1. Introduction

Dental implant treatment is widely available as an effective means of replacing missing teeth with long term predictability(1, 2). Several factors have been suggested to influence implant success and there are various theories for the aetiology of late implant failure. In this chapter a literature review will provide an overview of the natural dentition in health and disease, relate this to the dental implant as a replacement for the tooth and present competing theories for the aetiology of peri-implantitis as the main cause of late failure. The emphasis of this project will be on the potential role of particle release from implant biomaterials in the onset of peri-implantitis, and possible materials that can provide resistance to implant failure. The subsequent four chapters will be on the experimental work carried out with the concluding remarks in the final chapter. This work has resulted in three publications.

The rational for this research is to improve the gap in knowledge with regards to the role of dental implant biomaterials in the onset and progression of peri-implantitis, and to investigate preventative strategies. This can have clinical, manufacturing and future research implications.

1.1 Literature review

1.1.1 Human dentition in health and disease

In health, the tooth has an intact periodontal ligament which securely holds the tooth in the alveolar bone of the jaw. The transmucosal anatomy, where the tooth emerges from the alveolar bone through the mucosa into the environment of the oral cavity consists of connective tissue attachments between the tooth cementum and the adjacent alveolar bone and gingivae (gums), followed by the more superficial junctional epithelium with hemidesmosomal attachments between the tooth enamel and the adjacent gingivae Fig. 1 (3). The healthy tooth will have no carious cavities, no inflammation within the pulp of the tooth or in tissues supporting the tooth and there will be no loss of supporting gum and bone.

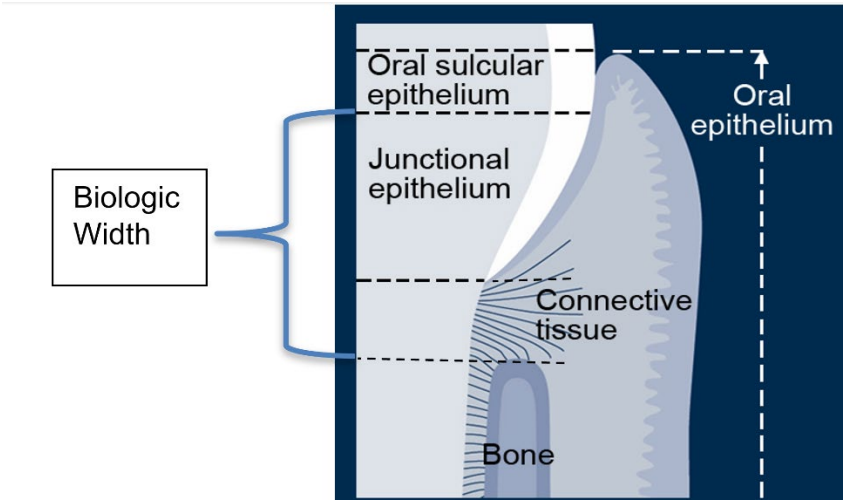

Figure 1. Dental gingival (gum) anatomy at the point of emergence into the oral cavity. Adapted from ITI speaker's library.

1.1.1.1 The transmucosal anatomy of a tooth

In nature, whenever a structure communicates between the deep tissues of the body, beyond the basal membrane zone (BMZ) of the skin or mucosa and the external environment, there is a potential for pathogens to invade. Hairs, nails and horns are structures that emerge from the dermis and do not penetrate the BMZ. Whereas teeth emerge from the jawbone into the hostile oral environment with its associated chemical, physical and microbial insults.

The anatomy of the junction between the tooth and the oral mucosa as the tooth emerges from the jaw into the oral cavity is depicted in Fig. 1. As the tooth emerges from the bony socket into the oral cavity there is a connective tissue attachment just above the crest of bone, which is then covered by the junctional epithelium. The collective name for the connective tissue attachment and junctional epithelium is the Biologic Width.

In healthcare, various devices that need to be implanted deep into tissues communicate with the external environment for various periods of time. The short-term use of intravenous catheters for a few hours to days does not need a strong connective tissue connection as that would complicate their removal. However, prostheses for replacing limbs, dental and maxillofacial implants, cochlear implants and long-term catheters for renal dialysis for example would benefit from having

collagen fibres from adjacent tissues inserting into the prosthesis at the entry point to provide a protective barrier against the external environment, as seen with natural teeth (Fig. 1).

Several processes or mechanisms are involved in the attachment process of the connective tissue fibres to the teeth and bone. Understanding these processes or mechanisms is paramount for the attempt to mimic this around artificial prostheses. The roots of teeth are held in place inside the jawbone via a fibrous joint (gomphosis) called the periodontal ligament (PDL), which is rich in blood supply and stem cells, with an important role in repair and regenerative processes around the tooth. The periodontal ligament allows tooth movement of 50-80 μm under occlusal load (biting) (4). This movement is possible due to the wavy pattern of the PDL fibres, which allow for elongation. Also, the PDL ground substance consists of 70% water allowing for a ‘shock absorbing’ property. Collagen fibres in PDL (mainly type I and III) have a high turnover compared to those in tendons and are therefore less mature and thinner in diameter (55 nm)(3). Type V collagen is found in the interstitial fibrils and between the fibril bands. Collagen IV, VI and XII are thought to play a role in remodelling of the PDL(3).

The PDL fibres insert in cementum at one end and bone at the other. The insertion is thought to be achieved via connections to non-collagenous proteins in the corresponding matrices which then calcify and ‘lock’ the fibres in. The calcified ends are referred to as Sharpey’s fibres. During bone remodelling, osteopontin and bone sialoprotein play an important role in detaching and reattaching of the fibres to the new bone laid down(3). Glycoproteins are thought to be of importance in the PDL attachment include fibronectin and tenascin, which is concentrated at the alveolar bone and cementum insertion sites. The rest of the ground substance consists of non-collagenous matrix proteins such as alkaline phosphatase, hyaluronate, glycosaminoglycans (GAG), proteoglycans and glycoproteins. These may play a role in the organisation of the ligaments(5). The main GAG is dermatan, a polysaccharide with anionic charge that can covalently bond to proteins to form proteoglycans. Therefore, there are important non-collagenous proteins (bone sialoprotein, osteopontin, fibronectin, tenascin) and extra cellular matrix (ECM) ground substances (dermatan GAG) that play an important role in the PDL fibre attachment

to the cementum and bone.

Fig. 2 depicts the junctional epithelium's very unusual anatomy in that there are 2 epithelial layers, a supra-basal layer connecting to the tooth via the internal basal membrane zone (epithelial attachment against the tooth) and basal layer with an external basal membrane zone separating epithelium from the underlying connective tissue.

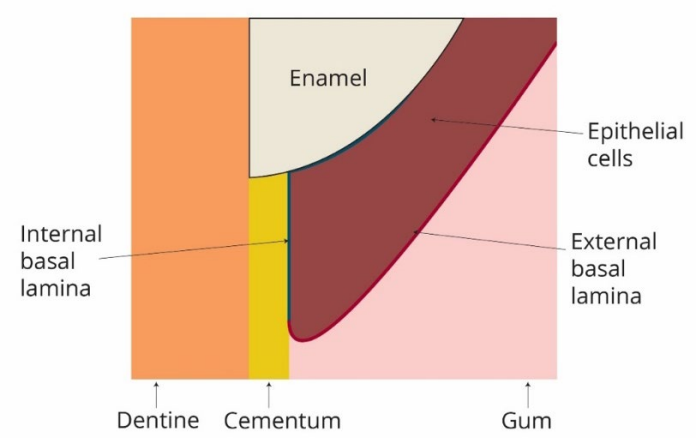

Figure 2. Junctional epithelium. Adapted from Kobayashi, K.(6).

The internal BMZ differs in its anatomy from other BMZ's in that hemidesmosomes attach to the tooth surface, the Lamina Reticularis is missing, and the ECM consists mainly of Collagen VIII and Laminin 332 (Fig. 3).

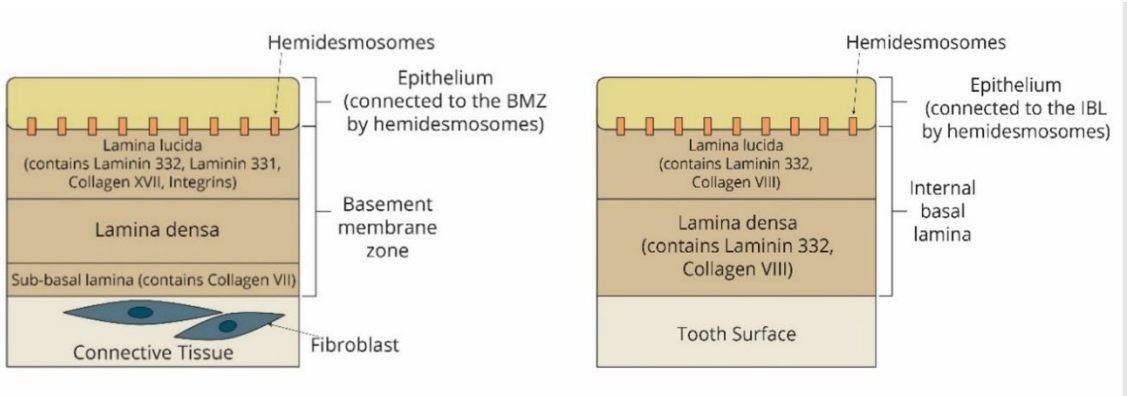

Figure 3. Left: Diagram of basement membrane zone in skin. Right: Diagram of basement membrane zone of gingival junctional epithelium. Adapted from Abdallah, M. N. et al 2017(7).

Laminin 332 is thought to be important in the soft tissue attachment mechanism; the ECM seems to play an important role in this (7). The collagen attachments between the root of the tooth and the gingival tissues (above the crest of bone) have a mechanical protective role for prevention of bacterial invasion as well as providing stability of the gum's position. This has important implications for dentists in the control of the cosmetic appearance of the gums around the tooth. If this barrier is disrupted by bacteria, inflammation and bone loss can result. The inflammatory process can cause release of matrix metalloproteinases (MMP) from macrophages and bacteria, resulting in the breakdown of collagen in the PDL and bone matrix, with subsequent tissue loss and pocketing around the teeth. This process is better known as periodontal disease.

Tooth loss can occur for a number of reasons including tooth decay, chronic periodontal disease (gum disease), trauma, and pathology such as cysts and head and neck cancer. Chronic periodontal disease will be expanded on further below due to its relevance to this research topic of peri-implantitis.

1.1.1.2 Chronic periodontal disease

Chronic inflammation of the gingivae (gingivitis) and the periodontal ligament (periodontitis) is caused by the buildup of plaque on teeth, restorative material such as fillings or crowns and dentures. Plaque is a bacterial biofilm in which stable communities of bacteria can be established when it forms on hard surfaces of the dentition as there is no shedding from the dental surface, unlike the oral mucosa (8).

The biofilm formation starts with the adsorption of salivary proteins and mucins on intraoral hard surfaces to form a pedicle onto which bacteria can directly attach. As the bacterial colony grows an extracellular polymer matrix is synthesised and deposited, allowing further attachment of microbes without direct access to the pedicle and the development of a thicker biofilm.

