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THE INTERFACE ZONE OF INORGANIC IMPLANTS *IN VIVO:* TITANIUM IMPLANTS IN BONE

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The interface zone between titanium implants and bone is considered at the macroscopic, microscopic, and molecular levels. A high rate of successful dental implants of pure titanium is associated with a very close apposition of the bone to the titanium surface, called osseointegration. At the macroscopic level, osseointegration allows efficient stress transfer from the implant to the bone without abrasion or progressive movement that can take place if a fibrous layer intervenes. At the microscopic level, surface roughness and porosity provide interlocking of the implant and bone tissue which grows into direct contact with titanium. Sections studied in the electron microscope show that calcified tissue can be identified within 50 Å of the implant surface. The interface zone includes a tightly adherent titanium oxide layer on the surface of the implant which may be similar to a ceramic material in relation to tissue response. The five year success rate of 90% in 2895 implants in clinical trials since 1965 is associated with the favorable behavior of bone tissue at the interface zone with pure titanium.

Keywords *— Interface zone, Titanium implants, Osseointegration, Dental implants.*

1. INTRODUCTION

Under certain circumstances implants manufactured from nonalloyed titanium and inserted into human bone will establish and maintain a direct contact of implant to hard tissue which we call *osseointegration.* In a computerized evaluation program (1), the 5-year success rate for osseointegrated dental implants has been approximately 90%. Most other clinical dental implants are surrounded by a connective tissue layer which thickens with time and reduces the long term success rates in humans, typically under 50% [for a review see Hench (19)].

In spite of a high level of success with osseointegrated implants the precise reasons for this favorable record are not completely known. The present overview is based on an interdisciplinary analysis of this problem. The main purpose of our discussion is to examine the interface zone of osseointegrated titanium implants, but many of the results and questions have a more general validity.

A more complete picture of the interface zone has been arrived at by a combined approach with participation of scientists from the area of solid-state physics, surface physics, biophysics, biochemistry and the medical sciences. This collaboration has made possible a many-faceted discussion of the interface zone from different points of view, including macroscopic, microscopic, and molecular aspects.

One important conclusion is that a crucial factor that determines the success or failure of a particular implant is the structure and chemical properties of the outermost atomic layers of the implant and the first molecular layers of biomolecules adsorbed on the implant. Another conclusion is that from a chemical–biological point of view there may be little difference between ceramic implants and nonalloyed titanium fixtures since the latter are covered by a tightly adherent oxide layer which is actually the only part of the implant, at least initially, in direct contact with the tissues.

Consider the situation when an inorganic implant is first being placed at the desired position in a bone. The chemical and biological processes that are initiated at the interface between the inorganic solid implant and the biological tissue will depend on a number of factors that determine whether the implant eventually becomes successfully osseointegrated or not. A useful but somewhat ambiguous classification of these factors is obtained by associating them with either the implant properties or with the biological environment. Additional factors may be associated with external perturbations during or after the healing process, such as mechanical stress, disease, medication, etc. We will discuss some of these different factors separately, emphasizing that the relative importance of any one factor may be strongly influenced by some of the others. This is one reason why an interdisciplinary approach to the subject is necessary.

Extensive animal experimental series aiming at investigating the cellular reactions to an implantation procedure were carried out at the Laboratory of Experimental Biology during the 1960s and 1970s. Brånemark *et al.* (10) studied cell differentiation phenomena in bone marrow of the rabbit and the interrelationship between the marrow compartment and the surrounding bone tissue. There is not only a vascular interdependence between bone and marrow (9), but also, at least partly, a tissue dependency on the same stem cells for repair. The importance of a minimally traumatizing surgical technique to avoid unneccessary vascular and cellular injury in the bone and marrow was demonstrated in a 5-year follow-up of canine dental implants (11). In contrast, if a traumatizing surgical technique was used, this inevitably led to loss of the implants. The function of vitallium implants was compared to that of titanium ones which led to the conclusion that the latter were better tolerated in the body environment. These findings have later been confirmed in several experimental works (2, 3, 25).

