Generalizations Regarding the Process and Phenomenon of Osseointegration.
Part I. In Vivo Studies

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The clinical success of endosseous implants is associated with the formation and maintenance of bone at implant surfaces. Histologic analyses have indicated that bone formation at a variety of implant surfaces is a continuous process that supports long-term functional integration. Based on in vivo observations, several generalizations have been derived regarding the nature of the interface. Experimental descriptions indicate that the implant-bone interface may be characterized in spatial and temporal terms as discontinuous. Biomechanical tests of the bone associations with implants demonstrate that the chemical composition and the surface topography of the implant influence the rate and extent of bone formation at implant surfaces. The precise character and functional attributes of this interface are the focus of this investigation. Many technical difficulties are associated with its structural and chemical characterization in vivo. Despite the technically difficult nature of this type of analysis and the limitations of current histologic examinations and biomechanical tests, in vivo models of osseointegration are necessary experimental tools for the continued empirical development of clinical implant applications.
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Implant placement in bone is presently associated with defined expectations of success based on defined clinical and radiographic endpoints.[1] This successful outcome has been correlated to the histologically represented bone-implant interface and is commonly referred to as "osseointegration."[2,3] Albrektsson and Sennerby[4] suggest that the experimental definitions are based on either biomechanical or structural considerations. A meaningful definition must also reflect the processes that produce and maintain bone at alloplastic interfaces. With regard to process, the actual determinants of osseointegration are not well defined. Attaining the result of osseointegration requires a prescribed surgical procedure that has been successfully applied using several specific implant designs and materials. It is presently unclear to what extent the surgical process of implant placement contributes to osteogenesis at implant surfaces; however, the bone's response to surgery may be a common determinant of success using different implants.[5]

It is also not known precisely how implant surfaces contribute to or modify the process of bone formation. Ceramic and metallic implant materials are not truly osteogenic—that is, capable of inducing bone forma-

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tion at ectopic sites by recruitment or stimulation of precursor osteoblasts. Current implant surfaces may not display the property of osteoinduction, a process that supports the mitogenesis of undifferentiated mesenchymal cells, leading to the formation of osteoprogenitor cells to create new bone.[6] Implant material surfaces may differ considerably in their capacity to support osteoconduction, a process that involves the ingrowth of sprouting capillaries, perivascular tissues, and osteoprogenitor cells from the recipient host bed into an implant or graft.[6] Can implant surfaces be custom-tailored for osteoblasts or bone?

To begin to address this question, the endosseous implant literature has been reviewed with the goal of allowing specific generalizations to be made regarding *(a)* the process of bone formation at various implant surfaces, and *(b)* the morphology and character of the bone-implant interface that is a hallmark of osseointegration. Part I of this report features a comprehensive review of published observations from in vivo studies of the bone-implant interface. Several generalizations regarding the structure and biome-

chanical attributes of the bone-implant interface have been derived. This review reiterates the observation that many in vivo models for the assessment of bone's response to implants reflect static measures of healed tissues that fail to identify many of the initial determinants of bone formation at implants.[7] In Part II of this review, in vitro studies that seek to define the interaction of cells at implant surfaces and the composition of an osteoblast-formed interface on implant surfaces have been reviewed. Together, this body of literature demonstrates that the precise molecular nature of the interface and the cellular process of bone formation at an artificial surface is still relatively undefined. Continued attempts to improve the application of osseointegration require careful attention to the molecular and cellular details that may represent critical determinants of the rate, extent, and maintenance of bone formed at implants.

Materials and Methods

This review is based on a survey of the current literature using the MEDLINE database (Fig 1), available through the Health Sciences Library at the University of North Carolina. The text word "osseointegration" and the text phrases "dental implantation" and "bone-implant interface" were used to search the database. The results indicate that an early interest in clinical "implantology" has been supplanted by a biologic interest in osseointegration and the nature of the bone-implant interface (Figs 2a and 2b). In addition to the information found in the MEDLINE database, the authors have identified and reviewed relevant symposium textbooks and monographs published since 1985.