As the thickness of the biofilm increases, the diffusion of oxygen becomes limited due to the matrix which results in an increasingly anaerobic condition in deeper aspects of the biofilm. This results in an increasingly gram negative and anaerobic

species which utilise periodontal tissues, blood supply and other microorganisms for nutrients, compared to the surface bacteria which tend to be more gram positive, aerobic or facultative anaerobic (such as streptococci), which utilise products in saliva as nutrients. Gram negative, anaerobic species that have been associated with periodontal disease progression include: Porphyromonas gingivalis, Prevotella intermedia, Tannerella forsythia, Treponema denticola, Fusobacterium nucleatum and Campylobacter species.

Prevention of plaque biofilm build up is the basic principle underlying oral hygiene protocols, such as brushing teeth in order to disrupt the biofilm and transition of bacterial species to the more gram negative and anaerobic species which are believed to be involved in the advanced periodontal disease. If the plaque buildup continues undisturbed tissue damage can take place as a result of direct bacterial action as well as the indirect host response, such as the release of proteolytic enzymes, pro-inflammatory cytokines (IL1β, IL6, TNFα) and complement activation. These can result in the loss of the periodontal collagen fibres and bone resorption. In an inflamed PDL, stem cells may have an impaired immunomodulatory function which can lead to an imbalanced immune response with an acceleration of osteoclastogenesis, inflammation and bone loss (9).

The loss of supporting tissue around the tooth gives rise to areas of pocket formation between the tooth and adjacent gums, resulting in micro-environments which are protected from the disruptive cleaning by tooth brushing. Other local factors such as poor restorative margins with an overhanging filling which the patient cannot access to clean also provide a micro-environment for undisturbed plaque buildup. This can cause tissue damage and further periodontal pocket formation, which in itself is a protected environment for the plaque and therefore allows for the propagation of the disease with further tissue loss until the eventual loosening and loss of the tooth (8).

The natural barriers around a tooth usually slow down or limit the pathological process through the physical collagen attachment barrier and the immune response from the well vascularised periodontal ligament. The result of this protective mechanism is to create fibrous encapsulation of the inflammatory process (10) and limit the spread of infection. Hence, in patients with periodontal disease there can be isolated pockets around the root of the tooth where there has been an invasion of the

barrier and tissue loss, but then the barrier is re-established lower down; this can slow the invasive process down.

The rate of disease progression is dependent on several factors, including the host's inflammatory response which can be an overreaction in individuals who are highly susceptible to periodontal disease. Other factors that affect the rate of disease progression include the patient's general health, smoking, oral hygiene, and immune status.

1.1.2 Dental implants in health and disease

1.1.2.1 Demand for dental implants

The demand for dental implant treatment as a means of replacing missing teeth is likely to grow with the ever-increasing population age and public awareness of this treatment modality(1). A general dental practitioner today cannot avoid dealing with dental implants in their day-to-day practice. Global figures for the number of implants placed per 10,000 population is summarised Fig. 4.

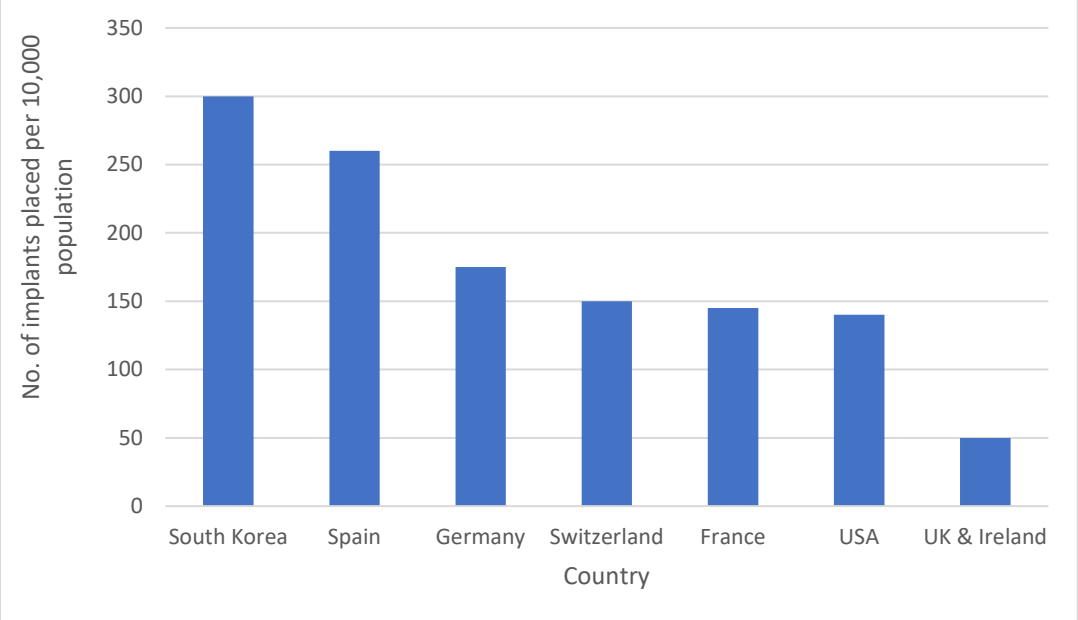

Figure 4. Dental implant placements per 10,000 population. Adapted from Straumann report 2016 (11).

The trend in the percentage of population over 65 years of age has increased dramatically and is forecasted to increase exponentially in the coming decades

(Fig.5).

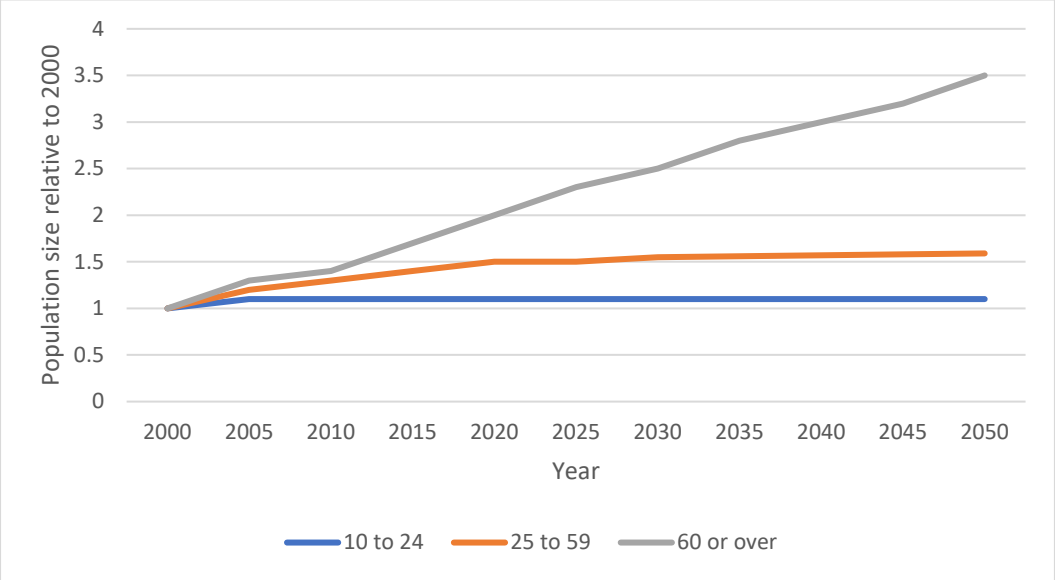

Figure 5. Increase in world population relative to 2000, by broad age group, 2000-2050. Adapted from United Nations (2015). World population Prospects: the 2015 revision (12).

The significance of the above projections includes:

1. As the percentage of the population over sixty is increasing, so is the need for implant treatment due to higher edentulism in the elderly.
2. Implant longevity is becoming more significant as patients are living longer.

Dental implant treatment can improve the quality of life for people with missing teeth. The improvement in chewing, speaking and patient satisfaction from placing two implants in the lower jaw to support the denture has been well demonstrated over the years (13, 14).

1.1.2.2 What is a dental implant?

A dental implant consists of two major components: the implant fixture which is designed to replace the root of a tooth and sits in the jawbone, followed by the crown which is the tooth part. The crown itself consists of the abutment, which is secured

inside the implant with a screw, and the ceramic (or metal-ceramic) cover/ crown, which has the appearance of a natural tooth.

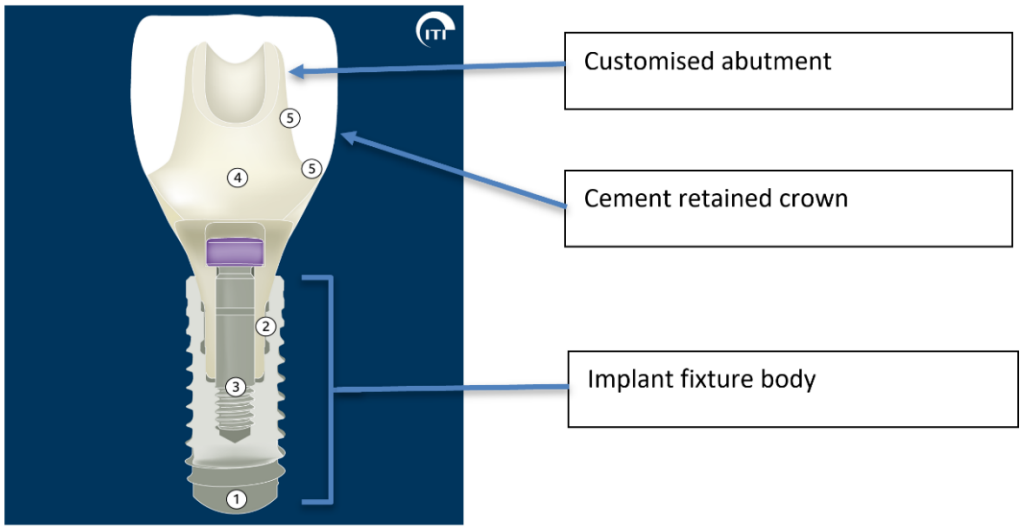

Figure 6. Implant components. Adapted from ITI speaker's library. 1. Implant fixture 2. Implant/ abutment interface (micro-gap) 3. Abutment screw 4. Abutment 5. Abutment fit surface.

Fig. 6 depicts a bone level implant which is the most commonly used implant; this refers to the fact that the implant fixture is set in bone, with the implant-abutment junction at bone level. This contrasts with the tissue level implant (Fig. 7 and 8) where the implant-abutment junction is at gum level and remote from the bone.

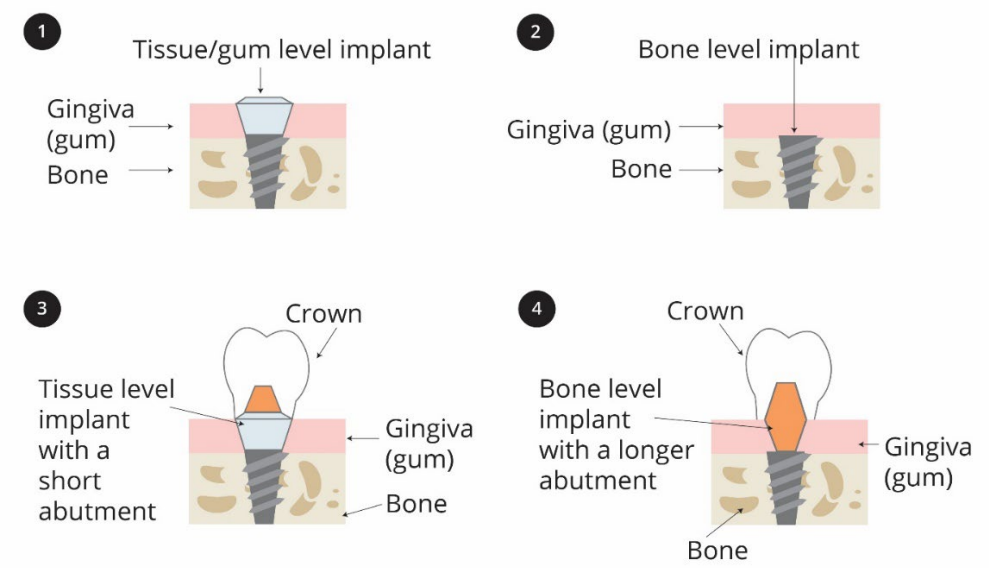

Figure 7. Schematic of a tissue level versus bone level implant: 1. Tissue level implant 2. Bone level implant 3. Restored tissue level implant with a short abutment 4. Restored bone level implant with longer abutment. Designed by Dr F Barrak, illustration by Kitty Lungley.