In 1977, Brånemark *et al.* (12) published a consecutive clinical material, study of 1618 implants inserted in the jaws of 211 edentulous patients and followed up for 1 to 10 years. Brånemark's presentation of 1977 differed from that of many other dental implant papers in the fact that the outcome of each and every single inserted implant had been analysed and included in the statistics. All patients had been reviewed at least once annually. Brånemark used threaded implants of pure (99.8%) titanium and of a defined surface microstructure. The implants were installed with a minimally traumatizing technique into a non-infected bone bed. Individual loading of the implants was allowed first after an initial healing-in period of 3–5 months. After that time the implants were fully load-bearing. Based on the results of this extensive clinical study it was concluded that the osseointegration method for direct bone anchorage of implants can provide a lasting anchorage for dental bridges and that the soft tissue around the penetrating abutments formed some sort of functional barrier, most important for the long term clinical success.

Albrektsson *et al.* (4) presented an interface analysis of dental implants (Fig. 1) belonging to the clinical material published by Brånemark *et al.* (12). The

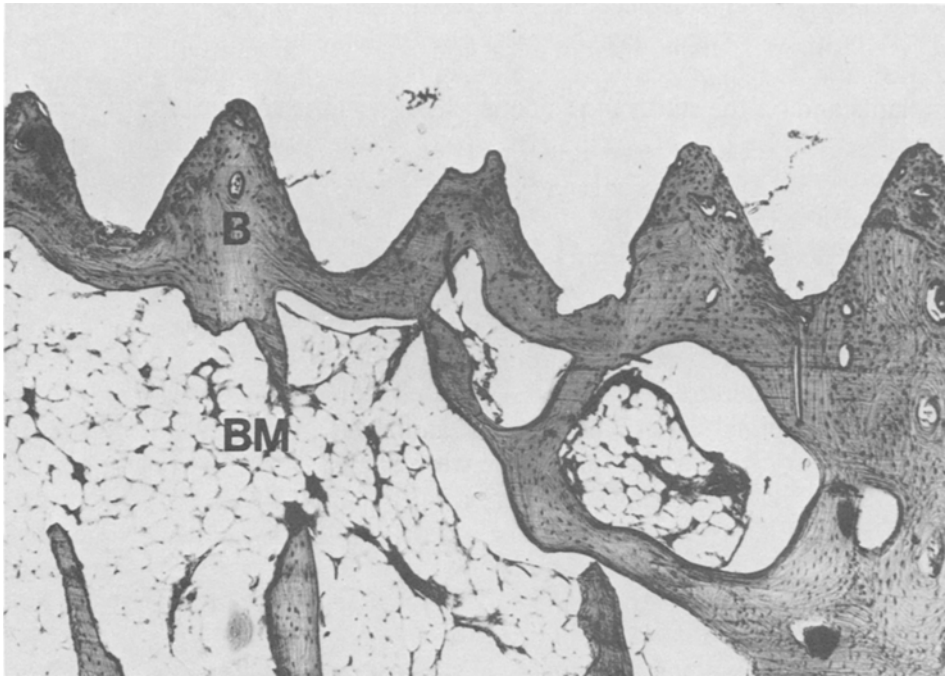


FIGURE 1a. Histological section of bone (B) detached from a titanium implant after 7 years of clinical function. The implant was removed in spite of an undisturbed bone anchorage. Bone Marrow = BM.

