface chemistry also enhances the wettability of certain material. Modification of surface texture will create different surface topography of dental implant material including abutment materials. Analysis of surface topography can be obtained from scanning profilometer (Figure 5) or SEM (Figure 6) in which

the surface details can be visualized three dimensionally. The definition of surface roughness of dental implant has been proposed by Albrektsson and Wennerberg [59, 60]. This definition can be used for study of osseointegration or implant-soft tissue interface. Accordingly, the characterization of surface topography is shown in **Table 2**. The values of Sa were determined by optical interferometry using Gaussian filters. There is a need to emphasize that **Table 2** shows a summary of several studies cited in this chapter.

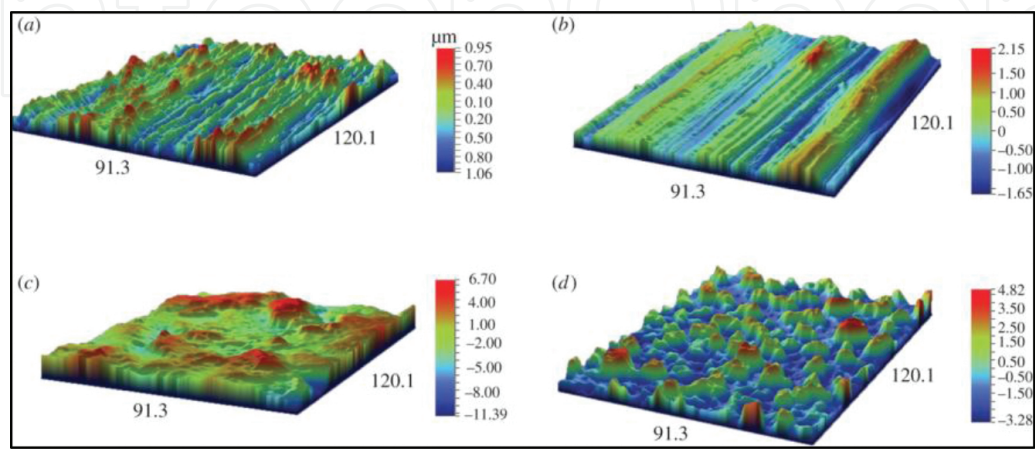


Figure 5. A light interferometry micrograph showing the surface topography of the four types of Ti surfaces. (a) Polished, (b) machined, (c) sandblasted, and (d) TiUnite. Scale bar: (a) 21.06–0.95 mm, (b) 21.65–2.15 mm, (c) 211.39–6.70 mm, (d) 23.28–4.82 mm. (Reproduced with permission from [52]).

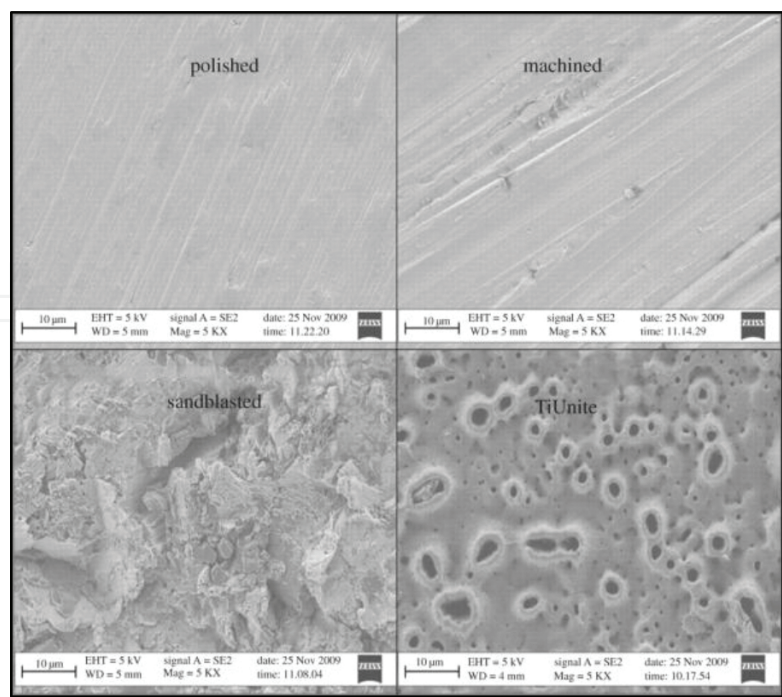


Figure 6. Scanning electron micrographs of the four types of Ti surface topographies.(Reproduced with permission from [52]).

