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Strategies for Optimizing the Soft Tissue Seal around Osseointegrated Implants

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Percutaneous and permucosal devices such as catheters, infusion pumps, orthopedic, and dental implants are commonly used in medical treatments. However, these useful devices breach the soft tissue barrier that protects the body from the outer environment, and thus increase bacterial infections resulting in morbidity and mortality. Such associated infections can be prevented if these devices are effectively integrated with the surrounding soft tissue, and thus creating a strong seal from the surrounding environment. However, so far, there are no percutaneous/permucosal medical devices able to prevent infection by achieving strong integration at the soft tissue–device interface. This review gives an insight into the current status of research into soft tissue–implant interface and the challenges associated with these interfaces. Biological soft/hard tissue interfaces may provide insights toward engineering better soft tissue interfaces around percutaneous devices. In this review, focus is put on the history and current findings as well as recent progress of the strategies aiming to develop a strong soft tissue seal around osseointegrated implants, such as orthopedic and dental implants.

1. Introduction

Percutaneous medical devices are increasingly used in several medical fields for a wide variety of conditions.[1–6] Percutaneous devices can be defined as foreign bodies crossing the epithelial barrier, and thus connecting the external environment to the internal structure of the body.[2–5] Examples of percutaneous devices include catheters and other long-term intubation systems or osseointegrated implants (e.g., orthopedic and dental implants), where the device is permanently implanted in the body.[7–10] Other examples include devices that are temporarily placed until the patient's condition resolves or a better alternative becomes available (e.g., fixator pins and prosthetic urethras).[1,11] Various terms also have been used to describe these devices including transcutaneous devices/implants, or in the case of dental implants, the term transmucosal/permucosal implants has also been used since dental implants penetrate the oral epithelium (**Figure 1**). **Table 1** summarizes the list of some of the currently used medical percutaneous/permucosal devices.

Despite all their benefits, percutaneous medical devices are not bulletproof solutions and may fail in a number of ways that might lead to serious complications or even death in severe cases.[2,3,5,12] According to von Recum, classical modes of percutaneous device failures include:[6] marsupialization, permigration, mechanical avulsion, infection, or any combination of the previous conditions. Marsupialization (also called "epithelial downgrowth) is the apical migration of epithelial cells along the surface of the percutanoues device, eventually creating a sinus tract, pocket, or a gap between the skin and the implant surface. Permigration is similar to marsupialization but it is more specific to completely porous materials. In permigration, the epithelial tissue (e.g., epidermis) migrates inward and then completely through the porous percutanoues component. Over time, the porous structure becomes filled with keratized, nonviable epithelial cells and cell debris, which gradually leads to extrusion of the implant. Mechanical avulsion (i.e., mechanical induced failure) is the extrusion of the devices due to excessive or inappropriate mechanical forces. Infection and abcess formation around the percutanoues devices is one of the most devastating complications.

2. Epidemic of Infections Related to Percutaneous Devices

Infections related to percutaneous tissues cause increased morbidity, mortality, and health care costs.[2,3,5,12] Indeed, the number of patients that suffer from infections related to

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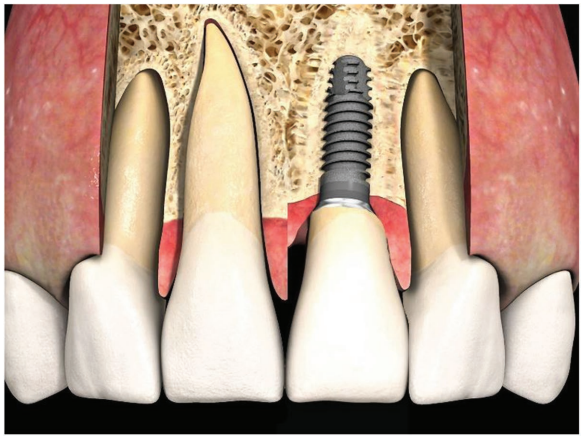

Figure 1. Schematic illustration of a cross section of a human maxilla showing an osteointegrated dental implant penetrating the oral epithelium (i.e., transmucosal/permucosal).

percutaneous devices is significant;[12–17] 38% of osseointegrated arm prostheses are infected within five years of implantation, and 17% fail.[14] These orthopedic-related infections can cost up to $50 000 per patient.[12] Similarly, there are over 1 million cases of catheter infections in the USA every year, accounting for over 40% of all nosocomial infections in hospitals, and in certain conditions, they could have an attributable mortality of up to 41%.[13] These catheter-related infections increase hospitalization expenses by $56 000 per patient and cost $450 million annually in the USA.[13] Moreover, bacterial mucosal infections (peri-implant mucositis) occur in up to 56% of the dental implants. Untreated, mucositis could lead to peri-implantitis, which is characterized by peri-implant bone resorption and pocket formation. The mean prevalence of peri-implantitis is estimated to be around 22%, and it could cause up to 2% failure after nine years of placement.[15–19]

Early bacterial contamination of percutanoues devices, particularly implants, is generally considered unaviodable and combinations of antibiotic prophylaxis and post-opreative hygience measures are applied, which vary upon the clinical scenario, patient, clinician, and nature of the implant.[20] Despite recent advances in biomaterials, standards for sterilization, and preoperative and postoperative protocols, infection remains the most common and devastating complication related to percutaneous devices.[5,12–14] Infection of percutaneous devices can occur due to several reasons, such as improper surgical techniques, an existing infection at the implantation site, the introduction of microorganisms during the surgical procedure, improper cleaning of the device after implantation, or misuse of the device.[21]

It should be emphasized that infections related to percutaneous devices are mostly, but not always, a result of the lack of integration at the soft tissue–implant interface.[1,6] This lack of integration causes a series of secondary complications starting with inflammation, swelling, accumulation of bursal fluids, and eventually the establishment of infection at the skin–implant interface.[22] In the majority of cases, the causative agents originate from the normal skin flora (e.g., *Staphylococcus aureus* and *coagulase-negative staphylococci*);[21] however, bacteria from other sources of contamination might be implicated in the infection process.[21,23] In case of dental implants, the major causative agents originate from the oral biofilms, microorganisms most commonly related to the failure of an implant are the Gram-negative anaerobes, like *Prevotella intermedia*, *Porphyromonas gingivalis*, and *Fusobacterium species*.[24]

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Table 1. List of percutaneous medical devices.[1,6]

Devices	Anchoring	Implantation time
Blood access devices		
Central venous catheter	Soft tissue and/or internal organ	Indefinite implantation or until alternative therapy becomes available
Heart assist device		
Dialysis catheter		
Body cavities access devices		
Prosthetic urethra	Soft tissue and/or internal organ	From months to years
Peritoneal dialysis device		
Osseointegrated prosthetic devices		
Dental implants	Bone	Indefinite
Limb prosthesis		
Hearing aids		
Power and signal conduits		
Pneumatic, hydraulic, or electrical power devices used for activation, control of internal natural or artificial organs or for recording electrical potentials from these organs	Soft tissue	Usually indefinite
Tissue access devices		
Probes for monitoring parameters like pO_2, pCO_2, pH, temperature, enzymes (e.g., insulin sensor, glucose sensor)	Soft tissues	Usually indefinite
Percutaneous access ports (e.g., snap button for connection)		

Some of the mild infections can be treated with local administration of antibiotics.[21,24] Whereas, long lasting-chronic-infections, particularly those associated with permanent percutaneous implants, are extremely difficult to manage with antibiotic therapy because once the bacterial film is formed on the percutaneous device, it is difficult, if not possible, for the antibiotic agent to eliminate the chronic infection. Another concern is the increased likelihood of developing antibiotic resistant bacteria due to drug overuse.[21,25,26] The progressive infection and deterioration of the soft tissue–implant interface might induce the formation of fibrous tissue and eventually implant failure.[1] In extreme situations, the infection may progress to deeper tissues and, thus, lead to the development of sepsis and osteomyelitis.[27,28]

In the subsequent sections, this review provides an overview of the anatomy of skin as a natural soft tissue barrier, with emphasis on the basement membrane zone (BMZ) which links the outer protective layer of epithelial cells to the underlying tissues. This would help the reader understand the molecular biology of the adhesion structures responsible for soft tissue attachment and strategies used to improve the soft tissue seal around osseointegrated implants. The structure of biological soft/hard tissue interfaces is also described to understand the biology and morphology of these natural interfaces, which in turn may provide insights into possible methods that could be employed to engineer soft tissue interfaces around percutaneous devices. Furthemore, the process of superficial wound healing is briefly discussed to comprehend how epithelial cells and fibroblasts migrate and adhere to the underlying structures, and understand the rationale behind some of the criteria used to evaluate soft tissue around osseointegrated percutaneous implants. After that, the review walks briefly through the early history of osseointegrated implants and then focus is put on the progress of strategies aiming to promote soft tissue integration around these implants.

3. Skin—The Dynamic Natural Epithelial Barrier

Skin is the largest organ, covering the entire exterior surface of the human body.[29] It enables the body to sense temperature, pressure, and pain, and regulates both body heat and moisture through perspiration and blood flow.[29,30] In addition, the skin provides an essential barrier against the external environment and protects the body from harmful bacterial, mechanical, and thermal insults.[30] The skin is a highly specialized and dynamic structure; typically, it constitutes of functionally distinct compartments: the epithelial tissue of the epidermis, a basement membrane (basal lamina), the connective tissue of the dermis, and the subcutaneous layer (**Figure 2**). The skin integrity and functionality are well maintained through complex functional relationships between these different compartments.[29] Furthermore, the skin contains several appendages (e.g., hair, sweat glands) that vary in shape, distribution, and function.[30]

3.1. The Epidermis

The epidermis is the outermost layer of the skin, which is impermeable to harmful organism and toxic substances.[30] Essentially, it is a keratinized stratified squamous epithelium, composed mainly of epithelial cells (keratinocytes) (85%) along with other cell types, including Merkel cells, Langerhans cells, and melanocytes, overlying a collagen-rich dermis.[31] Microscopically, four distinct strata can be identified in the epidermis;[30,31] these strata are (from the inner surface facing the dermis to the exterior): basale, spinosum, granulosum, and corneum. An additional thin strata of three to five layers of flattened translucent cells, known as stratum lucidum, provides extra protection in areas exhibiting a thick epidermis (e.g., foot soles, hand palms).[32]

The epidermis is regenerated and replenished from the basal layer by stem cells of epithelial cell linage that differentiate as they migrate through the layers toward the surface of the skin. During migration, cells change their appearance from one layer to another. The innermost layer, stratum basale, contains cells attached to the underlying basal lamina via adhesion structure knows as hemidesmosomes (HD), further details about these structures will be provided below.

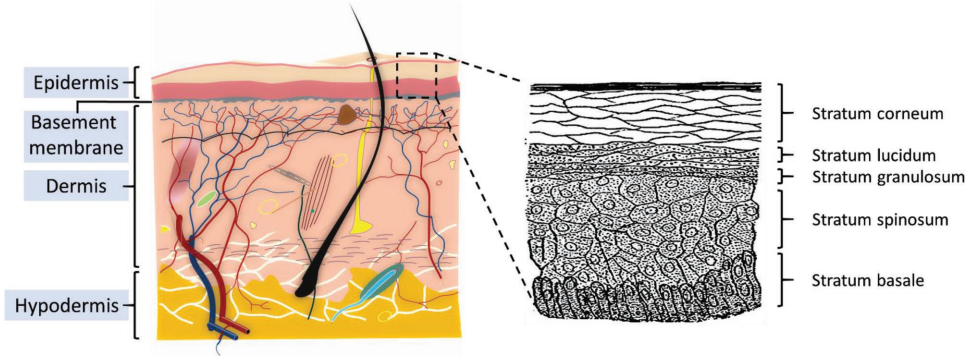

Figure 2. Schematic illustration showing the different layers of a human skin and the different strata of the epidermis layer. (Schematic illustration of the different strata is adapted from the original Grey's diagram, http://en.wikipedia.org/wiki/Image:Gray941.png, Wikimedia Commons, public domain).[276]

3.2. The Dermis

The dermis is the tough fibrous layer of the skin lying between the epidermis and the subcutaneous tissues. It consists of a variable type of fibrous connective tissue made of dermal fibers (collagen, reticular, and elastic fibers) that are embedded in an extrafibrillar matrix. The dermis cushions the body from stress and strain, and provides tensile strength and elasticity to the skin.[29,30] The dermis is ≈15–40 times thicker than the epidermis and can be further divided into the papillary dermis, a superficial layer adjacent to the epidermis, and the reticular dermis, a deep thicker part. The papillary dermis interdigitates with the epidermis and is mostly composed of loosely arranged thin collagen fibers, whereas the fibers of the reticular compartment are thick and densely packed.[33] The dermis is relatively acellular and is predominately composed of an extracellular matrix (ECM) of interconnected collagen fibers with some interspersed elastic fibers.[29,30] In the dermis, collagen type I and collagen type III (also known as reticular fibers) represent about 80% and 15% of the collagen fibers, respectively, while most of the remainder fibers are thought to be collagen type V. Collagen types IV, VII, and XVII are mainly located in the basal membranes beneath the basal epithelial cells of the epidermis.

