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Abstract: If dental implantology is an increasingly successful treatment modality, why should we still need to understand the mechanisms of peri-implant bone healing? Are there differences in cortical and trabecular healing? What does "poor quality" bone mean? What stages of healing are most important? How do calcium phosphate-coated implants accelerate healing? What is the mechanism of bone bonding? While there are still many aspects of peri-implant healing that need to be elucidated, it is now possible to deconvolute this biological reaction cascade, both phenomenologically and experimentally, into three distinct phases that mirror the evolution of bone into an exquisite tissue capable of regeneration. The first and most important healing phase, osteoconduction, relies on the recruitment and migration of osteogenic cells to the implant surface, through the residue of the peri-implant blood clot. Among the most important aspects of osteoconduction are the knock-on effects generated at the implant surface, by the initiation of platelet activation, which result in directed osteogenic cell migration. The second healing phase, *de novo* bone formation, results in a mineralized interfacial matrix equivalent to that seen in the cement line in natural bone tissue. These two healing phases, osteoconduction and *de novo* bone formation, result in contact osteogenesis and, given an appropriate implant surface, bone bonding. The third healing phase, bone remodeling, relies on slower processes and is not considered here. This discussion paper argues that it is the very success of dental implants that is driving their increased use in ever more challenging clinical situations and that many of the most important steps in the peri-implant healing cascade are profoundly influenced by implant surface microtopography. By understanding what is important in peri-implant bone healing, we are now able to answer all the questions listed above.

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It is just as true in dentistry as in other disciplines that the more useful a technology, the more rapidly are its limits challenged by the user and that, in turn, user demand drives the necessity for refinements and improvements in the technology. An obvious example would be the rapid evolution of computers over the last few decades that has led to our almost indispensable reliance on the ubiquitous microchip. Similarly, in dentistry, over the last few decades there has been an increasing use of endosseous (in-bone) implants as a means of providing a foundation for intra-oral prosthetic devices,[1] from full arch dentures to single crowns, or other devices for orthodontic anchorage[2,3] or distraction osteogenesis.[4,5] While there is no question that the popularity of endosseous implants for these treatment modalities is based on increasingly convincing data of long-term clinical success rates, it is this very success that has prompted the use of implants in more challenging clinical situations than were previously envisioned.[6]

Thus, single root implants previously employed in only anterior mandibular and maxillary sites are now commonly inserted into posterior regions where there is less cortical bone to provide initial mechanical stabilization. Indeed, the clinical success of implant therapy has led to the emergence of new operative procedures, such as sinus lifts, to increase local bone stock to accommodate implant placement. Similarly, implants that would previously only be placed into occlusal function after an extended initial healing period of several months are now loaded increasingly earlier in a matter of weeks, days, or even hours![7,8] These radical changes in the practice of implant dentistry have been made possible through the evolution of a more profound understanding of the essential requirements of individual case treatment planning, improvements in surgical procedures, and the evolution of the design of the implants themselves such that almost a million dental implant procedures are now conducted, annually, worldwide.

Therefore, the unequivocal success of endosseous dental implants is driving the need for continuing refinements in implant design and optimization of the biological healing response following implant placement.

The purpose of this paper is to examine some of the more important concepts and mechanisms that comprise the biological cascade of early peri-implant bone healing. It is not my intention to provide an exhaustive review of the litany of published work on peri-implant bone formation since, as we shall see later, by the time that bone is formed on the implant surface, the most important healing events have already occurred!

The Paradox of "Poor Quality" Bone

Since the clinical success of dental implant therapy is based on the anchorage of the endosseous component of the implant in bone tissue, it is incumbent upon implantologists to know something of both the macro- and microarchitecture of the host biological tissue.

Bone is a dynamic, vascular, living tissue that changes throughout life and is one of the so-called "connective tissues" of the body and thus comprises cells that become embedded in their own extracellular matrix. Bone tissue has evolved over a far greater period than the half million or so years that we, as *Homo Sapiens*, have existed on earth. Some believe that the evolution of our cranium can be traced back as far as the emergence of the protofish[9] or cyclostomes[10] some 540 million years ago. Indeed, it is neither trivial nor facetious to point out that the historical timeframe of dental implant development pales into insignificance when compared to the evolution of the tissue into which such implants are placed. Thus it is not unreasonable to assume that, not only do all bony architectures represent highly evolved biological structures, but also that an understanding of normal bone formation and remodeling, through which such architectures are achieved, may well provide an insight into both the healing of bone around implants and the influence of implant surface design on such healing mechanisms.

Bone tissue is arranged in two macroarchitectural forms—trabecular (or cancellous, or spongy) and cortical (or compact)—which are employed in various proportions and geometries to form the individual bones of the body. The latter can be broadly subdivided into four groups: long bones (e.g., femur, tibia, fibula, humerus, radius, and ulna); short bones such as those of the carpus (wrist) and tarsus (ankle); flat bones such as those of the calvaria (skull vault); and irregular bones (e.g., the remaining bones of the skull, the scapula, and pelvic bones). These differences in macroarchitecture have been employed to derive a clinical classification of bone type in the dental implant field, based on the relative proportion of cortical to trabecular bone (that is, where Class 1 bone is predominantly cortical as in the anterior mandible, while Class 4 bone is almost all trabecular as found in the posterior maxilla).[11] However, when such a classification is reinterpreted to indicate that Class 4 bone is "poor quality," it should be understood that this is a clinical judgment based on the initial limited success of placement of relatively simple machined implant designs in such anatomical areas rather than a judgment of the true biological quality of the bone.

Indeed, this clinical classification takes account of neither the biological function of such bony macroarchitecture in different anatomical sites nor the emergence of a wide range of surface textured implant designs that have unequivocally demonstrated improved clinical success in these previously challenging anatomical sites.[12,13] To illustrate this important difference between historical clinical pragmatism and basic biology, one only has to consider the tissue macroarchitecture of a typical long bone where the dense cortical structure is limited, essentially, to the diaphysis, while the articular extremities have almost no cortical covering but are packed with cancellous bone. In this case there would be general agreement that cortical bone has evolved to withstand torsional loading, while the function of cancellous bone is to withstand predominantly compressive loading, and there would be no question that the bone of such articular extremities is of poor quality simply because it is trabecular! Indeed, such a judgment would be considered ludicrous.

About 3-5 percent of one's skeleton in being eaten away by osteoclasts and replaced by osteoblasts, throughout life from six weeks *in utero*, when bone first forms, until we die. This constant remodeling of bone tissue provides a mechanism for scar-free healing and regeneration of damaged bone tissue, and results in the exquisite lamellar microarchitecture of both cortical and trabecular mature bone.[14,15] How-

ever, the macroarchitecture of bone tissue differs radically from one anatomical site to the next, and it is important to realize that the means by which the bone will heal in these different sites may also be radically different. Thus, the relatively slow regeneration of peri-implant cortical bone relies exclusively on lamellar remodeling as illustrated in Figure 1, while the generation of peri-implant trabecular bone may involve not only remodeling of existing lamellar trabeculae but can also include the rapid formation of new trabeculae by the recruitment of new populations of osteogenic cells within the healing compartment (Figure 2). These osteogenic cells are derived from both the endosteal trabecular surfaces and the marrow that fills the large interconnected pores between the struts and sheets of trabecular bone tissue. Thus, cancellous bone has a very high surface area, which is contiguous with the marrow compartment. Since marrow contains not only mesenchymal progenitor cells that can give rise to osteoblasts, but also a rich vasculature that can supply both the circulating mononuclear precursors to osteoclasts (needed for remodeling) and the endothelial population needed for angiogenesis, it is not surprising that trabecular bone can remodel far more quickly than cortical bone.

From this perspective, therefore, trabecular bone represents a biologically superior tissue, ideally evolved for rapid (peri-implant) bone healing, when compared to the slowly remodeling healing pattern typical of cortical bone, and should definitely not be considered as "poor quality" bone.