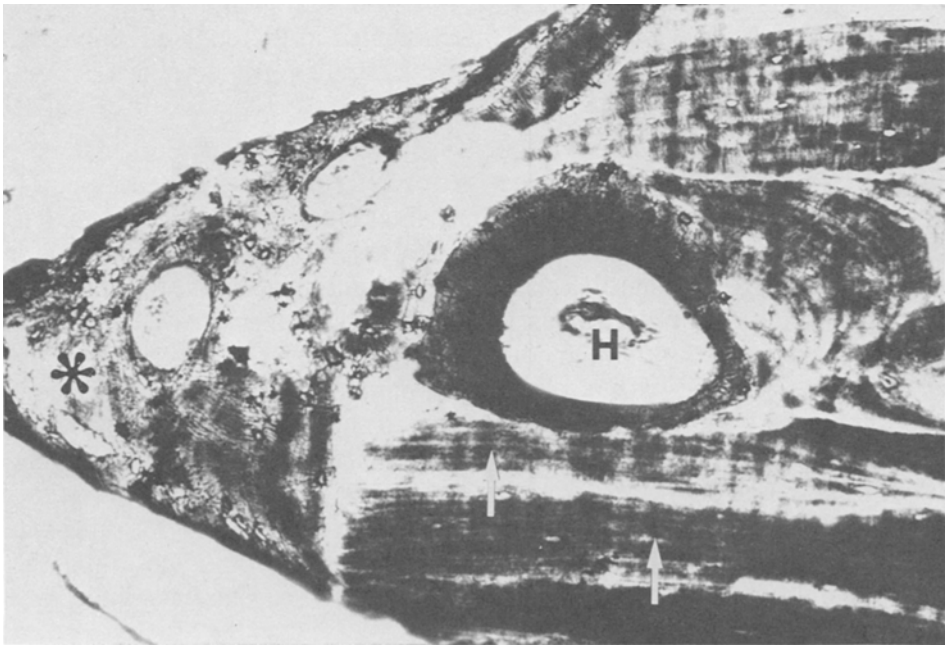


FIGURE 1b. Higher magnification of a bone thread from the same patient as Fig. 1a. A Haversian system (H) with concentric lamellae is seen. Arrows point at circumferential lamellae. The apical part (*) of the bone thread consists of a well organized bone with distinct osteocytes.

investigated implants had been removed in spite of an undisturbed bone anchorage after up to 7 years of load-bearing. The authors summarized that osseointegration of a foreign device depends on material, design and surface finish of the implants and on the status of the bone, the surgical technique and the loading conditions. From this and other studies (4, 12) we know that a direct contact between living bone tissue and titanium is achievable at the resolution level of the electron microscope (Figs. 2 and 3). In the middle of the sixties, however, most authorities were of the opinion that a direct metallic implant anchorage was an impossible goal (12). An implanted metallic object was thought inevitably to be surrounded by a connective tissue layer (29). However, a direct bone anchorage of implants is today not only regarded as possible but is generally accepted as a desirable goal for the surgeon (15, 18, 22, 23).

In the Gothenburg clinic up to 1981, altogether 2768 titanium implants have been inserted into 410 edentulous jaws to function as support for stable dental bridges (1). Figure 4 shows the titanium screw implants in the jaw. As only totally edentulous patients have been included in this clinical material, the entire masticatory load has been carried by the implants. All inserted implants, with no exception, have been included in a careful, computerized evaluation program with repeated clinical and roentgenological examinations. The five to nine year implant function frequency in the lower jaw amounts to 91%. Furthermore, most of the observed failures have occurred during the first year and if only the implants which have uneventfully passed the initial healing-in phases are included, a five to nine year success rate of 99.1% is found. These figures

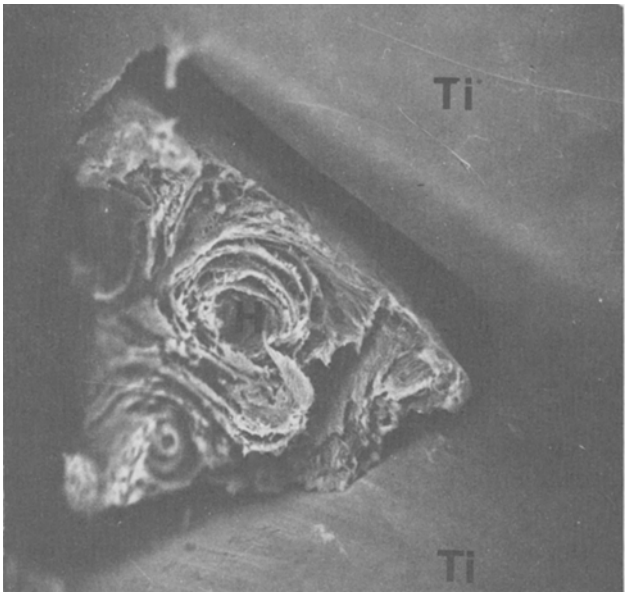


FIGURE 2. Scanning electron micrograph of a bone thread, partly detached from the Titanium (Ti) due to the decalcification procedure. A major Haversian system (H) is seen centrally in the bone tongue. Observe the regular organization of the collagen forming circumferential lamellae.