3.3. The Basement Membrane Zone—The Dermal–Epidermal Junction

The terms "basal lamina" and "basement membrane" are often used to refer to the same structure. The term "basement membrane" was first used to desrcibe the thin undulating line beneath the epidermis rich in glycogen, mucin, mucopolysaccharides, and collagen fibers.[34–36] With the emergence of electron microscopy, researcher used the term "basal lamina" to refer to the structure visible under the electron microscope underneath the basal epithelial cells, and it encompasses two layers: the lamina lucida (electron-lucent) and lamina densa (electron-dense). Whereas the basement membrane refers the structure seen under the light microscope, and it includes an additional layer known as lamina reticularis, which also appears as a radiolucent area under the electron microscope. To avoid confusion, we will refer the zone between the epithelial tissue and underlying structures as the BMZ. BMZ is a highly specialized component representing a dynamic link between two distinct skin compartments—the epidermis and dermis, or in case of the oral mucosa, between the oral epithelium and connective tissue.[37–39] It has a thickness ranging from 50 to 100 nm and contains highly organized interconnected ECM proteins. The BMZ can generally be divided into three layers or regions (**Figure 3**): (1) the lamina lucida, (2) the lamina densa, and (3) lamina reticularis or the sublamina densa. It should be noted that the distinctions between the layers of the BMZ on the molecular level are not as clearly defined as in the electron microscope, since many molecules extend through more than one layer. Therefore, it is more appropriate to visualize the BMZ as a dynamic structure adhering the basal epithelial cells to the underlying dermal tissue instead of a distinct separate structure. As mentioned earlier, the basal epithelial cells facing the lamina lucida form specialized junctional structures called HD. Ultrastructurally, HDs are small electron-dense domains or plaques (<0.5 µm) of the plasma membrane, and they serve as links between the keratin cytoskeleton of the epithelial cell and the lamina lucida below.[40] The HDs and the anchoring filaments originating from the lamina densa, and the anchoring fibrils arising from the dermis, are all interconnected and form one functional unit called "the hemidesmosomal adhesion complex."[40]

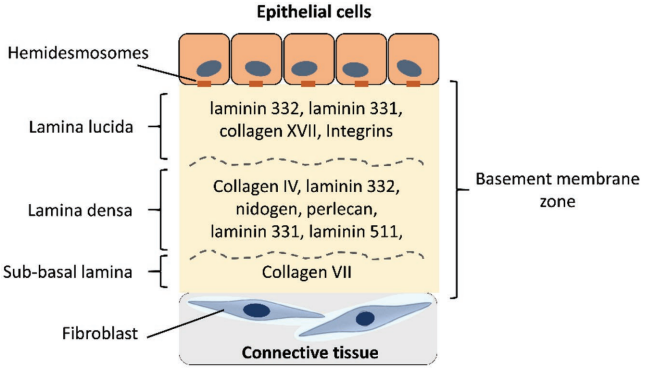

Figure 3. Schematic illustration showing the different layers of the basement membrane zone at the interface between epidermis and dermis of the skin.

Table 2. Summary of the main molecules identified within each region of the BMZ. IF: intermediate filaments; BPAG: bullous pemphigoid antigen.

Molecule	Basal epithelial layer	Lamina lucida	Lamina densa	Lamina reticularis
IFs	✓			
BPAG1[a)]	✓			
Plectin[a)]	✓			
Collagen XVII[a)]	✓	✓		
$\alpha6\beta4$ integrin[a)]	✓	✓		
$\alpha3\beta1$ integrin	✓	✓		
CD151	✓	✓		
Laminin 311		✓	✓	
Laminin 332		✓	✓	
Laminin 511		✓	✓	
Collagen IV		✓	✓	
Nidogen		✓	✓	
Perlecan		✓	✓	
Collagen VII			✓	✓

[a)]Components of the hemidesmosomes.

3.4. Molecular Structure of the BMZ

Even though the electron microscope demonstrated the basic overall structure of the BMZ, molecular biology techniques further refined the composition and detailed structural components of the BMZ.[37–39,41–45] These studies dramatically improved our understanding of the epidermal BMZ morphology, structure, and function. Currently, there are at least 14 distinct molecules with several functions characterized within the BMZ.[37–39,41–45] **Table 2** illustrates the main molecules that have been identified within each region of the BMZ.

The four major components of BM are laminin isoforms, collagen type IV, nidogen (or entactin), and perlecan.[39,40,46] BMZ also includes numerous other components, such as fibulin, collagen type XV, collagen type XVIII.[39] The biological importance and functions of these minor components are still under investigation.[39,47,48] The dynamic interactions between the BMZ molecules are crucial for maintaining the integrity and function of the BMZ.[49] For instance, laminins and collagen type IV individually assemble into suprastructures that are essential for the BMZ stability as they form its basic framework.[50–55] Perlecan and nidogen also contribute the BM stability and integrity by acting as bridges between the laminin and collagen type IV self-assembled networks.[39] Even though these two molecules play a supportive role, they are not required for the formation of the epidermal BMZ.[41,56] In fact, some studies showed that a functional BMZ was formed even in the absence of nidogen or perlecan.[41,57,58]

3.4.1. Collagen Type IV

Collagen type IV is the most abundant collagenous protein in the BMZ, making more than 50% of its mass.[45] It is a nonfibrillar structure and differs from other connective tissue fibrillar collagens by the presence of a noncollagenous rod-like or globular-like domains (N and C domains).[39] Similar to other collagen types, collagen type IV is composed of three α-chains that are self-assembled into a triple-helical structure.[40] In mammals, there are at least six distinct chains of collagen IV, known as α-chains ($\alpha1$–$\alpha6$).[39,40,59] In the epidermal BMZ, only two types of collagen IV heterodimers can be found ($\alpha1\alpha1\alpha2$ and $\alpha5\alpha5\alpha6$).[44,60,61] Collagen $\alpha5\alpha5\alpha6$ networks can bind to epithelial cells, laminins, nidogen, perlecan, collagen VII, and integrins.[39]

3.4.2. Laminins

Laminins are the most abundant noncollagenous proteins in the BM and are the most important integrin-binding structures within the lamina lucida and lamina densa.[39,62] Laminins are a family of large glycoproteins composed of heterotrimeric chains (α, β, γ) bound together by disulfide bonds in a pattern resembling a three-pronged fork. The laminin family encompasses eleven different chains ($\alpha1$–$\alpha5$, $\beta1$–$\beta3$, and $\gamma1\gamma3$) that can combine and form at least 15 isoforms of laminin.[63] Laminins were first named using Arabic numerals following their order of discovery, while the genes for laminin chains were named LAMA, LAMB, and LAMC for α-, β-, and γ-chains, respectively.[64] Recently, a new nomenclature has been introduced that identifies the chain composition of each laminin isoform in a simplified manner (**Table 3**).[63] The expression of these isoforms is tissue-specific, and it can even vary within the same tissue.[65] This structural diversity enables each laminin isoform to demonstrate highly specialized functions. Among all laminins, laminin 332 is of particular interest due to its crucial role in facilitating epithelial cell adhesion to the underlying dermis.[66] Laminin 332 is present in the BMZ of

Table 3. Nomenclature of the laminin protein family

Standard	Abbreviated	Previous
$\alpha1\beta1\gamma1$	111	1
$\alpha2\beta1\gamma1$	211	2
$\alpha1\beta2\gamma1$	121	3
$\alpha2\beta2\gamma1$	221	4
$\alpha3A\beta3\gamma2$	332, or 3A32	5, or 5A
$\alpha3B\beta3\gamma2$	3B32	5B
$\alpha3A\beta1\gamma1$	311, or 3A11	6, or 6A
$\alpha3A\beta2\gamma1$	321, or 3A21	7, or 7A
$\alpha4\beta1\gamma1$	411	8
$\alpha4\beta2\gamma1$	421	9
$\alpha5\beta1\gamma1$	511	10
$\alpha5\beta2\gamma1$	521	11
$\alpha2\beta1\gamma3$	213	12
$\alpha4\beta2\gamma3$	423	14
$\alpha5\beta2\gamma2$	522	–
$\alpha5\beta2\gamma3$	523	15

nearly all epithelial tissues in the human body.[67] Furthermore, laminin 332 is the main component found at the upper lamina densa/lamina lucida border at the base of the anchoring filaments.[49] Mutations in laminin 332 are linked to a condition that causes skin fragility and severe blistering, which is known as junctional epidermolysis bullosa.[68–70] Moreover, the absence of laminin 332 expression considerably disrupts the epidermal adhesion, as seen in patients having a lethal disease known as Herlitz junctional epidermolysis bullosa.[45,68]

4. Biological Percutaneous/Permucosal Interfaces

To date, a permanently successful percutaneous interface between living soft tissues and a synthetic material has not been described. On the other hand, nature has evolved to protect the internal structures of some mammalian species from the harsh outer environment through maintaining firm epithelial barriers. There are few structures that naturally penetrate these barriers, and unlike man-made percutaneous devices, nature has already overcome many of the problems associated with these structures. There are several junctions that are present naturally between soft tissues and solid structures that appear to penetrate the epithelial layers of skin including horns, hooves, hair, fingernails, and feathers. However, investigators believe that these structures essentially originate at the base of the epidermal invaginations within the underlying dermal layers, and thus do not disrupt the continuity of the epithelial barrier.[71,72] Whereas, deer antlers, babyrussa tusks, and teeth are examples of true naturally occurring percutaneous structures, in which nature has incorporated certain design criteria required to overcome the problems associated with percutaneous devices. Understanding how these structures adhere to the soft tissues, irrespective of the dynamic nature of epithelial tissue layers, could help researchers to artificially engineer the soft tissue–implant interface. The following section describes the structure of the percutaneous/permucosal natural analogs with emphasis on teeth, which are most investigated in the literature among these examples. **Figure 4** illustrates the periodontium and the peri-implant tissues and compares the structure of a human tooth and a dental implant.

