REVIEW ARTICLE

Peri-implant Bone Healing: Its Basic Osteogenesis and Biomarkers

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ABSTRACT

The continuous sequence of bone healing phases starts off with osteoconduction to the implant surface, depending on the migration of osteogenic cells. Osteoneogenesis ensues resulting in a mineralised interfacial matrix and is followed by bone remodelling to the implant interface at discrete sites. Dental implant drilling procedure and placement produce osseous defect which is filled by blood. Within seconds, blood proteins are adsorbed onto the implant surface and platelets are activated resulting in the release of cytokines and growth factors. Further platelet aggregation initiates osteoconduction to the surface, followed by osteoneogenesis, forming an extracellular matrix. Subsequently, remodelling creates a bone to implant interface which can be explained through distance and contact osteogenesis. The dental implant surface has been shown to influence osteoconduction by modifying protein properties and adsorption around the implant. Salivary biomarkers may be considered as a specific and sensitive diagnostic tool to detect these changes in protein expressions after implant placement. Thus, the purpose of this narrative review is to provide a detailed account of the bone healing mechanism associated with dental implant placement, as well as how the implant surface architecture and protein release play a role in bone healing, and the potential use of saliva to detect these biomarkers.
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INTRODUCTION

Endosseous implants are becoming more popular due to their long-term clinical effectiveness, which has led to their usage in more challenging clinical situations. As a consequence, single root implants are increasingly used in posterior sites with minimal cortical bone to provide early mechanical stability. Given the clinical efficacy of implant therapy, new surgical methods such as sinus lifts have been developed to increase the local bone quantity and enable implant placement. Similarly, implants that formerly required months of early healing are now loaded almost immediately (1, 2). The undisputed success of endosseous dental implants necessitates further refining of implant design and maximisation of the biological healing response (3). The macro architecture of bone tissue varies greatly across anatomical areas, as do the mechanisms by which bone heals in various locations. As a result, bone remodels at the peri-implant cortical bone site, on the cut bone surface, by distance osteogenesis, while the bone heals at the trabecular bone site, on the implant surface, via contact osteogenesis (4).

The osteocytes within the cortical bone die due to thermal necrosis during implantation in distance osteogenesis. The osteoclasts reabsorb the dead bone (5, 6) and the osteogenic cells differentiate into osteoblasts causing bone matrix formation. This process is highly reliant on the blood supply at the site; hence, angiogenesis is crucial here. Some of the osteoblasts submerge in the newly formed matrices forming osteocytes and the cellular connection between these osteocytes and other surface osteoblasts is preserved through the canaliculi. The new blood vessels change its position as the bone matrix deposition occurs and moves closer to the implant surface. The integrity of the newly formed bone is sustained through continuing synthesis, however, this bone never quite reaches the implant surface due to the presence of surface osteoblasts itself (3). In contact osteogenesis, the osteogenic cells colonisation begin first on the implant surface. New bone matrix is laid down directly onto the implant surface and the osteogenic cells migrate along the cement line and form a calcified, collagen-free matrix separating the old and new bones.

Subsequently, the osteoblasts form a collagenous and extracellular matrix.

Therefore, bone formation in healing occurs in two opposite ways, distance osteogenesis will cause bone approximating but not quite reach the implant surface while contact osteogenesis results in bone apposition directly on the implant surface. Osteoconduction is essential in contact osteogenesis, which is the recruitment and migration of osteogenic cells and simultaneous bone formation by these cells onto the implant surface (4). Contact osteogenesis appears to be 30 percent faster than distance osteogenesis (7, 8). This review will discuss how dental implants induce bone healing, how the implant surface affects bone healing, and whether proteins involved in bone healing can be detected in saliva and used as biomarkers for implant healing.

