Osseointegration: biological events in relation to characteristics of the implant surface

ABSTRACT
Osseointegration of titanium implants is a complex biological process involving interactions between immuno-inflammatory responses, angiogenesis and osteogenesis, all of which are influenced by the physical and chemical characteristics of the implant surface. An implant surface with moderately rough topography and high surface energy influences cellular activities, enhancing peri-implant bone wound healing. Primary mechanical stability of the implant is essential for osseointegration. In this article we review some of the more important biological events of peri-implant bone wound healing in the process of osseointegration, and discuss how the biophysical properties of implant surfaces influence cellular responses.

Key words: peri-implant bone wound healing, stability of implants, titanium osteopromotive properties

INTRODUCTION
Restorations borne on osseointegrated implants can predictably and successfully replace missing teeth. Their long-term success depends, among other factors, on the extent and on the integrity of the interface between the implant and the surrounding bone. Osseointegration can be defined as a healing process, the outcome of which is the establishment and maintenance of a clinically asymptomatic rigid fixation of an alloplastic material in bone under functional loading. Healing of bone which ultimately brings about osseointegration after implant placement, is a complex process involving several biological mechanisms including an early immuno-inflammatory response, angiogenesis and osteogenesis.

There are two types of bone, woven and lamellar. Whilst the early stages of bone healing results in woven bone, this is subsequently remodelled so it is lamellar bone that finally supports the implant. Lamellar bone exists in two macroscopically distinguishable forms: trabecular (cancellous) and cortical (compact). Cortical bone can provide greater mechanical support to an implant and can better withstand loading forces than trabecular bone. Their proportions in the jaws vary greatly, but there is generally more trabecular bone, except in the anterior mandible where there is more cortical than trabecular bone.

As judged by mechanical properties, there may not be optimal primary stability of an implant inserted into bone of low trabecular density, rich in bone marrow, as typically found in the posterior maxilla. There is evidence that the long-term rate of success of implants placed in such bone is lower than that of implants placed in bone of higher trabecular density. In the jargon of dental implantology, bone of low trabecular density is therefore often termed ‘poor quality’ bone. However, despite the fact that implants in cancellous bone may not have optimal mechanical stability, cancellous bone regenerates more rapidly than cortical bone, and therefore contributes substantially to
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the earlier phase of osseointegration. Osseointegration is brought about mainly by appositional deposition of newly formed bone by the osteoblasts residing in the remodelling wall of the bone osteotomy site. New bone is laid down on the bone surface facing the implant, a process termed distant osteogenesis, and this bone will ultimately surround and come into contact with the surface of the implant. On the other hand osseointegration of an implant placed in predominantly trabecular bone is brought about both by distant osteogenesis from the walls of the osteotomy site, and by direct contact osteogenesis on the implant surface, mediated by differentiating osteogenic cells recruited from the inter-trabecular, highly vascularised, bone marrow that is rich in mesenchymal progenitor cells. These cells differentiate into the osteoblasts, endothelial cells and osteoclasts required for bone formation, for neo-angiogenesis and for bone remodelling, all of which processes are essential for successful osseointegration.

Bone formation by contact osteogenesis occurs more rapidly than by distant osteogenesis, and the continuous recruitment of differentiating osteogenic cells to the implant surface with de novo bone formation establishes initial bone healing around implants. Moderately rough implant surfaces provide innumerable microniches for contact osteogenesis, resulting in an increased surface for bone-to-implant contact. This produces a superior anchorage of the implant in bone, compared with the anchorage achieved with smooth-surfaced implants.

Apart from the degree of surface roughness, the biophysical properties of titanium implants, including surface chemistry, topography and energy/wettability, influence cellular responses such as cell adhesion, proliferation, differentiation and migration, thus affecting the biological events of peri-implant bone wound healing.