4.1. The Human Tooth

The human tooth is one of the most investigated biologically anchored implants.[73] It penetrates the mucosa in the oral cavity creating a dental–epithelial interface that is under constant attack by bacteria, chemical substances, and physical insults.[74,75] The integrity of this interface is well maintained via a strong soft tissue seal to the tooth surface that included a specialized structure known as the junctional epithelium (Figure 4).[74,75] Even after an injury or dental surgeries, epithelial cells rapidly migrate and proliferate on the tooth surface, and thus re-establishing the epithelial barrier between the oral environment and the deep periodontium.[76] Furthermore, following the scaling/root planning procedure, commonly used to treat periodontitis, pocket healing re-establishes the formation of a long junctional epithelium.[77] The junctional epithelium is located at a strategically crucial interface between the gingival sulcus and the tooth surface.[75] The junctional epithelium is essentially made of flattened cells characterized as nondifferentiated, nonkeratinized, stratified squamous epithelium. The junctional epithelium contains cells directly connected to the tooth surface forming a collar-like attachment around the tooth. The cells facing the tooth provide the actual attachment to the tooth through an adherence complex called "epithelial attachment apparatus."[75] This attachment maintains the structural continuity and forms an efficient barrier against bacterial invasion at the tooth-gingival interface.[75,78] At the level of this apparatus, the junctional epithelium is essentially composed of two layers of epithelium: (1) a basal layer that is separated from the underlying connective tissue by a BMZ known as "the external basal lamina;" and (2) a suprabasal layer of epithelial cells tightly adhering to the tooth surface via a basement-membrane-like structure known as the internal basal lamina.[78] Often, the epithelial cells facing the tooth surface are referred to as DAT cells (directly attached to the tooth).[79] Since the connective tissue layer is absent at the tooth–epithelial interface, the components of the internal basal lamina are synthesized and renewed solely by the DAT cells.[80,81] Indeed, the biochemical composition of the internal basal lamina is different from the external basal lamina and other typical BMZs.[75] While the external basal lamina has a similar composition to the general BMZ, the internal basal lamina lacks the most common

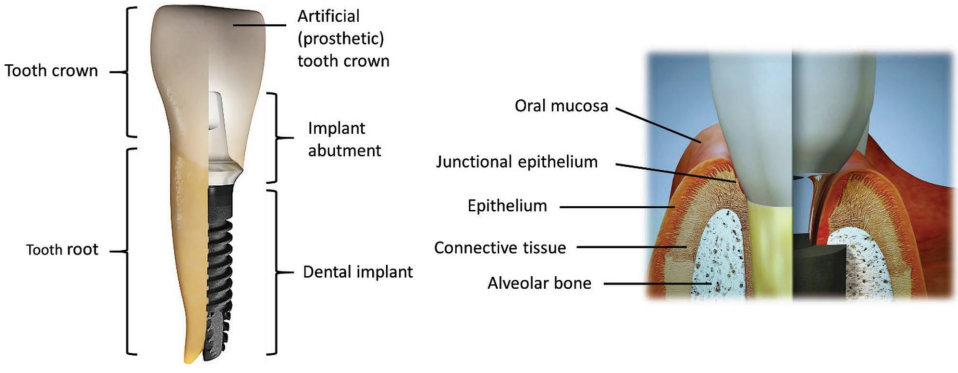

Figure 4. Schematic illustratiations comparing between the structures of a human tooth and a dental implant, as well as their respective surrounding tissues.

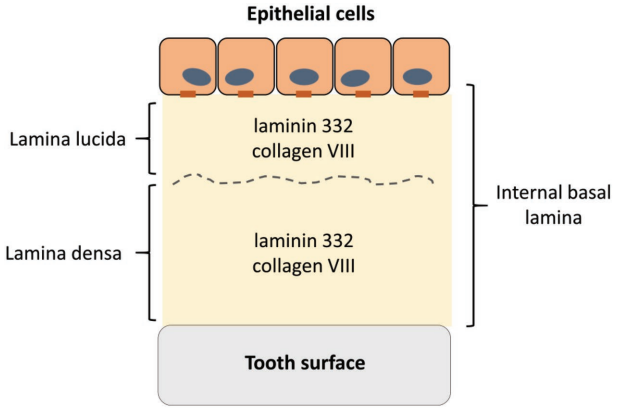

Figure 5. Schematic illustration showing the tooth and junctional epithelium. Schematic illustration showing the different layers of the internal basal lamina at the interface between epithelial cells and the tooth surface.

components, such as collagen types IV and VII, most laminin isoforms, and perlecan (**Figure 5**).[82–85] Furthermore, the internal basal lamina consists of two strata, lucida and densa, that mainly include laminin 332 and collagen type VIII.[86,87] The differences in composition between the external and internal basal lamina have led many investigators to investigate the role of these ECM proteins, particularly laminin 332 and different types of collagen, in maintaining the soft tissue attachment as detailed later.

4.2. Babyrussa Tusk

It is thought that the only permanent naturally occurring structure that penetrates the skin is the tusk of the babyrussa, a very rare pig originating from the Celebes in South-East Asia.[88] The upper canine teeth of these animals do not penetrate the gingiva as other teeth instead they grow in the opposite direction and penetrate the external skin layers of the snout immediately below the babyrussa's eyes. Histologically, the tusk is surrounded by thickened layer of keratin at the skin-penetrating interface.[88] In general, the epidermis is present in close adherence of the tusk without showing any signs of downgrowth. So far, no study has been able to characterize the ultrastructure of the attachment observed between the tusk and epithelium. It would be interesting to investigate whether there are common features between the soft tissue interfaces of the tusk and teeth that are responsible for promoting the strong soft tissue attachment.

4.3. Deer Antler

Deer antlers are considered transient percutaneous bony pedicles arising from the frontal bones of the skull of most members of the deer family.[89,90] The growing antlers are covered by a hair soft skin (velvet) and from the moment of exfoliation to the time of shedding, antlers penetrate all layers of the skin. In early life, the pedicle is a subcutaneous bone becomes pronounced around the onset of puberty and then grows from the frontal bone of the deer skull.[91] During the percutaneous phase, the pedicle pierces the skin and adheres to the dermal tissues with sufficient strength to prevent marsupialization and infection.[89] The dermal collagen fibers penetrate the surfaces of antlers in a perpendicular orientation, and it is thought they are responsible for reducing the epithelial downgrowth into the underlying tissues.[92] Previous studies developed specialized orthopedic implants mimicking the topography of deer antlers.[4,93,94] These biomimetic implants were successful in reducing the epithelial downgrowth by optimizing the dermal attachment; however, no consistent epithelial attachment was observed.

5. Superficial Cutaneous Wound Healing

Superficial wound healing is a tightly coordinated process that involves several controlled overlapping events: inflammation and formation of a provisional ECM (or clot formation), epithelial cell activation and migration, remodeling of the BMZ, and epidermal and dermal maturation.[95–98]

Several animal models, including mouse, rabbit, and pig, have been used to investigate epidermal wound healing at the cellular level.[98–102] In contrast to humans and pigs, mice and rabbit have loose skins with an extra thin sheet of striated muscle located beneath the epidermis known as the panniculus carnosus, which with its contraction can aid wound repair.[98] Despite this variation, the timing of re-epithelialization in animal models and humans is similar, and therefore these animal models provide helpful insights for understanding the mechanism of the wound healing process.[103] Unfortunately, the cellular events leading to epithelial cell migration and adhesion to the wound bed are far from being understood, due to the complex and hostile environment of the human skin and gingival wounds; an environment that is often hypoxic and under the influence of inflammatory mediators, proteases, and connective tissue fragments.

This section describes the current understanding of the re-epithelization process during wound healing, which is generally similar in the different epithelial tissues. However, it is believed that oral wounds are likely to heal faster with lesser possibility to form scar formation when compared to the epidermal wounds.[104,105] Re-epithelization is a term used to describe the process of new epithelial formation on the surface of a skin wound.[37,95] Re-epithelization includes ongoing dynamic cycles of interaction between cells and the surrounding environment. On one hand, ECM proteins affect cell behavior, while on the other hand, cells synthesize and modulate the surrounding extracellular environment.[106] Therefore, an appropriate balance between ECM synthesis and degradation is essential for proper cutaneous wound healing. Microscopically, the re-epithelization process involves multiple steps:[37,95,97] (i) migration of epithelial cells from the cut edges of the wound; (ii) proliferation of epithelial cells; and (iv) reestablishment of the BMZ that anchors the epithelium to the underlying structures.

After the disruption of the epithelial continuity by tissue injury, there is a short lag period that lasts for several hours. After 24–48 h, the epithelial cells from the wound periphery and the cut epidermal appendages begin to migrate rapidly to the wound bed to re-establish tissue integrity.[37,95,97] It has been

suggested that the stimulus leading to epithelial cell activation and migration is attributable to the breach of the normal intraepithelial electric potential.[107] In some cases, multiple islands of epithelial cells scattered throughout the wound bed also contribute to the re-epithelization process.[95]

Epithelial migration in both skin and oral mucosa follows somewhat a similar pattern, but the origin of the migratory epithelial cells is different.[95] In the skin, epithelial cells originate from the wound edge, and from hair follicles and sweat glands. Whereas, in the oral mucosa, they only arise from the wound edge. As the epithelial cells at the wound edge interact with the extracellular proteins present in the provisional matrix, their phenotype changes dramatically from stationary basal cells to migratory flat elongated cells with long cytoplasmic processes called "lamellipodia."[37] The migratory cells lose their hemidesmosomes and desmosomes, while their gap junctions become more prominent.[37] During this process, the migrating cells detach themselves from the basement membrane proteins and then regenerate new basement membrane proteins at the new site.[108]

Epithelial cells assemble a provisional BMZ beneath them during the migration process, which contains laminin 332, tenascin-C, and cell-derived EDA (extra domain A) fibronectin,[76,97] but common components such as collagen type IV and VII, heparan sulfate proteoglycans.[109,110] Interestingly, laminin 332 seems to be always deposited by the migratory epithelial cells against the wound bed matrix.[96,109] Moreover, epithelial cells can proteolytically process laminin 332 into different structural forms that can either serve as nucleators of hemidesmosomes that stop migration or can act as promoters of migration.[95] Therefore, it is evident that epithelial cells are capable of synthesizing their own ECM that supports and modulates the migration of epithelial cells according to the demands of the surrounding environment.[95,111]

As epithelial cells form the first layer covering the wound, they start to proliferate to guarantee an adequate supply of cells to close the wound.[96] Only the basal epithelial cells can proliferate, while the differentiated epithelial cells in the suprabasal layers have lost this ability.[112] The proliferation of the basal cells depends on the cell attachment to the underlying structure, degree of cell differentiation, and the presence of growth factors and ECM proteins.[96] Once the migrating cells from both edges of the wound join, they start forming hemidesmosomal adhesions with the provisional BMZ.[95] The final BMZ begins to form from the margins of the wounds inward in the skin, or at several locations in case of the oral mucosal wounds.[76] Despite the ability of epithelial cells to secrete the main components required for the maturation and the reorganization of the BMZ,[95,111] a significant portion of these proteins is also synthesized by fibroblast.[113] This indicates that an appropriate synergy between epithelial cells and fibroblasts is crucial during the reorganization of the final BMZ.[95]

The wound healing process is a dynamic environment and achieving a comprehensive understanding of the nature of these mechanisms is a challenging task. However, studying the molecular pathways governing the re-epithelization process and connective tissue integration at wound sites might point toward novel strategies to promote faster and better tissue regeneration. Moreover, understanding the tissue-specific characteristics that promote scarless wound healing in the skin and oral mucosa may provide valuable information that holds the potential for the development of more efficient approaches for tissue regeneration and engineering. For more comprehensive understanding, the reader is referred to the following reviews that discuss cutaneous and mucosal wound healing and describe in detail the biomolecular events at the nano/microscalar level.[96,98,114–116]

6. A Brief Walk through the Early History of Developing Osseointegrated Implants

According to our literature search, the first mention of a percutaneous device goes back to the beginning of the mid-1800s, when Malagaigne used external fixators for bone fractures.[117] These fixators resemble percutaneous osseointegrated devices that are inserted into the bone and then pierce the skin barrier. Malagaigne and other investigators reported a fairly good success of using external fixators and suggested that inflammation and infection around these devices, most probably, occurred as a result of repeated gross motion of the skin around the external fixator.[117] Later in the early- to mid-1990s, several attempts were made to restore the limbs of amputee patients with prosthetic skeletal attachments, especially during and after the period of World War II.[117] In one clinical setting, a general surgeon in Germany, Dr. Dümmer, placed osseointegrated limb prostheses in four human subjects. Even though three of the percutaneous implants did not show signs of infection, all implants were removed because one individual developed an infection.