BONE HEALING AFTER IMPLANTATION

During an implant surgical procedure, a drill punctures the naturally sculpted bone, tears blood vessels, and creates a large defect that quickly fills with blood. A titanium implant is inserted into the space and is held in place solely by mechanical friction. This phenomenon is referred to as primary implant stability. Osseointegration, also known as secondary implant stability which ensues after the first week of implant placement, necessitates a highly complex series of additional biodynamic processes (9). This is facilitated by highly regulated communication between the main cells of wound healing (10, 11) and will be described as the four phases of healing as summarized in Figure 1.

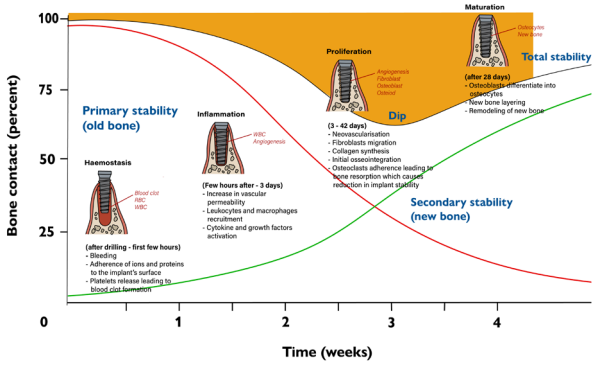

Figure 1: Stages of osseointegration and changes in implant stability with time

Phase 1- Hemostasis (minutes after surgery)

Blood immediately perfuses the surgical site, providing cues for subsequent healing. Ions and serum proteins such as fibrinogen, albumin and fibronectin begin adhering to the titanium surface within seconds or minutes (12). Blood platelets, also known as thrombocytes, then stop the bleeding. They aggregate and close the ruptured blood vessel when exposed to collagen and other proteins from the traumatized tissue and implant surface. Platelets release multiple messenger substances for cell-to-cell communication, including thromboxane, which promotes platelet aggregation, and platelet-derived growth factors (PDGF) and transforming growth factor-beta (TGF-β), which stimulates fibroblast cell division as well as vasoactive factors such as histamine and serotonin. Both TGF-β and PDGF have been shown to be chemotactic factors for neutrophils, fibroblasts, smooth muscle cells, and osteogenic cells (13-16). Arachidonic acid metabolites are released, following platelet degranulation, causing vasoconstriction. Tissue Factors VII and III in extravasated blood activate factor X, which, in conjunction with factor V, converts prothrombin to thrombin, which then cleaves the fibrinopeptides from fibrinogen to produce the clot's fibrin (17-19). Fibrin monomers spontaneously cross-link, resulting in the formation of a fibrin network. The blood clot permeates the wound space and adheres to the implant surface, forming a provisional matrix (20). This provisional matrix is crucial for subsequent bone healing processes on the implant surface.

Phase 2 – Inflammatory phase (hours after surgery)

During the early stages of healing, immune cells clean the wound of the very fine bone chips, tissue debris and oral bacteria that remain following the surgical procedure. In the first step, bradykinin from the platelets increases blood vessels permeability. As a result, the endothelial cells move apart very slightly. Endothelial cells on the inside of the vascular walls promote the attachment of polymorphonuclear leukocytes from the bloodstream. After digesting the basal lamina with proteases, the polymorphonuclear leukocytes squeeze their way through the gaps between the endothelial cells and are free to enter the wound (21). Polymorphonuclear leukocytes navigate chemotactically towards the wound along a molecular concentration gradient, which consists of bacterial proteins, fibrinopeptides, and pro-inflammatory interleukins. When they arrive, they kill bacteria by releasing reactive oxygen species. Polymorphonuclear leukocytes also secrete digestive enzymes like collagenase and elastase. The wound then heals normally, unless a toxic wound environment develops with elevated bacterial counts and toxic by-products, potentially resulting in wound breakdown and implant loss. Polymorphonuclear leukocytes can request help from other cells by releasing monocyte chemotactic protein or MCP-1. Macrophages respond and become the next players on the scene. They, too, use phagocytosis to eliminate bacteria (22).