**PRIMARY MECHANICAL STABILITY AND OSSEOINTEGRATION**

It is essential for early bone wound healing that rigid fixation is achieved immediately after insertion of an implant, with only minimal micro-movement allowed. Primary mechanical stability is achieved by screwing the implant into the osteotomy site with adequately tight implant-to-bone contact. Increased density of bone mineral and a wider diameter implant, which has a greater surface area, enhance primary stability. Poor primary stability with excessive micro-movement leads to the formation of fibrous tissue around the implant, with failure to osseointegrate. Very small micro-movements at the implant-bone interface optimally promote bone progenitor cell differentiation and bone formation; while greater micro-movements compromise osseointegration.

The mechanical interlocking between the implant and the bone creates lateral compressive forces on the bone which, together with the surgical trauma from the preparation of the osteotomy site, cause superficial tissue damage, releasing inflammatory mediators resulting in a very narrow zone of bone resorption. This temporarily decreases the initial mechanical intimacy of the implant-to-bone contact, tending to cause some loosening of the implant. However, that reaction is counteracted by woven bone formed by contact osteogenesis on the surface of the implant which also serves as an osteoconductive scaffold for the attachment and migration of newly recruited differentiating osteogenic cells from the bone marrow. The initial woven bone subsequently undergoes remodelling and is replaced by lamellar bone that ultimately provides secondary stability of the implant by osseointegration.

Although the bone-to-implant contact as a proportion of the total implant surface (bone-to-implant contact ratio) is one determinant of long-term implant stability, other factors play a role in the mechanical fixation of the implant and in its capacity to withstand occlusal forces. In fact the mineral density and the degree of maturation of the bone are more important to the stability of the implant than is the bone-to-implant contact ratio. Once osseointegration has occurred, compressive occlusal forces within the physiologic range promote osteogenic differentiation and an increase in bone density, while tensile forces and particularly shear forces are detrimental to osseointegration.

**THE OSTEOПROMOTIVE PROPERTIES OF TITANIUM**

It is evident that titanium implants have osteopromotive properties. Animal studies have shown that the expression of several extracellular genes related to bone matrix and to bone-resorption are more pronounced during the healing of an osteotomy site with an implant in situ than during healing of an osteotomy site without an implant. This suggests that titanium promotes new bone formation. Moderately rough titanium implant surfaces promote a greater degree of upregulation of genes related to bone healing than is seen with smooth titanium implant surfaces, leading to faster and more extensive osseointegration. It is possible that a moderately rough implant surface comprises innumerable bioactive niches which favour protein adsorption and release of biological signalling factors, leading to recruitment of differentiating endothelial and osteogenic cells to the implant surface and promoting osseointegration.

Hence, modification of implant surfaces by physical (surface energy, surface charge), chemical (inorganic or organic) and/or morphological (micro- and nano-topography) means may enhance osseointegration. Activation of implant surfaces with bone morphogenic proteins, or with platelet derived growth factor will further promote osseointegration.

**BIOLOGICAL EVENTS ASSOCIATED WITH PERI-IMPLANT BONE WOUND HEALING**

Early bone healing after implant insertion into a prepared site in the bone comprises several coordinated and sequentially overlapping biological events. Following clot formation in the micro-voids around the implant there is an initial immunoinflammatory response followed by neovascularisation and the formation of a transient fibrin-based structural matrix that serves as an osteoconductive medium. Recruitment occurs to the implant surface of mesenchymal progenitor cells from the bone marrow, and possibly also pluripotent pericytes.
Titanium implants have a stable titanium dioxide surface layer (TiO2), which in the peri-implant micro-environment it undergoes electrochemical changes, resulting in its thickening. This influences the interactions in the implant-micro-environment. When an implant is inserted, proteins from the blood and from the tissue fluids are immediately adsorbed onto the implant surface forming a ‘conditioning film’. Although cells in the peri-implant micro-environment interact with this film rather than with the surface of the implant itself, the characteristics of the titanium surface with regard to its roughness, porosity, and the thickness of the titanium oxide layer determine the molecular profile of the adsorbed protein layer. These factors influence the interaction of the cells in the micro-environment with the protein conditioning film and determine the nature of bone mineral precipitation on the implant surface.