In 1952, Brånemark first described the phenomenon of "osseointegration,"[118] and after a few years, in the mid-1960s, Brånemark et al. in Sweden revolutionized the field of percutaneous devices by combining the concept of percutaneous devices with that of osseointegration to develop dental implants as tooth replacements.[118] Furthermore, they used this concept for limb prosthetics, plastic reconstructive applications, and bone-anchored hearing aids.[118,119] To date, Brånemark et al. reported successful treatment of over 100 amputees with these osseointegrated prosthetics.[118,120] Conversely, others reported that using such prostheses as a permanent solution for amputees has limited success due to infection, implant loosening, and fixture failure.[121]

In the 1970s, Winter investigated skin reactions around porous and nonporous implants sticking out of the skin surface in an experimental pig model.[122] In their model, they collected implant specimens at separate time points up to 10 weeks after implantation. They concluded that epithelial tissue migrates downward along a nonporous surface and creates an unstable skin–implant interface leading to infection. Accordingly, they suggested that the percutaneous component of a prosthesis piercing the skin should be porous to enable fibrous tissue ingrowth, and thus creating a stable skin–implant junction.[122] Hall CW assessed for 14 months in a percutaneous goat model several biomaterials (nylon or Dacron velour, polypeptide with rough cast surface or nonwoven fabric, polyurethane foam, nylon foam, vitreous carbon buttons, and solid uncoated Silastic rod were used as a control).[123] Among the

different materials, he demonstrated that a percutaneous component made of nylon velour permits soft tissue ingrowth that hindered epithelial downgrowth and created, per his results, a "bacteriostatic" soft tissue seal around the implant.[123] In another clinical study including three amputees in the late 1970s, Mooney et al. included an unpolished carbon surface on the percutaneous component of stainless steel osseointegrated implants.[124] However, these implants never developed an efficient seal at the skin–implant interface and all prostheses were removed after 6 months due to chronic infection.

There were several significant advancements in the 1980s that established some of basic concepts related to the design of percutaneous devices and mechanisms of their failure, concepts that are still followed to this day, As mentioned previously, von Recum described five principle modes that lead to failure of percutaneous devices: marsupialization, premigration, mechanical avulsion, infections and abscess formation, or a combination of these mechanisms.[6] He suggested these mechanisms based on using percutaneous implants fabricated from nylon velour (Dacron) in different animal models (i.e., rabbits, dogs, goats). After a few years, von Recum and co-workers investigated species-related differences by using nylon (Dacron) mesh percutaneous implants.[125] Despite the lack of statistical evaluations, their results revealed no substantial differences between the animal species, but the rate of epithelial downgrowth was faster in rabbits than that observed in dogs or goats.[125] Grosse-Siestrup and Affeld proposed a general design criteria for percutaneous devices that reduce the stress at the skin–implant interface, or as they referred to it the "three-phase junction," which is the intersection of the skin, implant material, and air.[72] Implant design parameters were based upon investigating some relevant natural percutaneous analogs including deer antlers, horns, hooves, feathers, hair, fingernails, and teeth. One of their suggestions included adding a cone or a cuff to the percutaneous component at the skin interface junction in order to shift the interfacial mechanical stresses from the center of the implant. In addition, they proposed including a subcutaneous flange (below the skin–implant interface) to prevent soft tissue ingrowth and accommodate external stresses. Furthermore, they suggested different geometries and shapes for the subcutaneous flange, such as disks with small and/or large holes, disks with meshwork, disks in a shape of a leaf and flanges that resemble a snowflake.[72]

Two researchers, Squier and Collins, were also among the pioneers investigating the influence of surface porosity on soft tissue attachment and epithelial down growth.[126] They implanted Millipore filters made of cellulose ester of different pores sizes (0.025, 0.65, 1.0, 1.2, 3.0, 2.2, 7.0, and 8.0 μm) in the skin of a porcine model for a period of 8 weeks. Their findings showed that implants with larger pores (3.0–8.0 μm) significantly decreased the epithelial downgrowth more than those with smaller pore sizes (<0.3). Consequently, Squier and Collins concluded that implants with the larger pores promoted a substantial amount of soft tissue ingrowth that served as a barrier against further epithelial downgrowth.[126] In the late 1980s and following decades, the field of percutaneous devices expanded enormously as several research groups focused their efforts on developing strategies to prevent infections related to these devices. The following section will detail these different strategies and highlight their main results.

7. Strategies to Prevent Infections Related to Osseointegrated Implants

This review will focus on summarizing the literature involved with developing strategies for bone-anchored percutaneous/permucosal implants, particularly orthopedic and dental implants. These implants are mainly made of materials composed of metals or other nondegradable materials, with some exceptions, and are implanted with the purpose to function for the lifetime of the individual. These strategies to prevent infection related to these implants can be broadly categorized into strategies that aim at developing surfaces that could promote the adherence of the implants with the host soft tissues, strategies that prevent or reduce bacterial adherence and colonization or a combination of both.

7.1. Strategies to Promote the Soft Tissue Attachment around Osseointegrated Implants

The aim of these strategies is to produce implant surface properties that can promote soft tissue attachment around percutaneous/permucosal implants, which would not only provide the implant with certain stability but would also establish a permanent barrier against bacterial invasion and, thus, infection.

The soft tissue interface around osseointegrated implants consists of two zones, one of epithelium and the other of connective tissue. Both these tissues contribute to the establishment of soft tissue around these implants. Hence, previous studies attempted different strategies to promote epithelial cells adhesion, prevent epithelial downgrowth, and/or enhance fibroblasts attachment and connective tissue integration. Such strategies include engineering approaches (i.e., changes in the device structure/design, surface topography and/or chemistry alterations, application of different materials), biomolecular coating approaches, surgical approaches, or other strategies. In particular, the notion of modifying the implant surface has captured the interest of many scientists, clinicians, as well as manufactures. This is due to the fact that the reaction of cells and tissues to implanted foreign bodies relies on the material's surface properties and its behavior upon contact with the body fluids, which in turn governs the healing mechanism around the percutaneous/permucosal implants. **Tables 4–6** summarize the main studies that aimed to promote the soft tissue seal around osseointegrated percutaneous/permucosal implants.

7.1.1. Engineering Approaches

Previous studies applied several engineering techniques that involved application of different materials, surface chemistry modifications (e.g., binding functional chemical groups), or altering the device design and/or surface topography (e.g., addition of a subcutaneous flange, grooves, pits).

Surface Chemical Composition (or Type of Material): From a chemical point of view, the type of material used to fabricate percutaneous implants can be categorized into three main groups: metals, ceramics, and polymers. Polymeric materials have been used in solid and porous forms, as well

Table 4. Main studies assessing the influence of surface chemistry of implant/material on surrounding soft tissues or soft tissue cells. 1SS: single-stage-surgery; 2SS: two-stage-surgery; Al_2O_3: aluminum oxide; Au: Gold; cpTi: commercially pure titanium; DLC: diamond-like carbon; HA: hydroxyapatite; HGF: human gingival fibroblasts; HaCaT: cultured human keratinocytes; GC: glass carbon; PC: percutaneous; PETE: polyethylene terephthalate; PHEMA: poly(2-hydroxyethyl methacrylate); PM: permucosal; PMMA: poly(methyl methacrylate); SC: subcutaneous; TCP: tricalcium phosphate; N6: nylon-6; N12: nylon-12; TiZr: titanium zirconium; VP-DMMEP: N-hexylpyridinium copolymerized with 4-vinylpyridine and dimethyl (2-methacryloyloxy-ethyl) phosphonate; ZrO_2: Zirconia.

Material(s)	Type of evidence	Main conclusions	Reference
cpTi, HA	In vitro: HGF	cpTi promoted higher cell attachment than HA	[129]
cpTi, Al_2O_3	Clinical: human biopsies	No qualitative structural difference in soft tissues	[130]
cpTi, Ti_6Al_4V, TiTa30	In vitro: HGF	Cells showed more favorable response to cpTi and TiTa30 than Ti_6Al_4V	[131]
cpTi, PETE	In vivo: dorsum of goats/PC/1or2SS	PETE induced inflammatory reactions, and cpTi sheets had good biocompatibility	[132]
HA, TCP, GC	In vivo: dorsum of dogs/PC/1SS	Dense HA showed best soft tissue response, GC induced epidermal down growth and inflammation	[133]
cpTi, Al_2O_3, Au	In vivo: mandible of dogs/PM/1SS	Unlike Au, cpTi or Al_2O_3 had proper soft tissue attachment	[134]
Ti, Al_2O_3, Au	In vitro: HaCaT	Metallic biomaterial surfaces are optimal for epithelial cell adhesion and spreading	[135]
Ti, ZrO_2	In vivo: maxilla of monkeys/PM/1SS	No significant quantitative or qualitative differences	[136]
Ti, Au	Clinical: patients randomly received different mucosal abutment	No differences were observed	[137]
Ti, ZrO_2	Clinical: gingival biopsies/1SS	Inflammation was higher in cpTi than ZrO_2	[138]
Ti, HA, DLC	In vivo: tibia goats/PC/1SS	Surface chemistry had no effect on degree of epithelial downgrowth or dermal attachment	[4]
cpTi, Au	In vivo: mandible of dogs/PM/2SS	No differences in soft tissue dimensions	[139]
Ti, ZrO_2, Au	In vivo: mandible of dogs/PM/2SS	Healing was more favorable to abutments of Ti, ZrO_2	[140]
Ti, HA	In vivo: periauricular region of sheep/PC/1SS	HA enhanced the attachment of soft tissues more than Ti	[141]
Ti, TiZr [142]	In vivo: mandible of mini-pigs/permucosal/1 stage surgery	Surface chemistry had no effect on soft tissue dimension	[143]
HA-coated Ti	In vivo: periauricular region of sheep/PC/1SS	HA coating improved soft tissue integration and reduced pocket depth	[144]
Ti_6Al_4V, ZrO_2	In vitro: HGF and HNEpC	On comparable topographies, ZrO_2 enhanced higher fibroblast proliferation rates than Ti	[145]
Ti_6Al_4V coated with DMMPVP	In vivo: dorsum of mice/SC/1SS	Coating reduced infections but had no effect on epithelial downgrowth	[146]
Ti, TiZr	In vivo: mandible of mini-pigs/PM/1SS	Better epithelial attachment and collagen organization on TiZr surfaces	[147]
cpTi, PMMA, N6, N12 PHEMA	In vitro: primary HGE and primary HGF	PMMA promoted best epithelial cell behavior, PMMA and Ti promoted best fibroblast behavior Surface amination enhanced cell behavior	[148]

as coatings for soft and hard tissue attachment, replacement, and augmentation. Various polymers have been tested as materials for percutaneous implants including polyurethanes, polyamides, polyethylene terephthalate (PETE), silicones, and acrylics (e.g., polymethylmethacrylate (PMMA)).[127,128] They present a wide range of chemical and physical properties depending on the monomer units, polymerization reaction, and the formation of co-copolymers with tunable concentrations. Despite their benefits, polymers generally demonstrate lower strengths than the other classes of materials and some polymers showed unfavorable soft tissue reactions. In this review, focus is put mainly on metals and ceramics, whereas polymers are mentioned when relevant. Table 4 summarized the main studies that focused on investigating the influence of surface chemistry on the peri-implant soft tissues or soft tissue cells.