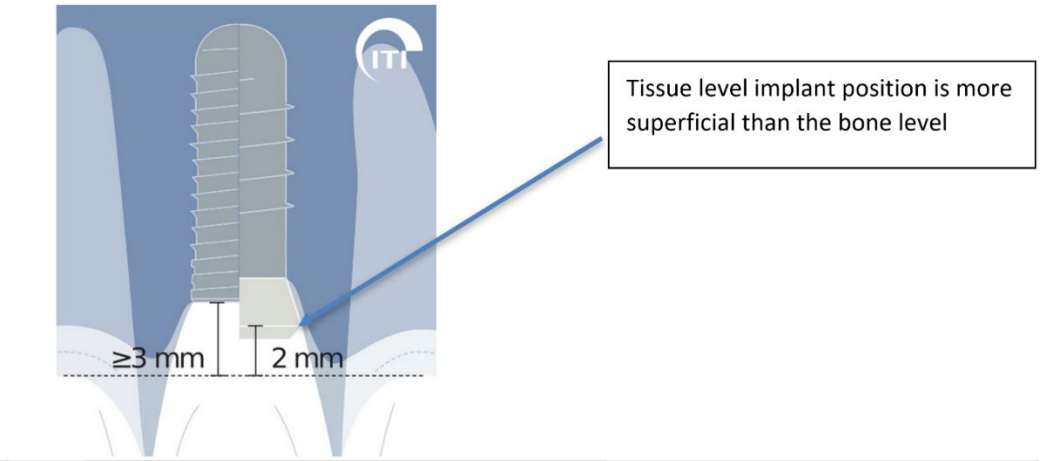

Figure 8. Bone level implant on the left half and tissue level on the right half of the image. Adapted from ITI speaker's library.

Bone level implants are much more widely used and available commercially because their deeper position below the gum allows for adjustment in angulation of the crown where necessary to improve the aesthetic and functional position. The tissue level implant positioning must be exact, as there is less room to compensate for the fixture angulation as depicted by the green 'safe' and red 'danger zones' in Fig. 9.

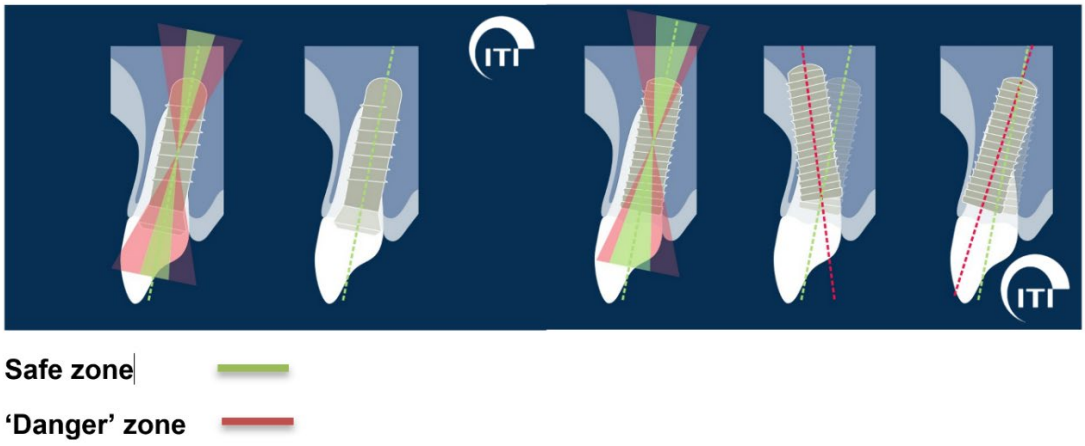

Figure 9. . Tissue level implant (left) has a much smaller 'safe' zone of varying angles of placement depicted in green, compared to a bone level implant (right). Tissue level implant placement needs to have more precise placement. Adapted from ITI speaker's library.

Dental Implants have high survival rates over a 10 year period in which patients have been followed up for (15). However, implants are still susceptible to failure secondary to inflammation in the supporting tissues, referred to as peri-implantitis (15). There has been a considerable amount of clinical research carried out on peri-implantitis, ranging from aetiology and risk factors to its management. Despite the sizable volume of work on the subject there are still no conclusively effective treatment modalities (16). There is still controversy on the aetiological origin of the onset of peri-implantitis (17).

1.1.2.3 Bone healing around dental implants.

Surgical trauma to the bone during the osteotomy drilling process causes bleeding from the alveolar bone marrow due to damage to the vasculature within the bone marrow. On insertion of the implant, its surface becomes impregnated with a fibrin coagulum, neutrophils and macrophages. Subsequently, fibroblasts infiltrate the site, forming granulation tissue and angiogenesis commences. Sequestrated, non-viable bone fragments from the surgery are then removed by osteoclasts migrating into the site; this starts the process of new bone formation mediated by factors released from bone matrix breakdown by the osteoclasts. Mesenchymal stem cells and fibroblasts from the adjacent alveolar bone differentiate into osteoblasts which lay

down immature bone matrix, known as osteoid. This is subsequently mineralised to form immature woven bone. Some of the woven bone is formed directly on the implant surface through differentiation of pre-osteoblasts to active osteoblasts, while some of the bone is generated by extension from local trabecular bone, referred to as distance osteogenesis. In humans, woven bone formation takes approximately twelve weeks and mature lamellar bone can takes six months to form (18).

At the time of implant placement, achieving stability of the fixture is required to ensure a stable surface on which new bone can grow. If the implant is mobile, fibrous tissue is likely to form rather than bone. This initial stability of the implant at the time of surgery is termed primary stability and is achieved through mechanical engagement of the implant threads in the alveolar bone. However, this stability is reduced during the healing process as a result of bone remodelling which involves resorption of the bone in contact with the implant and deposition of new bone. Formation of new bone and osseointegration of the implant brings about the secondary stability, which is long lasting in a healthy implant. Fig. 10 demonstrates the temporal relationship between primary and secondary stability of the implant graphically and highlights a period of dip in implant stability at approximately 3 weeks post-surgery when the stability is compromised as the primary stability is lost due to remodelling and the secondary stability is being established with new bone formation (18).

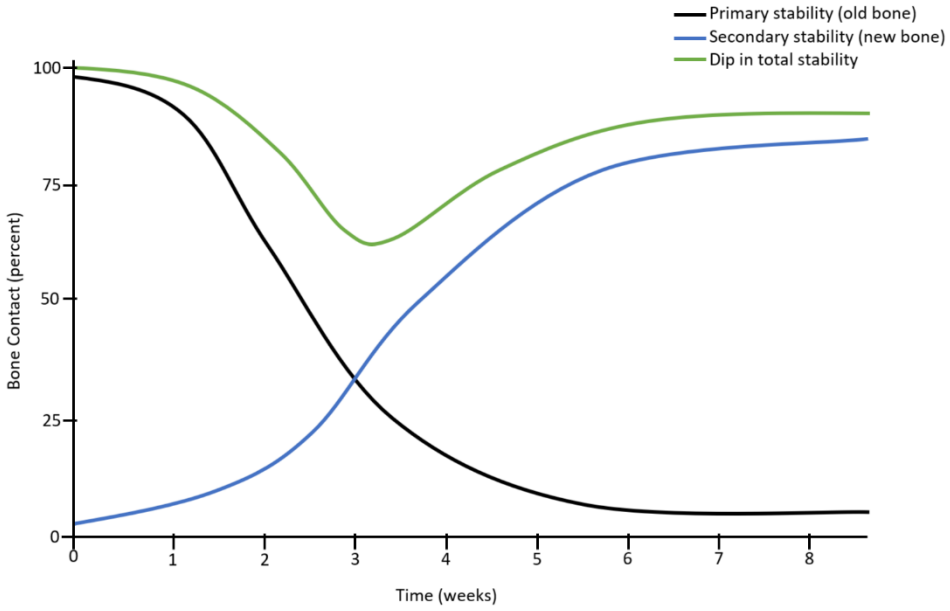

Figure 10. Graphical representation of the temporal relationship between primary mechanical stability (black line) and secondary osseointegration stability (blue line) of a dental implant. The period of drop in total stability when primary stability is reduced and secondary stability in still being established is depicted with the green line showing a dip in stability at 3 to 4 weeks post placement of implant. Adapted from Villar C.C (18).

1.1.2.4 Healthy implant

In health the peri-implant tissues will be free from inflammation, swelling, suppuration (pus discharge), the alveolar bone will be integrated with the implant surface and the transmucosal section will have weak hemidesmosomal connections with the implant surface. Clinically, there would be no distinguishing features between the gums around the tooth or implant. However, on examination the probing depths around the implant may be deeper than around the tooth and the papillae (gum between adjacent teeth) may be blunted (19).

1.1.2.5 Early and late implant failure

Implant failures that occur before the crown is fitted and loaded in occlusal function are referred to as early failures and are likely to be due to the failure of the implant to integrate following surgery. The causes of early implant failure include post-operative infection, the risk of which may be reduced with a prophylactic dose of antibiotics; there is some evidence to suggest a reduced rate of infection with the use of single dose of pre-operative antibiotics, however, there are conflicting opinions on this (2). Poor surgical technique with overheating damage to the bone resulting in osteonecrosis, and a lack of bone implant contact following implant placement can result in fibrous encapsulation as opposed to osseointegration (2). These can subsequently result in early implant failure. Systemic predisposing factors for early implant failure include smoking, immunocompromised patient, and medications such as selective serotonin reuptake inhibitors (20).
Late implant failure refers to the loss of an implant after successful integration has taken place. This research is in relation to late implant failures as a result of peri-implantitis which is the main cause of late implant failure and will be the focus of the rest of this work.

Comparative anatomy and disease progression of teeth and dental implants
As dental implants emerge from the jawbone through the mucosa into the oral cavity, the soft tissue surrounding the per-mucosal aspect follows the same sequence of having connective tissue over the bone, followed by epithelium (the biologic width) as in teeth. However, the key differences are that implants do not have a periodontal ligament and the connective tissue covering the bone does not attach to the implant (Fig.11) but runs parallel or circumferentially around the neck of the abutment in a bone level implant (or the smooth collar of the tissue level implant) (10, 21).