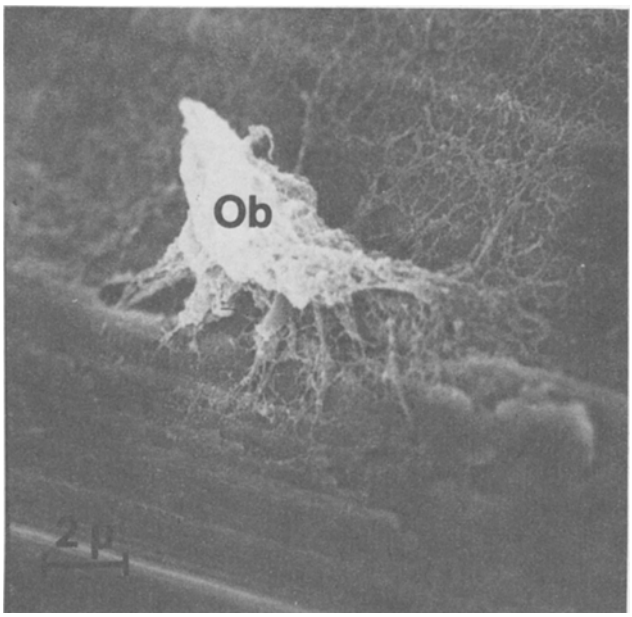


FIGURE 3a. A scanning electron micrograph of an osteoblast (Ob), via its processes remaining adherent to the titanium oxide surface irregularities. The bone was removed prior to the analysis. The implant was removed from a human jaw after 5 years of clinical function.

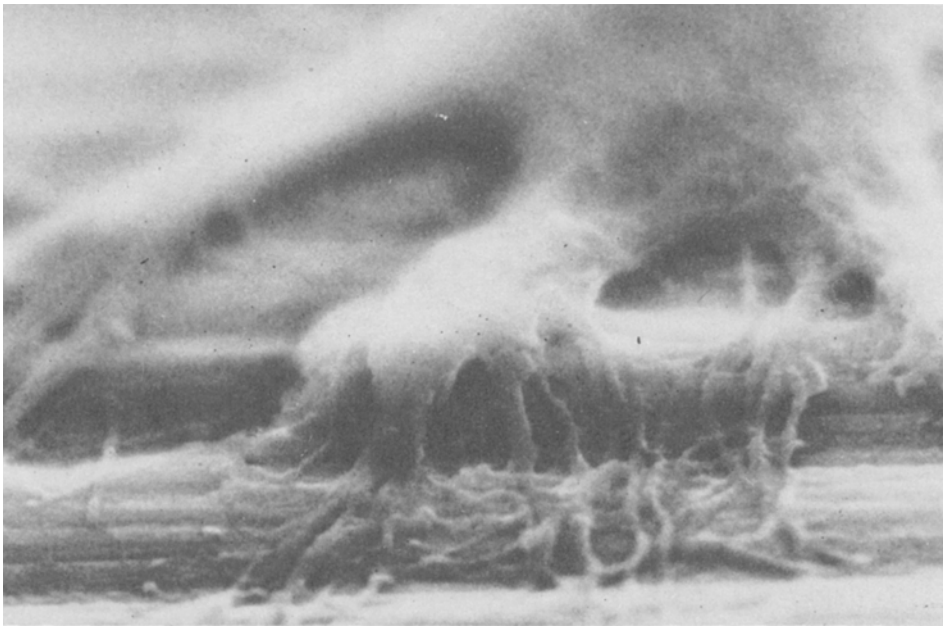
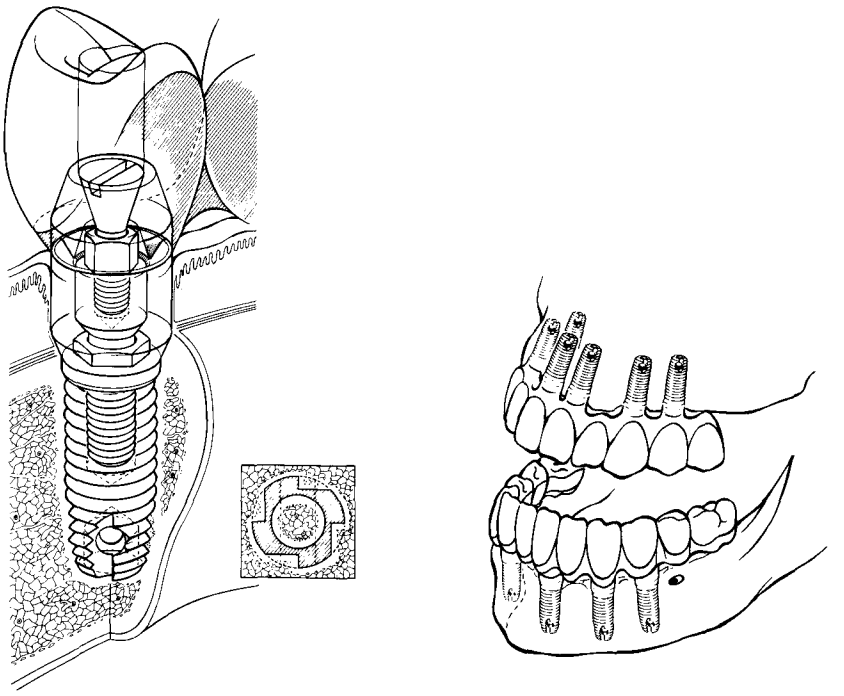


