



Leading Opinion

Bone bonding at natural and biomaterial surfaces[☆]John E. Davies^{a,b,*}^a*Institute of Biomaterials and Biomedical Engineering, University of Toronto, 164 College Street, Toronto, Ontario, Canada M5S 3G9*^b*Faculty of Dentistry, University of Toronto, 124 Edward Street, Toronto, Ontario, Canada M5G 1G6*

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Abstract

Bone bonding is occurring in each of us and all other terrestrial vertebrates throughout life at bony remodeling sites. The surface created by the bone-resorbing osteoclast provides a three-dimensionally complex surface with which the cement line, the first matrix elaborated during de novo bone formation, interdigitates and is interlocked. The structure and composition of this interfacial bony matrix has been conserved during evolution across species; and we have known for over a decade that this interfacial matrix can be recapitulated at a biomaterial surface implanted in bone, given appropriate healing conditions. No evidence has emerged to suggest that bone bonding to artificial materials is any different from this natural biological process. Given this understanding it is now possible to explain why bone-bonding biomaterials are not restricted to the calcium–phosphate-based bioactive materials as was once thought. Indeed, in the absence of surface porosity, calcium phosphate biomaterials are not bone bonding. On the contrary, non-bonding materials can be rendered bone bonding by modifying their surface topography. This paper argues that the driving force for bone bonding is bone formation by contact osteogenesis, but that this has to occur on a sufficiently stable recipient surface which has micron-scale surface topography with undercuts in the sub-micron scale-range.

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Keywords: Bone bonding; Cement line; Biomineralisation; Calcium phosphate; Bioactive; Implant**1. Introduction**

Bone bonding, or the ability of bone tissue to bond to the surface of a synthetic material, was a term first introduced into the biomaterials lexicon following the exciting experimental findings of Hench et al. [1] in the early 1970s on tissue bonding to bioactive glasses. However, the biological phenomenon of bone bonding is as old as the normal remodeling of bone itself; a tissue

which can be traced back in evolution to the agnatha of the early middle Paleozoic Era (543–248 million years ago) [2]. Indeed, because the extensive calcium phosphate cranial exoskeleton of these jawless protofish is preserved in the fossil record, we can track the evolution of our calvariae and demonstrate that our bone tissue has been evolving considerably longer than we have existed as a genus (*Homo*)! It is therefore startling that attempts to deconvolute the mechanisms of bone bonding have, generally, focused on the surface properties of biomaterials rather than the underlying biology which is the driving force for the phenomenon.

To achieve an understanding of bone bonding, my approach herein is twofold:

First, I describe the resorption surface created by an osteoclast in bone and the initial matrix synthesis which occurs during de novo bone formation at such a natural bone remodeling site. Thus, without unnecessary repetition of information which can be gained by other reviews of the broader biological cascades of bone remodeling [3,4], the

[☆] *Editors Note:* Leading Opinions: This paper is one of a newly instituted series of scientific articles that provide evidence-based scientific opinions on topical and important issues in biomaterials science. They have some features of an invited editorial but are based on scientific facts, and some features of a review paper, without attempting to be comprehensive. These papers have been commissioned by the Editor-in-Chief and reviewed for factual, scientific content by referees.

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focus herein is on the structure of the resorbed bone surface and the important “cement line” extracellular matrix that occupies the interface between new bone and old bone during such remodeling.

Second, I address, from a somewhat phylogenetic perspective, the conservation of the cement line and its relevance to our understanding of bone bonding to biomaterial surfaces. My aim here is not to provide treatises on either bone cell biology, or bone phylogeny, but to demonstrate that the cement line is a highly conserved extracellular interfacial matrix which has evolved to anchor new bone tissue, of which it is an integral part, to the highly three-dimensionally complex sub-micron scale surface of bone tissue created by an osteoclast; and that this biology can be recapitulated at the implant surface.

My underlying thesis is that it is only by understanding such fundamental biological processes that one can begin to comprehend how bone can bond to synthetic biomaterials and, critically, what surface features of such biomaterials are important in permitting bone bonding to occur. This argument is an extension of four previous reviews, on the principals of peri-implant bone healing, in which we have provided the foundations for what is discussed herein [5–8].

2. The bone remodeling process

Bone is a bloody, dynamic, living tissue that changes throughout life. Like other connective tissues of the body, bone comprises cells embedded in an abundant extracellular matrix. However, unlike most other connective tissues the extracellular matrix is mineralized to bestow unique physiological functions. As the major structural element of the skeleton, bone provides not only locomotor support and protection, but also a dynamic mineral and protein reservoir. The constant remodeling of bone tissue provides a mechanism for scar-free healing and regeneration of damaged bone tissue and also plays, through endocrine control, a vital role in the calcium and phosphate balance of the body fluids.

Bone remodeling is achieved through the resorptive activity of osteoclasts and the synthetic activity of osteoblasts. These two cell populations are constantly responsible for the turnover, at any one time, of approximately 3–5% of the human skeleton. Perturbations of this cellular activity, resulting in an imbalance between the activities of the two cell types, are the key element in many bone metabolic diseases, disuse atrophy, and microgravity-induced osteopenia. The process of remodeling of human bone can be witnessed histologically as soon as the first bone is formed (approximately 6 weeks in-utero) and continues throughout life, although the rate of remodeling decreases with age.