Once in contact with the adsorbed protein layer, the differentiating osteogenic cells initially secrete non-collagenous bone proteins such as osteopontin and bone sialoprotein which provide a medium for osteoblast attachment and for calcium phosphate nucleation with subsequent crystal growth. Adjacent to this collagen-free mineralized matrix on the implant surface, the mature osteoblasts form a collagogenous organic matrix that subsequently undergoes ossification. It is suggested that the bone grows into the irregularities of the micro-rough surface of the titanium implant, establishing a mechanical bond between bone and implant.

Occlusal forces generate stresses at the implant-bone interface, stimulating physiological peri-implant bone remodelling in response to the mechanical loading. The remodelling is characterised by osteoclastic bone resorption and subsequent formation of new osteons which are arranged parallel to one another and perpendicular to the long axis of the implant, extending one micrometre from its surface.

The role of platelets in peri-implant bone wound healing
Platelets play an essential role in the early stages of wound healing, producing and secreting a number of growth factors including platelet derived growth factor, transforming growth factors α1 and α2, insulin like growth factor and vascular endothelial growth factor. Other biological mediators include coagulative and vasoactive agents which promote tissue healing and bone regeneration. Although platelets do not possess intrinsic osteoinductive properties, they stimulate proliferation of undifferentiated mesenchymal cells, and enhance both the differentiation of osteogenic cells, and angiogenesis, thus promoting bone regeneration. In the context of dental implants, platelet-derived biological factors thus enhance peri-implant bone wound healing.

Peri-implant bone wound healing: The initial immune-inflammatory response
Within 24 hours of implant insertion, neutrophils can be observed in the peri-implant wound. Within two to four days, macrophages and monocytes appear in the peri-implant bone wound, their function being the removal of biological debris, and production of cytokines and growth factors that stimulate cell proliferation, angiogenesis and collagen synthesis. These actions of macrophages and monocytes are supported by CD4+ and CD8+ T cells which are present within the haematoma in the microvoids around newly inserted implants.

Peri-implant bone wound healing in the dog
Studies in dogs have shown that on the first day after implant insertion, the main feature of healing of a peri-implant osteotomy wound is the formation, stabilisation, and organisation of a blood clot. During the first 24 hours neovascularisation begins within the organised clot. By the fourth day, macrophages and monocytes have migrated into the healing wound, and there is a gradual replacement of the blood clot by fibrous connective tissue produced by differentiating mesenchymal cells recruited from the bone marrow which forms part of the wall of the osteotomy wound, and from pericytes around the developing blood vessels. In this vascularising maturing connective tissue, osteocalcin, a hydroxyapatite-binding protein is present, reflecting osteogenic cell differentiation that occurred between day one and day four.

By the seventh day after implant insertion, the process of contact osteogenesis has given rise to woven bone against the implant surface, and newly formed blood vessels sprouting from the walls of the osteotomy site have become incorporated into these trabeculae. The trabeculae formed by contact osteogenesis, and the trabeculae originating from the wall of the osteotomy wound (distant osteogenesis) will gradually merge. By the fourteenth day the maturation and the density of the woven bone, as well as the bone-to-implant contact ratio will have increased so that the implant is surrounded by newly formed woven bone. The density of the newly formed peri-implant bone increases during the four to six week period after implant insertion, with the woven bone being replaced by lamellar bone. After the eighth week, the ongoing process of bone remodelling begins.

Peri-implant bone wound healing in humans
The pattern of peri-implant bone wound healing in humans appears to be similar to that in dogs, but the process is slower. In humans, one week after implant insertion there is little bone formation in the peri-implant osteotomy wound and the newly formed osteoid is in contact with only 6% of the implant surface. After two weeks, early mineralisation of the osteoid is evident, and the bone to implant contact occupies about 13% of the implant surface. By the 28th day, newly formed bone which has bridged the space between the bone walls and the implant surface is evident, and the bone is in contact with about 40% of the implant surface. After six weeks 62% of the implant is in contact with mature bone.