A number of general features of the result termed "osseointegration" can be derived from representative in vivo studies (Table 1); these can be catego-
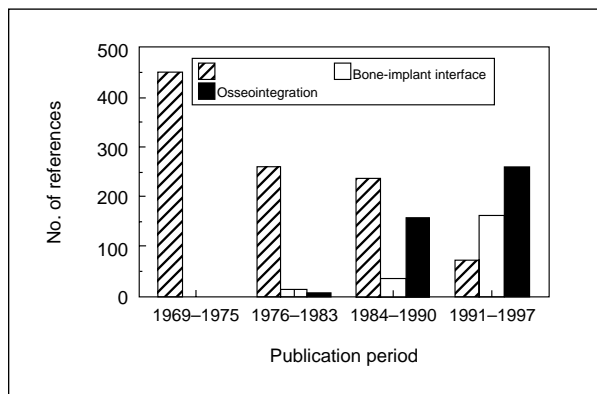

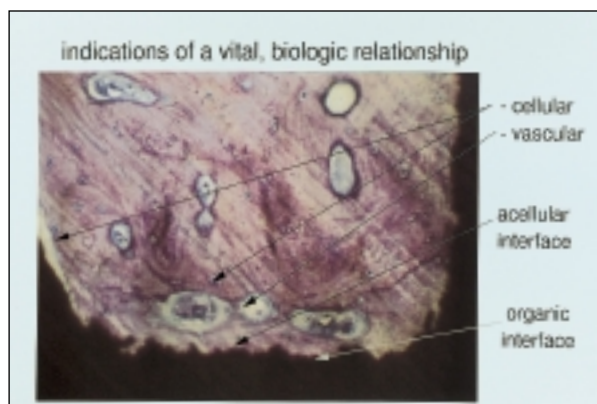**Fig 1** Implant-related literature, 1969 to 1997.

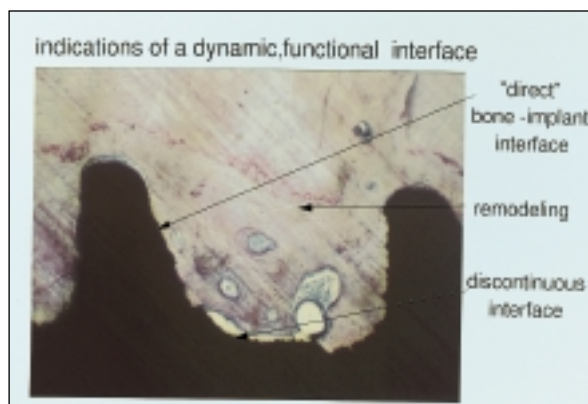**Fig 2a** Indications of a vital, biologic relationship between the implant and bone.

Fig 2b Indications of a dynamic, functional interface between the implant and bone.

Table 1 Summary of In Vivo Investigations of the Implant-Bone Interface

Site	Species	Duration	Materials	Observations	Reference
Mandible	Baboon	12 weeks–6 months	Ti, aTi	LM, SEM, TEM, biomech	52
	Dog	2 days–5 years	Ti, aTi, HA, AIO	LM, SEM, TEM, biomech	16,32,33,36,39,50,77,82,93,95,106,109,117
					7–122
			Ti, HA		
	Goat	2–4 weeks	HA	LM, biomech	51
	Monkey	2 weeks–16 months	Ti	LM	27,28,107,123,124
	Pig	6, 18 months	Ti, HA	SEM	45
	Human	1–7 years		LM, SEM, TEM, laser scan	11,13,15,113,125
Maxilla	Baboon	12 weeks–6 months	Ti, aTi	LM, SEM, TEM, biomech	52
	Dog	2–5 months	Ti	LM	46,119
	Goat	2–24 weeks	Ti	LM, biomech	51,118
	Monkey	1–3 months	HA	LM	123,124
	Rat	2–6 weeks	Ti, HA	LM, SEM, TEM	60,86
	Human	15 months	HA	LM, TEM	88
Femur	Cat	6 weeks	Ti, GI	LM	126
	Dog	4–32 weeks	Ti, HA, AIO, PMMA, Carbon	LM, SEM, biomech	12,30,53,68
	Rabbit	8 weeks–6 months	Ti, aTi, HA	LM, laser scan, biomech	25,35,43,49,70,115,127
	Rat	3 days–6 months	HA	LM, SEM, TEM,	87,96,97,100,116
	Pig	3–6 weeks	Ti, HA	LM	34
Tibia	Rabbit	3 days–11 months	Ti, aTi, TiO, SS, CoCr, HA	LM, SEM, TEM, biomech	37,40–42,44,49,70,84,85,92,97,112,128,129
					81,83,124
	Rat	1 day–5 months	Ti, SS, GI	LM, SEM, TEM	34,113,130,131
	Pig	3 weeks–4 months	Ti, HA	LM	111
	Mouse	1–18 months	Ti	LM	
Mastoid process	Human	89–175 days	Ti	LM, biomech	17
Hip prosthesis	Human	2–18 months	Ti, aTi, HA	LM, SEM, TEM,	20
Knee joint	Dog	16 weeks	Ti, HA	LM, biomech	99
	Rabbit	6 months	Ti, HA	biomech	49,69,78
	Pig	12 weeks	Ti, aTi, HA	LM, biomech	38
	Human	6–131 weeks	Ti, aTi, HA	LM	5,19