As stated above, it is not the purpose of this review to focus on the molecular and biochemical activities of the cells responsible for this remodeling process. But, there are

two specific issues that receive little attention in the bone biology literature and are of considerable importance from a biomaterials perspective. These are discussed below.

2.1. The bone surface created by osteoclasts

When osteoclasts resorb bone, which is known to be a two phase process of both the dissolution of the inorganic matrix and enzymatic degradation of the organic components, the result is the creation of a demineralized bone matrix which becomes the recipient surface for new bone formation. While it is common, for reasonable reasons of graphic expediency, to represent an osteoclast sitting on the surface of bone with its ruffled membrane of the resorptive organ falling into the resorption lacuna below the cell [9], such cartoons do not represent the biological reality where it has been reported that the ruffled membrane with its invaginated surface penetrates the bone matrix to a depth of approximately 1 μm [10]. This morphological feature of the osteoclast/bone matrix interface is important because it results in the demineralized collagen of the bone matrix presenting a resorption surface of three-dimensional complexity at the sub-micron scale range. Thus, the floor of a Howship's lacuna, a histological feature that can be visualized at the light microscopic level and which measures tens or even hundreds of microns in cross section, is highly topographically complex at the sub-micron level (Fig. 1).

Furthermore, because of the varying orientation of the collagen fiber bundles in bone, not only is this three-dimensional structure highly variable but it can also present a surface with undercuts. This morphological feature of the resorbed bone matrix is important because it presents a surface of three-dimensional complexity, at the sub-micron scale range, into which the matrix of the cement line can be deposited to form an anchoring mechanism of new bone to old. Thus, in normal bone remodeling, the resorption surface of old bone provides a highly topographically complex surface into which new bone matrix will be deposited, and with which the latter can interdigitate and interlock. Despite this, it is the opinion of some, based on in vitro experiments, that osteoblasts digest the remaining demineralized collagen in the osteoclast resorption lacuna prior to elaborating new collagen directly on the old bone surface [11]. However, this opinion completely fails to provide an explanation for the formation of a collagen-free cement line interface.

2.2. Elaboration of the cement line interface

The existence of this interfacial matrix has been known since the early observations of von Ebner who, in 1875, first reported that osteons were demarcated from the surrounding bone by a distinct matrix, which he called “Kittlinien” (Engl: cement line) suggesting the biological function of cementing a secondary osteon to the surrounding bone matrix [12]. However, it was more than a century