FIGURE 3b. Detail showing cellular processes closely following the surface of the titanium implant.



a **b**

FIGURE 4.(a) A titanium implant in the mandible to function as support for a dental bridge. To ensure osseointegration the implant has to be inserted with a gentle surgical technique and unrestricted loading should be avoided during the first months after implantation. (b) Usually 5-6 implants are inserted into each jaw and connected via a dental bridge.

make the Gothenburg material unique in comparison to other published long-term follow-up clinical reports on dental implants.

Haraldson (17) compared 35 patients with osseointegrated implant bridges and dentate controls with respect to maximal bite force levels and muscular reflex activity. No differences were found between the test and the control groups. It was concluded that the osseointegrated patients had been restored to a level of functional capacity of their masticatory system equal to that in individuals with a natural dentition.

Combination of an osseointegrated titanium screw and a transcutaneous passage (13) has led to the development of a bone transmission hearing aid (31) and also to clinical tests of a new type of direct episthesis attachment in cases of severe facial deformities (32). Parts of these studies have been followed up for 5 years. Various orthopaedic applications of the osseointegration principle are at present being clinically evaluated in the reconstruction of metacarpophalangeal joints (16) and in rehabilitation of tibial amputee cases.

The following sections (II, III, IV) cover three complementary views of the interface. In Sec. II, the macroscopic properties of implant and bone are discussed and the mechanical aspects of stress, deformation, etc., are considered.

The microscopic mechanical properties are then discussed with special attention to the role of surface roughness and porosity. In Sec. III, the microstructure of the interface is analyzed at the scale of molecular dimensions. The role of the surface composition of the implant and of treatment of the bone is discussed and the possible bonding mechanisms, structure and chemical composition at the interface are considered. In the last section, the dynamics of the interface on different time scales are briefly discussed. Then some of the most important questions that have been formulated during this research are reviewed and the problems that need to be solved are identified. Finally the experimental methods necessary for such further research are considered. Figure 5 represents a schematical interpretation of the boundary layers around threaded titanium implants.

II. MECHANICAL ASPECTS

A. Macroscopic Material and Mechanical Properties

The first consideration in the mechanical behavior of titanium implants in bone is the relative strength and stiffness of titanium and bone. The main fact is that titanium is much stiffer than bone and can carry much larger stresses. This means that any difficulties of mechanical failure are to be expected first in the bone or in the bond of the bone and titanium. This assumes, of course, that the implants are not made too thin and are not used in a way that produces excessive stress concentrations in the implant.

The relevant mechanical properties of titanium are the Young's modulus, E, which is about 1.1×10^{11} N/m^2 and Poisson's ratio which is about 0.3. The failure stress for titanium for our purposes may be taken to be the stress at which the material first yields and undergoes permanent deformation. In tension, the failure stress of titanium is about 3×10^8 N/m^2. The strain at which failure of titanium occurs in tension is about 0.2.

The above values for titanium are usually quite uniform and isotropic within any item made of titanium and will not usually vary much from specimen to specimen. In contrast, the properties of bone vary widely depending on the type of bone involved, the direction of loading, and history and condition of the individual. Nevertheless, the values to be expected in bone are clearly much smaller than for titanium.

The Young modulus of cancellous bone in tension or compression at low stress levels is of the order of 10^{10} N/m^2 (7,8,28). The ultimate strength of bone is much less than that of titanium. The tensile failure stress of cancellous bone is generally about 5×10^7 N/m^2 (7,8,28). Cortical bone has, of course, higher elastic modulii and strength than cancellous bone. The strain at which failure of cancellous bone occurs is thus of the order of 5×10^{-3}.