Site-specific experimentation listed according to species (columns 1 and 2). The duration of experiments and various materials investigated (Ti = titanium, aTi = titanium alloy, HA = hydroxyapatite, SS = stainless steel, CoCr = chromium cobalt alloy, GI = glass, AIO = alumina) are listed in columns 3 and 4. The histologic methods used (LM = light microscopy, SEM = scanning electron microscopy, TEM = transmission electron microscopy) or biomechanical testing are indicated in column 5. References are provided in column 6.

rized as either structural or biomechanical. The structural analyses have been conducted at macroscopic, microscopic, and ultrastructural levels. Biomechanical examinations of the physical character of the bone-implant connection have used mechanical means (pull off, push out/pull out, torque) to induce and measure bone-implant connection failure. To date, there have been few biochemical or molecular analyses of forming bone at endosseous implanted surfaces. Within this category of studies are a series of investigations using osteoblast matrix vessicle formation and content to examine implant effects on bone marrow ablation–related osteogenesis[7,8] and the measurement of bone alkaline phosphatase activity and calcium present in tissue surrounding healed implants in rat tibia.[9]

Light Microscopic Investigations and Interpretations

The most striking common characteristic of osseointegration is that bone opposes the implant surface without an intervening organized collagenous and fibroblastic matrix. This is the light microscopic histologic result classically associated with the clinical result of osseointegration.[10] This result has been observed in a wide variety of animal models, which all provide for the formation of bone at different types of implant surfaces (Table 1). The extent to which the surface is opposed by mineralized matrix is variable, depending on the implant surface character,[10] the time at which the sample was evaluated, and, perhaps, the animal model.

Light microscopic analysis of the bone-implant interface traditionally has focused on the relative presence of mineralized versus fibrous connective tissue opposing the implant surface. Ground sections (5 to 10 μm) provide ample demonstration of bone, osteoid, and fibrous connective tissues using stains that include toluidine blue with or without basic fuchsin[11–13] or hematoxylin and eosin. Osteoblastic cells, osteoclastic cells, multinucleate giant cells, eosinophils, monocytes, vascular elements, collagenous matrix, and mineralized matrix are identifiable. Morphometric analysis of the various cellular components within bone at implants could provide valuable insight into ongoing activities at measured interfaces. Many of these key aspects of the bone-implant interface are illustrated in Figs 2a and 2b.

Implants have been placed in a range of animals, including mouse, rat, cat, dog, pig, goat, and monkey (references summarized in Table 1). Osseointegration is achieved in diverse osseous locations. To study the bone-implant interface in vivo, implants have been placed transcortically in the mandible, maxilla, and in long bones. Cheng et al[14] quantified the relative amount of bone formed at rabbit periosteal, endosteal, and marrow stromal regions at commercially pure titanium implants (periosteal > endosteal >> marrow). Comparing results among many different models requires consideration of site selection. Many studies involve two-stage surgeries, while a few offer single-stage intraoral models. Generally, cortical fixation has been a goal of experimental surgeries. Human data have also been obtained through the retrieval of integrated and failed implants from human jaws,[11,13,15,16] mastoid process,[17] knees,[18,19] and hips.[20]

While there are clear similarities among the reported findings from this broad range of in vivo studies, some differences exist among these representations of osseointegration. The differences may represent variables among studies involving the characteristics of the implant and surgery, the temporal extent of healing, the location of the implant (anatomy of supporting bone), and the relative health and functional state of the implant at the time of analysis. Regarding the temporal ranges used in these investigations, the analytic endpoint appears to be somewhat arbitrary and suited to the animal model under investigation. This issue of time frame is important in that it may confuse the interpretation of data among studies. The temporal changes of bone at osseointegrated implants were examined in the rat tibia at 28 and 730 days. The rapid accumulation of bone (43% bone-implant contact) apparent at 28 days was followed by progressive bone apposition to 730 days (89% bone-implant contact).[21] The extent of healing

as a function of time has not been standardized to a biologic endpoint. A uniform molecular or cellular endpoint common to all animal models has not been identified or selected. Furthermore, healing at implant surfaces has not been standardized to a biologic control, such as the critical size defect used in bone regeneration studies.[22]