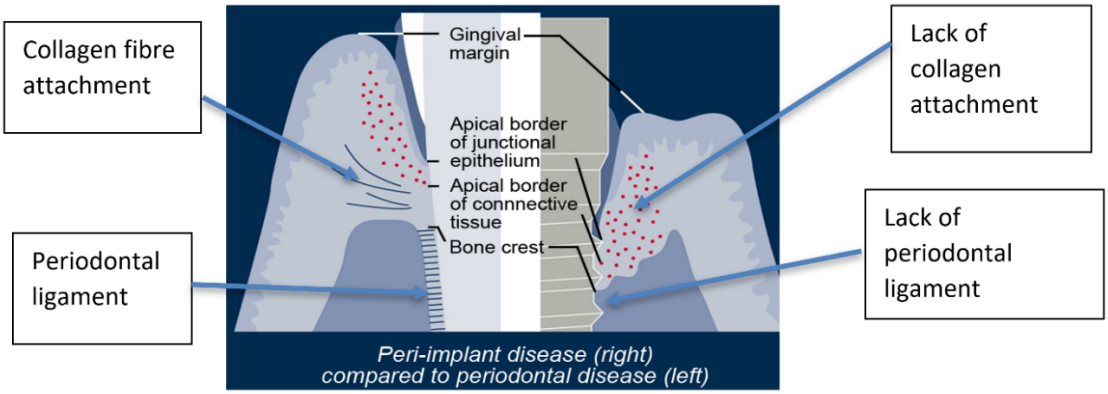

Figure 11. Comparative anatomy of tooth and implant. Adapted from ITI Speaker's library.

The lack of collagen attachment around the neck of implants means that the physical barrier against bacterial invasion, advancing inflammation and recession is missing (10). The rich blood supply and stem cells normally found around the natural tooth are also reduced due to the lack of the periodontal ligament (3).

Fig. 12 shows the contrast between the natural tooth where inflammation is encapsulated in pockets and an implant where the physical barrier of collagen attachment is lacking thereby allowing rapid spread of inflammation to the bone. Therefore, inflammation around an implant can progress much faster and spread around its circumference with significant bone loss. This process is referred to as peri-implantitis and can result in catastrophic bone loss as shown in Fig. 13 (10, 15, 21-23). The aetiology and clinical features of peri-implantitis are similar to that of periodontitis, in that inflammation as a result of bacterial colonies and the local host response results in the damage and loss of soft and hard tissue support for the implant (10). However, the disease onset and progression in peri-implantitis is different with an earlier onset of bone loss and a non-linear progression (15).

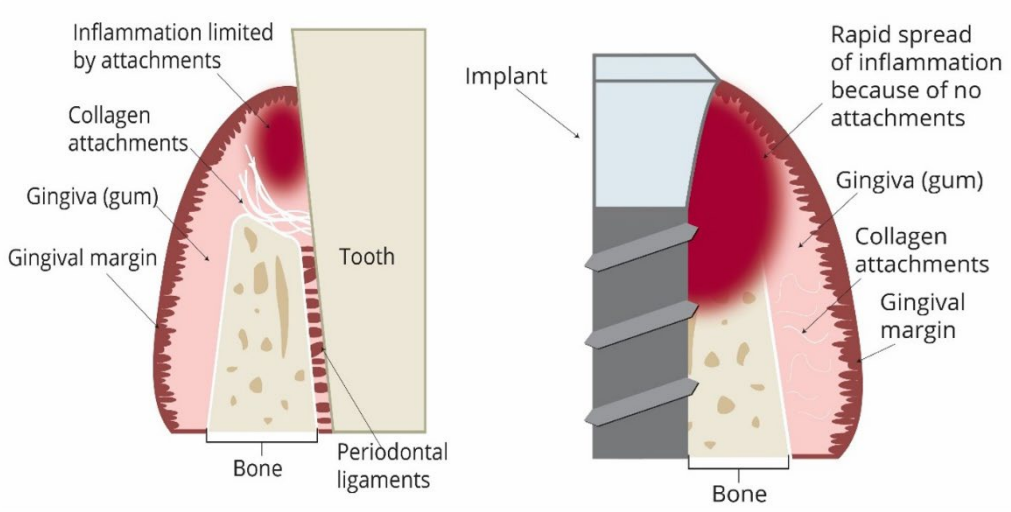

Figure 12. Diagrammatic comparison of the inflammatory infiltrate extension on a tooth surface (left) and an implant surface (right). Adapted from Salvi, G.E. (10).

(10)

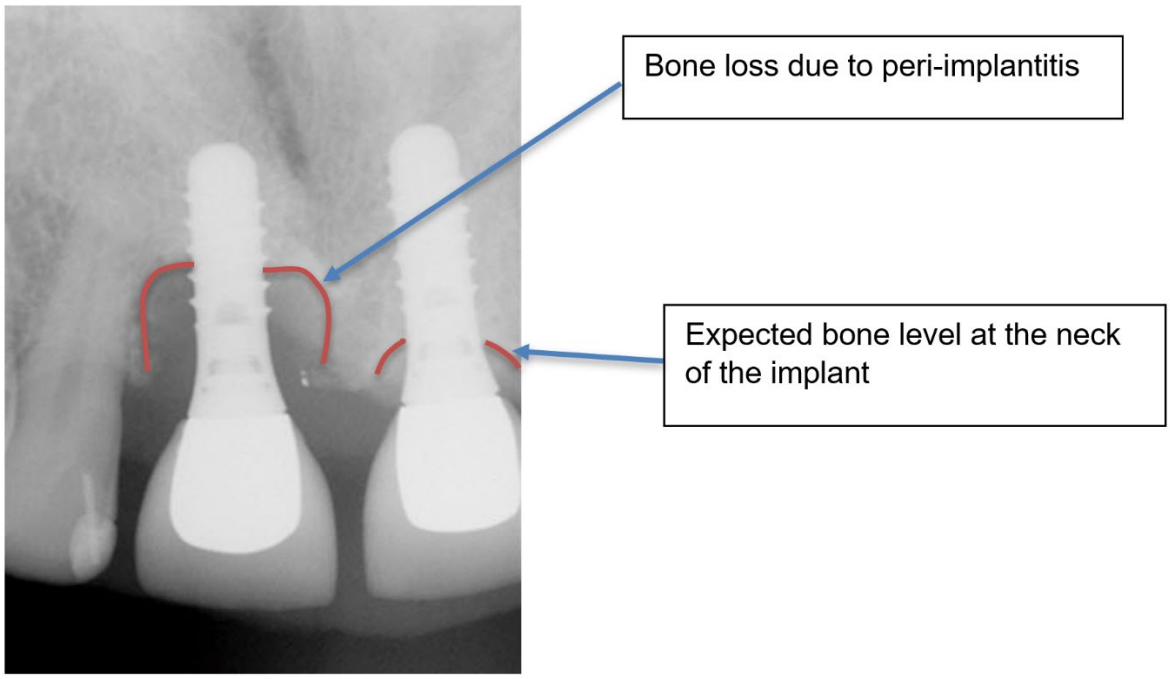

Figure 13. Radiograph of dental implants depicting the vertical bone loss appearance pathognomonic of peri-implantitis. Adapted from ITI Speaker's library.

Further complications with dental implants develop when there is bone loss around the neck of the fixture, including aesthetic compromise, further tissue loss with the advancement of the inflammatory process and increased risk of implant fracture

secondary to the loss of the bone support. This loss of bone can be very dramatic with significant consequences to the patients where they can become worse off from where they started as further reconstruction or restoration will be more complicated with compromised results, if at all possible (10, 15, 21-23).

1.1.2.6 Aetiology of peri-implantitis

The current consensus for the aetiology of peri-implantitis is that it is plaque associated implying a bacterial origin (19).

Some researchers believe that peri-implantitis may start as a foreign body reaction, which then develops into an infection with secondary bacterial invasion (24), as opposed to having a primary bacterial aetiology. There is, however, a consensus agreement that a history of periodontitis makes the individual more susceptible to peri-implantitis (19). There may be a complex interaction between the materials used, the bacterial biofilm and the patient's immune response, which may be over-reacting to the stimulus.

Other suggested causes of peri-implantitis include the presence of peri implant cement adjacent to the implant below the gum level which results in an inflammatory reaction to the cement material with subsequent bone loss. This would occur following restoration of the implant crown using any of the various dental cements to secure the crown on the implant abutment. Many clinicians use screw retained crowns in order to avoid cement, partly for this reason but also as the screw retained crown can make crown retrieval, if required, easier than with a cemented crown.

Limited evidence has also been linked peri-implantitis to poorly positioned implants that hinder oral hygiene measures. The evidence for occlusal overload and peri-implantitis is inconclusive. Although he evidence for the link to bio corrosion and particle release as a cause of peri-implantitis is considered as yet to be determined (19), there is some evidence to suggest that particles emanating from implants are deposited into the surrounding tissues, which may play a role in implant failure because of the tissue reaction (25, 26). This could be significant as one must consider the implant material and its constituent metals such as the presence of vanadium in titanium alloy (Ti6Al4V), termed Grade 5 titanium alloy. The Grade 5 alloy has been shown to cause a tissue inflammatory reaction in contrast to the

grade 4 commercially pure (CP) Ti, or the Roxolid® alloy of Ti (85%) and Zr (15%) (Fig. 14) (27).

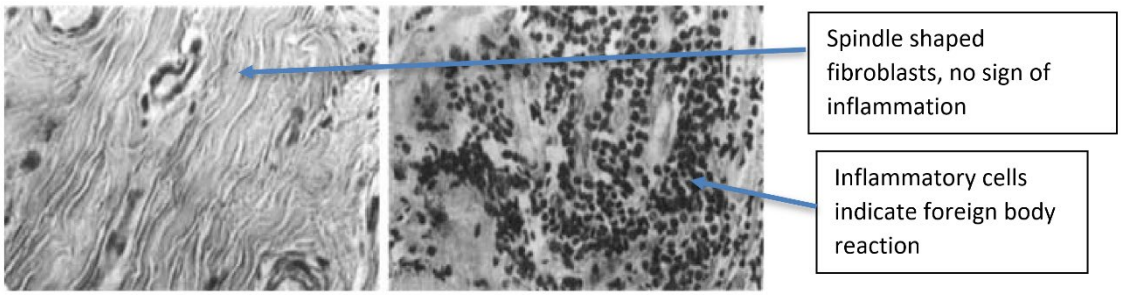

Figure 14. Micrograph of tissue in contact with implants made of commercially pure titanium (left) and Ti6Al4V alloy (right). Scale not available. Adapted from Steinemann 1998 (27).

Studies have demonstrated that fibroblasts proliferate successfully on Ti, Zr, Ta, Nb, but not when in contact with Cu, Mo and V. There was complete inhibition of osteoblast growth when cultured on pure discs of V, Ag, Zn and Fe and partial inhibition with Sn and Al. There was no inhibition on contact with Ti and Zr (27).

Although the consensus opinion for the aetiology of peri-implantitis is of a bacterial origin (19), it is important to consider other theories and the emerging evidence. Hence the importance of this research to investigate the potential role of particle release in peri-implantitis and the influence of the various materials currently used in implant fixtures.

The various theories put forward above have been summarised in Fig. 15 (17).