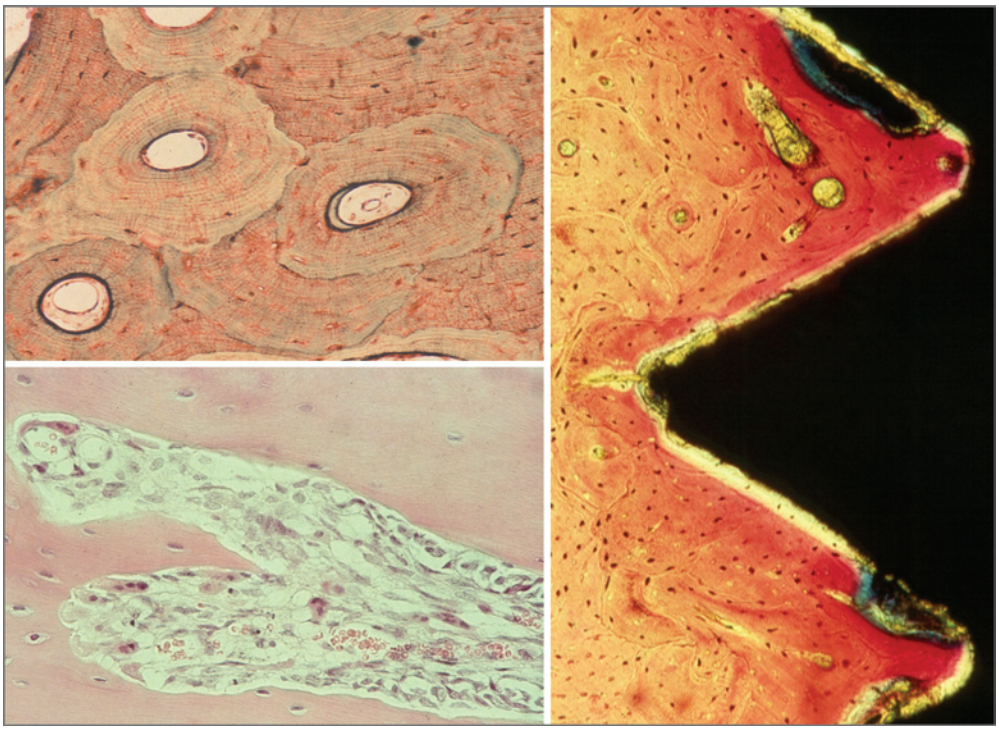

Figure 1. The exquisite microstructure of dense cortical human bone is seen (top left) where the vascularized remodeling units (osteons) are demarcated from the surrounding older bone by scalloped borders. This interface between new and old bone is occupied by the cement line. Osteocytes, buried in the bone matrix, are rarely found in contact with the cement line matrix. Indeed, their cell processes usually are directed away from the cement line and toward the central vascular supply of the osteon. Such osteonal systems are also seen in the cortical remodeling around the screw-threaded implant on the right. This replacement of the bone that has died, as a result of creating the site for implant placement, is a time-consuming process and is the result of the type of remodeling shown on the bottom left. The latter is an example from rat femoral bone, which remains as woven bone throughout the life of the animal. The image shows the beginning of a branching osteonal system in longitudinal section with two groups of osteoclasts creating two independent cutting cones (left side). The red blood cells clearly show the presence of the important blood vasculature that provides the mononuclear precursors to the osteoclast. To the right another population of cells is evident from their somewhat darker staining (more basophilic cytoplasm) and represents the developing osteogenic cells that will eventually form bone on the walls of the tunnel created by the osteoclasts.

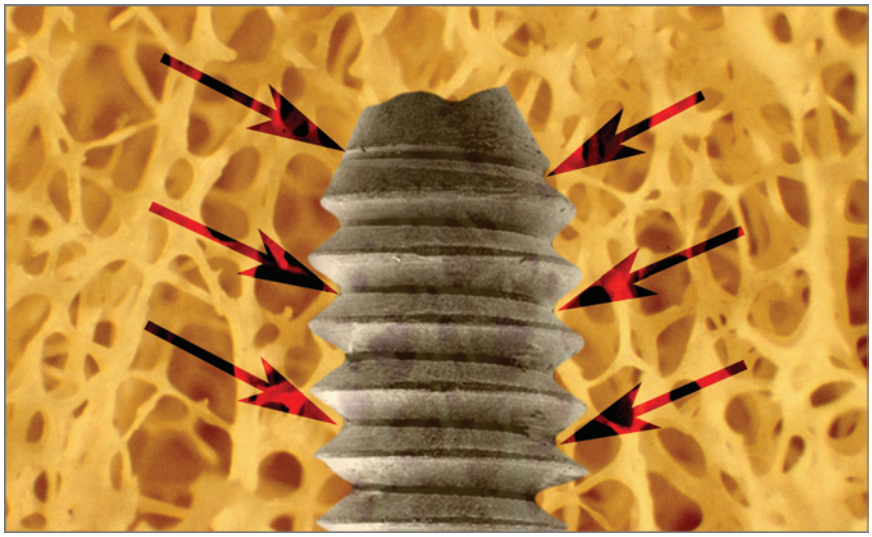

Figure 2. This collage represents the placement of a screw-threaded implant in trabecular bone. Here, the trabeculae will have been damaged due to the preparation of the site from implant placement and will provide little support for the implant itself. The only chance to stabilize this implant in new bone will be to recruit osteogenic cells to the implant surface (represented by the arrows) where they could elaborate bone matrix directly on the implant surface. This combination of recruitment and migration of osteogenic cells (osteoconduction) and bone formation by those cells on the implant surface is known as contact osteogenesis.

Remodeling and Osteogenesis

In examining Figures 1 and 2 and using them to explain peri-implant osteogenesis, we run the risk of focusing on only mature lamellar cortical, or trabecular, bone. But this is not the structure of rapidly healing bone. So it should be emphasized that the histological microarchitecture of human bone formed rapidly during fracture, or trabecular peri-implant, healing differs from that formed during lamellar remodeling since rapidly formed bone is of a woven, or irregular, microarchitecture. Woven bone is the result of rapid growth which, rather than the slower remodeling that results (in the majority of higher vertebrates) in lamellar bone formation, occurs either in the embryonic development (Figure 3) or during fracture healing, and for this reason structural immaturity should not be equated with a lack of functional utility. Although the microarchitecture of lamellar and woven bone differs, the underlying histodynamics of the formation of these two distinct types of bone tissue are remarkably similar. It is simply the rate of change that produces the significant differences in histological appearances of woven and lamellar bone, as we have discussed in more detail elsewhere.[16] Notwithstanding the emphasis on woven bone, it is the bone remodeling phenomenon, either in woven or lamellar bone, that provides a different, but equally important, focus for our attention, since it is during remodeling that new bone forms at the solid surface presented by resorbed bone.

Thus we can envisage that, around endosseous implants, osteoblasts may lay down bone on the old bone surface or on the implant surface itself. This distinction was explored by Osborn and Newesley,[17] who described the two phenomena, distance and contact osteogenesis, by which bone can become juxtaposed to an implant surface. For computer animations of distance and contact osteogenesis, see www.ecf.utoronto.ca/~bonehead/.