Characteristics	Roughness value (S_a)	Clinical Use	Findings (cell behavior or soft tissue response)			
			Monolayer studies	Animal studies	Human studies	3D OMM
Smooth	0.0–0.4 μm	Machined surface, abutments	Generally, two types of cell used (epithelial and gingival fibroblast cells)	Surface topography has no influence on soft tissue attachment	Surface compared: turned, oxidized and acid etched Soft tissue seal was almost the same for all surfaces compared	Surface compared: polished, machined, sandblasted and anodized No different found
Minimally rough	0.5–1.0 μm	Turned implant, Osseotite™ (dual acid etched)	No value of surface roughness stated	(epithelial and connective tissue).	The length of the junctional epithelium appeared higher/longer on smooth titanium (2.9 mm) than for rough surfaces (1.4–1.6 mm),	in term of contour of soft tissue attachment [52, 53].
Moderately rough	1.0–2.0 μm	Tioblast™ and Osseospeed™, sandblasted and acid etched (SLA), TiUnite™ (anodized) (most common implant topography)	Most studies compared polished, sandblasted and plasma-sprayed titanium surfaces	fibres were found parallel both at smooth (turned) and at rough (acid-etched) abutments	Connective tissue appeared higher/longer on smooth titanium (2.9 mm) than for rough surfaces (1.4–1.6 mm),	
Rough	>2.0 μm	Plasma-sprayed, hydroxyapatite-coated	Noted higher adhesion and proliferation of both cells on polished titanium surface [34, 35]	parallel both at smooth (turned) and at rough (acid-etched) abutments [42]. Conflicting evidence found that perpendicular fibres are in favour of more porous implant surface [13]	for the length of the connective tissue [49]	

Table 2. Implant surface roughness.

Surface texture is known to influence epithelial cells and fibroblast attachment, although there is no complete agreement in the literature on the exact effect. One report found no significant differences concerning soft tissue reactions between roughed or smoothed surface implant [13], whereas Cochran et al. [35] found that smooth surfaces were more favourable for epithelial cell proliferation, as the fibroblasts appear to attach and proliferate better on rough surfaces. Simion et al. [61] reported that epithelial cells adhered and spread better on metallic

surfaces than on ceramic surfaces with well-organized focal contacts and pre-hemidesmosomes found on metallic surfaces, but not on porcelain and aluminium oxide.

Brunete and Chehroudi [62] in their review have suggested that the micro-fabricated grooved surfaces are able to inhibit epithelial downgrowth on implants depending on the dimension of the grooves in vitro. Similarly, fibroblasts also exhibit contact guidance on grooved surfaces, although its shape in vitro differs from that found in vivo. Delgado-Ruiz and co-workers [63] noted that micro-grooved surfaces were able to induce transverse collagen fibre formation, thus supporting two studies [26, 64]. It is also important to include a study by Nevins et al. [26] who demonstrated that soft tissue in humans is attached mechanically by perpendicular collagen fibre bundles on a micro-grooved pulsed laser surface.

4.2.2. *Surface composition*

Over the years, many strategies have been explored to improve the biological seal of peri-implant tissue by changing the surface chemistry of dental implants and implant abutments. The surface chemistry of the materials may be altered by biological modification, or by changing the chemical composition of the materials. As for biological modifications, methods of surface modification available include adding or coating with biomimetic/bioactive substances such as fibronectin or intergrin onto the surface with the aim of promoting cellular adhesion and controlling cell behaviour. Fibronectin is a glycoprotein present on cell surfaces, found in connective tissues, basement membranes, and extracellular fluids, and is known to play a role in cell-to-cell and cell-to-substrate adhesion and enhances gingival fibroblast attachment. It is interesting to note that epithelial cells and fibroblasts have different affinities for adhesive proteins of the extracellular matrix. Dean et al [65] noted that higher number of fibroblasts bound to fibronectin coated implant surface than epithelial cells, while gingival epithelial cell binding on implant surface coated with laminin was higher in number than fibroblasts [66, 67]. Collagen Type 1 was also used to modify surface chemistry as it was found to improve initial fibroblasts attachment [68].

The chemistry of material surfaces can also be altered by using element such as calcium or magnesium coating. Hydrothermal treatment of titanium with CaCl_2 or MgCl_2 was found to enhance initial attachment of epithelial and fibroblasts cells, and may increase the quality of the soft tissue seal around dental implant [69]. In addition, surface chemistry of materials may also inadvertently be altered by the presence of impurities, surface contamination and saliva. A clean surface has a high surface free energy, while a contaminated one has a lower surface energy.

5. Conclusion

There is some controversy on the possible attachment of connective tissue fibres to implant, but current studies indicate the presence of a parallel orientation with no insertion of peri-implant connective tissue fibres. This difference in connective tissue attachment may affect the peri-implant tissue's susceptibility to disease. The gold standard for evaluating the connective

tissue and epithelial attachment is assessing the histological section using various modes of processing, staining and analyses. The 3D OMM mimicking the oral tissue is a promising technique to be considered for evaluating the connective tissue attachment, yet the processing of the tissue/implant block is still similar to the tissue block obtained from animal/human. The reaction of cells on biomaterials is affected by the surface topography and surface physico-chemistry of the materials. Various studies have shown that materials and surface modification of dental implants influence cell behaviour and interaction. Some of documented data were limited to cell response on the monolayer cell culture model and animal histological studies. Therefore, it is recommended that randomised controlled clinical trials are to be performed to determine the effects of dental implant materials and surface modifications on the peri-implant tissues.

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