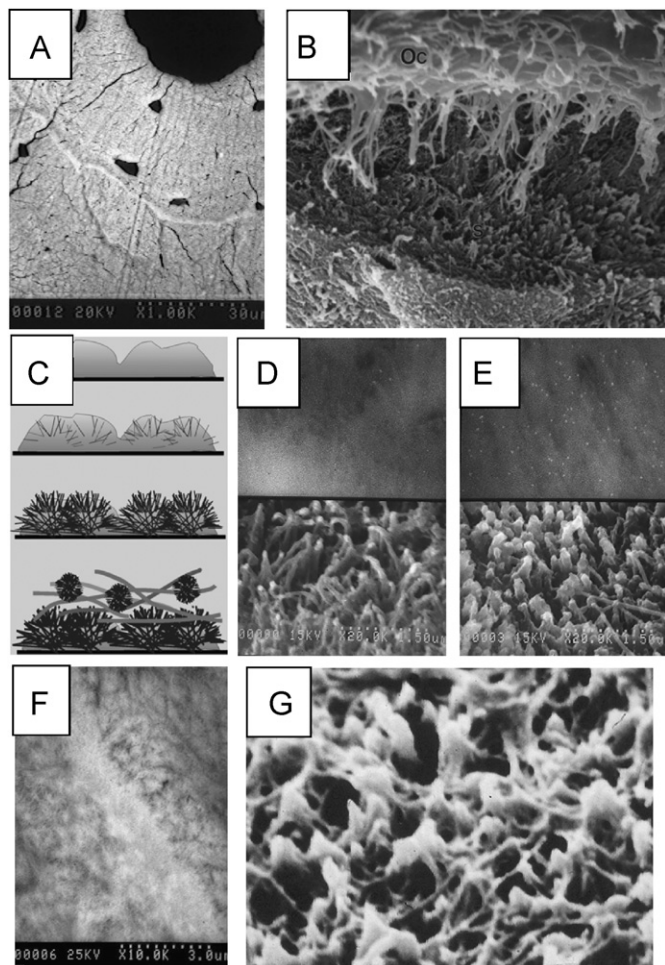


Fig. 1. The cement line interface in bone and the osteoclast resorption surface. (A) Backscattered electron image of a secondary osteon in rat femur clearly showing the relatively hypermineralized cement line forming the interface between the new bone (top) and the old bone (bottom). The scalloped appearance of the cement line is due to the precedent osteoclast resorption of the old bone. Field width = 72 μm . (B) The resorption surface (S) of a Howship's lacuna (rat) created by an osteoclast (Oc). Reproduced from Ref. [55] with permission. See also Refs. [10,31]. (C) Cartoon illustrating the four stages of de novo bone formation at a solid surface. See Ref. [6] for greater detail. (D and E) The first stage of cement line formation is the secretion, by the differentiating osteogenic cell, of bone sialoprotein and osteopontin on the osteoclast-resorbed bone surface. These scanning electron micrograph pairs comprise backscattered (above) and secondary (below) images of the same field of view. In each case the specimen has been labeled with a gold-conjugated monoclonal antibody against osteopontin. The secondary images show deposition (E) on the osteoclast-resorbed collagen in (D), while the BSEI in (E) shows this to be due to the presence of osteopontin. Unpublished images reproduced from Ref. [56], with permission. (F) Higher magnification of (A) showing the detail of the cement line, which in this field of view varies in width from 0.9 to 1.5 μm . The mottled appearance of the bone either side of the cement line is due to the space occupied by collagen fibres. Since the cement line contains no collagen, the line appears relatively hypermineralized. Field width = 7.3 μm . (G) Higher magnification of a field of view similar to that in (B) showing the detail of the three-dimensional complexity of the osteoclast resorption surface in bone. Note the undercut created by the varying orientation of the collagen fibres. Field width = 3.3 μm .

later when our interest in cement lines was ignited by almost simultaneous observations that evidence for cement line formation could be found both in osteogenic cultures and at implant surfaces [13–15].

Interestingly, while little attention has been paid to this morphological feature of bone in the bone biology literature associated with remodeling, it is critically important in understanding the structure and composition of bone-bonded interfaces. This is because it is the cement line, secreted as a non-collagenous mineralized matrix by differentiating osteoblasts, that invaginates, interdigitates and interlocks with the demineralized collagenous matrix left by the resorbing osteoclast and thus it plays a critical physical role in the establishment of the interface of new bone and old bone. Our observations over the last 15 years have allowed us to describe the cascade of de novo bone formation (this term is explained in [7]) at solid surfaces as a four-stage process (Fig. 1C) comprising: the adsorption of non-collagenous bone proteins to the solid surface; the initiation of mineralization by the adsorbed proteins (Figs. 1D and E); continued mineralization due to crystal growth; and finally the assembly of a collagenous matrix overlying the interfacial matrix with mineralization within the collagenous matrix. We have described this cascade in detail elsewhere in both animal [6] and human [16] models. It is only after the formation of the cement line that the, now differentiated, osteoblast assembles collagen fibres extracellularly which become encrusted in the forming cement line and, of course, themselves become mineralized to produce the vast majority of the volume of normal bone matrix. While there are other interfaces in bone such as resting lines and inter-lamella lines which have similar staining properties to cement lines, their dimensions are smaller and their composition significantly different in that there is continuity of collagen across these other interfaces while cement lines are devoid of collagen.

Unfortunately, there is considerable confusion with respect to the usage of the term cement line, as we have discussed elsewhere [7]. Weinmann and Sicher [17] introduced the term “cementing lines” to describe both resting and reversal lines in bone. Similarly, McKee and Nanci [18] provided an “operational definition” that grouped all matrix/matrix interfaces in bone together as “cement lines”. The situation has become even more complicated by the syncretizing of the terms lamina limitans and cement lines, as discussed previously [19,20]. However, employed in the spirit of its original coining by von Ebner [12] the term cement line recognizes both the unique genesis of this matrix and the differentiation state of cells responsible for its elaboration.

This interfacial matrix is seen in Figs. 1A and F, which are a backscattered electron images of the cement line interface at a remodeling site. In these figures, the cement line is easily visualized as a white line demarcating the border between new and old bone. It can also be seen from the image that the electron density is seemingly no different from surrounding bone. However, the line appears whiter

than the surrounding bone because there is no space being occupied by collagen and thus the cement line appears relatively hypermineralized. The structure of the cement line, and the difficulties inherent in its visualization by scanning electron microscopy, have been thoroughly treated in the scholarly review by Skedros et al. [21].

2.3. Maturation of the cement line

Every known biological mineralization system, whether calcium carbonate, or calcium phosphate based, is predicated on the precedent secretion of an organic matrix that provides the nucleation foci for the inorganic mineral phase. In all cases the inorganic volume percent increases with time, as the composite matures, and becomes the predominant phase. This is true of bone, tubular bone, dentin, cementum, enamel, enameloid, and both the calcite and aragonite phases of shells. It is also true of the cement line in bone. It is evident, therefore, that the elaboration of the cement line can itself be arbitrarily divided into three phases: the initial secretion of non-collagenous proteins; mineral nucleation within these proteins and crystal growth or maturation. In this manner the cement line becomes a relatively hypermineralized matrix which matures in and around the submicron topography presented by the resorbed bone surface.

Fortunately, as has been shown by Hosseini and colleagues [22–24] the cement line can be modeled in vitro to the exclusion of the collagenous matrix of bone, which facilitates the study of its composition and structure. By employing ascorbic acid-free culture conditions collagen formation is prevented while the cement line is still formed (which is not surprising as the latter contains no collagen).

