Understanding and controlling the bone–implant interface

D.A. Puleo, A. Nanci

Abstract

A goal of current implantology research is to design devices that induce controlled, guided, and rapid healing. In addition to acceleration of normal wound healing phenomena, endosseous implants should result in formation of a characteristic interfacial layer and bone matrix with adequate biomechanical properties. To achieve these goals, however, a better understanding of events at the interface and of the effects biomaterials have on bone and bone cells is needed. Such knowledge is essential for developing strategies to optimally control osseointegration. This paper reviews current knowledge of the bone–biomaterial interface and methods being investigated for controlling it. Morphological studies have revealed the heterogeneity of the bone–implant interface. One feature often reported, regardless of implant material, is an afibrillar interfacial zone, comparable to cement lines and laminae limitantes at natural bone interfaces. These electron-dense interfacial layers are rich in noncollagenous proteins, such as osteopontin and bone sialoprotein. Several approaches, involving alteration of surface physicochemical, morphological, and/or biochemical properties, are being investigated in an effort to obtain a desirable bone–implant interface. Of particular interest are biochemical methods of surface modification, which immobilize molecules on biomaterials for the purpose of inducing specific cell and tissue responses or, in other words, to control the tissue–implant interface with biomolecules delivered directly to the interface. Although still in its infancy, early studies indicate the value of this methodology for controlling cell and matrix events at the bone–implant interface.

Keywords: Orthopedic implants; Dental implants; Ultrastructure Cell–biomaterial interactions; Surface modification

1. Introduction

Over 300 000 hip and knee implants [1] and between 100 000 and 300 000 dental implants [2] are used each year in the United States to replace or restore function to diseased and damaged tissues. The variety of devices available is purportedly because modifications in design or materials will enable better performance of the implant. Events leading to integration of an implant into bone, and hence determining the performance of the device, take place largely at the tissue–implant interface. Development of this interface is complex and involves numerous factors. These include not only implant-related factors, such as material, shape, topography, and surface chemistry, but mechanical loading, surgical technique, and patient variables, such as bone quantity and quality, as well. A goal of current implantology research is design of devices that induce controlled, guided, and rapid healing.

More specifically, in addition to acceleration of normal wound healing phenomena, implants should result in an interfacial matrix with a composition and structure characteristic of bone, and the matrix should have adequate biomechanical properties. These outcomes would allow not only faster recuperation for the patient, but also stable fixation between bone and implant that would permit early or immediate loading of the device. This latter point has great significance, in terms of decreased patient morbidity, improved patient psychology, and decreased health care costs. To achieve these goals, however, a better understanding of tissue healing events is needed.

This paper reviews current knowledge of the bone–biomaterial interface and methods being investigated for controlling it. Because of their predominant use as load-bearing implants, emphasis is placed on metallic biomaterials.

2. Events at the bone–implant interface

The performance of biomaterials comprises two components, the response of the host to the implant and the
behavior of the material in the host [3] (see Fig. 1 for summary). Although this paper emphasizes the host response, a brief review of relevant components of the material response is also given. For more information on the latter aspect, readers are referred to the papers referenced below.

2.1. Material response

The almost immediate event that occurs upon implantation of metals, as with other biomaterials, is adsorption of proteins [4,5]. These proteins first come from blood and tissue fluids at the wound site and later from cellular activity in the periprosthetic region. Once on the surface, proteins can desorb (native or denatured, intact or fragmented) or remain to mediate tissue–implant interactions [4,5]. In fact, the nature of this ‘conditioning film’ deposited on biomaterials is believed to be responsible for the host response (described later).

In addition to protein adsorption on the implant’s surface, significant changes also occur in the material’s surface. There is ample literature that describes oxidation of metallic implants both in vivo and in vitro [6,7]. Although these materials were selected because of their stable oxide films, they still undergo electrochemical changes in the physiological environment. For example, depending on the method of sterilization, commercially pure Ti implants have an oxide thickness of 2–6 nm before implantation [8]. Films on implants retrieved from human tissues are two to three times thicker [6,8,9]. Furthermore, surface analytical studies show that the chemical composition of the oxide film changes by incorporating Ca, P, and S [8,9]. Continued oxide growth reflects ongoing electrochemical events at the tissue–implant interface. Another consequence of these events is release of metal ions into tissues [10]. These corrosion byproducts accumulate locally, but they also spread systemically. Significantly elevated metal contents have been measured both in periprosthetic tissues [11,12] and in serum and urine of patients with implants [13–15]. For example, metal levels of up to 21 ppm Ti, 10.5 ppm Al, and 1 ppm V around Ti–6Al–4V and up to 2 ppm Co, 12.5 ppm Cr, and 1.5 ppm Mo around Co–Cr–Mo have been measured in the fibrous membrane encapsulating implants [11,12]. The tissue may include some particulate metal, but the ratios do not reflect the bulk composition of the alloys. Trace metals are essential for health, but they can also be toxic [16] or cause hypersensitivity reactions [17]. In vitro studies have revealed that metal ions, even at sublethal doses, interfere with differentiation of osteoblasts and osteoclasts [18–20]. It remains to be determined if these effects on bone cells also occur in vivo.