Metal and Metal Alloys: Metals possess good biomechanical properties, are easy to process and modify, and can be generally sterilized by the common sterilization approach, and thus are easy to use. Historically, gold, stainless steel, cobalt–chromium, and titanium have been used as implant materials. Implants made from gold, stainless steel, or cobalt–chromium demonstrated less success rates than those made of Ti.[127,149] Nevertheless, some prosthetic components of the implants are still made from gold alloys, stainless steel, cobalt–chromium, or nickel–chromium alloys.[127]

Currently, commercially pure titanium (cpTi) and Ti alloys are the materials of choice for orthopedic and dental implants.[127,149] Ti is a biologically inert material with a high resistance to corrosion due to the spontaneous formation of surface oxide layer (TiO_2), which separates the metal from the surrounding environment.[150] Typically, Ti forms a surface

Table 5. Main studies assessing the influence of surface topography of implant/material on the surrounding soft tissues or soft tissue cells. 1SS: single-stage-surgery; 2SS: two-stage-surgery; cpTi: commercially pure titanium; DLC: diamond-like carbon; HA: hydroxyapatite; HDF: human dermal fibroblasts; HEK: human epidermal keratinocytes; HGF: human gingival fibroblasts; HNEpC: human nasal epithelial cells; PC: percutaneous; PHEMA: poly(2-hydroxyethyl methacrylate); PM: permucosal; RSF: rat skin fibroblasts; SC: subcutaneous; Ti: titanium; TiZr: titanium zirconium; ZrO_2: Zirconia.

Surface topography	Material(s)	Type of evidence	Main conclusions	Reference
Porosity	PHEMA	In vivo: dorsum of mice/PC/1SS	Good epidermal and dermal integration on all rods regardless of treatment	[180,182,183]
			Epithelial migration was shorter on 20 µm pores than both 40 and 60 µm pores	
Porosity	Ti_6Al_4V	In vivo: sheep/PC/1SS	Porous cpTi coating decreased marsupialization rate by four times	[142]
Porosity	Ti_6AlV_4	In vivo: dorsum of rats/SC/1SS	Small (40–100 µm) & nanopores had less extrusion rate than implant with large pores (100–160 µm)	[184]
Roughness	cpTi	In vivo: mandibles of dogs/PM/1SS	Soft tissue reaction was similar in all surfaces (polished/fine or rough sandblasted)	[185]
Roughness	cpTi	In vivo: mandible of dogs/PM/2SS	Soft tissue attachment was not influenced by surface roughness (acid-etched or polished)	[186]
Roughness	Ti, Epoxy	In vivo: parietal of rats/PC/2SS	Failure time was longer for implants with grooved surfaces versus smooth and pitted surfaces	[178]
Roughness	cpTi	Clinical: human biopsies	Oxidized & acid-etched implants revealed less epithelial downgrowth & longer connective tissue seal than machined ones	[187]
Roughness	Ti, Epoxy	In vivo: parietal of rats/SC/1SS	Roughness provides stable connective tissue attachment	[179]
Roughness	cpTi	In vivo: dorsum of rats/SC/1SS	Roughness improves connective tissue attachment	[179]
Roughness	Ti_6Al_4V	In vitro: HaCaT	Smoother surface showed increased cell adhesion proliferation	[94]
Roughness	cpTi	In vitro: HaCaT	Nanotexturing increased cell adhesion, proliferation, and spreading	[188]
Roughness	Ti, TiZr	In vivo: mandible of mini-pigs/PM/1SS	Soft tissue dimensions were similar on all surfaces	[143,170]
Roughness	cpTi	In vitro: rat OEC	Smoother surfaces showed higher OEC adhesion and stronger epithelial seal but rougher surfaces showed less EDG	[189]
		In vivo: maxilla of rats/PC/1SS		
Roughness	Ti_6Al_4V and ZrO_2	In vitro: HGF and HNEpC	Rough surfaces improved fibroblasts attachment but no effect on epithelial cells	[145]
Nanotubes	Ti	In vitro: HEK and HDF	Nanotube arrays increased HDF but decreased HEK adhesion and proliferation	[190]
Groove	cpTI	In vitro: HGF	Microgrooves with widths of 15 or 30 µm enhanced fibroblast behavior	[191]
Groove/porosity	Ti, HA, DLC	In vivo: tibia of goats/PC/1SS	Porosity or groove has no effect on epithelial downgrowth or connective tissue attachment	[4]
Groove/roughness	cpTi	In vitro: RSF	Roughness decreased cell number and strength of adhesion	[192]

oxide layer within seconds after exposure to air and the thickness of this layer is ≈3–10 nm. It is suggested that this passive layer has a protective role and maintains its stability even in biological systems as hostile as the oral cavity, and thus provides a favorable environment for osseointegration.[150] However, it is unclear whether this oxide layer is favorable for soft tissue attachment around implants. Unalloyed cpTi is produced in various degrees of purity that are graded from 1 to 4. This grading is related to the corrosion resistance, strength, and ductility. cpTi grade 1 presents the highest purity, highest corrosion resistance and formability, and the lowest strength. While, grade 4 exhibits the highest strength and moderate formability. Most dental and orthopedic implants are fabricated from cpTi grade 4 because it is stronger than the other grades.[150] Ti alloys are metals containing a mixture of Ti and other chemical elements and the most commonly used Ti alloy is Ti_6Al_4V, sometimes referred to as grade 5 Ti. Ti_6Al_4V is alloyed by weight with 6% aluminum and 4% vanadium, 0.25% iron, 0.2% oxygen, and 90% Ti. Such alloys demonstrate high tensile strength and toughness with greater yield strength and fatigue properties than cpTi. However, Ti_6Al_4V alloys are more expensive than cpTi and exhibit poor wear resistance, which can result in the

Table 6. Main studies assessing the influence of biomolecules on the soft tissues or soft tissue cells attachment to implant/material surfaces. 1SS: single-stage-surgery; 2SS: two-stage-surgery; CaP: calcium phosphate; Col: collagen; cpTi: commercially pure titanium; EMD: enamel matrix derivative; EVOH: ethylene-vinyl alcohol copolymer; FGF-2: fibroblast growth factor-2; FN: fibronectin; HA: hydroxyapatite; HaCaT: cultured human keratinocytes; HDF: human dermal fibroblasts; HEK: human epidermal keratinocytes; HOE: human oral epithelial cells; HGF: human gingival fibroblasts; IHGK: immortalized human gingival keratinocytes; IOK: immortalized oral keratinocytes; LN: laminin; PC: percutaneous; PDGF: platelet-derived growth factor; PM: permucosal; SC: subcutaneous; Ti: titanium.

Biological coating	Material(s)	Type of evidence	Main conclusions	Reference
LN-332	Ti_6Al_4V	In vitro: IHGK	Soluble LN-332 promoted cell attachment	[238]
LN-332	Porous Ti	In vitro: IOK	LN-332 improved cell adhesion in vitro but not in vivo	[3]
		In vivo: jaws of dog/PM/1SS		
LN-332	Ti_6Al_4V	In vitro: HaCaT	LN-332 increased the cell attachment	[241]
LN-111, LN-332	Ti_6Al_4V	In vitro: HEK	LN-332 enhanced cell attachment and spreading, LN-111 did not enhance cell attachment	[239]
LN-111	EVOH, CaP	In vitro: epithelial-like cells (BSCC93)	LN-111 increased the number of adherent cells by 10 times	[242]
LN-111	EVOH, HA	In vivo: scalp rats/PC/1SS/	LN-111 immobilization on HA increased the strength of the soft tissue attachment	[243]
FN	cpTi, HA	In vitro: HGF	FN coating showed minimal increase in cell attachment only in porous HA	[129]
FN	Ti	In vitro: HGF	FN improved adhesion and growth of cells	[247]
FN	Ti	In vitro: HDF	FN improved cell adhesion and spreading	[244]
FN	Ti_6Al_4V, HA	In vitro: HDF	FN increased fibroblast cell attachment strength and CT attachment	[248,251]
		In vivo: tibia of Mule ewe/SC/1SS		
FN	Ti_6V_4Al	In vitro: HDF	FN coating showed favorable cell alignment in vivo	[249]
		In vivo: tibia of sheep/SC/1SS		
FN	HA	In vitro: HDF	FN increased HDF attachment strength	[250]
Col I	Ti, PS	In vitro: primary HGF	Col I improved the activity of HGF	[252]
Col I	Ti	In vivo: jaws of dogs/PM/2SS	Col I coating had no effect on soft tissue healing	[253]
Col I	cpTi with different topographies	In vitro: HDF	Immobilized col I increased HDF adhesion and activation	[262]
LN, FN, Col I, Col IV, VN	Ti	In vitro: primary HOE	Col IV coated Ti showed the best cell attachment, while VN coated Ti hindered cell attachment	[254]
FGF-2	Ti	In vitro: primary HEK	FGF-2 increased cell density on all surfaces	[225]
PDGF, EMD	Ti	In vivo: dorsum of Agouti/SC/1SS	Coating with PDGF or EMD increased the speed and quantity of soft tissue healing	[257]
E-cadherin	Ti_6V_4Al, HA	In vitro: murine keratinocytes	E-cadherin increased metabolic activity and attachment of cells	[260]
Vit E, 7-DHC	cpTi	In vitro: primary HGF	Both coatings had a positive effect on HGFs	[261]

release of potentially toxic metal debris into tissues.[150] These alloys are often preferred over cpTi in situations where good mechanical properties are the main concern.

In one in vitro study,[135] the epithelial cells attachment was assessed on five different materials: three metallic surfaces (cpTi, Ti_6Al_4V alloy, gold alloy) and two ceramic surfaces (dental porcelain and aluminum oxide). By using scanning electron microscopy and immunofluorescence microscopy, this showed that the metallic surface promoted better epithelial cells adhesion and spreading than the ceramic surfaces. In addition, only the metallic surfaces demonstrated epithelial cells with well-organized prehemidesmosomes and focal contacts. Titanium–tantalum alloys have been investigated as possible implants materials. When two Ti alloys, Ti_6Al_4V and TiTa30, were compared with cpTi, gingival fibroblasts exhibited an unfavorable round shape and less spreading on the Ti_6Al_4V alloy, presumably due to minor toxicity to aluminum or vanadium.[131] Ti and Ti alloys can establish and maintain direct contact with bone through the process of osseointegration; however, no direct permanent attachment with soft tissue has been achieved

so far. Research attempted to improve their integration with soft tissues using various modification techniques including modifying their texture, coating with biological molecules or ceramics.

Ceramics: There has been increasing concern regarding Ti sensitivity, as there were some reports of allergic reactions (e.g., contact dermatitis) when using Ti in orthopedic or dental implants.[151,152] In fact, the prevalence of allergic reaction to Ti dental implants was estimated at 0.6%.[151] Furthermore, Ti undergoes some corrosion when contacting some ions and metals present in the saliva or demonstrates increased oxidation in the acidic environment of bacterial biofilms.[152,153] However, the clinical implications of the previous findings remain unclear.[154] Another disadvantage of Ti is its dark gray color; which is a major concern when considering restoring teeth in the visible esthetic areas. When Ti dental implants are inserted in the anterior or maxillary region a gray shadow sometimes appears under thin peri-implant soft tissues leading to aesthetic impairment, especially in cases where the soft tissue condition is not optimal.[149,155] The increasing fear of allergic reactions to Ti and the high esthetic demands nowadays have led the notion to find alternatives to metallic implants. Accordingly, ceramic materials were suggested as potential surrogates.

Ceramics can be either coated or plasma sprayed on the metallic surfaces with a ceramic material, or the ceramic can be used as a bulk material, especially in the case of zirconium, to fabricate the entire implant or some of its components (e.g., percutaneous/permucosal component).[149] The main types of ceramic materials used in the field of percutaneous/permucosal implants are calcium phosphates, aluminum oxide, and zirconium.

Calcium Phosphates: Calcium phosphates ceramic materials are widely used as bone replacement and regeneration in the dental and orthopedic surgical fields.[156] There are a variety calcium phosphates compounds but the most thoroughly researched and characterized calcium phosphate in percutaneous/permucosal implants is primarily hydroxyapatite (HA).

HA is a mineral which naturally occurs in the form of apatite and it is the main organic component of hard tissues, such as bone and teeth. HA has the chemical formula of $Ca_5(PO_4)_3(OH)$, but is often described as $Ca_{10}(PO_4)_6(OH)_2$ to refer that the crystal unit comprises two entities.[157] It exhibits excellent biocompatibility and direct integration with living tissue; however, due to its brittle nature and poor mechanical properties, HA is often used as a coating on metallic prosthesis.[157–159] So far, studies have shown that implants coated with HA enhance bone implant;[127] however, there were conflicting results concerning their influences on peri-implant soft tissues.