Local anatomic considerations affect outcomes; the relative contribution of cortical versus cancellous bone is one such detail.[18] These major issues, which affect our interpretation of data and our hypotheses concerning the process of osseointegration, may not be reconciled by this attempt to review the literature concerning in vivo experimental techniques that employ different animals, different implants (materials, shapes, surfaces), different surgical and retrieval techniques, and varying types of analyses. Finally, age may be another variable worthy of consideration. Bone formation at implants in young rats was greater than in mature rats. Bone contact at implants in young rats was mediated by a thick amorphous zone, while a well-developed connective tissue was seen at the implant surface of mature rats.[23,24]

The histomorphometric analytic methods analyses based on "best thread" data, often reflecting a predominant contribution of cortical bone, are meaningful, but require careful comparison to less-defined data. A cell-based index (osteocytes/unit area)[17,112] has not been adopted or further developed. Included are measures of total bone-metal contact (as percentage of total surface), bone-metal contact at the three best consecutive threads in the cortical passage, and the total bone area within the threads, or a "mirror-image" analysis[25] used to compare bone area occupying the "inside" of a thread to that "outside" the thread. Noninvasive methods of quantifying the amount of bone at the implant-bone interface are advantageous. Hollister et al[24] have demonstrated that computed tomography (CT) provides a clear representation of trabecular bone architecture adjacent to transcortical implants. Additional development of three-dimensional analyses[26–28] may contribute to a definition of a minimal value for bone formation at implants. In addition to the histomorphometric analysis of bone surrounding cylindrical implants, bone ingrowth models utilize bone harvest chambers[29] and ingrowth coupons.[30] Such models may prove to be suitable for quantifying rates of bone formation.

It can be concluded from many investigations that bone formation at titanium implant surfaces in experimental models is less than 100% and can vary considerably among test surfaces. A recent comparison of bone-implant contact at niobium, commercially pure titanium (cpTi), Vitallium, and titanium-aluminum-vanadium (Ti-6A1-4V) surfaces in rabbit

bone indicated that there was 18.5 to 78% contact.[10] Other studies in experimental animals support the observation that less than 100% contact is typically achieved. Values of 60 to 85% bone apposition (best thread analysis) at functioning implant surfaces were obtained for functioning implants removed from humans. On the basis of this preliminary evaluation limited to anterior edentulous jaws, it was tentatively suggested that "osseointegration" corresponds to 60% or more bony contact and 70% or more bone filling of individual threads at the cortical passage.[31]

Bone formation at implant surfaces is not a dichotomous phenomenon. This appears to be true even for the osteoconductive hydroxyapatite surfaces. While de Lange and de Putter,[32] de Lange and Donath,[33] and Cook et al[12] claimed 100% interface, several others, including Buser et al,[34] Piatelli et al,[35] Kohri et al,[36] Jansen et al,[37] and Wong et al,[38] have demonstrated that the interface of integrated hydroxyapatite cylindrical implants is in contact with bone from 60 to 85%. "Discontinuous" may be a better description of the interface than "incomplete" and may be interpreted in both temporal and spatial terms, thus reflecting a bias toward an understanding of osseointegration as a process and not as a result.

While qualitative assessments at the light microscopic level indicate that many implants achieve a direct bone-to-implant contact, quantitative assessments continue to demonstrate differences in the amount of interfacial bone at different implant surfaces. Albrektsson and Johansson[10] indicate that the proportion of direct bone-to-metal contact varies with the material and design of the implant, as well as the state of the host, the surgical technique, the loading conditions, and time.[4,39–46]

Relationship of Histologic Findings and Biomechanical Attributes of Implants

The amount of bone formed at surfaces may be related to biomechanical attributes of the implant. The scope of this review cannot include the biomechanical theory of bone and the bone-implant interface; however, it is important to review the biomechanical findings in relation to histologic interpretations of the bone-implant interface. How do the biomechanical attributes reflect the histologic attributes of the bone-implant interface?