In distance osteogenesis, new bone is formed on the surfaces of old bone in the peri-implant site. The bone surfaces provide a population of osteogenic cells that lay down a new matrix that encroaches on the implant. An essential observation here is that new bone is not forming on the implant, but the latter does become surrounded by bone. Thus, in these circumstances, the implant surface will always be partially obscured from bone by intervening cells, as illustrated in Figure 4A. Distance osteogenesis can be expected in cortical bone healing since vascular disruption of the cortex caused during implant site preparation is known to lead to death of the peri-

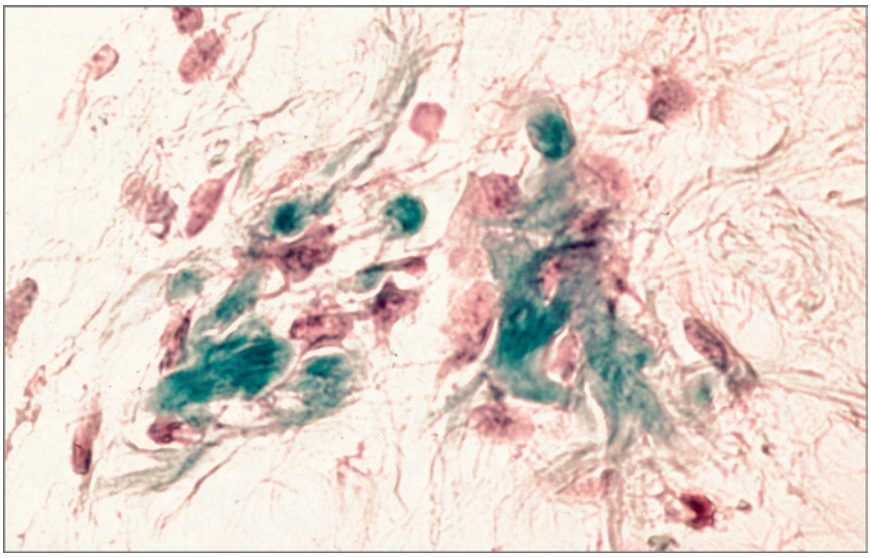

Figure 3. A light micrograph of human woven bone from the developing human maxilla (5 months i.u.). The dark extracellular matrix is the initial bone matrix elaborated by the cells seen here, which are osteoblasts, but are in the process of becoming embedded in their own matrix as osteocytes. The less dense surrounding tissue is embryonic mesenchyme (or fetal connective tissue).

implant cortical bone[18] and its subsequent slow remodeling by osteoclast invasion from the underlying medullary compartment.[19] This type of healing has been reviewed thoroughly elsewhere[20] and has also been explicitly described to explain the phenomenon of so-called "osseointegration" of machined metallic implants. In the latter, initiation of mineralization of the healing bone tissue did not occur on the implant surface, but bone grew towards the implant,[21] subsequent to the death of the intervening tissue.[22]

In contrast, in the process of contact osteogenesis, new bone forms first on the implant surface (Figure 4B). Since, by definition, no bone is present on the surface of the implant upon implantation, the implant surface has to become colonized by bone cells before bone matrix formation can begin. This is also what happens at bone remodeling sites where a resorption surface of old bone is populated with osteogenic cells before new bone can be laid down. The common factor linking normal tunneling remodeling and contact osteogenesis is that bone is formed for the first time at the appropriate site by differentiating osteogenic cells. We call this *de novo* bone formation.[16,23] We use the term "differentiating osteogenic cell" to denote a cell that still has migratory capacity but will become an osteoblast. This is different from the terms "pre-osteoblast" or "osteoprogenitor," which are often given connotations related to intracellular expression of bone-related proteins. Clearly an essential prerequisite of *de novo* bone formation is that bone cells must first get to either the old bone or implant surface respectively, before extracellular matrix synthesis is initiated. The result of *de novo* bone formation is that the implant/bone interface, in an identical fashion to remodeling bone surfaces, is occupied by a cement line matrix (Figure 4B) as first described by von Ebner in 1875.[24] I have described elsewhere the formation of such new or *de novo* bone formation in some detail,[25] and also emphasized the importance of understanding *de novo* bone formation as one of the three phenomenologically distinct mechanisms that comprise endosseous implant integration[26] (see below).

Therefore, distance osteogenesis will result in bone approximating the implant surface while contact osteogenesis results in bone apposition to the implant surface. Although it is inevitable that both distance and contact osteogenesis occurs in every endosseous healing site, the biological significance of these different healing reactions is of critical importance in both attempting to unravel the role of implant design in endosseous integration and elucidating the differences in structure and composition of the bone/implant interface. Obviously, in Class III and Class IV bone, optimizing contact osteogenesis by implant surface design to ensure early stability is of great importance because, in the absence of suffi-

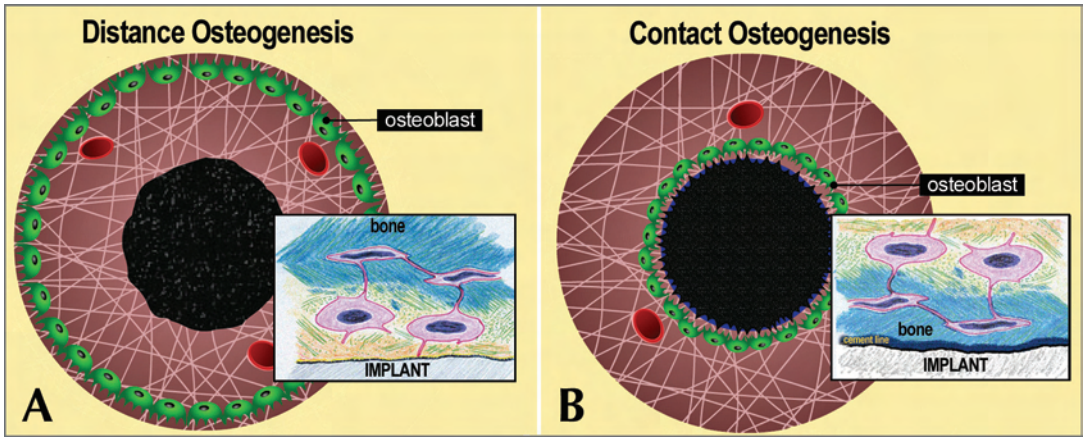

Figure 4. Drawings to show the initiation of distance osteogenesis (A) and contact osteogenesis (B) where differentiating osteogenic cells line either the old bone or implant surface respectively. The insets show the consequences of these two distinctly different patterns of bone formation. In the former the secretorily active osteoblasts, anchored into their extracellular matrix by their cell processes, become trapped between the bone they are forming and the surface of the implant. The only possible outcome is the death of these cells. On the contrary, in contact osteogenesis, *de novo* bone is formed directly on the implant surface, with the cement line in contact with the implant (insert) and is equivalent to the osteonal interface illustrated in Figure 1. These drawings are still images from computer animations of distance and contact osteogenesis, which can be viewed at www.ecf.utoronto.ca/~bonehead/ (follow buttons to osteogenesis and osteoconduction).

cient cortex to provide stability,[16,27] recruitment of osteogenic cells to the implant surface and subsequent bone formation is the only way in which implant stability can be achieved in such bony sites.

Deriving a Maxim for Endosseous Healing

As important as it may be to understand something of the microstructure of bone, three essential facts govern our current concepts of endosseous healing.

First, bone matrix is synthesized by only one cell: the osteoblast. While some would argue that osteoblasts from different anatomical sites represent different end members of this osteogenic differentiation pathway, the outcome remains the same: it is the osteoblast that makes bone matrix. Indeed, like most secretory cells, osteoblasts are polarized cells, and the direction of their secretory activity is away from the nuclear end of the cell (this is also true for the so-called reverse polarization of ameloblasts). Since the matrix secreted by osteoblasts becomes mineralized as bone tissue, the cell processes of osteoblasts become surrounded by mineralized matrix (and, with their canaliculi, form the only means of vital communication between surface osteoblasts and those that have become completed surrounded by matrix as osteocytes). Thus the osteoblast is irrevocably attached to the bone-forming surface.