2.2. Host response

The host response to implants placed in bone involves a series of cell and matrix events, ideally culminating in intimate apposition of bone to biomaterial, i.e., ‘osseointegration’. For this intimate contact to occur, gaps between bone and implant must be filled, and bone damaged during preparation of the implant site must be repaired. During this time, unfavorable conditions, e.g., premature loading leading to micromotion (discussed later), will disrupt the newly forming tissue, leading to formation of a fibrous capsule [21–23]. Morphological studies have revealed the heterogeneity of the bone–implant interface. One feature often reported is an afibrillar interfacial zone, comparable to cement lines and laminae limitantes [24–28]. Although its thickness and appearance vary, this zone forms regardless of the type of biomaterial implanted, including cp Ti, stainless steel, and hydroxyapatite (Fig. 2; reviewed in Nanci et al. [29]). Early reports indicated that the interface was rich in glycosaminoglycans [25]. More recent high resolution immunocytochemical studies demonstrated that the electron-dense interfacial layer is rich in noncollagenous proteins, such as osteopontin (OPN) and bone
sialoprotein (BSP) (Figs. 2 and 3), as well as certain plasma proteins, such as α2 HS-glycoprotein [29,30]. The absence or relative paucity of serum proteins, such as albumin, indicates a selective accumulation/deposition of molecules at the interface [29]. Because they contain Arg–Gly–Asp and polyacidic sequences, OPN and BSP are believed to play roles in cell adhesion and binding of mineral [31–33]. It has been postulated that this interfacial zone provides a mechanism for ‘bonding’ between natural hard tissue and biomaterial, but the inherent weakness of cement lines argues against this possibility [29,34,35].

Osteoblasts, osteoid, and mineralized matrix are observed adjacent to the lamina limitans-like layer [24–28], suggesting bone is deposited directly on the surface of the implant, extending outward from the biomaterial. Thus, bone formation in the periprosthetic region occurs in two directions; not only does the healing bone approach the biomaterial, but bone also extends from the implant toward the healing bone (Fig. 4). Interestingly, fluorochrome labeling indicates that the bone extending away from the implant forms at a rate about 30% faster than that moving toward the biomaterial (Fig. 5).
Understandably, because of the complexities of the in vivo environment, the bone–implant interface is not fully characterized. The heterogeneity and patchy immunolabeling observed in morphological studies suggest that, even though several biomolecules have been identified at the interface, they are likely not the only ones present. These other biomolecules may have essential roles in directing the bone response to the implant, and further work is needed to identify them and determine their functions at the interface.

2.3. In vitro studies

Bone cell culture models are increasingly employed to study bone–biomaterial interactions. Most of the cultures have utilized osteoblastic cells (reviewed by Cooper et al. [36]), with only a few using osteoclastic cells [20,37]. Primary and passaged cells from several species and anatomical locations have been used, as well as several osteosarcoma, clonal, and immortalized cell lines. Substrate-dependent differences have been reported (Figs. 6–7), however, the variety of models used makes it difficult to draw consensus conclusions.

Whether the different bone cell models would be expected to give comparable results is still a subject of debate. There are few side-by-side comparisons of different bone cell culture models on the same substrates. Taking into account the different microenvironments from which the cells come, there is no a priori reason to expect that all ‘osteoblastic’ cells, e.g., those derived from bone marrow or calvaria, would behave the same, at least during the initial phases of culture. In fact, unpublished observations indicate differences in adhesiveness of bone marrow-and calvaria-derived osteogenic cells on certain substrates (Nanci and Puleo). Nonetheless, in vitro models have the potential to help elucidate events at the bone–implant interface (reviewed by Davies [38]), by providing morphological, biochemical, and molecular information regarding osteoblastic development and synthesis of matrix at the interface with various biomaterials. An important consideration, however, is that the information obtained reflects in vivo events. That the interfacial behavior of some bone cell cultures can be similar to that in vivo is exemplified by the formation of a lamina limitans-like layer (described earlier) and appropriate organization of mineralized matrix during culture on various substrates (Fig. 8) [38–40].
2.4. Biomechanical considerations

In addition to the biomaterial and biological events described above, the biomechanics of the bone–implant interface must also be considered. Although a thorough discussion is beyond the scope of this paper, two important biomechanical aspects will be addressed. The reader is referred to other works for more information [21–23].