The physical association of implants with enveloping bone has been measured for various implant types, and the existing data have recently been reviewed (see Thomas[47] and Brunski[48]). While some data suggest that integrated surface area is well correlated with biomechanical behavior,[25,38,49] other data demonstrate that the strength of association does not

increase with increased bone apposition.[50] That is, the amount of bone at a surface does not fully characterize the biomechanical attributes of the interface. More sophisticated assessment of the relationship of histology to function is presently required. The biomechanical analyses are also difficult to interpret, in part because the relative contributions of cortical versus cancellous bone to the biomechanical parameters measured have not been fully evaluated.[49,51,52]

Although specific conclusions are difficult to derive, several general features of osseointegration have been illustrated by these biomechanical studies. First, the strength of interaction is significantly higher for osseointegrated implants than for fibrous encapsulated implants. Second, the strength of interaction between the implant and enveloping bone increases shortly after implant placement (0 to 12 weeks).[46,53] The initial processes of woven bone formation and its replacement of fibrous connective tissue may take place during this period. This initial rate of increasing strength of association is greater for hydroxyapatite (HA) surfaces than for titanium surfaces. Further gains in the strength of interaction continue over a minimal follow-up period of 1 year.[54] In humans, measured increases in bone-implant interactions occur for at least 3 years.[55] Thus, a threshold value for this interaction is only achieved following a protracted healing period. The temporally defined changes in biomechanical attributes of bone around implants suggest that modeling and remodeling of bone contribute to both the formation and the maintenance of osseointegration.[56] The direct application of biophysical stimuli to transcortical cylindrical implants conclusively demonstrated the significance of biophysical stimulation to the process of bony ingrowth at implants.[57]

The strength of association or connection of implants to surrounding bone may reflect the amount of bone at implant surfaces. This appealing hypothesis has received some support, but direct, concise proof has been difficult to attain. The rat model has yielded compelling support for this hypothesis.[58] The type of bone may further influence the biomechanical behavior of implants. For example, the relative value of cortical bone may confound simple interpretations of implant design.[59]

Interfacial strengths may reflect an adhesive parameter that should be related to the degree of surface attachment to bone. Studies of the tensile strength of titanium implants to bone suggest the importance of adhesive phenomenon to the physical connection of implants with bone.[60,61] When rabbit bone at transcortical implants was detached by an Instron-type device, detaching failure loads at titanium implants were virtually 0 kg at 8 weeks, and increased

to 2.85 kg at 25 weeks. Corresponding light microscopy and scanning electron microscopy (SEM) analysis revealed an equal amount of direct bone-implant contact at both 8 and 25 weeks. Techniques for assessing the physical quality of bone and attachment at implants are therefore required. In comparison, tensile failure at surface active bioglass and hydroxyapatite implant surfaces at 8 weeks was equal to or severalfold greater than that measured at 25 weeks for titanium implants.[56] Any adhesive interaction is dependent on bone formation at the implant surface and is affected by both implant surface chemistry and time.

Both macrostructural features, such as threads and grooves, as well as microstructural features imparted through plasma spraying, grit blasting, acid etching, machining, and polishing can significantly alter the biomechanical behavior of transcortical implants. The resulting microenvironment is now recognized as a key determinant of cell behavior at implants.[62] Nanostructural changes, such as intentional alteration of the oxide layer, affect bone responses to implants.[63,64] Surface properties, such as topography and roughness, oxide thickness, composition (purity), and microstructure, vary considerably among implants in use. For example, modification of implant oxide layers altered oxide thickness and surface roughness, which improved the interaction of implants with bone.[65] Current indications from animal studies suggest that these properties influence bone formation and bone adaptation to physical strain.

Surface roughness has been considered in the context of bony ingrowth, appositional bone formation, and tensile or torsional shear strength effects. For surface variables significantly larger than a cell (eg, 100 μm), bone ingrowth might be a significant parameter affecting osseointegration.[66] This magnitude of surface character, for example, as imparted by plasma spraying, provides a mechanical interlocking of bone and enhances tensile shear strength of the bone-implant interface.[67]