HA in the form of dense ceramic bulk was first applied as part of percutaneous devices in the late 1980s by Aoki et al. in a dog model.[160] In this study, sintered HA had good compatibility and long-term stability with skin tissue. In another percutaneous dog model, researchers investigated three types of HA (dense, porous with open pores, porous with closed pores) along with tricalcium phosphate (TCP) and glassy carbon.[133] As per their results, dense HA was the best percutaneous material of those tested, whereas both types of porous HA induced acute inflammation within 1 month, and glassy carbon caused a serious epithelial downgrowth and inflammatory response. Other in vivo studies also showed favorable soft tissue response to dense HA.[161,162] More recently, two studies by Larsson et al. investigated the influence of four experimental configurations, differing in type of material (Ti vs HA) and/or macroscopic configuration (round vs concave outer surface) on soft tissue in a sheep model.[141,144] The studies concluded that HA enhanced the attachment of soft tissues and resulted in significant reduction in pocket depth compared to Ti, and HA-coated concave surface achieved the most stable soft tissue integration.

On the other hand, results from few studies in the literature contradict the previous observations. One in vitro study showed that human gingival fibroblasts attachment to cpTi was significantly higher than porous and nonporous HA.[129] In a more recent study, Pendergrass et al. assessed in a percutaneous goat model the influence of different coatings including HA, diamond-like carbon, and Ti. The study revealed that the type of coating did not affect the degree of epithelial downgrowth or dermal attachment to straight or flanged implants.[4] Besides the conflicting results, there has been some concern related to the degradation of HA and crack propagation, which might affect the longevity of implants made from or coated with HA.[163]

Aluminum Oxide and Zirconium: The first generation of ceramic implants was fabricated from aluminum oxide.[155] Aluminum oxide materials are inert and possess desirable mechanical properties (high compressive strength, hardness, and elastic modulus). Furthermore, they demonstrate good biocompatibility, corrosion resistance, wear resistance, and stability in a physiological environment.[127] Abrahamsson et al. investigated the mucosal attachment around cpTi, gold, and aluminum oxide ceramic.[134] The results showed that, unlike gold, both cpTi and the highly sintered aluminum-based ceramic allowed the formation of a soft tissue (mucosal) attachment that encompassed both epithelial and connective tissue components. Furthermore, a previous study in dogs observed the presence of hemidesmosal adhesion of mucosal epithelial cells to implants fabricated from a single crystalline form of alpha-alumina oxide ceramic (sapphire, α-Al_2O_3).[164] Likewise, Hashimoto et al. confirmed the observation of a well-ordered basal lamina with cell membrane hemidesmosomes at the interface between epithelial cells and aluminum oxide.[165] Further observations also revealed no qualitative structural differences in soft tissues surrounding single-crystal sapphire (α-Al_2O_3) implants and Ti implants.[130] Despite showing good osseointegration and promising soft tissue integration results, aluminum oxide implants were withdrawn from market because of their heterogenous and poor survival rates (65–92%),[149] and because of the rising concern regarding their superstability to catastrophic failures.[127,155] It is suggested that alumina implant ceramics are prone to failures due to their low tensile strength, brittleness, and long-term aging.[155]

In response to the previous concerns regarding alumina oxide failures, yttrium-stabilized tetragonal zirconia polycrystals (Y-TZP) were introduced in the recent decades. Zirconium belongs to the same group as Ti in the periodic table and it is often called "cement steel" due to its excellent mechanical properties.[127] Y-TZP exhibits a fracture toughness and flexural strength that are two to three times larger than that of alumina oxide, which makes Y-TZP one of the strongest ceramics

in the medical field.[127] Moreover, it is suggested that zirconia has a characteristic property, sometimes termed phase transformation toughening, that enables it to change its crystalline reticulation when force is applied to its surface, and thus causing a volumetric change that stops the crack caused by the stress.[149,166] One drawback of zirconia is its low-temperature degradation or aging that occurs due to slow transformation from the tetragonal phase into the monoclinic in the presence of water or water vapor. This leads to slow development of roughness and eventually progressive deterioration of the material.[149] To overcome this problem, Y-TZP was reinforced and toughened with the addition of alumina and experiments showed improvements in the stability of the tetragonal phase of zirconia and increase in hardness. One short-term clinical study showed promising bone and peri-implant soft tissue integration around alumina-toughened zirconia.[167] Still, further research is needed to assess the biological behavior of these materials and compare them directly to the Ti implants.

In clinical settings, zirconia has been used successfully in orthopedic surgeries and its current main application is to be used as ball heads for total hip replacements.[168] Besides its excellent mechanical properties and biocompatibility, the low affinity to bacterial colonization and the toothlike color of zirconia have intrigued researchers and clinicians to consider it for dental applications as a substitute for metallic restorations.[149,155] Even though several studies on zirconia reported good interactions with bone, limited evidence is available regarding the integration of soft tissues to zirconia surfaces.[149,155]

One study evaluated the soft tissue integration around rough Ti and zirconia implants using a monkey model.[136] Some of the limitations of the previous study are the small samples which might preclude reaching significant differences and the two types of material did not receive similar surface treatment (different topographies). Within the limitations of the study, it showed that both types of implants formed a somewhat similar mucosal attachment. Even though the previous study did not evaluate thoroughly the soft tissue around implants and included a relatively small sample size, it is noteworthy to mention that the nonhuman primates used in the experiment resemble the human oral anatomy and histology more than any other animals.[169] Welander et al. confirmed the previous observations and showed by using a dog model that soft tissues dimensions were not statistically different between implant made of Ti and zirconia.[140] In a more recent study, similar surface topographies were created on both zirconium oxide and Ti alloy in order to assess the influence of material chemistry on human gingival fibroblasts in vitro.[145] Interestingly, fibroblasts demonstrated higher proliferation rates on comparable surface topographies of zirconium oxide compared with the Ti alloy. Using a minipig permucosal model, Linares et al. compared the peri-implant soft tissue healing around implants made from zirconia, Ti alloyed with zirconia, and Ti implants with different topographies.[143,147,170] Irrespective to the surface topography, clinical indicators for tissue healing revealed no significant differences between the different groups at the end of each study. Nevertheless, higher collagen organization and significantly lower epithelial downgrowth were observed in zirconia implants as opposed to Ti implants with somewhat similar microtexture, thus suggesting a more mature and pronounced soft tissue integration around ceramic implants.[147]

Several clinical studies assessing the use of zirconia implants have been published in the past decade.[149,155] Few short-term clinical studies reported good osseointegration with zirconia implants; however, the clinical data regarding soft tissue integration are scarce.[149,155] Some clinical reports indicated favorable soft tissue response to surfaces made of zirconia,[167,171–174] but the evidence from these studies is not conclusive, as there was no direct comparison with Ti implants. One randomized clinical trial concluded no significant differences in both the success rates and soft tissue clinical outcomes between two-piece Y-TZP implants and Ti implants.[175] However, the results of the study reported that out of the 16 zirconia implants and 15 Ti implants investigated, one Zr implant was lost 8 months after restoration. On the contrary, one clinical study conducted on five patients showed that inflammatory levels were statistically higher in mucosal tissues surrounding Ti gingival caps than those made of zirconia.[138]

In summary, zirconia could potentially be a surrogate to Ti as a nonmetallic implant solution, and shows promising peri-implant soft tissue integration. However, at present, long-term data on the clinical outcomes of using zirconium are scarce and there are still critical concerns regarding the possible catastrophic failures. One recent systematic review on zirconia dental implants revealed that early failure of one-piece zirconia implants ranged between 1.8% and 100%, with a calculated overall early failure rate of 77%.[176] Further improvements and clinical investigations could overcome the present limitations. For instance, some researchers investigated the use of titanium zirconium (TiZr) alloys.[143,147,170] Some investigators showed that TiZr enhanced epithelial attachment and connective tissue organization more than Ti,[147] whereas other findings indicated that TiZr had no influence on the peri-implant soft tissue attachment when compared to Ti.[143,170]

It should be noted that the chemical composition of the bulk material usually varies greatly from that of the surface, which is at the interface with the soft tissues. For instance, some materials exhibit a surface oxidation layer, whereas the preparation process or the sterilization mode (e.g., autoclaving) can significantly alter the surface chemistry of a material. Currently, surface characterization techniques, such as X-ray photoelectron spectroscopy (XPS), are commonly used to characterize the surface chemistry of the implant. For example, XPS characterizes the specific elements of a surface and their chemical and thus can be used to assess the thickness of the Ti oxide layer or the presence of surface contaminants.[177] Even though higher standards on surface characterization are being applied now, not all studies of the older literature presented here used such advanced characterization techniques. This could be one of the reasons of variability between the different studies, and why it is difficult to draw a conclusion solely based on surface chemistry. Furthermore, not all manufactures release the exact chemical composition of their marketed implants companies.

Taken altogether, it seems that until more clinical evidence is available, cpTi and Ti alloys will remain the preferred bulk material for orthopedic and dental implants. Indeed, the vast majority of marketed percutaneous/permucosal implants are made from cpTi or Ti alloys, whereas a smaller group is made

out of zirconium or surface-coated HA. However, neither of these materials is fully capable of forming a tight permanent soft tissue seal. Accordingly, modification to these surfaces is constantly being made for the purpose of enhancing the soft tissue attachment around percutaneous implants. These modifications include altering the surface topography and design, coating the surface with biomolecules or other strategies.

Surface Topography and Implant Design: There are many available surface treatment approaches for altering the surface topography (or texture) of implants, such as mechanical methods (e.g., blasting, grinding), chemical methods (e.g., acid-etching, anodization, vapor deposition), or physical methods (e.g., ion deposition, sputtering). These techniques lead to surface topographic features that can range from millimeters to nanometers. In general, modifying the surface topography of the implant by creating micromachined grooves,[4,178,179] porous surfaces,[180,181] or pits[178] increases the surface area of the implant, and therefore increases the available area for soft tissue attachment. Table 5 summarizes the main studies investigating the influence of surface topography of implant/material on the surrounding soft tissues or soft tissue cells.

Presence of a Percutaneous Collar or Flange: Promising results toward achieving a soft tissue barrier were shown by using subcutaneous components made of solid bar with a flexible mesh collar and holes[193,194] or a collar that compromises nylon hooks or a stainless steel spring.[195] Furthermore, Pendegrass et al. showed that placing a subcutaneous porous disk or flange immediately below the epithelium increases the surface area for host soft tissue integration, thus enhancing the implant–soft tissue attachment.[4] However, the skin attachment to the solid core was not strong because the cells did not penetrate perpendicularly to the wall of the central core. In addition, including connecting collars or flanges to the implant design renders its positioning relative to the subcutaneous tissues very technique sensitive and the implant may not tolerate any shifting in the position implant/soft interface due to shrinkage during the healing process, possible recession or any junction shifting when the distance from the bone to the soft tissue interface changes.

Grooved Surfaces: Surface geometry may influence the adhesion and proliferation of cells. Contact guidance refers a phenomenon to which cell locomotion is directed or guided by the orientation of the dominating geometrical surface patterns, such as microgrooves on the implant surface. Several researchers investigated the influence of contact guidance on soft tissue cells of the skin and mucosa. Among the first studies investigating this phenomenon were conducted by Brunette, which indicated that grooved surface influence the orientation of epithelium and fibroblasts.[196,197] In addition, Inoue et al. showed that fibroblasts tended to form capsule-like structures on Ti surface with circumferential grooves, whereas porous surface had no influence on the orientation of cells.[198] Early in vitro and in vivo studies by Chehroudi et al. suggested that epithelial cells seem to orient themselves along the long axis of the grooved (V-shaped grooves, 10 μm deep) Ti surfaces.[199,200] In addition, clusters of epithelial cells were markedly more attached on the grooved Ti surface than adjacent flat surfaces. Moreover, these studies indicated that micromachined horizontal grooves hinder epithelial downgrowth on Ti-coated epoxy implants.