Macrophages, which produce proinflammatory cytokines and proteases, take up tissue debris and biochemically degrade it. During the late inflammatory phase, macrophages predominate to produce endogenic inhibitors of proteinases, also known as tissue inhibitors of metalloproteinases (TIMPs) to aid in the halting of the polymorphonuclear leukocytes -initiated round of tissue

destruction. This protects the wound's matrix proteins and proteoglycans, which in turn protects important growth factor messenger substances like VEGF, PDGF, and FGF, which stimulate fibroblasts and angiogenesis and initiate the proliferative phase (22).

Phase 3 – Proliferative Phase (days after surgery)
Fibroblasts appear on the third or fourth day. They use amoeboid movement to migrate into the wound healing process. They produce the extracellular matrix's protective and stabilizing components, such as elastin, collagen and proteoglycans. When the circulation stops at the broken ends of the capillaries, ischemia and bone necrosis occur (23). Necrosis is caused by a deficiency in oxygen supply to the osteocytes (24). Necrosis is an intricate phenomenon that involves feedback mechanisms between mitogens, signaling factors and chemoattractants and is a precursor to leukocyte clot destruction. Factors that increase the adhesion of inflammatory cells to endothelial cells (leukotrienes) and chemoattractants cause diapedesis of leukocytes into the clot from post-capillary venules. The majority of these factors are released by activated platelets and endothelial cells, as well as leukocytes. The tissue's low oxygen concentration affects both macrophages and endothelial cells, stimulating them to produce the intracellular transcription factor, hypoxia inducible factor, or HIF (25, 26). Following that, newly formed VEGF influences perivascular cells. They migrate along the VEGF gradient into low partial oxygen pressure areas. They form new blood vessels here, which eventually integrate into the existing vascular network. Angiogenesis restores oxygen supply and serves as the foundation for bone healing. Beginning around the seventh day, activated osteoclasts attach to the fractured edges of the residual bone, resorbing it and making room for bone healing. However, this will initially reduce the implant's primary stability.

Here, osteoclasts dissolve the bone with HCl acid and proteases, releasing BMP, TGF-β, and PDGF from the bone matrix and initiating the formation of new bone (27). Perivascular cells not only form new blood vessels, but they also migrate toward existing trabeculae and the implant surface, where they differentiate into new osteoblasts in response to BMPs released by dissolved bone. Adsorbed proteins, such as fibronectin, play an important role in the attachment of bone progenitor cells to the implant surface (28, 29).

The osteoblasts form an organic matrix on the implant surface (30). This thin protein layer which becomes mineralised by incorporating calcium phosphate provides mechanical stability by interlocking the surfaces of the implants with the bone (31). At the end of the first week following surgery, woven bone forms on the implant surface. As a result, the implant's secondary stability improves, compensating for the implant's progressive loss of primary stability. The formation of woven bone concludes the proliferative phase of wound healing.

Phase 4 – Remodelling phase (weeks after surgery)
The site's stability is restored through orderly and coordinated bone remodeling. In this situation, local adaptation is critical. Initially, the woven bone will have grown in the valleys and parallel to the implant surface. After remodeling, most bone will be structured perpendicular to the peaks of the implant threads and at right angles to the implant surface. The architecture and organization of this bone become trabecular. The structure is thought to be directly responsive to forces applied to the interfacial tissues via the implant. This is made possible by the interaction of osteoblasts and osteoclasts (32). The woven bone is resorbed by osteoclasts, which are activated by the osteoblast RANKL messenger. The osteoblasts then lay down highly organized lamellar bone. The osteocyte and its messengers, such as sclerostin, primarily coordinate the work of both cells. Lamella bone structures are formed similarly to the arches and vaults in a gothic cathedral – they absorb occlusal load stresses perfectly adapted to the new situation. The molecular events around the implant surface are depicted in Figure 2 and the list of proteins involved in bone healing and their functions is summarised in Table I.