Great interest in the roughness and topography associated with grit-blasted or acid-etched substrates is presently demonstrated in the dental implant marketplace. Surface roughness imparted by pits ranging in magnitude from approximately 1.0 to 10.0 μm in diameter and depth has been shown to improve bone formation and torsional shear strength of the bone-implant interface.[38,68–70] Hansson[71] has described an ideal implant surface in mathematical terms that model the ideal topographic form to resist shear stress separated from bone by an interface of defined dimension. Imparting a surface topography related to this topographic ideal by titanium dioxide grit-blasting was shown to increase bone adaptation and bone fixa-

tion when compared with turned titanium implants.[50,72] Wennerberg et al[70] applied mathematical characterization of surface topography and roughness to examine surface roughness parameters on cpTi implant osseointegration. In a series of detailed and well-controlled studies, a surface created by aluminum oxide blasting with an average surface roughness of 1 to 1.5 μm and an ideal peak spacing of 9.6 to 11.1 μm displayed the greatest torsional shear strength in rabbit bone. In summarizing this work, Wennerberg et al indicated that superior bone fixation was obtained for blasted implants compared with as-machined implants. This work demonstrated that an optimal range of surface roughness exists; an implant surface may be too rough. The ideal surface may represent a balance of increased surface area, altered Ti ion release, and relative homogeneity of surface structure, resulting in improved load transfer and better mechanical interlocking.[70,72–75] The nanostructural attributes of these different surfaces were recognized as additional features for further investigation.

Surface roughness may have direct effects on the strength of interaction or may indirectly increase the strength of interaction by supporting greater bone formation or subsequent adaptation at implant surfaces.[38,69] Buser et al[34] indicated that, following HA-coated implants, grit-blasted surfaces supported more appositional bone formation than plasma-sprayed, machined, or electropolished materials. The biomechanical attributes were not defined. In another study of grit-based versus machined titanium implants, parallel histomorphometric analyses indicated that the amount of bone at the interface did not correlate with biomechanical behavior.[38,50,76–78] The delayed responses associated with bone adaptation must be added to the list of potential mechanisms by which surface roughness evokes favorable responses from bone at implants. Speculative caution regarding implants bearing rough surfaces has to do with the potential for the associated increased surface areas to lead to increased ionic leakage.[79] Promising data support the notion that engineering of implant surfaces positively affects bone formation and/or the biomechanical response of bone at implant surfaces.

Ultrastructural Characterization of Bone Formation at Implants: The Interface

The interface at metallic implants is a histologically distinct and significant structure, described as an organic layer interposed between the alloplastic surface and mineralized bone matrix.[80] Based on ultrastructural analyses, the interface is defined as distinct from mineralizing bone matrix or osteoid.[81] While

the composition of this interface has not been fully defined in biochemical terms, histologic evidence from in vivo and in vitro studies suggests that it is enriched in proteoglycans and glycoproteins. Much of this analysis has been performed at the morphologic level in dog,[32,82] rat,[83] and rabbit[84,85] models. Molecular analyses have recently been derived using immunologic probes in rat models.[60] The presence of osteopontin and α_2HS-glycoprotein clearly demonstrate the contribution of osteoblastic matrix proteins to the formation of the interface.[81,86] This electron-dense interfacial zone is proposed to represent a glycoprotein-rich, collagen-free region of extracellular matrix that resembles cement lines of bone.[82,87,88] A functional analogy to the cement line or reversal line of bone requires additional investigation.

The interfacial zone of metallic implants may not be homogenous. There are at least three types of interfacial morphologies that can be observed during histologic analysis of metallic implant samples. (For an excellent graphical comparison of results obtained from a number of investigations, refer to Albrektsson et al.[89]) Linder et al[84] suggest that these include (a) an acellular and amorphous collagen-free zone that is approximately 500 nm in thickness, (b) a 50-nm zone of amorphous material separating the implant from an organized collagenous matrix, and (c) a 500- to 600-nm zone containing a loosely organized filamentous material separating the implant surface from a collagenous matrix. Most importantly, this distribution of interfacial morphologies was observed for different metallic implant materials, including cpTi, stainless steel, and Vitallium.[44,84] Osseointegration of metallic implants involves the formation of an acellular, amorphous interface interposed between the implant surface and vital tissue. The observed heterogeneity may reflect multiple stages of a continuous process of interface remodeling.[56] In addition to reflecting the age of the interface, variations in the formed bone-implant interface may also reveal animal age effects.[23,90]

Ericson et al[91] suggest that a meaningful interpretation of the interface requires dissection of its molecular anatomy using analytic ultrastructural methods. They recently reviewed the problems and solutions that allow for careful analysis of metallic implants in bone. A survey of these methods includes sputter-coating of plastic plugs,[92] fracture techniques,[83–85,93] and electrochemical removal of the implant from embedded sections[94]: the character of sputtercoated materials may differ considerably from bulk materials; fracture methods may damage the intact interface; and electrochemical methods can result in demineralization of the interface and potentially alter the interface.[91] Interpretation of any result must

therefore be applied within the limitations of the sample preparation method.