Later, the same group investigated in vivo the effect of different groove parameters including spacing, depth, and horizontal/vertical orientation on the attachment of epithelial cells and fibroblast.[201,202] Results revealed that epithelial downgrowth was inhibited on the surfaces with horizontal grooves and was accelerated on the ones with vertical grooves. Additionally, epithelial cells were closely attached on the smooth, 3 and 10 μm deep grooves (horizontal or vertical) but bridged over the 22 μm deep, horizontally grooved surface. By contrast, fibroblasts inserted obliquely into the 22 μm deep, horizontally oriented grooves as opposed to the formation of capsules on most of the other surfaces.

Furthermore, separate subcutaneous and percutaneous components were used in order to separate the effects of surface topography on connective tissue from the effects on epithelium.[178] This study revealed that subcutaneous micromachined grooves improved the performance and longevity of percutaneous devices more than smooth and pitted subcutaneous surfaces by promoting connective tissue integration. More recently, Kim et al. showed that increasing the roughness or introducing grooves on the implant surface resulted in greater soft tissue attachment and less fibrous capsule formation when compared to the smooth surfaces.[179]

Porous Surfaces: Porosity of the implant surface and pore size are possible factors that should also be taken into consideration when designing osseointegrated implants. Porosity increases the available specific area for cell attachment and tissue ingrowth, and facilitates adequate transport of nutrients and cellular waste products. Whereas, appropriate pore size is essential for cell adhesion and spreading with optimal pore sizes varying according to the cell type and tissue engineering application.[184,203] Still, the influence of porosity and pore size on epithelium and connective tissues is not fully understood as evident from the limited available literature.[184,203–205]

Researchers using porous surfaces have demonstrated that it is possible for the skin cells and fibers to distribute or grow throughout the pores and maintain a suitable metabolism inside the implant.[206,207] In a series of studies, one research group focused on modifying surfaces made of poly2-hydroxyethyl methacrylate) (PHEMA) to assess the effect of different surface properties including porosity on epithelial cells in vitro[208,209] and soft attachment in vivo using a mouse model.[182,183,210,211] PHEMA shows resistance to protein and cell adhesion,[148,212] and this nonadhesive quality makes it an excellent negative control in studies designed to evaluate the effects of surface chemical and/or topographical surface modification on cellular adhesion. The following was concluded in the previous studies: (1) PHEMA with pore size of 20 μm and interconnecting throat sizes of 5 μm prevented epithelial incorporation in vitro, (2) increasing the pore and throat sizes to 40 and 8 μm, respectively, enhanced protein adhesion and epithelial incorporation in vitro irrespective to other surface modifications, (3) in vivo percutaneous implantation of porous PHEMA rods (pore size 40 μm and throat size 16 μm) showed consistent soft tissue incorporation with healing times extending up to 28 d irrespective to other surface modifications. Interestingly, surface

adhesive modification had no effect on epidermal incorporation when the pore size was increased to pore size 40 μm.

Farrell et al. also investigated the effect of pore size by subcutaneous implantation of porous Ti rods (pylons) with two ranges of pore sizes, 40–100 μm and 100–160 μm.[184] Implants with smaller pores size demonstrated fewer signs of extrusion when compared to those with the larger pores. Furthermore, their results showed that porous Ti implants promoted higher maximal soft tissue ingrowth as opposed to previous studies using porous PHEMA implants, and the authors suggested that skin has higher affinity for Ti than for PHEMA.

Rough Surfaces versus Smooth Surfaces: Other research groups focused on investigating the effect of surface roughness on the implant/soft tissue interface. In a dog model, it was shown that the soft tissue behavior was similar on Ti surfaces with different topographies (smooth, fine sandblasted, coarse sandblasted).[185] Similarly, another study in dogs showed that soft tissue integration was the same on both smooth and acid-etched Ti surfaces, and the connective tissue fibers had a parallel orientation on both surfaces. Therefore, the authors concluded that soft tissue adhesion was not influenced by this level of roughness.[186] In agreement with the previous study, Linares et al. showed in series of published studies using a minipig permucosal model that clinical indicators of preimplant soft tissue healing were similar on implants made from zirconia, Ti alloyed with zirconia, or Ti with different topographies (e.g., machined, acid-etched, sandblasted).[143,147,170]

Generalized conclusions from the previous studies should be drawn with caution, since sometimes similar overall peri-implant soft tissue behavior does not necessarily indicate similar epithelial and connective tissue outcomes. Indeed, one study investigating human biopsies compared histologically the soft tissue around experimental mini-dental implants with different topographies (machined, acid-etched, oxidized microporous).[187] Even though the overall width of the soft tissue attachment was approximately similar on all surfaces, the connective tissue component was longer on the rough surfaces as opposed to the smooth ones.

In vitro studies offer the advantage of allowing investigators to assess the effect of surface roughness on each cell type separately, and studies have shown that epithelial cells and fibroblasts demonstrate different behaviors to similar topographical features present on the implant surface. For instance, Cochran et al.,[213] investigated in vitro attachment and proliferation of human gingival fibroblasts and epithelial cells on Ti surfaces with varying degree of roughness (electropolished, acid-etched, sandblasted). Results demonstrated that the initial adhesion of fibroblasts was higher on the smooth surfaces and their proliferation was good on all surfaces. Whereas, epithelial cells only proliferated on the smooth surfaces. The effect of surface topography on fibroblast attachment has been also investigated in vitro[145,192,196,198,214–218] and in vivo.[161,179,219–221] Most literature suggested that roughened implant surface topographies would be beneficial for fibroblast attachment.

On the contrary, it is generally accepted that epithelial cells proliferate better on smooth surfaces rather than on rough surfaces; for this reason, most of the commercially available Ti implants present a smooth surface at the soft tissue/implant interface, particularly in dental implant.[145] However, the influence of surface topography on epithelial cells has been controversial in the literature. In 1978, Baumhammers et al. reported that there was no difference in the growth gingival epithelial cells between smooth or rough (sandblasted) surfaces.[222] In general, studies showed that smoother surfaces are better for epithelial cell proliferation and attachment than rougher surfaces.[135,145,189,213,218,223,224] Some of these studies demonstrated that smooth Ti surfaces are optimal for epithelial cell attachment and spreading.[135,218,224] on the contrary, other studies showed that epithelial cells adhered and spread in a similar manner on smooth (electropolished) and acid-etched Ti surfaces, but they adhered and spread less on sandblasted surfaces.[213,223]

Furthermore, with the recent advancements in nanotechnology, some studies propose that a certain degree of surface nanoroughness is required for soft tissue healing.[184,188,190,225] The argument of these studies is based on reports showing that nanoscale topography could facilitate a favorable template for adhesion and proliferation of different cell types. This could be due to the fact that cells in vivo interact constantly with nanometer size structures present in the surrounding environment (e.g., fibers, pores, protrusions); therefore, providing similar size scale features on the implant surface may trigger favorable cellular behaviors.[226] Puckett et al. demonstrated that Ti surfaces with nanotexture features have a positive effect on epithelial adhesion.[188] Specifically, it was shown that nanorough and nanotubular Ti surfaces increase epithelial adhesion more than the unmodified and micrometer-rough Ti counterparts,[188,225] whereas only the nanorough surfaces increase the proliferation and spreading of epithelial cells.[188] on the contrary, another study showed that nanotube arrays decreased the adhesion, proliferation, and differentiation of epithelial cells but increased these activities for fibroblasts up to four days in vitro compared to control Ti surfaces.[190] Furthermore, the findings of another study using a rodent model revealed that nanotubular treatment of Ti surfaces did not affect skin ingrowth and it seemed to only increase cellular inhabitation.[184]

Taken together, it seems that the literature contains plentiful information regarding the effect of surface topography, especially microscale texturing, on peri-implant soft tissue and cells. So far, it is generally accepted that fibroblast cells prefer a certain degree of surface roughness, whereas epithelial cells tend to behave better on smooth surfaces as opposed to rougher surfaces. Still, it is difficult to draw a definitive conclusion about the effect surface topography on cellular behavior due to variability in surface treatment protocols, cell culture studies, and animal models.

Free Surface Energy and Surface Wettability: Other important surface parameters that could be crucial for dictating the behavior soft tissue cells are the free surface energy and surface wettability.[227] Indeed, Hallab et al. and Ponsonnet el al. demonstrated that surface free energy seems to be a dominant factor when it comes to cellular adhesion and proliferation.[228,229] It is well documented in the literature that hydrophilic surfaces promoted bone apposition to implants surfaces,[230] but information about the effect of surface energy and wettability on soft tissue integration is scarce.

Early in vitro studies showed that the attachment and spreading of fibroblasts are positively influenced by increasing the surface wettability.[231,232] Further in vitro studies showed that moderately hydrophilic surface (20°–40° water contact angle) promoted the highest levels of fibroblast attachment,[233] and cells adhered and spread more into regions with moderate hydrophilicity of the wettability gradient surface than the other regions.[234] Kloss et al. investigated in a subcutaneous rodent model the integration of connective tissues to polished Ti disks with different synthetic coatings presenting variable degrees of hydrophilicity.[235] Their findings revealed that hydrophilicity influences the connective tissue healing and attachment at polished implant surfaces positively, and the inflammatory response decreased at the hydrophilic surface. In agreement with the previous study, our group found a positive correlation between hydrophilicity and the proliferation of gingival human fibroblasts seeded in vitro on different synthetic materials (e.g., Ti, PMMA, nylon 6, nylon 12, PHEMA) with comparable surface roughness values.[148] However, the same study showed that the behavior of epithelial cells was not significantly correlated to surface hydrophilicity. Interestingly, Puckett et al. demonstrated that the response of epithelial cells was more influenced by nanorough Ti surfaces with intermediate surface energies than those that had the lowest (unmodified Ti) and highest (nanotubular Ti) surface energies.[188]

In summary, even though the surface free energy and wettability of orthopedic and dental implants may prove to be a crucial factor for establishing a successful soft tissue seal, they are not the main focus of most surface characterization studies of implants. This could be due to the difficulty in establishing a standardized protocol that accurately measures free surface energy or an experimental design that would allow a more definitive correlation between an observed cellular activity and a single surface parameters (e.g., surface chemistry, surface topography, or surface wettability).[227,236] Furthermore, the values of contact angle measurements of clinically marketed implants vary along a wide range[237] or not disclosed by the manufacturer; therefore, it is currently difficult to gather enough conclusive evidence regarding the influence of surface wettability on soft tissue attachment around percutaneous/permucosal implants.

7.1.2. Biomolecular Coating Approaches

The host tissue response to the implant surface is mediated by a sequence of interactions between the cells surrounding the ECM or other bioactive molecules. Accordingly, coating the implant surface with such molecules has attracted the interest of several researchers. Such approaches aim to immobilize different bioactive molecules, such as proteins or peptides on the implant surface. The main concepts behind utilizing these molecules are: (1) to reduce or eliminate nonspecific protein adsorption that would result in the adhesion of unspecific cells; (2) to promote the selective attachment of soft tissue cells; (3) to provide integrin-mediated signals that trigger the soft tissue healing mechanism around implants. For instance, if a material is coated with an adhesion peptide, such as laminin 332, then it would be expected to trigger and enhance cell adhesion. Some early studies investigating the effect of laminin 332 coating demonstrated promising results; as epithelial cells seemed to form more adhesion structures on laminin 332 adsorbed on Ti surfaces as opposed to uncoated surfaces.[238,239] In the previous experiments, Ti samples were immersed in a solution encompassing a whole protein extracted from a rat bladder carcinoma cell line (804G), which might impose infection risks and undesirable immune reactions, as well as could accelerate proteins degradation. To overcome such limitations, Werner et al. coated Ti surfaces with a short synthetic peptide presenting a cell binding motif derived from the laminin 332 sequence.[3] Compared to whole protein extracts, adhesion peptides display higher stability toward conformational changes, heat treatment, pH variations, storage, and sterilization conditions, as well as they are cheaper and easier to characterize.[240] Even though the synthetic laminin 332 improved epithelial cells adhesion only in vitro, no significant effect was observed in the in vivo using a percutaneous dog model. Whereas, another in vitro study showed that covalently binding laminin 332 to Ti_6Al_4V by salinization significantly enhances the attachment of epithelial cells.[241]

Laminin 111, another component of the epithelial basal lamina, was also utilized by studies trying to promote epithelial attachment. Oyane et al. showed that laminin 111 coated on polymer/HA composites enhanced the attachment of epithelial-like cells by approximately ten times in vitro[242] and increased the strength of soft tissue attachment in vivo.[243] on the contrary, a previous in vitro study contradicts these results by showing that coating Ti_6Al_4V with laminin 111 has no effect on epithelial attachment, spreading or hemidesmosome assembly.[239] The observed disparity in cell behavior between the two research teams could be because laminin 111 was coated on materials with different surface properties (i.e., surface chemistry and topography).