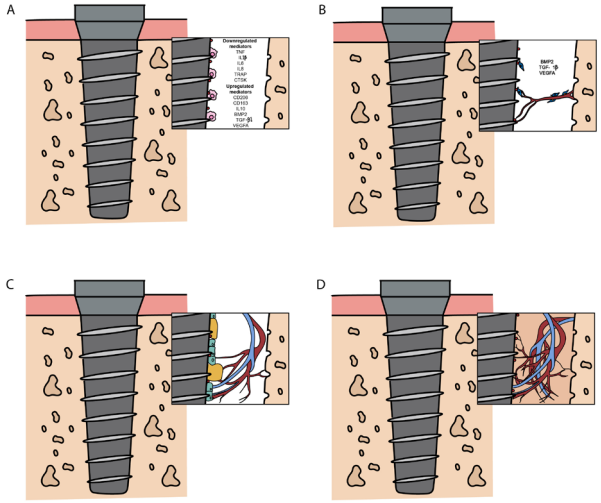

Figure 2: The molecular events around the implant surface during bone healing. (A) Migration of neutrophils, β and T cells, macrophages. Cytokine release leading to increase vascularisation, collagen synthesis and osteoclast activation. (B) Vascular neogenesis. (C) New bone deposition around vessels from deposition of osteoblasts. (D) Woven bone forms along scaffold of dead trabeculae.

EFFECT OF IMPLANT SURFACE ON BONE HEALING

Platelets play an important role in the early stages of wound healing because their activation causes the release of cytokines and growth factors that have been shown to induce and accelerate healing. The presence of an implant material may have tremendous effects

Table I: Proteins and their main functions in bone healing

Proteins	Main functions
Albumin	Induces mesenchymal stem cell growth (Yadav)
Bone Morphogenetic Protein-2 (BMP-2)	Promotes cell recruitment, angiogenesis and extending cell survival (rady)
Fibrinogen	Involves in fibrin-clot formation. (kim&lee)
Fibronectin	Mediates the communication between the intra and extracellular environment. (parisi)
Immunoglobulin (Ig)	Involves in immune response (Schmidt2009)
Interleukin-1β (IL-1β)	Initiate proliferation of osteoblasts and production of mineralised bone matrix (lange)
Interleukin-6 (IL-6)	Initiates proliferation of B cells and plasma cells and enhances IgG, IgM, and IgA production (takeuchi)
Interleukin-8 (IL-8)	Enhances bone regeneration and stimulates osteogenesis (yang)
Interleukin-10 (IL-10)	Reduces inflammation (ono)
Growth Factors (VEGF, PDGF, and FGF, TGF-β)	Initiates proliferative phase and activates fibroblasts and angiogenesis.
Matrix Metalloproteinases (MMPs)	Involves in remodeling process of mostly collagenous molecules (henle)
Monocyte Chemotactic Protein-1 (MCP-1)	Recruits mesenchymal progenitor cells (ishikawa)
Tumour Necrosis Factor-α (TNF-α)	Initiates neo-angiogenesis and stimulates mesenchymal stem cells (Timmen)
von Willebrand Factor	Regulates platelet exposure

on initial blood cell reactions, including red blood cell agglomeration, and substrate corugation influences the number and degree of activation of platelets, though the exact mechanisms are unknown. The early adhesion of platelets has been shown to be mediated by GPIIb/IIIa integrin binding to surface adsorbed fibrinogen (33-35). As a result of the increased microtopography, surfaces with a larger surface area absorb more fibrinogen, which could explain the pragmatic increase in platelet adhesion. Furthermore, the von Willebrand Factor has been shown to be a regulator of platelet exposure of CD62 (P- selectin) as a result of granule release (36), making it important for platelet-neutrophil interactions at biomaterial surfaces (37). According to one study, platelets activated on microtextured implant surfaces upregulate neutrophils - the first leukocyte cluster to enter the wound site during the acute inflammatory healing (38) to a greater extent than platelets activated on smoother implant surfaces (39).