Second, as a result of the polarized synthetic (meaning the synthesis of bone matrix) activity of osteoblasts, bone grows only by apposition. This means that, in a way similar to that in which a plasterer will coat a wall with a layer of plaster, bone can only be deposited by laying down matrix on a pre-existing solid surface. Of course, there is a caveat to this statement that must allow for the formation of bone where no pre-existing surface exists, as in the initiation of intramembraneous bone formation as shown, in the developing human maxilla, in Figure 3. Here, osteogenic cells differentiate into osteoblasts in the foetal connective tissue matrix and initiate bone matrix formation. This can be also demonstrated in the intramembraneous ossification of the calvarial bones, as we have illustrated and discussed elsewhere.[16] However, since each osteoblast may become a completely entombed osteocyte, the osteoblast is incapable of migration away from the bone surface, and the only method by which this surface can receive further additions (beyond the synthetic capacity of a single osteoblast) is by the recruitment of more osteogenic cells to the surface, which then differentiate into secretorily active osteoblasts.

Third, bone matrix mineralizes and has no inherent capacity to "grow." This is quite different from

other connective tissue, for example, cartilage, which can grow both interstitially (increasing extracellular matrix volume by the ongoing secretory activities of matrix-entombed cells) and by apposition. Thus, once bone formation has been initiated, the matrix and the cells that have synthesized that matrix have almost no ability to govern the ongoing pattern of bone growth on the implant surface.

These apparently simplistic notions are of vital importance in understanding endosseous healing because if we require new bone to be formed on an implant surface, the only means by which this can happen is that osteogenic cells must first migrate to that surface. Then, if we require that bone "grows" around the implant to establish functional endosseous integration, this too can only be achieved by the continued recruitment around, and migration of osteogenic cells to, the implant surface. Thus, we can derive the following important maxim: "The most important stages of endosseous healing *precede* bone formation."

Thus by the time bone grows on, or is juxtaposed to, an implant surface, the most important healing mechanisms have already been invoked! This is because not only will such early healing phenomena determine if bone will be formed on the implant surface but also that, as explained above, the continued growth of bone over the implant surface will be the result of continued recruitment and migration of osteogenic cells to the implant rather than an inherent ability of bone matrix to grow. Furthermore, we now understand that implant surface microtexture has a profound effect on these early healing stages (see below).

Thus, the most important aspect of early peri-implant healing is the recruitment of osteogenic cells and their migration to the implant surface. We employ the term "osteoconduction" to encapsulate these important early events that will position the osteogenic cells on the surface of the implant where they can than make bone matrix. The *de novo* formation of bone itself can therefore be considered as a separate and distinct phenomenon which, in time, will be followed by the remodeling of the peri-implant bone. The combination of osteoconduction and bone formation will result in contact osteogenesis. The long-term remodeling of the tissue is influenced by different stimuli, the most important being the biomechanics of the developed healing site, and thus should also be treated separately.

These three phenomena—osteoconduction, *de novo* bone formation, and bone remodeling—are not unique to the peri-implant environment *per se,* but also occur, as an outcome of evolutionary development, during both bone remodeling and fracture healing, and can thus be considered as critical hallmarks of bone healing and regeneration. Indeed, since trabeculae are damaged during implant site preparation, it is not surprising that bone fracture healing and peri-implant healing exhibit many similarities, although this view has engendered much debate since some authors have emphasized this notion[28-30] while others have suggested that the presence of an implant induces a different mode of healing.[31]

Lessons from Fracture Healing

Bone tissue was responding to injury long before the advent of iatrogenic damage caused prior to implant placement. It is reasonable to assume, therefore, that not only can we learn a great deal from understanding the normal mechanisms of bone healing and regeneration, since these have evolved over an extended period of time, but also that we could apply this knowledge to an understanding of peri-implant endosseous wound healing. Thus, in both fracture healing and peri-implant healing, blood vessels are damaged, and this results in hemorrhage and the formation of a blood clot, or hematoma. The amount of blood loss from the circulation depends on the location of the fracture. In the femoral shaft, up to one liter of blood can be lost due to extravasation,[32] whereas the amount lost in dental implant placement is significantly less, with the healing site volume measured in milliliters rather than liters.

Figure 5 illustrates the cellular composition of 1 mL of circulating human blood, which comprises predominantly red blood cells (erythrocytes) and platelets. While erythrocytes are clearly important in oxygen transport, they can reasonably be assumed to be of little importance in the mechanisms of early peri-implant healing. Platelets, however, are of considerable importance since their activation leads to a rearrangement in cell shape and to centralization of storage granules followed by the release of their contents into the extracellular environment (degranulation); and platelet degranulation releases a number of growth factors, such as platelet-derived growth

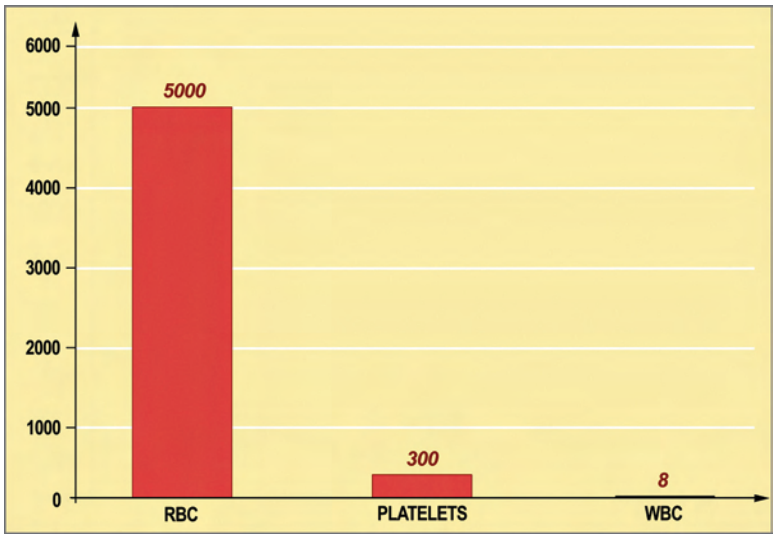

Figure 5. The relative proportions of cells in human peripheral blood. In 1mL there are 5,000 million red blood cells, 300 million platelets, and 8 million white blood cells.

factor (PDGF) and transforming growth factor beta (TGF-b), together with vasoactive factors such as serotonin and histamine. Conventional wisdom holds that these factors play an important role in the regulation of the wound-healing cascade,[33,34] based on in vitro and in vivo evidence of their stimulatory effects on the proliferation and migration of various cell types.[35-37] For example, both PDGF and TGF-b have been shown to be not only mitogenic for fibroblasts but also chemotactic factors for fibroblasts, neutrophils, and smooth muscle cells,[38-40] as well as osteogenic cells.[41-44] Indeed, not only has PDGF been shown to stimulate the proliferation of human osteoblasts,[45,46] but we have recently shown that platelet releasate is able to stimulate the recruitment, migration, and profileration of bone marrow-derived cells.[47] Following platelet degranulation, arachidonic acid metabolites are secreted that cause vasoconstriction. Within the surrounding tissue, factors VII and III (tissue factor) in the extravasated blood cause the activation of factor X that, together with factor V, causes the conversion of prothrombin to thrombin, which cleaves the fibrinopeptides from fibrinogen to produce the fibrin of the clot. For detailed historical reviews on hemostasis, see Colman et al.[48] and Halkier,[49] and for a recent treatment, see Gemmell and Park.[50]

Cessation of circulation at the broken ends of the fragments causes local ischemia and necrosis.[51] Necrosis is due to the lack of oxygen supply for the osteocytes that in living bone are no more than 0.1 mm from an intact capillary.[52] Necrosis is a complex phenomenon that involves feedback mechanisms between signaling factors, mitogens, and chemoattractants and is a prelude to clot demolition by leukocytes, but also emphasizes the importance of angiogenesis as the only means of providing a nutrient supply to the peri-implant healing compartment. Diapedesis of leukocytes into the clot from the post-capillary venules is caused by factors that enhance adhesion of inflammatory cells to endothelial cells (leukotrienes) and chemoattractants. Most of these factors are released by the activated platelets and endothelial cells as well as by the leukocytes themselves. Thrombin[53] and tissue degradation products[54,55] also serve as chemoattractants for leukocytes. Initially, neutrophils are the most numerous peaking at 24-48 h,[56] but macrophages rapidly become predominant.[57,58] Both cell types are involved in clot and necrotic tissue, demolition through both extracellular and intracellular phagocytic digestion mechanisms. A diminishing oxygen concentration gradient towards the center of the wound provides the chemotactic signal for endothelial and mesenchymal cells.[59]