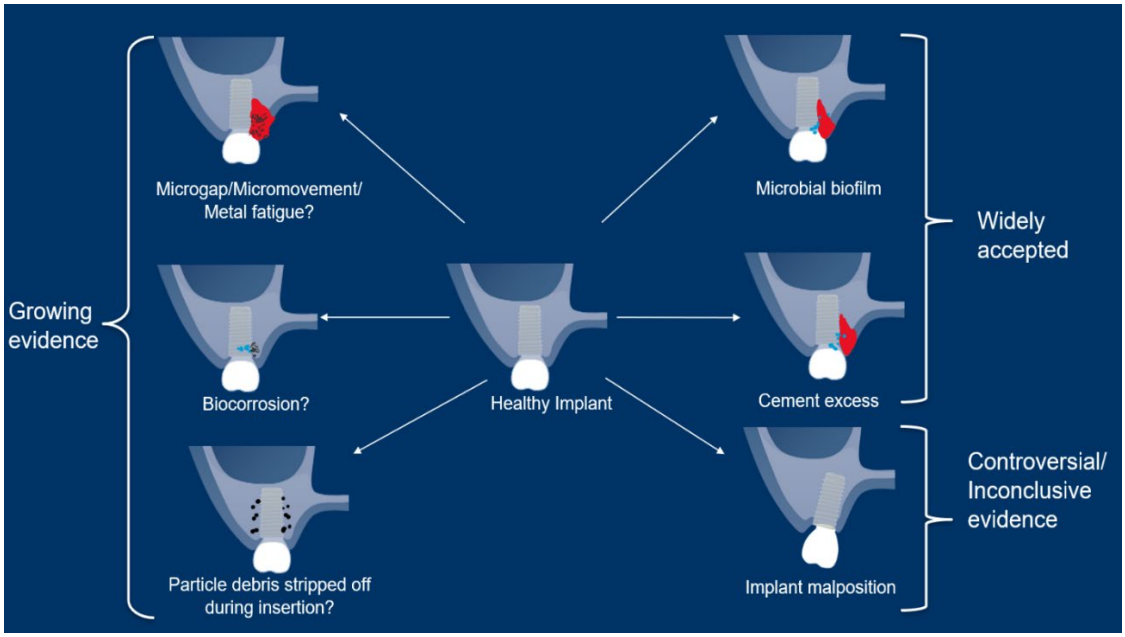

Figure 15. . Diagram summarising the theories for the aetiology of peri-implantitis. Adapted from Fretwurst et al. 2017 (17).

Although implants are considered to have very high survival rates, 94% with 10 years or longer follow up periods (28), increasing numbers being placed globally is causing a growing awareness of their limitations: the lack of connective tissue attachment in the biologic width and peri-implantitis.

There is no agreed or effective protocol for the treatment of peri-implantitis which, once established, has a high rate of recurrence (16). Derks and Tomasi's (15) epidemiologic review revealed that 1 in 5 implants placed develop peri-implantitis after 3.4 to 11 years in functional loading. However, the definition of peri-implantitis has historically been inconsistent; this was due to disagreement on the threshold bone loss before a diagnosis of peri-implantitis can be made (varied from 0.5 mm to 4 mm of bone loss between different groups). More recently a consensus was reached for the diagnosis of peri-implantitis (19). Future studies on peri-implantitis will hopefully be more standardised with the agreed diagnostic criteria, even though the aetiology is still debated.

1.1.3 Titanium properties

Titanium is found naturally as an oxide in either the stable Rutile phase, or a metastable phase such as Anatase or Brookite. Irreversible phase transformation from Anatase to Rutile takes place at 600°C as a result of reconstructive breaking and reforming of bonds, which results in 8% volume contraction and hence higher density of Rutile phase. Ti is a transition metal with a hexagonal closed packed (HCP) crystal structure (alpha phase) up to 882.5°C (the transus temp) beyond which it is transformed to the body centered cubic (BCC) (beta phase). Titanium's melting point is 1678°C. Due to its incomplete d-shell Ti is able to form solid solutions with certain elements such as Zr (15).

In implant dentistry titanium is used as an alloy, or in its commercially pure grade 4 form containing traces of Fe, C, O, N, which provide more yield strength compared to lower grades, without changing the Young's modulus which is dependent on the chemical bonds rather than the microstructure. Grade 4 commercially pure titanium (Ti-CP4) mainly consists of the alpha phase with spontaneous TiO_2 layer formation, rendering it biocompatible.

The most commonly used Ti alloys are the Ti6Al4V (Grade 5) and Ti15Zr (Roxolid® Straumann Group). Ti6Al4V has dual alpha and beta phases, with Al as an alpha phase stabiliser and V as a beta phase stabiliser, which improves the strength and physical properties. Ti6Al4V has higher strength with a lower coefficient of elastic mismatch with bone, however, it is more prone to corrosion due to the reduced surface oxide layer (15, 17, 29).

Zr is a transition metal with phase transition temperature similar to Ti. Ti15Zr has a binary alpha phase with smaller grain size, thereby increasing the surface area for layer of oxides, which enhances the material's corrosion resistance when compared to Ti6Al4V, but has reduced strength (30). Table 1 summarises the properties of the currently commonly used materials in dental implants.

Table 1 - 1. List of dental implant material properties (31-33)

Material	Modulus of elasticity (GPa)	Tensile strength (MPa)	Yield strength (MPa)	Reference
Ti-CP4	104	550	483	(31-33)
Ti6Al4V	113	930	860	(31)
Roxolid	98	850	849	(32, 33)
Zirconia (Y-TZP)	200	>1200	900	(32, 33)

Some metals are susceptible to corrosion, following interaction with tissue fluids resulting in hydrolysis (decomposition of a substance by water). An electrochemical reaction which results in ion release can give rise to hydroxides, hydrous oxides and oxides, which can result in a foreign body reaction, giving rise to tissue loss. With CP titanium, an oxide layer (3-10 nm thickness) forms within seconds of being exposed to air or tissue fluid; this oxide layer may inhibit Ti dissolution (27).

Titanium particles have been shown to start an inflammatory response with the release of IL1β, IL6 and TNFα from macrophages. This inflammatory reaction is accompanied by increased bone loss through osteoclastic activity and reduced osteoblastic activity (34). The inflammatory response results in macrophage recruitment with their subsequent release of MMP and activation of the complement cascade. Particles can be released for various reasons, including friction during fixture insertion, wear between the implant and abutment interface, corrosion and following use of metal instruments on the implant surface for cleaning and debridement (35, 36).

1.1.4 The role of tissue response in peri-implantitis

Consensus opinion suggests a bacterial aetiology for peri-implantitis (19). However, there is a growing school of thought and research efforts to investigate the potential role of titanium and titanium alloy particles in the onset of the inflammatory process in peri-implantitis (37). The orthopaedic community has accepted the role of particle release in aseptic osteolysis around hip replacement implants (38-40). The mechanical and tissue dynamics of orthopaedic prosthesis are very different to the oral environment, nevertheless this is worth investigating as certain alloys and

surface properties may play a significant role. The bacterial involvement could be due to the fact that dental implants are transmucosal, emerging from the alveolar bone through a thin layer of weakly connected soft tissue and into the oral cavity with its rich bacterial flora and the various fluids that moderate the chemistry and pH of the local environment. The orthopaedic and veterinary surgeons encounter similar challenges when they are working with transcutaneous prosthesis for replacement of limbs. This issue of bacterial invasion is also of importance in other fields whenever a prothesis such as a canula or catheter needs to be transmucosal for long periods. Despite the clear bacterial involvement in peri-implantitis, there is a possible foreign body inflammatory reaction as the aetiological origin which then allows bacterial invasion as a secondary, or synergistic factor (41).

In order to understand the potential factors involved in the aetiology of peri-implantitis it would help to consider the tissue response to the fixtures from the time of surgery and implant placement, as this is where the initial tissue response and subsequent healing takes place, both of which may be influenced by the reaction to the materials used. The initial response to surgery and implant fixture placement involves the innate immune system whereby the trauma to the soft tissues and the alveolar bone from the surgical procedure causes damage to blood vessel and ECM. This results in an inflammatory reaction mediated by a cascade of events, the initial part of which is summarised in Fig. 16 (42).

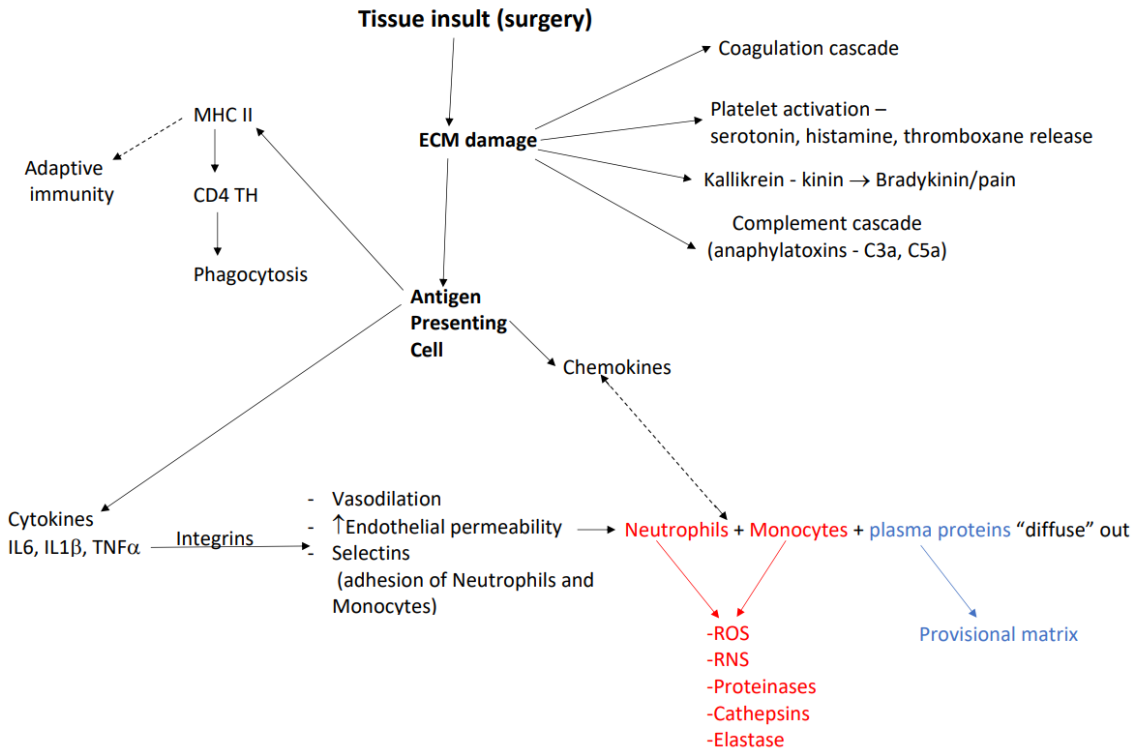

Figure 16. Flow diagram of the initial inflammatory response to implant placement. Design and illustration by Dr F Barrak.

At the time of surgery, the drilling process results in in the exposure and release of matrix proteins which activate the innate immunity cascades including the coagulation pathway, platelet activation, kallikrein - kinin pathway and the complement cascade (37, 42, 43). The latter results in enhanced inflammatory response through C3a, C5a which act as anaphylatoxins by activating histamine and serotonin release from mast cells resulting in vasodilatation, increased permeability, lymphocyte chemotaxis as well as opsonisation of contaminating pathogens during the surgery.