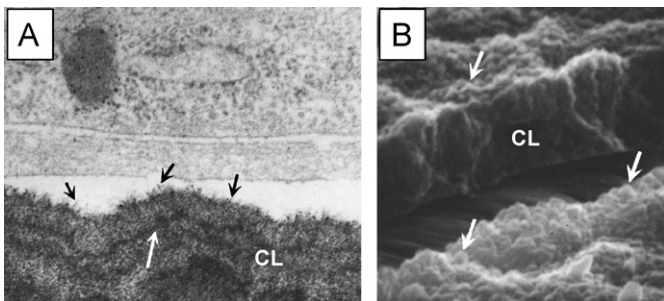


Fig. 2. Electron micrographs of cement line matrix formed in osteogenic cultures conditions devoid of ascorbic acid (AA). Reproduced from Ref. [22] with permission. (A) Transmission electron photomicrograph (TEM) of the isolated cement-line (CL) matrix can be seen at the interface with the tissue culture polystyrene substratum (below). No collagenous matrix is seen in the space overlying the cement line matrix due to the AA-free culture conditions. Electron-dense lines (white arrow) can be seen within the approximately 0.5- μ m-thick cement line (CL) which indicates incremental growth. The crystalline structure of the matrix is clearly visible (black arrows). Field width: 1.5 μ m. (B) Scanning electron photomicrograph (SEM) of the isolated cement line (CL), which has been exposed by removal with compressed air of the overlying cell layers, is seen at a fracture site generated during specimen preparation. Field width = 1.5 μ m. Compare the remarkable similarity of surface appearance (white arrows) with Fig. 4B in Ref. [11].

Figs. 2A and B are taken from [22] and show that, upon maturation, the cement line appears as a densely mineralized crystalline mass when fractured vertically. This represents the natural biological matrix of bone which is available, during contact osteogenesis, to provide the means by which new bone will bond to either an old bone surface or a biomaterial.

3. The interface in bone and at implant surfaces

3.1. The cement line in human bone

It was in human bone that von Ebner first described cement lines and, like other species (see below), this feature of bone represents a critical interfacial matrix at remodeling sites whether they be in woven, lamellar, compact or cancellous bone. However, they are most easily observed at the periphery of osteonal systems in cross-sections of compact lamellar bone. While it is human bone that is, of course, of primary interest to implantologists, some skepticism has been raised with respect to reliance on animal models to deconvolute the events taking place at the bone/biomaterial interface. However, I make a case below that the cement line interface has been conserved as an integral morphological structure in bone since bone first started to be remodeled, and since all findings regarding this interface have now been confirmed in human studies, both in vitro and in vivo, there should be no reasonable hesitation in employing other vertebrate species to investigate these interfacial phenomena.

3.2. Phylogenetic conservation of the cement line

Why this emphasis on phylogeny? The reason is simple and, again, twofold.

First: As we have stated earlier, the structure of bone has been exhaustively compared across both species and time. Bone remodeling did (does) not always occur as witnessed by the osseous elements of the largest group of vertebrates, the advanced teleosts, whose tissue lacks osteocytes (anosteocytic or acellular bone), a fact first exposed by Kölliker in 1859 [25] who also recognized osteoclasts as the cells responsible for bone resorption in 1873 [26], a couple of years before von Ebner described the cement line. Interestingly, and contrary to the elasmobranchii which have cartilaginous skeletons that can mineralize but do not remodel or heal [27], bony fish with acellular bone do have the ability to produce osteoclasts under experimental conditions [28] and do, in some circumstances, exhibit remodeling with attendant Haversian systems as is seen in the bony rostrum of the swordfish [29]. But, in the cellular bone of tetrapods remodeling does occur, is an essential adaptation to the rigors of calcium and phosphate homeostasis in a terrestrial environment (placoderms and ostracoderms of the fossil record may also have employed their cellular bone as a mineral reservoir), and a structural feature of both primary bone as seen in the rapidly growing

bone of the king penguin chick [30] or the laboratory rat [31] or the more slowly forming secondary bone of cows, dinosaurs (see Fig. 3) or humans. Thus, in all vertebrates which exhibit bone remodeling, evidence exists for the cement line providing the interfacial matrix between new and old bone.

Second: The detailed provenance and composition of the cement line matrix itself has been known for over 15 years, and can be modeled *in vitro* by the culture of osteogenic cells from several species, including human, as discussed above.

Therefore, since the cement line has been structurally conserved across Craniata, and recent evidence shows that it's cellular provenance and composition is the same across species, it is reasonable to assume that the composition and synthesis of the interfacial cement line matrix is essentially the same now as it has been throughout evolution. Indeed, this is reflected in the evolution of bone more generally with evidence that bone-specific proteins such as osteocalcin have been conserved from swordfish to humans [32] and the osteogenic master gene *Runx2* is derived from a gene family which may be a metazoan invention [33]. It is very surprising therefore that even in some very recent, and influential, dental biomaterials reviews it has been stated that “the process by which bone formation occurs and the way in which surface topography can influence that process is not clearly defined” [34]. Hopefully, the present text will provide the required clarity.