Angiogenesis is initiated predominantly from post-capillary venules, where endothelial cells degrade the subendothelial basement membrane and migrate and proliferate to form hollow capillary buds or sprouts. We now know a great deal concerning the molecular mechanisms of angiogenesis,[60] but, at present, we understand little of how the patterns of angiogenesis may be affected by the presence, or surface design, of an endosseous implant, although

some attempts have been made to image the vasculature that develops around endosseous implants.[61,62] Interestingly, Matsuo et al.[63] showed some differences in the neovasculature in cortical bone around machined and plasma-sprayed implants that exhibited distance osteogenesis. Recent (unpublished) work in our laboratory has also demonstrated that implant surfaces, which are known to promote contact osteogenesis, also exhibit a richer immediate peri-implant vascular supply within the healing compartment.

Osteoconduction: The Key to Contact Osteogenesis

As mentioned above, contact osteogenesis relies upon osteoconduction, or the recruitment and migration of differentiating osteogenic cells to the implant surface, together with *de novo* bone formation by those cells on the implant surface. Osteoconduction also occurs during normal tunneling remodeling in bone. In such remodeling, differentiating osteogenic cells are derived from undifferentiated peri-vascular connective tissue cells (pericytes),[64] just as soft tissue fibroblasts have long been recognized as being derived from the mesenchymal cell populations of blood vessel adventia.[65] However, a more complex environment characterizes the peri-implant healing site since this will be occupied, transiently, by blood.

Blood Cells in the Peri-Implant Compartment

Thus, we have to consider not only the effects of the interactions of blood cells with, but also the role of the transient fibrin-based structural matrix of, the blood clot through which osteogenic cells must migrate to reach the implant surface. Platelets can be expected to be of particular importance in these early stages of healing (as discussed above) since their activation results in the release of cytokines and growth factors that are known to accelerate wound healing. Although the exact mechanisms have yet to be elucidated, a small number of reports have emerged that show that the presence of an implant material may have profound effects on early blood cell reactions, including the agglomeration of red blood cells,[66] and that substrate rugosity influences the number, and degree of activation, of platelets.[67-70] In particular, the initial adhesion of platelets has been shown to be mediated by GPIIb/IIIa integrin binding to implant surface adsorbed fibrinogen.[71] Thus, surfaces of greater microtopography will exhibit an increased surface area and a resultant increase in fibrinogen absorption, which could explain the observed increase in platelet adhesion.[66] Furthermore, the von Willebrand Factor has been shown to be a specific regulator of the exposure of CD62 (P-selectin) by platelets, as a result of α-granule release,[50] and hence important for the interaction between platelets and later arriving neutrophils at biomaterial surfaces.[72] Indeed, in some recent preliminary work, we have shown not only that platelet activation is a function of substrate surface topography,[33] but also that platelets activated on microtextured candidate implant surfaces will upregulate neutrophils—the first leukocyte population to enter the wound site during the acute inflammatory phase of healing[56]—to a greater extent than platelets activated on smoother implant surfaces.[73]

Thus, as in fracture healing, the migration of osteogenic cells in peri-implant healing will occur through the transient three-dimensional biological matrix formed as a product of the coagulation cascade—the fibrin of the blood clot—and may be both potentiated and directed, either directly or indirectly through knock-on stimulatory events involving leukocytes,[74] by the release of cytokines, growth factors, and microparticles from platelets activated by contact with the implant surface.

Fibrin: The Transitory Matrix

Since fibrin, the reaction product of thrombin and fibrinogen released into the healing site, can be expected to adhere to almost all surfaces, osteogenic cell migration may be expected towards any implanted material. However, as is well known in dermal wound healing models, connective tissue cell migration is concomitant with wound contraction, which usually begins around the fifth day postwounding.[75,76] Indeed, the migration of fibroblasts has been recognized as responsible for wound contraction,[77] with individual cell adhesive contacts transducing a contractile force of approximately 3 nN.[78] This ability of cells to contract the matrix through which they migrate can be modeled in vitro[47,79,80] and, in the bony peri-implant site could possibly cause retraction of the transitory fibrin scaffold away from the implant surface (see Figure 6A). The fact that primary osteogenic cells can cause fi-

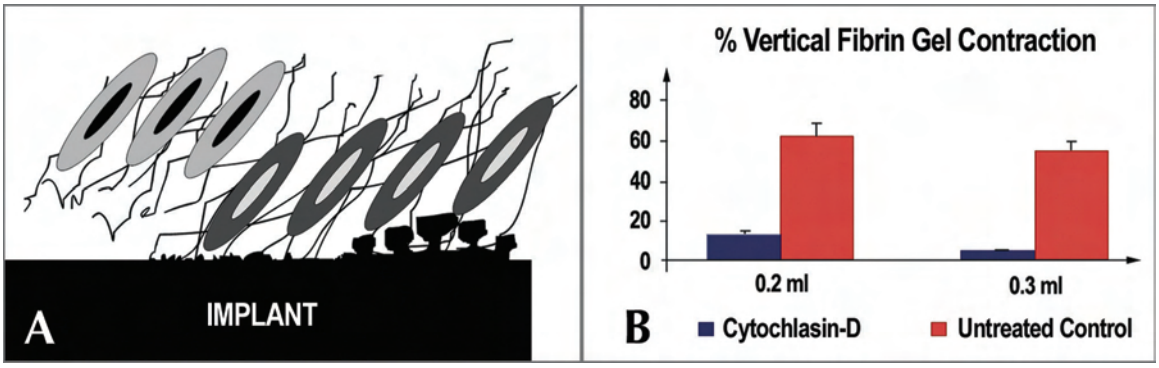

Figure 6A. Drawing to illustrate the migration of cells through the transitory fibrin matrix of the blood clot toward the implant surface. The central issue here is not whether fibrin will adhere to the implant surface, but whether the strength of attachment of fibrin will be great enough to withstand the contractile forces imposed on the fibrin by the active migration of osteogenic cells. These tractional forces can, theoretically, cause detachment of the transitory matrix from the implant surface. The latter, as illustrated, could be of many different surface geometries to ensure fibrin detachment does not occur. Again, a computer animation of this process is available at www.ecf.utoronto.ca/~bonehead/ (follow buttons to fibrin retention).

Figure 6B. Experimental evidence shows that intracellular forces are responsible for creating the traction mentioned in (a) can be gained from allowing primary osteogenic cells to migrate through fibrin in vitro in the presence, or absence, of cytochalsin-D. Migrating cells cause contraction of the fibrin gel (untreated cells shown in the presence of 2 different volumes of fibrin gel). However, if the cell cultures are treated with cytochlasin-D, which blocks actin-dependent cell processes, little fibrin contraction is seen. Modified from Reference 45.

brin contraction can be demonstrated by treating primary osteogenic cell cultures with cytochlasin-D, which blocks actin-dependent cell processes, such as cell migration, by capping actin filaments (Figure 6B).[81] Thus, the ability of an implant surface to retain fibrin during this wound contraction phase of healing is critical in determining if the migrating cells will reach the former. The implant surface design will play an important role in this fibrin retention. Indeed, if implants are recovered a few days after implantation, the adhesion of this transitory matrix to some surfaces can be easily visualized (Figure 7).