The damaged ECM components and bacterial Lipopolysaccharides (LPS) also activate the Antigen Presenting Cells (APC) locally through Toll-like receptors (TLR). The activated APC will release Cytokines (IL1β, IL6, TNFα) which will cause vasodilation and interact with the local endothelial cell integrins to bring about increased permeability and release of selectins to provide adhesion for circulating neutrophils and monocytes and enable their 'seeping' out into the tissues at the injury site. Once the cells are in the ECM chemokines released by the APC (CXCC8)

will 'guide' neutrophils and monocytes from the blood vessels to the APC at the injured location. The neutrophils and now macrophages will engulf foreign bodies, pathogens and damaged cells by endocytosis.
The APC will also activate the appropriate CD4 cells by presenting the phagocytosed antigens on the MHCII receptors which will result in phagocytosis as well as developing adaptive immunity. The latter can cause release of Reactive Oxygen Species (ROS), Reactive Nitrogen Species (RNS), proteinases and cathepsins.

The increased permeability of the local vessels also allows for proteins from plasma to 'seep' out into the tissues and aid in the formation of a new provisional matrix. Biomaterial surface properties can affect, or through design, guide the subsequent tissue response. The initial phase following the insertion of a device involves the attachment of proteins from the blood stream and local tissues to the substrate surface. The type of protein that is attracted to the surface will, to a large extent, depend on the surface properties of the biomaterial. The initial proteins may be displaced by others with a higher affinity to the surface, the Vorman effect. The surface proteins play an important role in the local cell interactions and can be used to guide this initial response to the implant (37).

The inflammatory process is a complex network involving cross reacting factors which can have positive and negative feedback loops affecting different parts of the network by amplification or limiting the responses. Although certain elements of the inflammatory response are identified and better understood, the overall process is far from being a linear cascade of events which can be systematically worked out and controlled in isolation and we still do not have the complete picture (42). This may partly explain the lack of agreement on the aetiology of peri-implantitis, the potential role of particles in the onset of the inflammatory process around dental implants and the potential influence of the biomaterials we are currently using in our daily clinical practice. Fig. 17 is an outline of some the events that may contribute to the emergence of peri-implantitis, including the potential role of Ti/ Ti alloy particles.

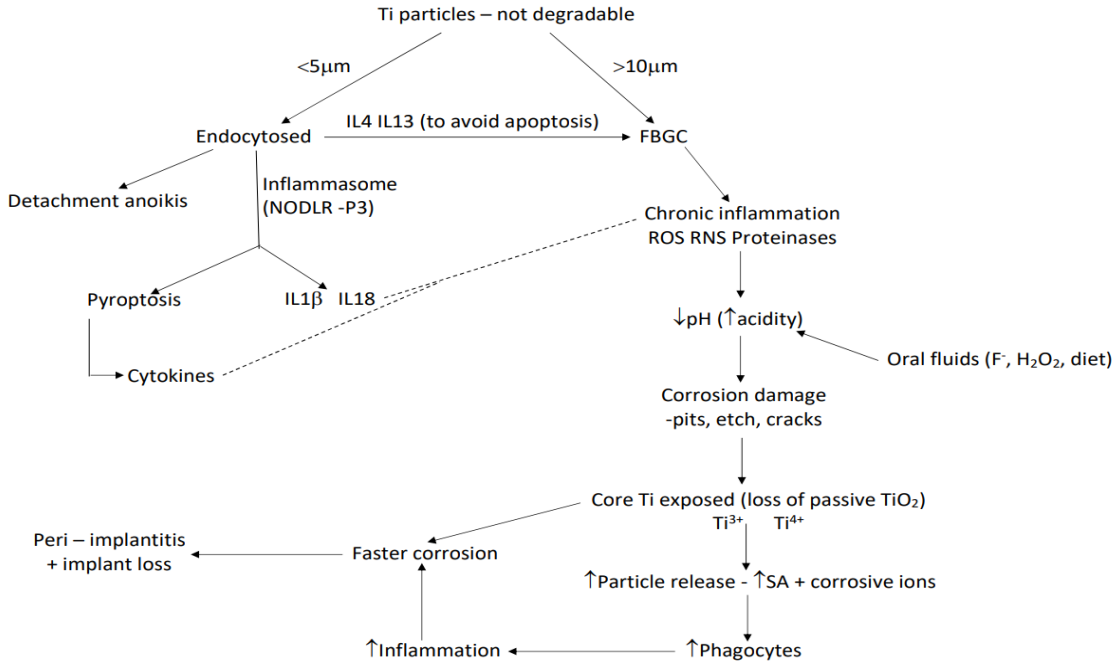

Figure 17. Flow diagram of sequence of events leading to corrosion and inflammation in tissues adjacent to implants. Design and illustration by F Barrak.

At the time of implant placement titanium particles have been shown to be released due to shear forces on the micro roughened implant surfaces (44). Particle release can also take place as a result of wear at the implant – abutment junction and due to corrosion. These particles are not degradable and those of less than 5 µm can be phagocytosed by macrophages in local tissues and on the implant surface. This may result in the disruption of cellular function and the detachment of the phagocyte from the matrix or substrate and the resultant anoikis (cell death as a result of detachment). The lysosome can rupture and release ROS, RNS and proteinases, activating the nucleotide oligomerisation domain like receptor-P3 (NODLR-P3) inflammasome formation and activation of Capsase 1 with the subsequent conversion of pro-IL1β and pro-IL18 to active IL1β & IL18 (Fig. 17). The inflammasome may also result in pyroptosis (inflammatory cell death) which will also result in the release of cellular components including cytokines. The cytokines thus released from the affected cell will result in enhancing the inflammatory response (37, 40, 45).

Phagocytosed particles larger than 10 µm can result in apoptosis, or the cell may avoid apoptosis and instead through the release of IL4 and IL13 promote the fusion

of the cells and formation of foreign body giant cells (FBGC) resulting in chronic inflammation (37).

Particle endocytosis and inflammasome activation, or FBGC formation result in enhancing the inflammatory process and potential chronic inflammation which results in ROS, RNS, proteinase release. The chronic inflammation results in a reduced pH in the local environment, which is often exacerbated by oral fluids including fluoride, H_2O_2 and dietary acids. The chronic drop in pH can cause corrosive damage to titanium resulting in surface damage in the form of pitting, etching and cracks (46, 47). This process can, over time, result in the loss of the passive TiO_2 layer, which can be permanent, exposing the titanium core which become ionised (Ti^{3+}, Ti^{4+}) with reduced potential for osseointegration (47).

The loss of the passive layer can take place at pH 6, very close to the salivary resting pH of 6.3-7.0 which can drop to pH 3.5 for short periods in the presence of acidic drinks (46). Exposure of the core titanium to electrolytes results in further corrosion. Once crevices and cracks develop, the area of stagnant fluid becomes depleted of oxygen with a further drop in pH, resulting in a potential propagation of the corrosive process with pitting and delamination of the surface (47). Macrophages and FBGC (and bacteria) can adhere to the implant surface where they provide a 'protected' environment between the cell membrane and the implant surface for the release of ROS, enzymes and acids which in phagolysosomes can have as low as pH 4 (37).

The corrosion products influence the macrophages to release inflammatory cytokines. Also, the increased number of particles and surface area encourage further phagocytosis. The process of inflammation, corrosion, surface damage and further inflammation can become self-propagating and further amplified in the presence of physical stresses such as the micro motion of the implant and mobility at the micro gap of the implant-abutment junction. This can result in faster corrosion and eventual clinically detectable peri-implantitis, bone loss and implant loss (47).

1.1.5 Possible inflammatory pathway at cellular level

The activation of the inflammasome is thought to be as a result of the APC/ macrophage being primed first via the TLR activation by extracellular pathogen associated molecules (PAMP) such as LPS. Activated TLR will result in nuclear factor kappa B (NFkB) transcription with the resultant synthesis of pro-IL1β, pro-IL18 and TNFα ready for their release which requires caspase 1 activation. Internalisation of material (e.g. Ti particles) directly into the cytosol, or by endocytosis (which results in rupture and release of the lysosome contents) causes disruption and activates the NLR-P3 inflammasome complex assembly and subsequent activation of caspase 1 to convert pro-IL1β and pro-IL18 to their active forms to be released and enhance the inflammatory response. There is a possibility that priming of macrophages can take place by TNFα via cytokine receptors, in addition to the priming via TLR activation by PAMP (Fig.18) (40, 45, 48). An initial inflammatory response in a sterile environment with no bacterial components can still result in priming the cell for inflammasome activation. This is thought to be a possible mechanism for the sterile osteolysis seen in orthopaedic prosthesis loosening.

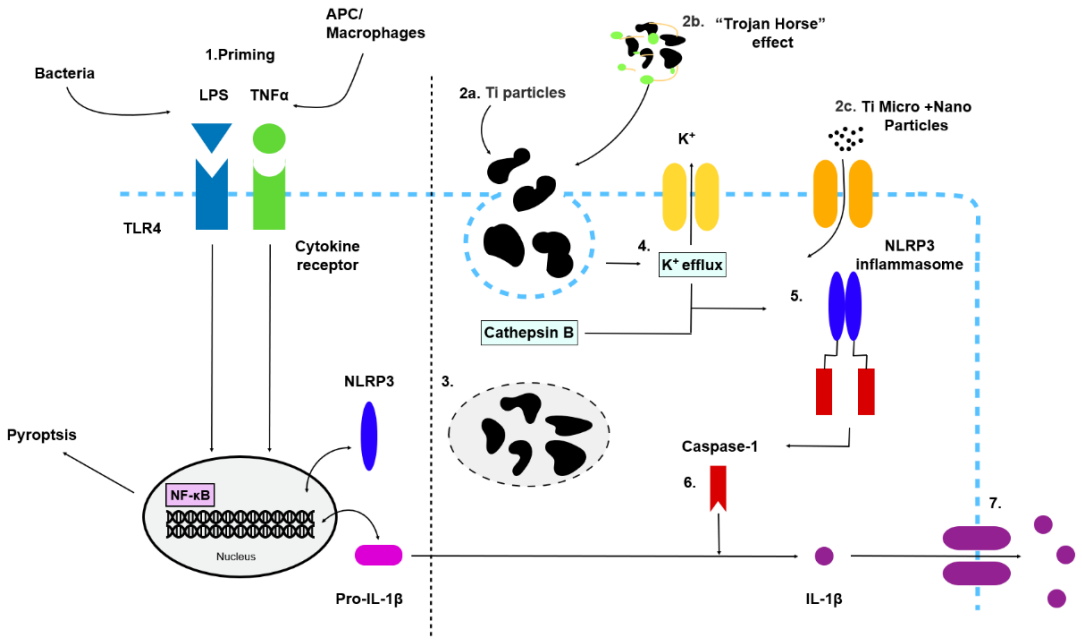

Figure 18. . Particle induced inflammasome activation pathway diagram. 1. Priming of the NFκB transcription via LPS/ TLR4 or TNFα/ cytokine receptor. 2. Particle internalisation pathways a. phagocytosis b. adhesion of proteins, calcium and other ions from ECM resulting in trojan horse effect c. particles passing into the cytoplasm via membrane receptors. 3. Leakage of cathepsin protease from the lysosome. 4. Outflow of K+. 5. K+ efflux is sensed by NLRP3 receptor leading to Inflammasome component assembly. 6. Caspase-1 is activated by NLRP3 inflammasome;

Caspase-1 converts pro IL1β to the active form. 7. IL1β release from membrane pores. Adapted from Jämsen et al. 2020 (48).