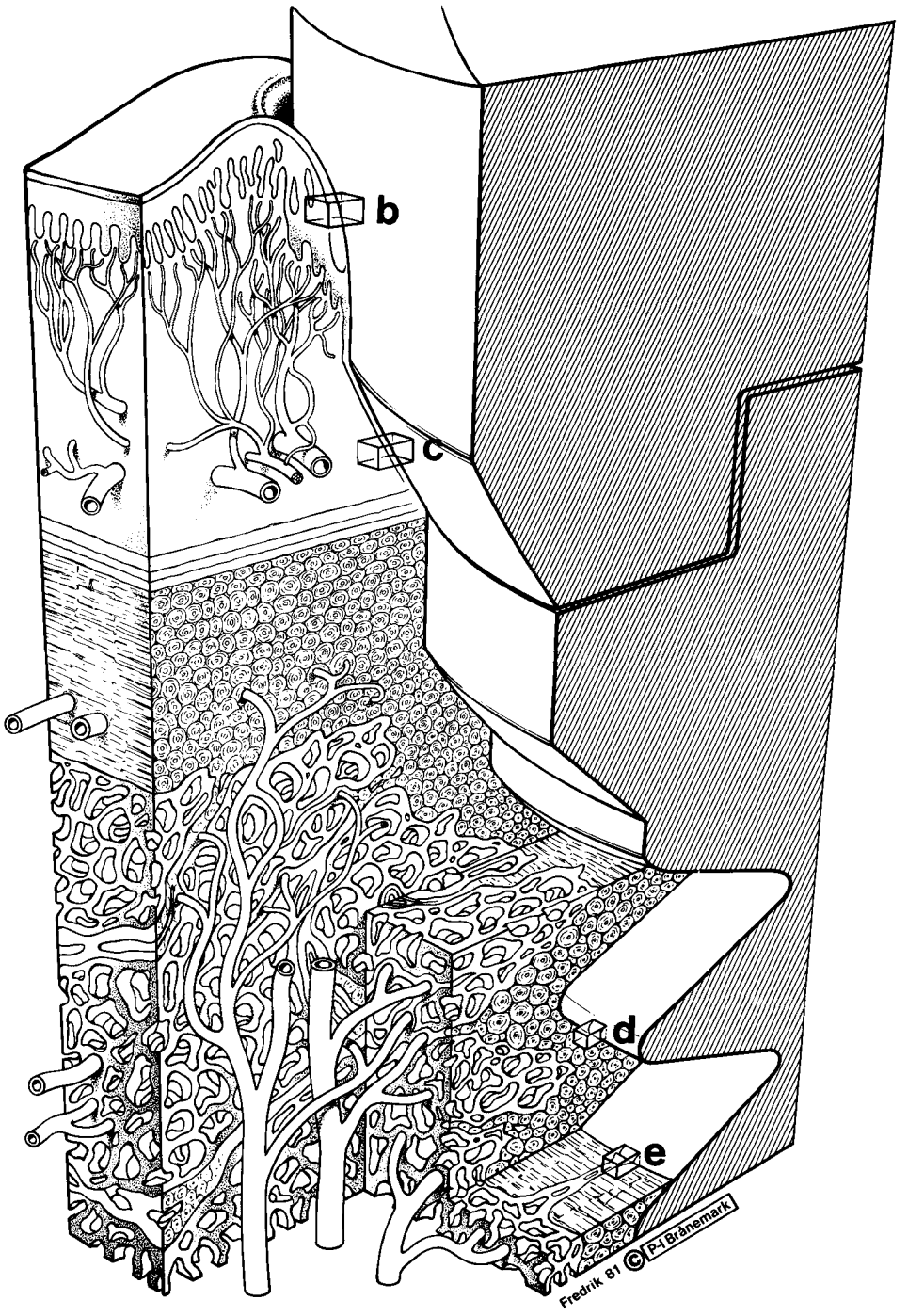


FIGURE 5a. Schematic drawing of tissue-titanium interrelationship showing an overall view of the intact interface zone around osseointegrated implants. The letters b-e indicate the localization of the detailed interface descriptions visualized in Figs. 5b-5e.