Such simple in vivo assays also illustrate another important, clinically relevant, issue. If fibrin retention is so critical to osteogenic cell migration to the implant surface, as a prelude to contact osteogenesis, then diluting the implant-attached fibrin could compromise the attachment of fibrin to the implant surface. The clinical practice of wetting an implant prior to insertion could therefore be expected to dilute implant-contacting fibrin and compromise osteogenic cell migration by reducing fibrin retention to the implant surface. This can be demonstrated experimentally and has resulted in some implantologists omitting the implant-wetting step prior to implant placement.

Thus, bone cells will reach the implant surface by migration through fibrin (and other early structural matrix proteins which are omitted here for simplicity), and these cells will then be available to synthesize *de novo* bone on the implant surface itself. In so doing they also stop migrating, and other cells, still in migratory mode, will gain the contiguous implant surface and secrete bone (see www.ecf.utoronto.ca/~bonehead/ for computer animations of these important events). The histological result will present itself as the apparent "flowing" of bone over the implant, although the bone matrix itself has no inherent capacity to "flow," and this histological effect is created by the matrix secretory activities of previously migratory osteogenic cells.

Therefore, the phenomenon of osteoconduction critically relies upon the migration of differentiating osteogenic cells to, and over, the implant surface. The implant surface design can have a profound influence on osteoconduction not only by modulating the levels of platelet activation, but also by maintaining the anchorage of the temporary scaffold through which these cells reach the implant surface.

It can be predicted that microtopographically complex surfaces would promote osteoconduction by both increasing the available surface area for fibrin attachment and providing surface features with which

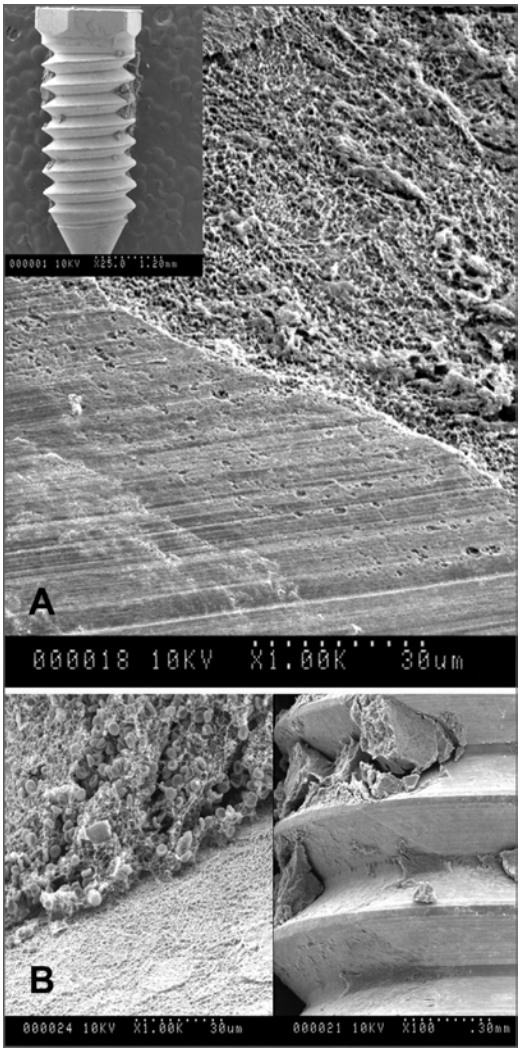

Figure 7A. Scanning electron micrograph (SEM) of the surface of a small (2mm diameter) titanium implant where half of the machined surface (lower left) has been dual acid etched (longitudinally) to create a microtopographic surface (upper right). The implant shape is shown in the inset. These are the same implants as employed in the human studies reported in Reference 25.

Figure 7B. An implant, such as that shown in (A), was recovered after three days' implantation in rat distal femur. The screw thread is shown on the left, and the junction between the machined and acid-etch surfaces is just visible running vertically. Far more tissue is attached to the acid-etched surface (left side) than the machined surface (right side). At higher magnification (right micrograph) of the acid-etched surface, it can be seen that the tissue adhering to the surface is a 3-D structural network (the transitory fibrin matrix) packed with red blood cells (the blood clot). Examination of the machined surface, of the same implant, showed that the blood clot had become detached as a result of specimen preparation for SE

fibrin could become entangled; they could also be potentiating the activation of platelets, which will produce density gradients of cytokines and growth factors through which leukocytes and osteogenic cells will enter the healing compartment.

De Novo Bone Formation

Finally, when the osteogenic cells reach the implant surface, they can initiate bone matrix formation. As mentioned previously, the initial stage of bone formation at remodeling sites is the secretion, by osteogenic cells, of the cement line matrix.[24,25] This is a collagen-free, mineralized interfacial matrix laid down between old bone and new bone. Despite the early description of this prominent histological feature in bone, the cement line interface eluded both structural and compositional characterization for over a century,[23,82,83] but is now becoming accepted as the tissue that occupies the bone/implant interface formed through contact osteogenesis.

This *de novo* bone formation cascade can be arbitrarily subdivided into a four-stage process, which has been confirmed by both in vitro and in vivo experiments.[25,73] Briefly, differentiating osteogenic cells initially secrete a collagen-free organic matrix that provides nucleation sites for calcium phosphate mineralization. We have identified two non-collagenous bone proteins, osteopontin and bone sialoprotein, and two proteoglycans[84] in this initial organic phase, but no collagen. The lack of collagen in this matrix concurs with the original observations of Weidenreich.[85] Calcium phosphate nucleation is followed by crystal growth and the initiation of collagen fiber assembly. Finally, calcification of the collagen compartment will occur. Thus, in this process of *de novo* bone formation comprising the second key element of contact osteogenesis, the collagen compartment of bone will be separated from the underlying substratum by a collagen-free calcified tissue layer containing non-collagenous bone proteins. This layer can be seen from the retrieved implants shown in Figure 8.

Implant Surface Microtexture and Chemistry

But why focus on the microtexture, rather than the surface chemistry of an implant? This question