First, consider the presence of a cement line/lamina limitans at the interface between bone and biomaterial, similar to that between mineralized matrices or bone cells and mineralized matrix. Because mechanical failure of bone frequently localizes at cement lines, they are generally thought of as weak points [41,42]. As such, the a fibrillar mineralized layer at the bone–implant interface would not be expected to have substantial tensile or shear strength. Mechanical testing of a variety of implanted biomaterials confirms that the tissue–implant interfacial strength is significantly inferior to the intrinsic strength of bone [43–47].

Second, excessive interfacial micromotion during bone healing is detrimental to osseointegration [21–23]. Current estimates put the threshold at about 100 μm [23]. There is speculation that relative motion between bone and implant damages the fibrin network and new vasculature that are part of the early bone healing process, consequently rerouting the healing response into repair by scar tissue. Considering the complexity of the tissue–implant interface and reports of mechanical loading altering cell responses in vitro and in vivo [48–51], this explanation may be too simplistic. Definitive studies determining effects of micromotion on ultrastructure and composition of the interface are still lacking and absolutely essential.

3. Controlling the bone–implant interface

Different approaches are being used in an effort to obtain the desired bone–implant interface. The ideal
implant should present a surface conducive to or that will induce osseointegration, regardless of implantation site, bone quantity, bone quality, etc. As Kasemo and Lausmaa [52], among others, have described, biological tissues interact with mainly the outermost atomic layers of an implant. Although secondary and other by-product reactions will occur, the ‘primary interaction zone’ is generally about 0.1–1 nm. Consequently, much effort is being devoted to methods of modifying surfaces of existing biomaterials to achieve desired biological responses. As described by Ito et al. [53] with respect to polymers, the approaches can be classified as physicochemical, morphological, or biochemical.

3.1. Physicochemical methods

Surface energy, surface charge, and surface composition are among the physicochemical characteristics that have been altered with the aim of improving the bone–implant interface. Glow discharge has been used to increase surface free energy in order to increase tissue adhesion [54,55]. Considering the role of electrostatic interactions in many biological events, charged surfaces have been proposed as being conducive to tissue integration [56,57]. Calcium phosphate coatings have been extensively investigated because of their chemical similarity to bone mineral [58,59]. Each approach, however, has drawbacks. Increased surface energy does not selectively increase the adhesion of particular cells or tissues, and it has not been shown to increase bone–implant interfacial strength [60]. Contradictory results with charged materials in bone have been reported; indeed both positively [56] and negatively [57] charged surfaces were observed to promote bone formation. Although short-term clinical results have been encouraging [59,61], dissolution of coatings as well as cracking and their separation from metallic substrates remain concerns [62,63].

3.2. Morphological methods

Alterations in surface morphology and roughness have been used to influence cell and tissue responses to implants. Porous coatings were developed with the rationale that, because of mechanical interlocking, bone ingrowth would increase fixation and stability of the implant. Data from retrieval studies of orthopedic implants, however, indicate that only a relatively small portion of the available pore volume is filled with bone [64–66]. In addition to providing mechanical interlocking, surfaces with grooves can induce ‘contact guidance’, whereby the direction of cell movement is affected by the morphology of the substrate [67]. This phenomenon has applications in preventing epithelial downgrowth on dental implants and directing bone formation along particular regions of an implant. Mineral deposits in bone cell cultures can also be altered by surfaces with pits and grooves [68].

3.3. Biochemical methods

Biochemical methods of surface modification offer an alternative or adjunct to physicochemical and morphological methods. Biochemical surface modification endeavors to utilize current understanding of the biology and biochemistry of cellular function and differentiation. Much has been learned about the mechanisms by which cells adhere to substrates [69], and major advances have been made in understanding the role of biomolecules in regulating differentiation and remodeling of cells and tissues, respectively [70]. The goal of biochemical surface
modification is to immobilize proteins, enzymes, or peptides on biomaterials for the purpose of inducing specific cell and tissue responses or, in other words, to control the tissue–implant interface with molecules delivered directly to the interface.

Although there are several reports of biochemical surface modification for modulating tissue responses to cardiovascular materials [71–74], this approach has received comparatively little, but increasing, consideration for orthopedic and dental applications [75–79]. This methodology has great potential for controlling initial bone–implant interactions. In contrast to calcium phosphate coatings, biochemical surface modification utilizes critical organic components of bone to affect tissue response.