Interpretation of interface morphologies must include consideration of the difficulties encountered in maintaining an intact interface throughout the processing of the bone-implant interface. Fixation, dehydration, and embedding of sections for processing have inherent problems that include incomplete fixation, incomplete dehydration, incomplete resin penetration, and incomplete polymerization of resins at the interface. Even if the interface is well fixed and embedded, sectioning by diamond saw methods can result in distortion of the interface; titanium is ductile and may be pulled across the interface. When high-quality, mineralized ground sections are obtained, their utility is limited because of (a) the thickness of the section (> 10 μm), and (b) limitations of various stainings and immunohistochemical analyses to the section. Alternately, implants can be removed from the bone following fixation to allow for subsequent sample demineralization and embedding. More detailed histochemical and immunohistologic analyses can be obtained. Immunohistochemical identification of collagen expression represents one example of biochemical assessment used to define bone formation in relationship to implants.[24] Continued effort must be made to pursue broader molecular and ultrastructural investigations.

The interface zone at HA implant surfaces is unique in that a continuity of the mineral phase of forming bone and the HA surface has been revealed. This continuity of mineral phases may form the basis of HA implant "bone bonding."[12,16,95,96] The process of epitaxic crystal growth has been implicated in chemically linking the HA implant surface with mineralizing matrix.[97] This may occur through an organic phase that contributes to the biochemical control of mineralization at surfaces. More rapid and direct mineralization at HA surfaces may reflect surface reactive phenomena. The osteoconductive behavior of HA may be related to its protein adhesive characteristics.[98] In a single study, a remarkable potential for osteoconduction was suggested by the preferential formation of bone at mobile HA-coated implant surfaces versus titanium implant surfaces.[99] Confirmation of this phenomenon is needed.

Observations of an electron-dense layer at the bone-HA interface are somewhat controversial. The organic interface at HA implants may display a similar 200- to 1000-nm thick amorphous zone separating the implant surface from a collagenous matrix.[32,100] While an uncalcified region at the interface has been attributed to demineralization protocols[32,86,87] or to a relatively immature interface, there is sufficient evidence to suggest that an organic interface, derived in

part from the extracellular matrix produced by osteoblasts, exists between forming bone and Ti as well as HA implant surfaces.[86,87] Structural differences in interface composition among Ti and HA surfaces could account for the relatively high reactivity (epitaxy, dissolution) observed at HA surfaces. There is continued need to seek correlations between surface properties and biologic responses observed in vivo. Two separate studies have recently reported that calcium ion modification of cpTI implants enhanced bone-implant contact, suggesting that a titanium implant surface may assume osteoconductive traits through chemical modification.[101,102]

Based on the present description, several functional attributes have been ascribed to the bone-implant interface: *(a)* molecular absorption, *(b)* cellular adhesion, *(c)* adhesion of mineralized matrix to the implant surface, *(d)* modulation of bone remodeling and thus maintenance of bone at the implant surface, *(e)* control of osteoconduction, *(f)* control of epitaxic crystal growth, and *(g)* modulation of stress transfer from a loaded implant to the host bone. Until the structural, biochemical, and dynamic nature of the interface is more comprehensively defined, consideration of the significance of these attributes will remain largely theoretical.

Cell-Implant Relationships

Understanding of both the process and the result of osseointegration may be improved by consideration of the relative location of cells in bone forming or formed at the implant-bone interface. Connectivity of cells with implants through cell processes contacting the implant surface have been elegantly demonstrated.[93] While cells have been shown to be in direct contact with implant surfaces in vivo,[103] the overwhelming consensus from light microscopic evaluations of bone at metallic implants is that such cell-implant contacts are limited in both number and interaction. One exception is the recent report of fluoride pretreated titanium implants, which displayed new bone with osteocytes directly lining the implant surface.[93] The pretreated implants demonstrated improved bone adaption and biomechanical attributes.