The importance of the grade of titanium used in the biomaterial becomes clear when we consider the chemical and physical influences on the surface. It becomes even more important to consider the potential toxicity of certain biomaterials when they are in particles especially if micro and nano sized. Implant companies carry out the standard testing of the materials using the entire fixture, with no current standards available for testing the implant material particles. Grade 5 titanium alloys contain vanadium and aluminium, which are toxic (38). This also raises the question of whether certain grades of Titanium and Ti alloys are more susceptible to corrosion and degradation, and therefore more likely to release particles with potential toxic influence in the tissues. Although some studies have suggested there is no difference between Ti-CP4 and Ti6Al4V (49), the *in vivo* conditions are very different to the experimental models where the many variables cannot be replicated accurately, such as the protein adhesions on the implant surface, the subsequent cellular response, the bacterial contamination in the presence of the occlusal loads and the implant- abutment micromovements, all of which can play a role in the conditions at the implant surface at various sites.

1.1.6 Biomaterial considerations for connective tissue attachment

Whether foreign body reaction or infection is the primary cause of peri-implantitis, having strong connective tissue attachments could be beneficial in disease limitation and maintenance of the soft tissue levels for function and aesthetics. Several attempts have been made to achieve connective tissue attachment for transcutaneous and transmucosal prostheses with the aim of reducing infection rates and their longevity.

Materials used for attempting soft tissue attachment include polymers such as polyurethanes, polyethylene terephthalate (PET), silicones and polymethyl methacrylate (PMMA). However, these polymers are too weak and have unwanted tissue reactions. Several metals have been used for tissue integration. Metals have free electrons, unlike in living tissue. Gold and silver are resistant to corrosion in air,

but not in body fluids. Stainless steel and cobalt-based alloys also become polarised and undergo redox reactions in living tissues. Other metals such as iron and aluminium are more susceptible to corrosion whereas nickel, vanadium, molybdenum and copper can be toxic to cells (27).
Ti has the right combination of the desired properties of strength, being inert and highly resistant to corrosion in living tissue as a result of the oxide layer. This process applies to Ti and Zr in that the oxygen atoms move into the metal oxide layer and the metal ions do not migrate to the surface, unlike other metals e.g. gold and stainless steel which is why they corrode. Water molecules are attracted to the oxide surface, and a hydroxyl group is formed, either as a lone group or as a bridging group. Therefore, the TiO_2 layer has amphoteric and zwitterion properties.

TiO_2 properties allow it to interact with various functional groups in the extracellular matrix, such as hydroxyl (OH), carbonyl (CO), carboxyl (COOH), amine (NH), sulfonate (SO_3H), and have physiological effects. Understanding these interactions may be the key to achieving the desired strong connective tissue attachment, e.g. interactions with ECM molecules such as osteopontin, bone sialoprotein, Dermatan and Fibronectin. The latter is a key ECM protein that enables attachment of cells and substrate via the functional groups on its arginine-glycine-aspartate (RGD) sequence. This sequence interacts with integrins on cells and influences cellular activity.

The question is whether Ti, as the currently preferred metal substrate, can promote connective tissue attachment. Although Ti remains inert in tissues, it can cause allergic reactions (0.6% prevalence), and undergo corrosion and increased oxidation in acidic conditions of bacterial biofilm (27). The oxide layer may not be favourable for soft tissue attachment. CP titanium is graded 1-4 based on the following properties - strength, corrosion resistance, ductility with Grade 1 being the most resistant to corrosion and most ductile while Grade 4 is the least resistant to corrosion but the strongest and least ductile. Grade 4 is used in dental and orthopaedic implants due its higher strength compared to the other lower grades.

The most commonly used Ti alloy commercially is Ti6Al4V, also known as Grade 5 Ti alloy with 6% Al, 4% V, 90% Ti. This has higher strength than the Ti-CP4 , but has

poor wear resistance, and is more expensive. The poor wear resistance is very important in long term prostheses, as it can result in the release of particles in the tissues which can have toxic effects, such as vanadium particles in a Grade 5 implant. Even at 4% vanadium can cause an inflammatory response with a foreign body reaction (Fig. 14) (27). Studies have shown that epithelial cells spread and attach more favourably on Titanium Grade 4 and 5 alloy compared to porcelain and aluminium oxide based on scanning electron microscopy (SEM) and immunofluorescence results; but gingival fibroblast attachment was unfavourable on Grade 5 alloy (Ti6Al4V) compared with grade Ti-CP4, as evidenced with rounded cell shapes indicating lack of attachment (50). This may possibly be due to the toxicity of Al or V.

For the purposes of this study, the main interest is in the positive interaction of the epithelial cells and fibroblasts to the substrate with regards to their proliferation and attachment. As far as metal substrates are concerned, the evidence supports Ti-CP4 over other metals and Ti alloys.

Other materials to consider for transmucosal prostheses include ceramics such as calcium phosphate, aluminium oxide or zirconium oxide, which can be applied as a plasma spray or used as bulk material and are discussed next.

Hydroxyapatite (HA), which is calcium phosphate ($Ca_{10}(PO_4)_6(OH)_2$), has excellent biocompatibility having a similar composition as mineralised bone. However, its brittle mechanical properties with long-term degradation and crack propagation, have resulted in increased complication rates as the HA coatings can shear off during placement or function. There are conflicting reports regarding the effect of HA on soft tissue attachment (7). Different morphologies seem to affect the soft tissue attachment (7). When porous and solid HA, as well as concave and round surfaces were compared to the solid HA showed better attachment and the concave surface showed more stable attachment of soft tissues. CP Ti has been shown to have superior attachment to HA.

Aluminium oxide has been shown to have good bone and soft tissue integration, both at epithelial and connective tissue level with hemidesmosomal attachments

(structures that enable cells to attach to underlying surface such as the basal lamina, ECM) and the presence of BMZ (51). However, failure rates of aluminium oxide implants were high (survival of 65-92%) possibly due to its brittleness, low tensile strength and long-term aging. Hemidesmosomal attachments may not be sufficient for the barrier effect which is achieved with the connective tissue attachment to the cementum of teeth.

Zirconia, yttrium-stabilized tetragonal zirconia polycrystals (Y-TZP), is stronger than aluminium oxide and is thought to have reduced tendency to collect plaque which is another desirable property for dental implants. However, in the presence of water it undergoes ‘low temperature degradation’ from tetragonal to monoclinic phase, which is brittle. The preparation of zirconia is very technique sensitive (52). Zirconia implants are more brittle with less flexion in response to load. Failure rates have been reported to be higher than Ti, however, more studies are required for conclusive results on the long-term survival and success of Zirconia implants compared to Ti. One of the weak points of the Zirconia implants are the threads where high stresses can result in fractures, which can be in response to high torque forces during the implant insertion. Zirconia in thin sections, as is desired on a thread, is weak. Two-piece Zirconia implants have an even higher risk of fracturing due to the internal abutment threads. The evidence for soft tissue integration to Zirconia or Ti and Zr alloys is lacking with some conflicting results.

1.1.7 Attempts at connective tissue attachment

1.1.7.1 Design

Various design modifications have been used to promote soft tissue attachment; these include surface treatments, collars (or flanges), grooves, porous structures,

surface roughness manipulation, as well as surface energy and wettability. Current surface treatments attempted include:

- Mechanical e.g. blasting with Ti, Zr, Al;
- Chemical e.g. acid etching, anodisation;
- Physical e.g. sputtering, ion deposition.

Although these rough surfaces increase surface area for bone integration, they do not necessarily enhance soft tissue attachment. The summary of current evidence is (7):

- Epithelial cells attach and proliferate better on smooth surfaces;
- Fibroblast attachment and proliferation is better on rough surfaces;
- There was no difference in connective tissue response to different rough material between Ti, Zr and TiZr alloys;
- Research on the nanoscale roughness is inconclusive so far for soft tissue attachment, although there is evidence for its effectiveness in osseointegration, potentially through affecting direct cell-substrate interactions e.g. via RGD motif.

1.1.7.2 Collars and flanges

Studies have looked at increasing the surface area for soft tissue attachment by adding a mesh collar, nylon hooks, steel springs and solid flanges Fig. 19 (53). These have shown better soft tissue ‘integration’; however they still do not offer strong connections due to the direction of the soft tissue attachment (collagen fibril alignment) not being perpendicular to the implant core. The surgical technique for positioning of the implant becomes very sensitive to ensure that the collar is at the correct height in relation to the appropriate aspect of the skin or mucosa.

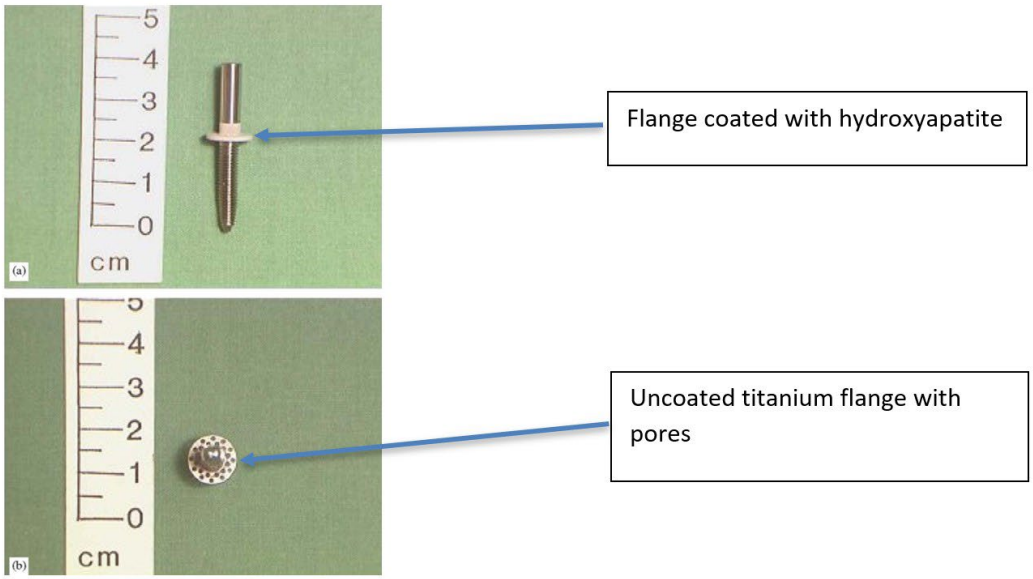

Figure 19. Images depicting transcutaneous implants with pores in flanges (a) coated with hydroxyapatite coating; and (b) plain titanium. Adapted from Pendegrass, C.J. (53).

1.1.7.3 Grooves

Groove orientation, depth and spacing can affect the position and growth of epithelial cells and fibroblast response. Contact guidance is a term to describe the ability of controlling epithelial cell and fibroblast orientation by altering the groove characteristics on an implant (54). Studies have shown that epithelial cell and fibroblast attachment and orientation can be influenced by the titanium surface microgroove morphology (53, 55, 56).

Contact guidance can be seen in studies by Nevins et al. (57) with the histological images (Fig. 20) demonstrating the orientation of the collagen fibres being ‘guided’ towards the implant surface with the grooves created by the Laser Lock technology. However, orientation does not mean attachment, and so far, the latter has not been demonstrated.