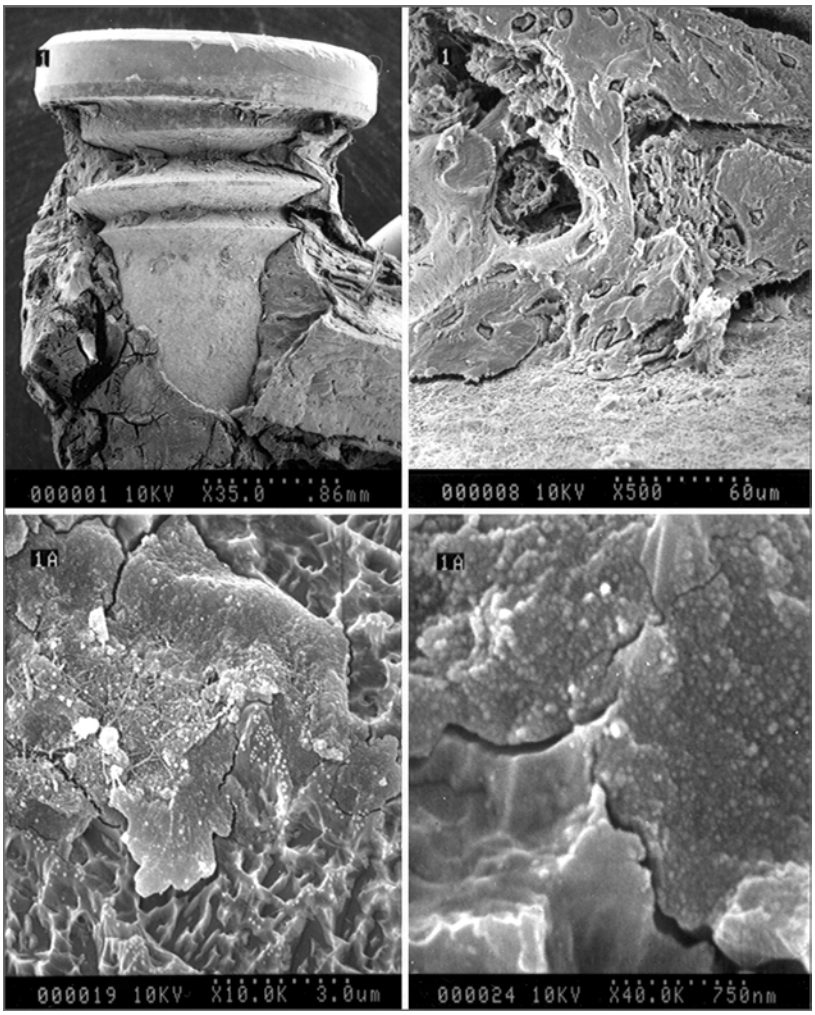

Figure 8. Scanning electron micrographs of mini-implants retrieved from rat femur, showing the apposition of bone to the implant surface. The image at top left shows the custom-made implant with a short thread to engage cortical bone and a parallel-sided cylindrical shaft that is acid etched to create the topography seen bottom left. This implant surface topography is equivalent to that of the implant illustrated in Figure 7. The image at top right shows bone, with osteocyte lacunae and vasculature, juxtaposed to the implant surface. The lower images show more detail of the bone growing on the implant surface in the medullary healing compartment comprising a thin extracellular matrix interdigitating with, and closely adapted to, the micron-scale topographic features of the acid-etched surface. Collagen can be seen to be distinct from the interfacial matrix (lower left) that separates the collagen from the implant surface.

clearly requires some explanation not only to throw some light on the reasons for the increasing development of dental implants with microtextured surfaces, which all have slightly different surface chemistries, but also because it has long been reported that early healing can be accelerated when the surface of a metallic implant is coated with calcium phosphate.[86] With respect to metallic implants, there are two distinct issues here.

First, many reports have been published comparing the surface chemistry of groups of commercially available metallic dental implants by methods such as Auger spectroscopy (AS), X-ray photoelectron spectroscopy (XPS),[87] or Time-of-Flight Secondary Ion Mass Spectrometry (TOF-SIMS), which have shown differences in the surface chemical composition of commercially available dental implants.[88] XPS, for example, provides exquisite chemical information generated from a 10nm deep surface zone of the sample material (1000nm = 1micron), and the use of this and other techniques applied to titanium has been exhaustively reviewed elsewhere.[89] However, it has also been recognized that there is no biological relevance to differences in surface chemical

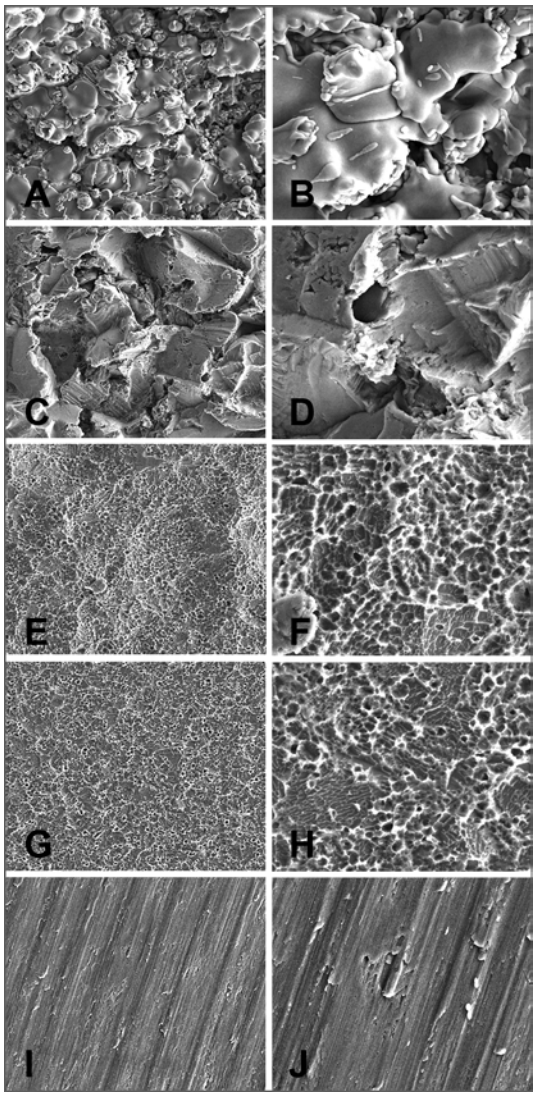

Figure 9. SEM photomicrographs of candidate cpTi surfaces. Low (left) and high (right) magnification of cpTi surfaces as used for surface characterization. (A, B): Plasma–sprayed cpTi (TPS); (C, D): Grit-blasted cpTi (GB); (E, F): Grit-blasted and dual acid-etched cpTi (GBAE); (G, H): Dual acid-etched cpTi (AE); (I, J): Machined (turned) cpTi (M). Field widths: (A, C, E, G, I): 234 μm; (B, D, F, H, J): 58 mm. From Reference 85, with permission.

composition of, for example, titanium alloys with respect to commercially pure titanium.[90] Nevertheless, microtextured surfaces do promote early healing and, as shown in Figure 6A, many different surfaces can be expected to effectively retain fibrin during the critical osteogenic cell migration stage of osteoconduction. This would suggest that implant surface topography plays a more important role in early healing than small variations in surface chemistry.

Second, it is equally obvious that surfaces of different surface topography may be more or less efficient at retaining fibrin. A selection of candidate implant surfaces is shown in Figure 9, each of which is commercially pure titanium of diminishing surface topographic complexity.[91] Fibrin retention can be measured by interposing a fibrin clot between two discs of similar surface topography and separating the discs using an apparatus that can measure the force needed to separate the discs and, ultimately, cause the failure of the fibrin/implant interface. So, while the surfaces in Figure 9 have remarkably similar surface chemical composition, as shown in Figure 10A, some of these surfaces demonstrate significantly different fibrin retention forces as illustrated in Figure 10B. Again, these results emphasize the importance of implant surface microtopography in orchestrating the biological cascades of early peri-implant endosseous healing. Interestingly, in Figure 10B, grit blasted, blasted and acid etched, and acid etched render (with this assay) no statistically different values in fibrin retention, yet their topographies are of decreasing complexity. This indicates that the least topographically complex of these surfaces is sufficient not only to retain fibrin but, as we have also shown in vivo (Figure 6), can also retain fibrin during the important initial stages of healing, which are concomitant with cell traction (see the fibrin retention animation at www.ecf.utoronto.ca/~bonehead/).

Nevertheless, this still doesn't help us understand the effects of calcium phosphate coating of implant surfaces. It is generally accepted that calcium phosphate materials, whether they are employed as lithomorphs or coatings, provide two advantages over most other endosseous materials. First, they accelerate early healing. Second, they "bond to bone."[92] We shall deal with the second issue, bone bonding, separately below, but how do calcium phosphates accelerate healing? An argument is sometimes made that this acceleration of healing is a result of the bone-bonding character of calcium phosphates; but this circular, and useless, argument relies on the formation of bone—a phenomenon that, we now understand, occurs after the most important stages of early endosseous healing have occurred! Clearly, therefore, a bone-bonding argument cannot be invoked to explain the acceleration of early healing.