Direct and physiologically relevant signaling from the interface to adherent cells underscores the importance of the extracellular matrix molecule(s) that mediate the interaction observed in vivo. In this context, it is hypothesized that surface-related alterations of local environments may influence mesenchymal cell differentiation to osteoblasts.[105] However, the process of bone formation at implant surfaces may not be wholly dependent on or directed by osteoblast attachment to implant surfaces. Careful examination of healing bone at HA surfaces did not reveal osteoblast or precursor cell attachment to the HA surface as a requisite step in healing.[106] That bone formation occurred toward the implant surface and not on the surface was demonstrated by the [^{3}H]-proline autoradiographic results of Clokie and Warshawsky.[83] Linder et al[84] indicated that osseointegration represented a gradual mineralization process directed toward, not initiated at, the implant surface. Active bone formation proceeds from the surgical margins of bone toward the implant surface.

One important observation seldom made regarding the formation of the bone-implant interface is the requisite process of neovascularization. The formation of new blood vessels at implanted surfaces occurs from the surgical margins of bone and within the loose connective tissue formed initially in microgaps along the implant surface. This process has been revealed by a plastic injection method for histologic analysis of tissue surrounding implants.[107] With regard to neovascularization after implant placement, the formation of blood vessels represents an important determinant of complete bone formation following surgery; new vessels are maintained and not resorbed during osseous regeneration.[108] In fact, bone formation can be significantly delayed if the environment is unsuitable for neovascularization (eg, reinforcement of bone with porous HA granules).[109]

Another observation made at the histologic level is the absence of an acute inflammatory process following implant placement. While granulocytes and plasma cells have been identified in the associated non-mineralized connective tissues near implant surfaces, inflammatory cells are not prevalent. Investigations of tissue responses to cp titanium implants support the original observations that a limited inflammatory response is present surrounding the implant and that this response typifies a temporally limited wound-healing response.[103,110] The association of immune cells with implanted surfaces has been considered in a murine model examining bone marrow–implant interactions.[111] However, the inflammatory responses must be examined at the earliest stages of osseous healing at implant surfaces.

An interesting observation regarding cellular interactions at implant surfaces is the presence of macrophages, which have been found to adhere to both Ti and Ha implant surfaces or to surrounding implant-related debris.[18,112,113] Mononuclear cell control of bone formation and repair has not yet been considered in the context of osseointegration. More significant, perhaps, is the known role of macrophages in mediating fibrotic diseases.[70] The presence of macrophages at implant surfaces should be considered with respect to both positive and nega-

tive outcomes for osseointegration. Importantly, the effect of surface configuration, cleanliness, and composition may significantly influence the number and activity of macrophages in immature extracellular matrix of bone.

Sennerby et al[112] suggest that, in a manner analogous to bone surface preparation by the osteoclasts during cutting cone resorption and remodeling, the multinucleate cell on the implant surface may produce components of the interface that direct future biologic activity. For example, one major noncollagenous protein of bone shown to be a product of the osteoclast is osteopontin.[114] In fact, osteopontin is presently defined by transmission electron microscope immunohistochemistry to be in relative abundance at the intact bone-implant interface of both Ti and Ha implants.[81,86] The special importance given to high rates of bone remodeling at implants[56] suggests that osteoclasts should be present in this interface region.

The biologic reactivity of any surface must be considered in terms of both positive and negative consequences; bone mass represents a dynamic state of formative and resorptive phenomenon mediated by distinct cell types. The surface of HA and HA-coated implants is resorbed. This has been demonstrated repeatedly.[18,34,78,113,115,116] The resorption rate of HA depends on various factors, such as its micro- and macroporosity, its sintering temperature, the nature of the resultant amorphous phase or the extent of crystallinity, and the content of ions such as fluoride and carbonate. Although alterations of the HA-coated surface (such as the use of fluoridated apatites[117,118]) may practically limit this degradation, an essential aspect of this degradation is that the bone-implant interface is a site of cellular activity.

Summary

In vivo investigations provide evidence for the formation of bone at implant surfaces. An organic component exists between the formed bone and the implant surface. Both the bone and the interface appear to be dynamic structures, subject to biologically and biomechanically induced change during the life of the implant. In addition, implant surfaces are subject to biologic modification. The current literature hints at this dynamic state, but has failed to fully demonstrate the processes that form and then continually maintain the bone, the interface, and the alloplastic surface. While the aggregate data indicate that the amount of bone formed at implant surfaces may be altered by surface-related factors, the interaction among fundamental alloplastic and biologic determinants of artificial surfaces is not completely understood. The interfacial zone may represent a key determinant of the dynamic state of bone formation, modeling, and remodeling that occurs during the lifelong process of osseointegration.

Acknowledgment

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