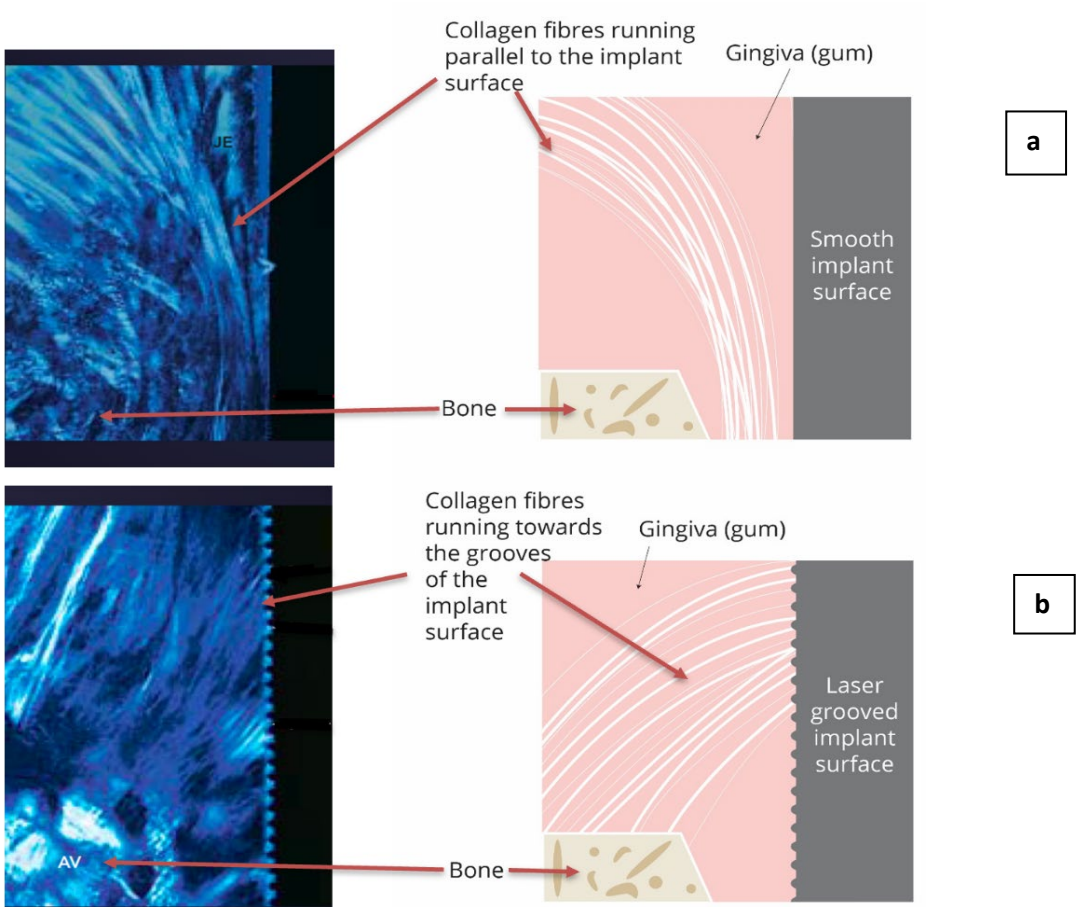

Figure 20. Histology and illustrations depicting connective tissue in contact with BioHorizons® (grade 5 Ti alloy) implant surfaces, (a) smooth surface depicting parallel orientation of collagen fibres to the implant surface and (b) laser grooved surface depicting collagen orientation towards the implant surface. Adapted from Nevins et al. (57) Illustrations designed by Dr F Barrak and drawn by Kitty Lungley.

1.1.7.4 Porosity

Different cell types will need different porosity for the growth and establishment of blood supply for tissue metabolism. Poly(hydroxyethyl methacrylate) has been used as a negative control as its surface does not allow protein attachment and so the results will reflect the pore size effect as opposed to the surface reaction. Results from studies have shown that (7): although the effects of porosity are not fully understood, a pore size of 40 μm and throat size of 16 μm allowed incorporation of soft tissues predictably irrespective of surface characteristics; pore sizes of > 100 μm demonstrated higher rates of epithelial downgrowth and implant exposure (58).

1.1.7.5 Surface energy and hydrophilicity

Fibroblasts have a positive response to increased hydrophilicity with the highest attachment found at 20-40° water contact angle. Epithelial cells did not respond to wettability, but more to nanotopography of intermediate surface energy compared to the unmodified Ti (with lowest energy) and nanotubular Ti (with the highest energy).

1.1.7.6 Biomolecular coating

Biomolecular coating e.g. peptides, and seeding of mesenchymal stem cells are further techniques being studied for the achievement of connective tissue attachment. The aims of biomolecular coating include:

1. Reduction of non-specific protein attachment and cell adhesion;
2. Enhancement of selective tissue cell attachment;
3. Integrin mediated cell stimulation for healing.

Fibroblasts and epithelial cells have ECM surrounding them with different constituents (e.g. epithelial cells are in contact with laminin) and therefore we can expect them to respond to different proteins. Components of ECM include collagen type I, collagen type IV, laminin, fibronectin, vitronectin. Collagen type I and IV have been shown to improve epithelial attachment (59). Laminin 332 has also been shown to improve epithelial cell attachment and reduce epithelial down growth. Fibronectin, on the other hand, promotes fibroblast attachment and has binding sites for fibrin, thrombin, heparin sulphate and integrins (arginine, glycine, aspartic acid – RGD sequence) which play a role in cellular interaction with the ECM.

Other biomolecular factors to be considered include: platelet derived growth factor (PDGF) which speeds up healing in response to injury physiologically, and as demonstrated in experiments around implants (60); E-Cadherins, which are calcium dependent trans-epithelial adhesion proteins have also been considered for biomolecular treatments (61); and vitamins D and E, which have beneficial effect on fibroblasts *in vitro* (62).

Various methods have been used for coating of medical devices such as implant fixtures and bone substitutes to improve bone healing via targeted cell activation. These include the use of growth factors such as BMP 2, 7 and various peptides with the aim of targeting specific cells and specific functions within them (63). The use of growth factors has had much attention with conflicting results in relation to their effectiveness for the stimulation of bone growth predictably (64). The application of growth factor proteins onto substrate surfaces is via adsorption methods and has disadvantages. The large protein molecules are susceptible to folding and structural changes in response to small environmental fluctuations such as pH and temperature. These can alter the protein's activity by altering the active sites. Also, the adsorption of the protein molecules involves weak forces which results in dissipation of the growth factors with reduced concentrations in the intended target site as well as ectopic bone growth, hence the need for the higher concentrations used in medical devices to compensate for the loss of activity from some of the factors.

In contrast to the use of growth factor proteins which are adsorbed, the application of small peptide chains to medical device surfaces can be attained via covalent bonding which allows a more stable, controlled and targeted approach, both in terms of dosage, site of activity and cellular responses (63). Hence, with small chain peptides lower doses are required and they are also less likely to have undesirable immunogenic effects compared to proteins, while still maintaining their intended targeted cellular action. Reyes et al. 2017 (65) showed the effects of using biomolecular treatment using selective amino acid sequences for stimulating osteoblasts via α2 β1 integrins and Runx2/Cbf1a expression, as demonstrated in Fig. 21 where the bone to implant contact as well as the pull-out forces were much higher with the implants coated with amino acid sequence (GFOGER) compared to the implants coated with collagen, which in turn were higher than the uncoated implants.

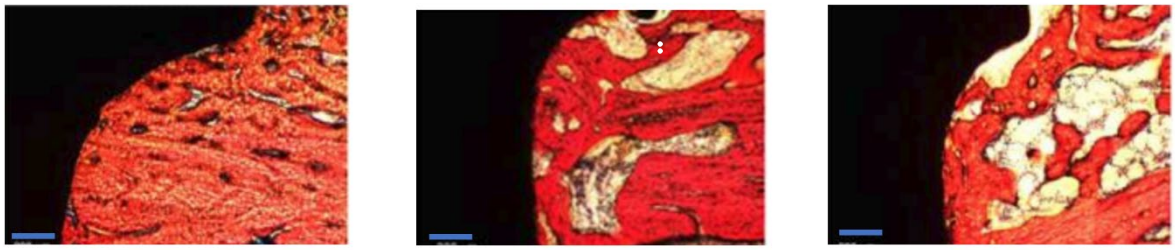

Figure 21. Histology images depicting the difference in bone to implant contact with different coatings - left: GFOGER peptide coating, middle: collagen I and right: no coating. The blue bar depicts scale of 200 µm. Adapted from Reyes, C.D. 2017 (65).

The choice of the peptide chain can influence the cellular interaction for the desired effect. Several peptides have been identified to have different cellular influences. For example RGD, is an amino acid sequence found on fibronectin, vitronectin and collagen, which interacts with integrins on various cells in a non-specific way to enhance adhesion. Other peptide sequences are believed to be important in cell adhesion and spreading such as KRSR and FHRRIKA (Phe-His-Arg-Arg-Ile-Lys-Ala) which was shown to be effective on silanised titanium surfaces (63).

Various scaffolds have been considered for application of small peptide chains. The use of peptides incorporated in hydrogel scaffolds may provide a structure to allow physical and chemical fixation of collagen fibers. Although hydrogels have lower mechanical strength than bone, the transmucosal application would not involve the loading forces encountered in the intra-osseous aspect of the implant. However, a disadvantage of hydrogels is the rate of degradation which would be of significant importance for the desired long-term physical connective tissue attachment.

Other materials considered for the application of small chain peptides include bioactive glasses, as well as direct application to titanium implant surfaces. This methodology has been focused on bone healing mainly, however, there may be applications for controlling connective tissue attachment.

1.1.7.7 Summary of biomaterial properties required for achieving connective tissue attachment

Based on the above literature analysis and the natural tooth anatomy the suggested criteria for an ‘ideal substrate’ may have the following characteristics:

- Porosity with pore size of 40 μm and throat size of 16 μm;
- Surface energy with a of 20-40° water contact angle;
- Roughness - micro or possibly at nano level;
- Specific affinity for integrins and cell selectivity for fibroblasts possibly through biomolecular surface treatments;
- Bioactive surface;
- Anti-microbial properties;
- Ability to calcify to ‘lock’ collagen attachments - mimicking the tooth cementum/ PDL relationship (Fig. 22).

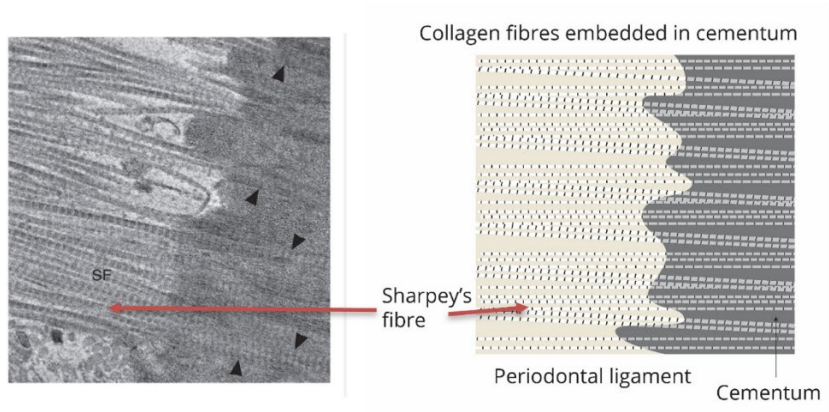

Figure 22. . SEM image and illustration showing the collagen fibres inserted in the calcified cementum matrix. Adapted from Beertsen, W. 1997 (66). Illustration designed by F Barrak and drawn by Kitty Lungley.
(66).

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