3.3. The cement line at the implant surface

The relevance of the bony cement line when considering the bone/implant interface was discussed, to my knowledge for the first time, by Hench and Paschall [35], although at that time they recognized that neither the composition nor structure of this extracellular matrix had been determined. From this early work, the mechanism for the bone-bonding phenomenon has been generally accepted to be a chemical interaction that results in collagen, from the bony compartment, being incorporated in the chemically active surface of the implant (reviewed in Ref. [36]). To be fair, the emergence of this dogma is hardly surprising given that even recently published work on bone remodeling completely discounts the histological reality of the cement line and adopts the view that the first formed matrix in bone at remodeling sites is collagen [3,11,37,38]. Thus, in the absence of relevant and necessary information from bone biologists, Hench assumed that the role of the cement line was replaced by that of the calcium phosphate-rich surface reaction layer on bioactive glass [35], paving the way for the dogma adopted ever since, by the majority of the biomaterials community, that the material surface provides the driving force, through surface chemical reactions, for the bonding mechanism to bone. Indeed, this is probably the reason why many published articles describe a bone-bonding biomaterial as one which possesses an ability to bond to bone, rather than one to which bone can bond.

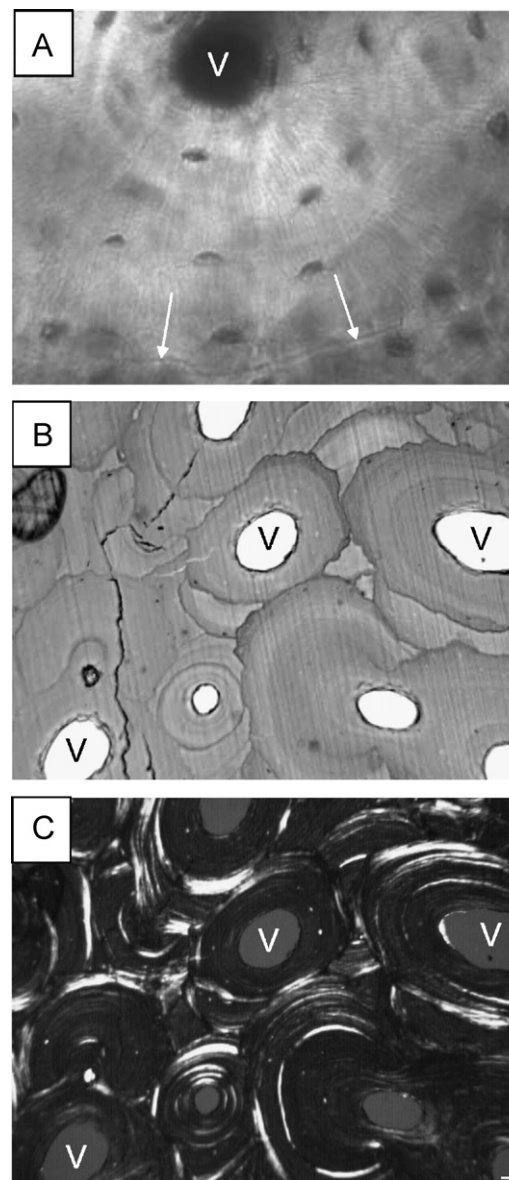


Fig. 3. Light micrographic examples of evidence for cement line formation in bone from both extant (cow—top) and extinct (dinosaur—bottom) species, respectively. (A) A specimen prepared from bovine (cow) bone which would otherwise have been disposed of following a family dinner one evening! Prepared by hand using a plumber's saw and several grades of wet and dry paper to achieve a thin plano-parallel section which was then mounted with water, coverslipped and photographed. The majority of the image is taken up with osteocyte lacunae and their radiating canaliculi, but the white arrows mark the junction of this osteonal system (with its central vascular channel-V) with the old bone by a distinct parallel line feature which represents the cement line. Specimen preparation courtesy of Luke Davies. (B and C) A similar, but machine-ground, preparation of the fossilized cortical bone of a newly discovered species of *Pachyrhinosaurus*, *Iddesleigh Ceratopsian*, clearly showing the demarcation of osteonal structures, with their central vascular channels (V), from the surrounding bone by the linear cement line feature. The cement line, in this *unstained* specimen appears darker due to a greater extent of inorganic substitution during the process of fossilization—again emphasizing the difference in original composition to that of the collagen-containing compartment of bone. The circular arrangement of the original collagen fibre orientation is clearly seen in the same field of view photographed with crossed polars in (C). The original specimen was generously provided by Don Brinkman, Vertebrate Palaeontology and Senior Curator Royal Tyrrell Museum, Drumheller, Alberta Canada. Specimen preparation courtesy of David Lickorish.

This polarization of opinion is exemplified by the work of Neo et al. [39] who described “two main theories” to explain the formation of an apatite-rich layer on the surfaces of bioactive materials.

The first, proposed by Kokubo et al. [40], was exclusively based on the ability of some materials to form a surface apatite layer when immersed in a simulated (but protein and cell-free) body fluid *ex vivo*; but neither Kokubo nor Neo provided any explanation of how the bonding mechanism would actually occur and what the relationship was to the apatitic surface reaction layer. They simply stated that “Subsequently, calcification of the surrounding bone matrix occurs, thus completing the material-bone bonding” and described the surrounding bone matrix as collagenous [39]. This approach mirrors that of Hench and co-workers [1,35,36] the only difference being that bioactive glass generated the surface apatitic layer through ion leaching from the bulk, whereas A-W glass facilitated calcium and phosphate ion deposition from the surrounding tissue fluid. Clearly, in the case of *de novo* bone formation, either during bone remodeling or contact osteogenesis on implant surfaces, the collagen-bonding mechanism is inconceivable since the first extracellular matrix elaborated by bone cells at either the bone, or implant, surface is collagen-free!