The reaction product of fibrinogen and thrombin that is released into the healing site adheres to almost all surfaces and this makes osteogenic cell migration to any implanted material to be possible. Connective tissue cell migration, on the other hand, occurs concurrently with wound shrinkage, which typically starts around the fifth day after wounding, as demonstrated in dermal wound healing models (40). Indeed, fibroblast migration has been identified as the cause of wound shrinkage (41), with specific cell adhesive contacts producing a contractile force of roughly 3 nN (42). The ability of cells to contract the matrix may result in retraction of the transitory fibrin scaffold away from the implant surface in the bony peri-implant site. The treatment of primary osteogenic cell cultures with cytochalasin-D, which inhibits actin-dependent cell developments such as cell migration by capping actin filaments, shows that primary osteogenic cells can cause fibrin contraction (43). As a result, the ability of an implant to retain fibrin during the wound contraction phase of healing is precarious in determining whether migrating cells reach the former. The implant surface design will have an impact on fibrin retention.

As a result, the migration of differentiating osteogenic cells over the implant surface is critical to the osteoconduction phenomenon. The implant surface's design can have a significant impact on osteoconduction, not only by modulating platelet activation levels but also by preserving the anchorage of the temporary scaffold that these cells use to reach the implant surface. Microtopographically complex surfaces are predicted to stimulate osteoconduction by increasing the surface area for fibrin attachment and providing surface features with which fibrin can become intertwined; they may also potentiate platelet activation, resulting in cytokine and growth factor density gradients through which leukocytes and osteogenic cells enter the healing site.

Thus, osteogenic cell migration in peri-implant healing will occur through a three-dimensional biological matrix formed as a result of the coagulation cascade, the fibrin of the blood clot and may be directly or indirectly initiated and guided through causal stimulatory events involving leukocytes (44), cytokines, growth factors and platelets activated by implant surface contact.

How do proteins bond to an implant surface? A protein that arrives first on the surface interacts through an intramolecular bond, ionic bond and charge transfer. The protein properties affect this interaction and consequently the protein adsorption onto the surface. Proteins comprise mainly of amino acids which may be charged and are more polar. Charged proteins are more readily adsorbed with an oppositely charged surface, meaning positively charged protein domains are attracted to negatively charged surface areas and vice versa (45). Blood proteins are mainly negatively charged, hence, a net negative charge of implant surfaces reduces protein adsorption. Amino acids are also more hydrophilic and tend to adsorb to water molecules available on the surface. Hydrophobic domains of the proteins, on the other hand, tend to

adsorb to hydrophobic surface areas. Commercially available implants generally have hydrophobic surfaces due to manufacturing contaminations. To create more hydrophilic surfaces, methods such as plasma and UV-light treatment can be applied to increase the wettability or protein adsorption of the implant (46).

Protein size influences the rate of diffusion and affinity of the proteins towards the implant surface (46, 47). The smaller the protein size, the faster it diffuses and arrives on site. However, a smaller protein size is related to fewer contact points with the surface compared to larger protein size. This means smaller proteins have a lower affinity towards the implant surface. Thus, what tends to happen is the smaller proteins will bond first and then be replaced by larger, high-affinity proteins. The process of the replacement of proteins over each other with time, based on the rate of diffusion and affinity is referred to as the Vroman effect. In addition, the structural stability of proteins influences the bond. Weak proteins or proteins with less internal body stability have a greater, easier and faster unfolding of protein molecules which tend to bond strongly to surfaces. These proteins with less thermodynamic stability bond with the surface before other proteins arrive at the site (46).