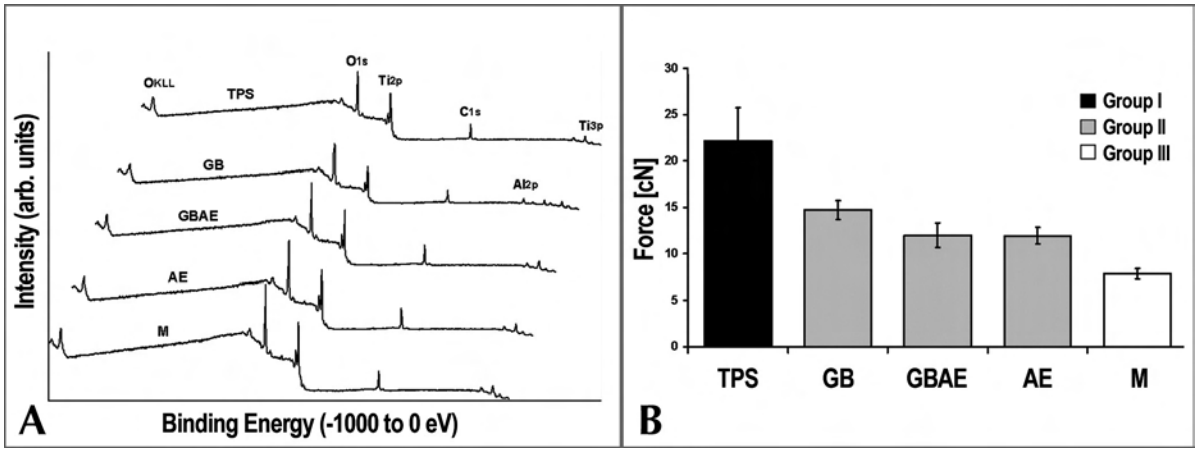

Figure 10 A. Low resolution X-ray photoelectron (XPS) spectroscopy of cpTi surfaces. Staggered depiction of the XPS binding energy profiles for cpTi surfaces. TPS: plasma-sprayed; GB: grit-blasted; GBAE: grit-blasted and dual acid-etched; AE: dual acid-etched; M: machined. Titanium oxide (Ti2p, Ti3p, OKLL, O1s), as well as residual carbon (C1s) are detected at all surfaces. At the GB surface, the additional presence of alumina (Al2p) is detected, which is due to residual Al_2O_3 particles from the grit blasting procedure.

Figure 10 B. Mean peak retention forces of fibrin clots with physiological concentration to cpTi surfaces. Mean peak retention forces (±SEM) are depicted for the different cpTi surfaces. Three statistically significant groups ($p < 0.05$) are discernible: (TPS) >p = 0.0056 (GB, GBAE, AE) >p=0.020 (M). TPS: Plasma-sprayed; GB: Grit-blasted; GBAE: Grit-blasted and dual acid-etched; AE: Dual acid-etched; M: Machined. Both (A) and (B) from Ref.85, with permission. The higher values attained with the TPS surface can be explained by the wrapping of fibrin bundles around the large, undercut, topographical features of this surface. Interestingly, the micron-scale features of the AE surface (see also Figure 9 G, H) have proven sufficient to clinically retain fibrin and permit contact osteogenesis.

However, what is known about calcium phosphates is that they readily adsorb proteins to their surfaces. Potentiating protein adsorption on calcium phosphate surfaces (with respect to uncoated metal oxide surfaces) could be expected to increase the binding of, for example, fibrinogen that would lead to increased platelet adhesion and, possibly, result in increased platelet activation that would accelerate healing. Increasing protein adsorption could also include an increase of, or improvement in, fibrin binding to the implant surface resulting in an earlier establishment of the three-dimensional matrix through which osteogenic cells have to migrate to reach the implant surface. Thus, calcium phosphate coatings could have a biphasic effect on both platelet activation and fibrin binding. The question remains whether such effects would be the result of the chemical composition of the calcium phosphate coating. Results (unpublished) emerging from our laboratory would suggest that platelet activation on calcium phosphate surfaces is a function of the surface topography of the calcium phosphate, rather than due to the presence of calcium and phosphate ions in the surface of the material. Such experiments are particularly difficult to design because, at the sub-micron scale range, it is almost impossible to vary substrate surface chemistry without altering the substrate topography. This is particularly important when one considers that most commercially available calcium phosphate coated implants are plasma sprayed and have an exceedingly complex topography which approximates to that of the TPS surface shown in Figure 9A,B but is more complex still as a result of selective dissolution of the various phases of calcium phosphates that are always present in plasma-sprayed calcium phosphate coatings. Thus reports that suggest that the presence of calcium and phosphate coatings on the surface of a titanium implant increase bone contact are unable to deconvolute the effects of surface chemical composition from those of the changes in surface topography.[93]

One question remains: What is the nature of the bond between calcium phosphate-coated implant surfaces and bone? The biological cascade of *de novo* bone formation has been shown to occur *in vivo*, as described above with the formation of the cement line interfacial matrix, on many candidate implant materials including metals and ceramics.[94-96] Indeed, after 540 million years of evolution, it would be presumptuous to assume that the presence of an implant

could generate a physiologically more refined interface than that which occurs in our bodies, throughout life, at remodeling sites. Nevertheless, since the pioneering work of Hench, two classes of endosseous implants have been identified: bone-bonding and nonbonding.[97] Metals, such as titanium, are nonbonding, whereas calcium phosphate materials are considered bone-bonding. However, we should be cautious. The phrase "bone-bonding" is quite misleading since it implies that an inanimate implant material can bond to bone or create the driving force for the bonding mechanism with bone. Therefore, more accurately, one should say that bone bonds to calcium phosphates. This, of course, then begs the question: What is the mechanism of bone-bonding?

The mechanism for the bone-bonding phenomenon is generally accepted to be a chemical interaction that results in collagen, from the bony compartment, interdigitating with the chemically active surface of the implant. Clearly, in the case of *de novo* bone formation and contact osteogenesis, this mechanism is inconceivable since the first extracellular matrix elaborated by bone cells at the implant surface is collagen-free. As cement lines are found on both nonbonding and bonding biomaterials, then a reevaluation of the phenomenon of bone-bonding is essential. In brief, while chemical hypotheses to explain bone-bonding have been generally adopted in the literature, experimental evidence demonstrates than in cases of *de novo* bone formation at implant surface, bonding is achieved by micro-mechanical interdigitation of the cement line with the material surface.[98] Thus, again, we now realize that implant surface microtopography is critical to not only the generation of contact osteogenesis, but also whether the elaborated bone matrix will bond to that surface.

Concluding Remarks

There are still many aspects of peri-implant healing that need to be elucidated, but we can now state that the healing patterns in cortical and trabecular bone are different and reflect the evolved form and function of this exquisite tissue. Cortical healing relies on osteonal remodeling, while trabecular healing can invoke the phenomena of osteoconduction and *de novo* bone formation that, combined, result in contact osteogenesis. Indeed, one can state that trabecular bone, previously characterized as "poor quality" bone, is far better adapted to rapid healing than cortical bone. Osteoconduction is a term that encompasses the recruitment and migration of populations of osteogenic cells to the implant surface through the residue of the peri-implant blood clot. Blood cell activities in this initial clot, particularly the activation of platelets and leukocytes, are a function of implant surface microtexture. The latter is also of key importance in the retention to the implant surface of the fibrin through which the cells migrate. Peri-implant angiogenesis will also be important at this stage, although currently we know little about the effect of implant surface design on this aspect of peri-implant healing. Nevertheless, it can be concluded that treatment outcomes employing endosseous implants are critically dependent upon implant surface designs that optimize the biological responses of early endosseous peri-implant healing.

Acknowledgments

The ideas expressed herein have evolved over several years as a result of interactions with generations of my graduate research students and fellows who have carried out our contributions to the work reported, together with an ever increasing population of dental implantologists who have enthusiastically showered us with questions of direct importance to their clinical practices. I want to acknowledge the stimulus that these national and international colleagues have provided for our research. The work has been funded by the Medical Research Council Canada (now the Canadian Institutes of Health Research), the Natural Sciences and Engineering Research Council of Canada, the Ontario Research and Development Challenge Fund, and Implant Innovations Inc. in Palm Beach Gardens, Florida.

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This re-issue of Dr. Davies' paper has been produced as some of the figures appearing in the original publication of the Journal of Dental Education were indistinct.

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