The second theory was attributed to my group [19] and dismissed by Neo et al. [39] for two reasons. First, they felt that we were speculating on the synthetic activity of osteogenic cells based on *in vitro* experiments—and that these did not accurately recapitulate all aspects of the *in vivo* environment. However, they ignored our reporting, in the same volume, of equivalent findings *in vivo* [14]. Second, they described the work of de Bruijn et al. [41] who employed the same cell culture system but found different interfacial results with different materials. Specifically, the latter authors were unable to find evidence of an interfacial apatitic layer when osteogenic cells were cultured on tricalcium phosphate (TCP). This sound experimental evidence was used by Neo et al. [39] to support their theory that bone bonding was a material-driven phenomenon. However, they overlooked the fact that TCP is highly soluble and thus presents an unstable surface for cell growth and tissue elaboration. Indeed, the same explanation could have been used to explain their own *in vivo* findings. The latter were further compounded by the fact that they did not take into consideration the differences between contact and distance osteogenesis, and thus their *in vivo* results clearly showed a lack of contact osteogenesis on the more soluble substrates (see the cells remaining at the interface in their Figs. 13 and 15, signifying distance osteogenesis associated with TCP).

However, Neo et al. [39] did recognize that both biology and material surface play a part in the ultimate bonding phenomenon. This is true, but it is important to distinguish between the driving force for bonding which is the formation of new bone on the implant surface, through the individual phenomena of osteoconduction and bone

formation (see Refs. [6–8] for reviews of these phenomena), and the role played by the material surface—biological or otherwise. The latter must provide a highly topographically complex, and sufficiently stable, surface with which the initial cement line matrix can interdigitate, and subsequently interlock as cement line maturation occurs. Thus, in retrospect, it is easy to explain why “bioactive” materials are bone bonding since they present highly topographically complex surfaces at the sub-micron scale range. The question remains: Can we now explain why some “bioactive” materials are not bone bonding and, similarly, why some “non-bioactive” materials are bone bonding.

The answer is: “Yes”.

First, as all bioactive materials so far described have been calcium phosphate-based (or generate a calcium phosphate rich surface), we have to be able to explain why some calcium phosphates do not exhibit bone bonding. The case of a highly soluble TCP has already been mentioned above, so one could predict that less soluble formulations of TCP could be bone bonding while highly soluble forms of calcium phosphates would not provide a sufficiently stable surface to which bone could bond. This is borne out in the literature already referenced. However, there is another case where it has been shown that calcium phosphate ceramics, when fabricated to be devoid of surface porosity, are not bone bonding. Dziejcz et al. [42] showed that when tape-cast HA ceramic lithomorphs were fabricated with controlled degrees of porosity, bonding only occurred (as monitored by the position of fracture planes occurring in the samples during specimen preparation for scanning electron microscopy) when the sample presented surface pores. Ceramics of identical composition but presenting no surface pores showed no evidence of bone bonding. This robust evidence clearly demonstrated the necessity for a surface topography (in that case 1 μm size pores of a “keyhole” cross-section) to provide a substrate with which the cement line matrix of bone interlocked. Similarly, where materials exhibit contact osteogenesis, we find evidence of cement line formation on both non-bonding and bonding biomaterials. Thus, while contact osteogenesis is an essential prerequisite of bone bonding, the mechanisms can be considered independently from both phenomenological and experimental points of view.

Second, if bone bonding, which critically depends on contact osteogenesis, is a function of the interdigitation of the cement line with a surface that presents a three-dimensionally complex topography, with undercuts at the submicron scale range, then it should be possible to find examples of traditionally “non-bonding” biomaterials which could be rendered bone bonding. This is indeed the case, with the first example coming from the work of Takatsuka et al. [43] on titania surfaces which had been modified with strong alkali treatments. They showed that such complex metal surfaces—generally, sodium titanates—were capable of generating interfacial strengths which would exceed that necessary to fracture the overlying

bone (a robust experimental demonstration of bone bonding). We have also produced such materials and corroborated their bone-bonding behaviour. We were able to show, by close examination of the interface which remained in tact following a mechanical disruption test, that the globular cement line matrix was interdigitating, and interlocked, with the complex reticulate surface of the modified titanium oxide (see Fig. 4 and [6]). Thus a traditionally non bone-bonding material can be rendered bone bonding by the formation of a three-dimensionally complex surface.

This bonding mechanism is quite distinct from bone apposition as can be seen in Fig. 5 where bone has grown on a commercially pure titanium (cpTi) surface which has been modified by acid etching to render its surface microtopographically complex. In this case, it is obvious that the bone has grown in close proximity to the surface and that the cement line matrix has interdigitated with the surface features of this implant. However, no bonding has occurred since the surface presents no undercuts around which the cement line can mature during the crystal growth phase (see above).