SALIVA AS A DIAGNOSTIC INDICATOR OF BONE HEALING

Saliva has emerged as a diagnostic tool in determining any activity changes within the oral cavity (48). It holds clinically relevant information of the human body (49, 50) and this information may be used for diagnostic purposes to detect oral or general diseases early (51, 52). Saliva is found to be rich in albumin, antimicrobial products and immunomodulatory proteins (53, 54) and protein adsorption on biomaterials and proteomic studies have been carried out with multiple methods including liquid chromatography, electrophoresis, nuclear magnetic resonance, mass spectroscopy or spectrometry and immunoassays such as an enzyme-linked immunosorbent assay (ELISA) (55). Previously, the trend was to rely on the gingival crevicular fluid (GCF) to identify the presence of proteins and their functions (56). Currently, the collection of whole saliva, stimulated or unstimulated, and saliva from the parotid glands are also reliable means in saliva collection to detect the expression of these proteins (57).

Saliva collection has obvious advantages over blood as it is easy, non-invasive and theoretically uneventful; reducing anxiety and discomfort (58). Transportation and storage of saliva are also simpler and more economical as the collection armamentarium can be obtained at a lower cost for analysis as well as less manipulation is needed throughout the diagnostic procedure (59). Additionally, saliva does not clot which makes the handling of the samples more convenient. Saliva collection, as compared to blood, also does not necessarily require trained medical staff, produces a minimal risk of cross-contamination, and prevents needle prick and other sharp-related injuries (58).

Studies have shown that oral cavity changes may influence systemic diseases such as cardiovascular disease and diabetes mellitus. This is because the detached plaque biofilm can flow into the bloodstream causing inflammation and infection and indirectly affecting the body system (50, 60, 61). Proinflammatory cytokines or acute phase proteins release may trigger a disease to occur (60, 61). It was found that 27% of blood proteins are detected in saliva and 40% of them can act as disease biomarkers (62). Also, the level of hormones in saliva reflects the serum or plasma-free active hormones; the biological activity of hormones is a function of their free fraction (55, 57, 63). The salivary mRNA biomarkers were also used to detect pancreatic cancer without chronic pancreatitis side effects (64). More importantly, in the osseointegration process after dental implant placement, proteins undergo changes to accommodate the injury occurring around the bone (65, 66).

The transcription and growth factors of the protein outcomes or products are responsible for differentiation of osteoblasts (67). This process may also show crucial markers around the healing area of the dental implants when taking the cellular events into consideration (68). Protein expressions have been linked together with bone healing phases even though their certain functions to osteoblasts are unsettled. For instance, in selective laser melting (SLM) implant, the elevated level of inflammatory response of tumour necrosis factor-alpha (TNF-α), interleukin-6 (IL-6) and interleukin-1-beta (IL-1β) pro-inflammatory genes can be visualized after placement (69, 70). Ogawa (2006) in his implant research on rats found that there are three genes that plays an important role in implant bone healing. The genes are apolipoprotein E, prolyl 4-hydroxylase alpha-subunit and an unknown transcript (71). Fine et. al. (2009) found that macrophage inflammatory protein – 1-alpha (MIP-1α) can be the early biomarker to detect localised aggressive periodontitis (LAP) as that specific macrophage is very sensitive with radiographic evidence of bone loss (72). Looking at the cellular events around bone formation sites, this protein product may also act as a crucial marker in saliva. The study of proteins during the healing phase after implant placement is still limited and there is a need to enhance the understanding of this bone healing and possibly provide a basis for the incorporation of protein biomarkers onto the implant surface to accelerate bone healing.

CONCLUSION

A fundamental understanding of peri-implant bone healing is required to optimise implant surface design. The architecture of the surface can have a substantial

effect on protein adsorption and, eventually, on osteoconduction and therefore it is critical to understand how altering the surface design might transform and expedite bone healing. Additionally, development in salivary analysis technology may help further understanding of the role of protein in bone repair.

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