One approach to controlling cell–biomaterial interactions utilizes cell adhesion molecules. Since identification of the Arg–Gly–Asp (RGD) sequence as mediating attachment of cells to several plasma and extracellular matrix proteins, including fibronectin, vitronectin, type I collagen, osteopontin, and bone sialoprotein [80], researchers have been depositing RGD-containing peptides on biomaterials to promote cell attachment. Cell surface receptors in the integrin superfamily recognize the RGD sequence and mediate attachment [69]. Because of redundancy in the affinity of integrins for adhesive proteins and because a variety of cells possess the same integrins, nonspecific attachment of cells to RGD-modified surfaces is a concern. Some groups are attempting to circumvent this problem by using longer peptides, having a particular conformation, rather than short tetra-, penta-, or hexa-peptides [81]. Others are examining non-RGD peptides that may be more specific for bone cells [82,83]. Furthermore, a combination of immobilized peptide and soluble growth factor(s) might be needed to elicit specific responses [84]. Presently, more studies are needed to develop surfaces, modified with cell attachment peptides, that are selective for only osteoblastic cells.

A second approach to biochemical surface modification uses biomolecules having demonstrated osteotropic effects. A great amount of information has been obtained about biomolecules involved in bone development and fracture healing. Many growth factors have been cloned and are recombinantly expressed. They have effects ranging from mitogenicity (e.g., IGF-I, FGF-2, and PDGF-BB) to increasing activity of bone cells (e.g., TGF-β1 enhances collagen synthesis) to osteoinduction (e.g., BMPs) [85,86]. By delivering one or more of these molecules, which normally play essential roles in osteogenesis, directly to the tissue–implant interface, bone formation may be promoted.

Two considerations about delivering biomolecules to the tissue–implant interface are: (1) local cell populations must interact with the biomolecules for a period of time to initiate cellular events and (2) concentrations of bio-
molecules must exceed threshold levels for cellular activity [87]. Data regarding the duration of exposure or concentration needed for optimal activity of osteotropic biomolecules are lacking, however.

To control exposure and concentration, retention and/or release of biomolecules from implant surfaces can be altered using different methods, including adsorption, covalent immobilization, and release from coatings (Fig. 9). The simplest way to deliver biomolecules to the tissue–implant interface is by dipping the device in a solution of protein before inserting it. Studies using simple adsorption indicate that delivery of TGF-β to the tissue–implant interface can improve bone formation in the periprosthetic gap [88,89] and can enhance bone ingrowth into porous coatings [76]. Using a similar approach, alkaline phosphatase adsorbed on titanium implants enhanced periprosthetic bone formation [90]. One drawback with the adsorption method, however, is that it provides little control over the delivery, including release/retention and orientation, of molecules. Proteins are initially retained on the surface by weak physisorption forces, then, depending on the implant microenvironment which varies between anatomical sites and between patients, they desorb from the surface in an uncontrolled manner to initiate desired responses.

Considering the necessity of specific receptor-ligand interactions for activity of many relevant biomolecules, appropriate presentation of protein may also be needed. Although positive responses have been observed using this simple approach, there is no indication they are optimal for clinical applications.

Bonding biomolecules to implants is an alternate way of delivering them to the tissue–implant interface, albeit protein will not be released. This approach is more complicated than adsorption, because of the chemistry involved, but the activity of molecules immobilized on plastics has been shown to equal or exceed that of soluble
In addition to an initial release over 1–4 days that can initiate cell and tissue responses, biomolecules are retained in the collagen matrix coatings and would be available for later release to sustain responses [102]. The amounts of protein released and retained can be controlled by the amounts of collagen and/or biomolecule. Additionally, collagen coatings will be turned over in vivo and replaced with new tissue during the healing response.

Recently developed injectable, absorbable calcium phosphate cements may also be useful for biochemically modifying the tissue–implant interface [106,107]. These materials solidify in situ to temporarily stabilize the implant and allow early loading, while providing an osteoconductive environment as the cement is replaced with bone. A further development of the cements would be to use them as a medium for delivery of bioactive agents to the bone–implant interface.

4. Conclusion

Many advances have been made in understanding events at the interface between bone and implants and in developing methods for controlling these events. Several important questions, however, still remain. What is the relationship between tissue structure, matrix composition, and biomechanical properties of the interface? Do surface modifications alter the interfacial tissue structure and composition and the rate at which it forms? If surface modifications change the initial interface structure and composition, are these changes retained? Do surface modifications enhance biomechanical properties of the interface? As understanding of the bone–implant interface improves, so will development of proactive implants that can promote desired outcomes.

Acknowledgements

The bone marrow culture used for Fig. 6 is a courtesy of Dr. Cedric Minkin, University of Southern California, Los Angeles, CA. Dynamic histomorphometric analyses (Fig. 5) were carried out by Dr. Louis-George Ste-Marie, Centre de recherche André Viallet, Montreal, QC. Supported by the National Science Foundation (DP), The Whitaker Foundation (DP), Medical Research Council of Canada (AN), and Theratechnologies Inc. (AN).

References