Finally, we have recently shown that if such an acid-etched non-bonding metallic surface is modified by the deposition of nanometre size range calcium phosphate crystals, which only cover approximately 50% of the metal oxide surface, then the undercuts presented by such crystals are sufficient to render the surface bone bonding [44]. Interestingly, we also showed that titanium alloy (Ti6Al4V) when acid etched can exhibit smaller etching features than cpTi, although the features also present undercuts, rendering the alloy somewhat more bone bonding than the cpTi. The details of the cpTi surface are shown in Fig. 6, where it is clear that the distribution of the discrete calcium phosphate crystals is similar to dropping common salt crystals on a table top. However, at higher magnification it is also clear that the crystals are stacked upon each other providing a surface with undercuts in the nanometre scale-range. From Fig. 6B, it is easy to imagine that the initial proteins of the cement line would completely envelop these

crystalline surface features. However, as the cement line matured, with crystal growth, the biomaterial crystals on the implant surface would be completely encased with the biological crystallites of the cement line matrix. This is beginning to happen in Fig. 6C where the crystallites on the left are the cement line matrix (represented in the cartoon in Fig. 1C and the TEM in Fig. 2A) yet the biomaterial crystals can still be seen on the right of the micrograph. To realize the next stage of the cement line maturation process, it is necessary to compare this image with that of Fig. 2B and the manner in which the implant surface will become both interdigitated, and interlocked, by the cement line becomes evident.

4. Bone bonding and implant surface design

Clearly, the formation of bone requires not only the recruitment and/or migration of a potentially osteogenic

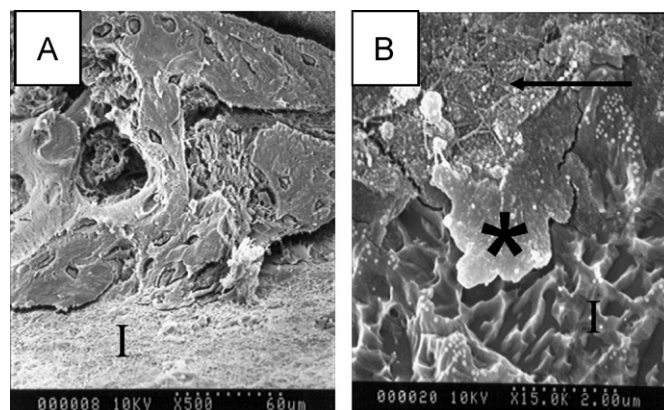


Fig. 5. Scanning electron micrographs of acid etched titanium samples fractured out of rat femoral bone. (A) Bone, identified by the osteocyte lacunae and vascular canal, has grown in close approximation to the underlying titanium implant (I). Field width = 183 μm . (B) At higher magnification the detailed surface of the implant (I) is more easily seen. The asterisk marks the cement line matrix which has grown into the micron sized pits in the metallic surface and the arrow marks collagen being deposited in the cement line matrix. Field width = 6.1 μm .

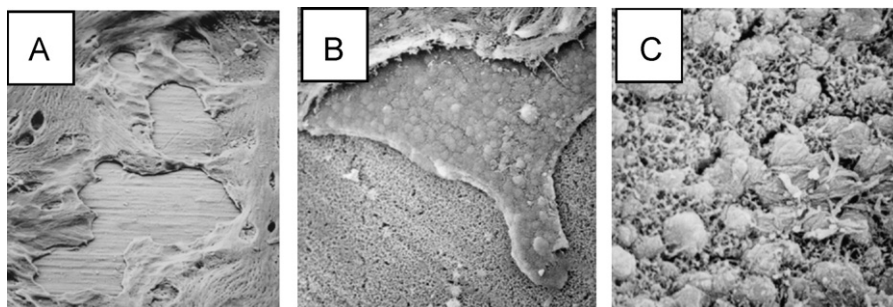


Fig. 4. Scanning electron micrographs of titanium samples following a tensile strength test. Modified from Ref. [6]. (A) Bone remaining on the sample following the tensile test is easily distinguished by the presence of osteocyte lacunae. The test samples can be seen beneath the bone. Field width = 184 μm . (B) At higher magnification, the reticulate surface of the sodium hydroxide treated titanium is now discernable. The globular matrix in contact with the implant surface is the cement line, above which the collagen compartment of bone can also be seen. Field width = 28.3 μm . (C) At this magnification the individual globular accretions of the cement line matrix can be seen interdigitating with the reticulate surface of the implant. A few collagen fibres are already attached to the cement line matrix. Field width = 7.92 μm .