From the previous studies, it can be concluded that the laminin-coated materials, particularly laminin 332, might be used to limit epithelial downgrowth by strengthening their attachment to the surface. Nevertheless, more investigations are required to clearly elucidate whether coating implants with laminin 332 is a feasible option for promoting the soft tissue seal. On the other hand, the percutaneous soft tissue attachment around the implant is constituted by an epithelial barrier and a zone of connective tissue attachment. Since epithelial cells and fibroblasts are surrounded by a different composition of ECM, it could be speculated that they would require different bioactive molecules. Interestingly, one of our in vitro studies revealed that, unlike fibroblasts, the attachment and proliferation of epithelial cells correlated significantly with laminin adsorption profile on a material surface.[148]

Some investigators explored the use of another bioactive molecule, fibronectin, to promote the attachment of fibroblasts or connective tissues in general. Fibronectin is a principal component of ECM and pertains binding sites for fibrin, thrombin, heparin sulfate, as well as cell integrin-binding sequences, such as those that include the amino acid sequence arginine–glycine–aspartic acid (RGD), through which it promotes cell–matrix adhesion.[244] Early in vitro studies showed that fibronectin is readily adsorbed into Ti and promotes

fibroblast attachment and proliferation.[245–247] However, in vivo observations revealed that adsorbed fibronectin onto Ti surfaces does influence connective tissue attachment around percutaneous devices.[244] Accordingly, it was presumed that adsorbed fibronectin might have been removed from the surface during implantation due to competitive adsorption from other serum proteins or physical abrasion. Further studies investigated the use of chemical methods to create a more durable attachment between fibronectin and Ti surfaces. Middleton et al. used salinization to covalently bind fibronectin to Ti surfaces, and their in vitro results showed that salinized fibronectin showed no significant loss of fibronectin upon soaking in fetal calf serum.[244] Furthermore, they demonstrated that salinized fibronectin enhanced dermal fibroblast behavior more than Ti with adsorbed fibronectin and untreated Ti surfaces.[244] Other studies investigating salinized-fibronectin onto Ti were also in agreement with the previous results that showed enhancement of fibroblast behavior in vitro,[248] and a better dermal attachment in vivo.[249]

The porous nature of HA was also utilized to facilitate higher fibronectin adsorption than Ti. Indeed, it was shown that HA adsorbed with fibronectin exhibited similar dermal attachment to that observed on Ti-salinized with fibronectin.[249] Furthermore, other findings revealed that HA adsorbed with fibronectin enhances fibroblast attachment in vitro[129,250,251] and dermal attachment in vivo.[251]

Other researchers also used other bioactive molecules, including collagen type I, collagen type IV, platelet-derived growth factor (PDGF), and E-Cadherins. Early in vitro studies showed that coating Ti surfaces with collagen type I improved epithelial cell and fibroblast attachment;[252] however, Welander et al. demonstrated in a dog model that coating Ti surface with collagen type I had no significant effect on soft tissue healing.[253] Researchers also attempted to understand the influence of different ECM constituents (collagen type I, collagen type IV, fibronectin, laminin, and vitronectin) on the epithelial tissue–implant interface in vitro.[254] Among the tested coatings, collagen IV-coated Ti surfaces exhibited the best proliferation and attachment of human oral epithelial cells. Despite showing the most promising results, collagen type IV was derived from rodent tumor tissues (Engelbreth-Holm-Swarm (EHS) sarcoma) and thus presents homogenates that differ in composition from human ECM.[255] On the other hand, the other ECM were extracted from different sources (collagen type I from calf-skin, laminin for human placenta, fibronectin and vitronectin from human plasma).

PDGF is secreted locally by blood platelets during clotting at the site of soft (or hard) tissue injury and it triggers a cascade of events that regulates the mechanism of soft tissue healing.[256] Furthermore, applying PDGF topically at the injury site showed promising therapeutic results.[256] However, its application in the field of percutaneous medical devices was limited. Bates et al. demonstrated in a subcutaneous rat model that coating implant with PDGF did not alter the orientation of fibroblasts and collagen fibers; however, it improved the depth of connective tissue penetration around implant within a shorter healing period compared to uncoated controls.[257] Therefore, it was proposed that coating implant with PDGF can increase the quantity and speed of soft tissue healing around percutaneous implants.

In general, squamous epithelial cells adhere to each other (cell–cell adhesions) via two main types of cell attachments, desmosomes and adherens junctions.[258] In adherens junctions, E-Cadherin (prefix "E" for epithelial) is a calcium dependent adhesion transmembrane protein that has a critical role in epithelial cell–cell adhesion and tissue formation.[259] Hence, the effect of E-Cadherin on epithelial attachment was assessed. In vitro observations showed that adsorption of E-Cadherin to Ti Alloys improves the attachment and metabolic activity of epithelial cells, with a fourfold increase in cell attachments via adherens junctions when compared to uncoated controls.[260] The influence of vitamin D precursor and vitamin E on human fibroblasts was also investigated. Satue et al. showed that coating Ti implants with ultraviolet (UV)-irradiated 7-dehydrocholesterol (7-DHC) and vitamin E has a beneficial effect on fibroblasts in vitro.[261] Table 6 summarized the main studies that investigated the influence of biological coatings on the behavior of soft tissue healing or soft tissue cells surrounding the material's surface.

7.1.3. Surgical Approaches

Besides surface properties, some investigators have argued that the type of surgical approach and number of implant components influence the healing of soft tissues around implant. Generally, in single-piece implants, the surface facing the soft tissues of skin or oral mucosa is part of the implant body, which is placed immediately during a one-stage surgery. By contrast, in the two-piece systems, the percutaneous/permucosal surface is part of separate component that can be placed following a one-stage surgery similar to single-piece implants, or placed by means of a second surgery that exposes the submerged component after a waiting period for tissues to heal (two-stage surgery). The two-component implant system has the advantage that it enables investigators to assess the influence of surface on percutaneous and subcutaneous components separately.

Even though several in vivo studies using one- and/or two-stage surgical implantation approaches showed promising soft tissue attachment results, only few studies directly compared the influence of the surgical approach (one-stage vs two-stage).[263–266] In one direct comparison study utilizing a rodent, it was shown that following a two-stage surgical approach promotes more connective tissues around Ti percutaneous devices as opposed to the one-stage approach.[264] In another study using a percutaneous goat model, the investigators reported that more one-stage implants were extruded than the two-stage one during a 4-month period.[265] After excluding these devices from the analysis, histological findings revealed no statistical differences in epidermal downgrowth of the survived devices between the two different approaches. However, if the extruded devices (4 out of 11) were included in the analysis and considered as a 100% downgrowth, results could have been different between the two groups. It was argued that the reported poor survival of one-stage implants could be attributed to the onset of early healing cues that might have been further compounded by exposure to bacteria at the implant exit sites. In order to

address this issue, Mitchell et al., unlike the previous two studies, the percutaneous site was immediately covered by a wound dressing following the surgery, which may facilitate early healing and reduce initial bacterial biofilms.[266] Their findings showed reduced epithelial downgrowth in the two-stage approach compared with the one-stage counterpart. It was hypothesized that the preexisting interdigitation of connective tissues before exposing and installing the second component in the two-stage surgery might have contributed to the reduced shear forces and thus limited the epithelial downgrowth. In a permucosal dog model, Weber et al. did not find any significant differences between one stage or two-stage surgical approaches regarding the connective tissue or the level of mucosa border; however, two-stage surgeries demonstrated longer epithelial attachment.[263] on the contrary, other researchers using a permucosal dog model showed similar soft tissue dimensions and position in both surgical approaches.[267,268]

7.1.4. Other Strategies

Negative pressure wound therapy (NPWT) was utilized to prevent soft tissue downgrowth around percutaneous implants coated with a porous commercially pure titanium in a Guinea pig model.[266] NPWT limited the soft tissue downgrowth in all implants, and the authors suggested that it could be due to the previously reported ability of NPWT to remove edema and inflammatory factors from wound beds, increase vascularity, and mechanically draw the wound edges closer together.

Another strategy was using mesenchymal stem cell (MSC) therapy, which has been explored for treating several clinical conditions due to its promising role in tissue repair and regeneration. Since it was shown that MSC therapy can accelerate wound healing, increase vascularity and collagen content, and enhance wound strength, researchers exploited its use for enhancing the soft tissue seal around implants.[269] Porous percutaneous Ti seeded with bone marrow-derived MSCs were implanted in a rat model, and findings indicated that stems cells accelerated early wound healing and resolution of inflammation. However, there were no significant differences in epithelial downgrowth or late wound healing between implant with or without MSC treatment.[269]

7.2. Strategies to Prevent or Reduce Bacterial Adherence and Colonization

In 1987, Gristina pictured the fate of a biomaterial upon implantation as a race between host tissue integration against bacterial adhesion and biofilm growth, which he referred to as "the race to the surface."[270] If the race is won by host tissue cells, then the implant surface gets covered by tissues and becomes less vulnerable to infection. This concept has been embraced by many researchers in the field of percutaneous/permucosal implants, and several antibacterial approaches have been designed to prevent or reduce bacterial adherence and colonization on the implant surface. These strategies include coating the surface with antibacterial agents or modifying the physicochemical properties of the implant surface. Detailing the numerous antibacterial strategies that have been used over the past decades is beyond the scope of this review and the readers are referred to other reviews that covered this topic in more depth.[271–275]

8. Concluding Remarks

Establishing a soft tissue seal around percutaneous and permucosal devices is a vital requirement for preventing bacterial infections and enhancing the implant long-term success. The present review provided the recent findings and challenges concerning establishing a strong permanent soft tissue attachment around bone-anchored implants, especially orthopedic and dental implants. In particular, this review pointed out the different strategies used to achieve this soft tissue seal as well as the relevant challenges and summary that can be concluded from the findings of each particular approach.

Despite all the aforementioned strategies and progress, researchers were not able, so far, to develop an osseointegrated implant, or a percutaneous device that enables a strong permanent attachment with the surrounding soft tissues. This is perhaps due to the difficulty in establishing valid comparisons between the previous studies and formulating concrete conclusions. This could be attributed to the great variation between study designs (e.g., different cell types, animal models), differences in implantation locations, incorporation of a wide range of materials with various designs, or the assessment of various outcome measurements that are rarely similar between studies. Another challenge is to improve the experimental designs in order to provide a more definitive correlation of observed biological responses to single surface parameters, such as surface chemistry or surface topography.

Emerging technologies have enabled the fabrication of nanoscale topographies that could hold the solution for establishing the peri-implant soft tissue seal; however, the number of studies employing such technologies remain limited in this field and future studies are required to explore the influence of nanoscale features on the soft tissue integration around implants. Moreover, the recent major advancements in the proteomics field might help in deciphering the correlation between the proteins adsorbed on the implant surface and the surrounding soft tissues as well as enhance our understanding of the molecular structure of biological soft/hard tissue interfaces that could provide insights into approaches for engineering soft tissue interfaces around osseointegrated implants.

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Conflict of Interest

The authors declare no conflict of interest.

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