Bone particles left by after the surgical preparation of the osteotomy, and by the insertion of the implant, remain in the peri-implant space and on the implant surface. Some of these residual bone particles enhance new bone formation, most probably by releasing osteoinductive factors such as...
bone morphogenetic proteins and also by serving as an osteoconductive scaffold. Ultimately some of these bone particles are incorporated into the newly formed mineralised bone. Therefore, these microscopic bone particles should not be flushed away during the procedure of implantation.

Osseointegration as a function of gene expression

The biological events of peri-implant wound healing described above are driven by an orderly sequence of gene expression that determines bone healing and remodelling. By the 4th day after implant insertion there is substantial expression of osteogenesis-and angiogenesis-related genes and a decrease in the expression of immuno-inflammatory genes. Between the 7th and the 14th day there is expression of neurogenesis-related genes of neural transduction pathways. This is by far more pronounced than is the expression of the osteogenesis/angiogenesis-related genes. In light of the recent reports that neural system related biological mediators, such as neuropeptide-Y, promote osteoblast differentiation, it is very likely that neurogenesis-related genes play a role in peri-implant bone wound healing. The alveolar process of bone originates from the neural crest cells, and many neurogenesis-related genes that are expressed during the embryonic development of craniofacial bones play a role in peri-implant bone wound healing. It is therefore possible that these genes also contribute to the differentiation of progenitor mesenchymal cells into osteogenic cell lines culminating in osseointegration.3

IMPLANT SURFACE CHARACTERISTICS AND OSSEOSTEONTEGRATION

Moderately rough titanium implant surfaces, and, in particular, chemically-modified moderately rough implant surfaces that exhibit high energy/wettability, are better than smooth implant surfaces in promoting osteogenesis; in increasing bone-to-implant contact ratio; and in increasing the bonding strength of the bone-to-implant interface. Such surfaces also upregulate genes associated with osteogenic cell differentiation and with bone matrix mineralisation. The nano-structural topography of moderately rough implant surfaces further promotes cell adhesion, cell proliferation, cell migration, and selective recruitment of osteogenic cells, all of which contribute to contact osteogenesis, and to the promotion of adsorption and retention of non-collagenous proteins, thus indirectly favouring cell interactions with the implant surface. Both the dimensions and the density of the nanostructure topography of the implant surface influence cellular activity, but the exact nanostructural topography which will bring about optimal osseointegration is yet unknown.

It is clear that the nano-topography of a titanium implant surface accelerates and enhances osseointegration. However, the beneficial effect of the micro-meter scale topography and wettability/ surface energy on early peri-implant bone wound healing is substantially greater than is the effect of nanometer topography.

SUMMARY

This paper does not purport to be a comprehensive review, but its aim is to describe the more significant biological events taking place during peri-implant bone wound healing which ultimately lead to successful osseointegration. Also to be borne in mind are general health, age, nutritional, hormonal and immune status, and medication as factors that might affect peri-implant bone wound healing and consequently osseointegration.

Declaration: No conflict of interest declared.

References


SADA Congress 2014
HIGHLIGHTS
Mark Hochman
Smile: Exposure of papillae

Dr Hochman and his colleagues explored a particular aspect of the Smile and he reported at the Congress on their findings.

“The smile has become the objective endpoint evaluation by which practitioners define aesthetic treatment outcomes. Aesthetics embraces and encompasses all specialties of Dentistry; therefore, proper diagnosis of a patient’s aesthetic smile line before any treatment is undertaken is critical for success.”

...“the importance of interdental papillae display during dynamic smiling should not be left undiagnosed since it represents a common and important aesthetic element that needs to be assessed during smile analysis of the patient.”

...“the aesthetic smile line before any treatment is undertaken is critical for success.”