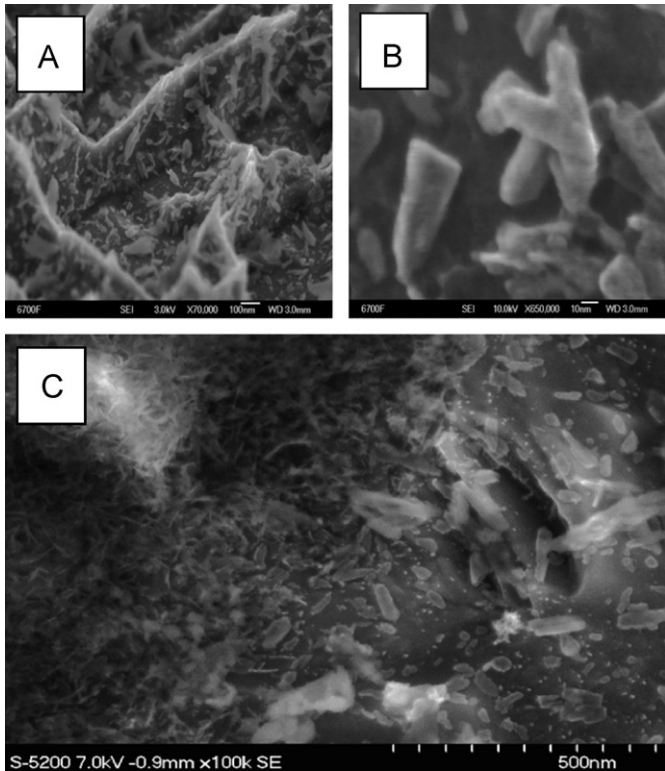


Fig. 6. Field emission scanning electron micrographs of acid etched titanium samples which have been modified by the deposition of discrete nanocrystals of calcium phosphate. (A) A control sample to show the appearance of the calcium phosphate crystal modified surface. Approximately 50% of the sample surface is still the original metal oxide. Field width = 1.37 μm . (B) Higher magnification of surface showing that individual crystals are stacked on each other creating undercuts at the nano-scale. Field width = 183 nm. (C) This sample was used in a tensile test evaluation described in detail in Ref. [44]. Some bone remained bound to the surface post-test. This micrograph was taken at a position representing the border between the bone and the underlying implant. The latter can be recognized by the presence of the discrete calcium phosphate crystals and reference to (A) and (B). However, the smaller crystallites on the left of the field of view are of the initial cement line formation as illustrated in Figs. 1(C) and 2(A). As the cement line matrix matures through crystal growth, it will completely interlock with the underlying biomaterial nanocrystals. Field width = 1.3 μm .

cell population (osteoconduction—see above), but also the differentiation of this population into mature secretory cells [6,8]. In either cortical or endosteal remodeling, this will involve the migration, through primitive connective tissue matrix, of peri-vascular cells to the resorbed bone surface. Similarly, in fracture and peri-implant healing, the potentially osteogenic population will migrate through the resolving blood clot and, assuming clot retention, will reach the surface of bone fragments, or the implant, within the wound site. In each of these cases, cells that reach the solid surface, provided by either the old bone or implant, will initiate matrix synthesis at the solid, or “target”, surface. Those cells that differentiate before reaching the target surface will secrete matrix. As a result, they will stop migrating and will not reach the target surface. Thus, osteoconduction will result in a bony spicule advancing

toward the target surface. The progression of such bony spicules has been observed, in optical bone chambers, to occur at an average rate of 73 μm [45] to 85 μm per day [46]. However, those cells which migrate onto the implant surface will differentiate and form de novo bone by secreting the first proteinaceous matrix of the cement line directly on the implant surface. [It is assumed that the implant surface will have already been modified by ion exchange, the adsorption of serum proteins and the secretory products of cells which migrate into the healing compartment before osteogenic cells—but these surface modifications will be at the atomic or molecular level and will be orders of magnitude in size below the sub-micron features of either the resorbed bone surface at bone remodeling sites, or the candidate implant.]

Given the dimensions of the cement line in normal bone and its component parts (non-collagenous bone proteins, proteoglycans and calcium phosphate crystallites), one can therefore expect bone bonding to occur to an artificial material if the latter exhibits a surface topography with features in the same scale-range as that seen on the old bone surface at bony remodeling sites (see Fig. 1). Thus, a candidate bone-bonding material should exhibit a surface microtopography in the micron scale range with undercuts at the sub-micron, or nanometre, scale range.

Bone bonding was defined during the Second European Society of Biomaterials conference in 1991 as “the establishment, by physico-chemical processes, of continuity between an implant and bone matrix” [47]. While it is obvious that interfacial chemistry will occur (see the statement in parentheses above), it is important to realize that bone bonding is judged as the outcome of some mechanical disruption test where the strength of the interface exceeds the cohesive strength of either bone tissue or implant [48,49].

Interfacial chemistry cannot explain such a bone-bonding phenomenon and atomic continuity, as invoked by Takatsuka et al. [50], or calcium phosphate epitaxy as discussed by others [51,52] fails to address either the fact that calcium phosphate crystallites can form on any surface in vivo given that body fluid is supersaturated for both calcium and phosphate, or the fact that bone crystallites are nucleated at calcium-binding domains on non-collagenous bone proteins. However, evidence has emerged to indicate that the mechanism of bone bonding is one of micro-mechanical interdigitation of the bone tissue with the implant surface [53,54] corroborating the findings of Dziedzic et al. [42] and recapitulating what has evolved over the millennia as the interface established during normal bone remodeling.

5. Conclusion

While chemical hypotheses to explain bone bonding have been generally adopted in the literature, experimental evidence demonstrates that bonding is achieved by micro-mechanical interdigitation of the cement line with the

material surface, at both natural remodeling sites and biomaterial surfaces.

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