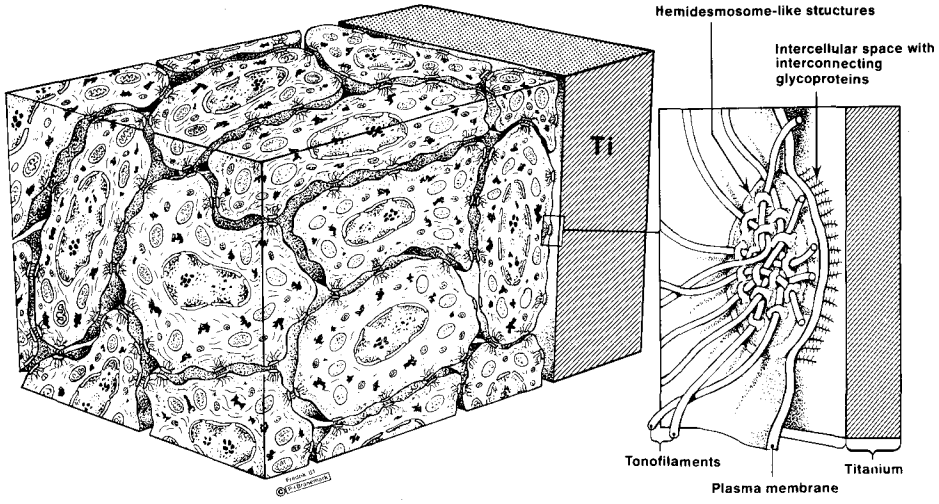


FIGURE 5b. Enlarged, schematical representation of gingival-titanium oxide contact zone. Inserted area demonstrates a hemidesmosome-like structure anchoring the epithelial cells to the implant surface. Ti = titanium.

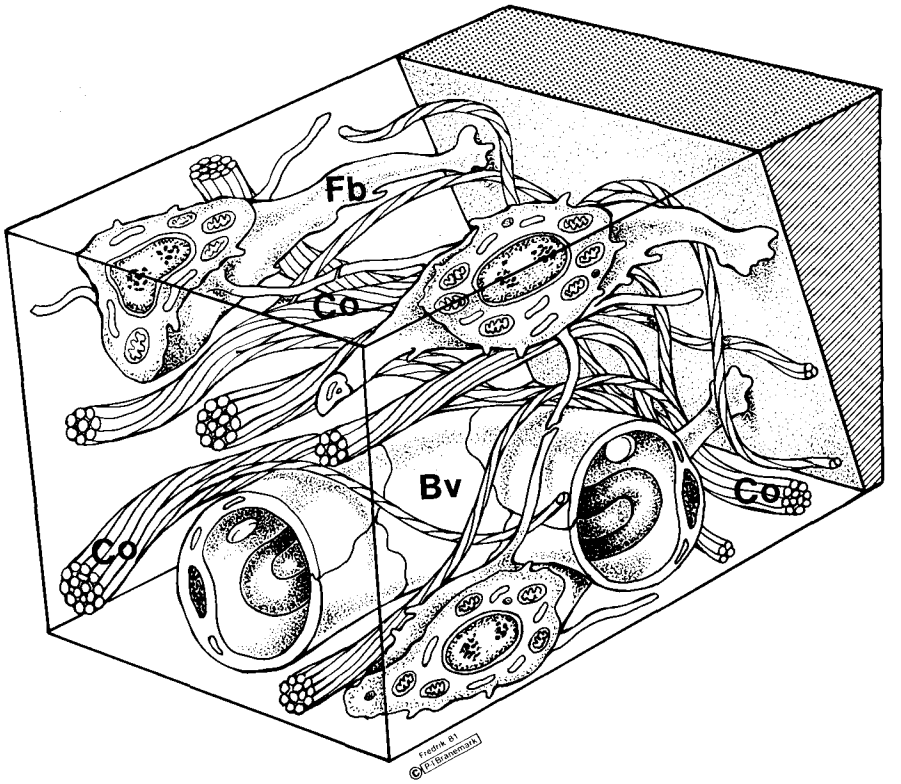


FIGURE 5c. High resolution drawing of subgingival connective tissue at the boundary zone. Fibroblast (Fb) processes are seen in immediate contact with the titanium oxide surface, in reality though separated from it by a thin layer of proteoglycans. Network of collagen = Co, blood vessel = Bv.

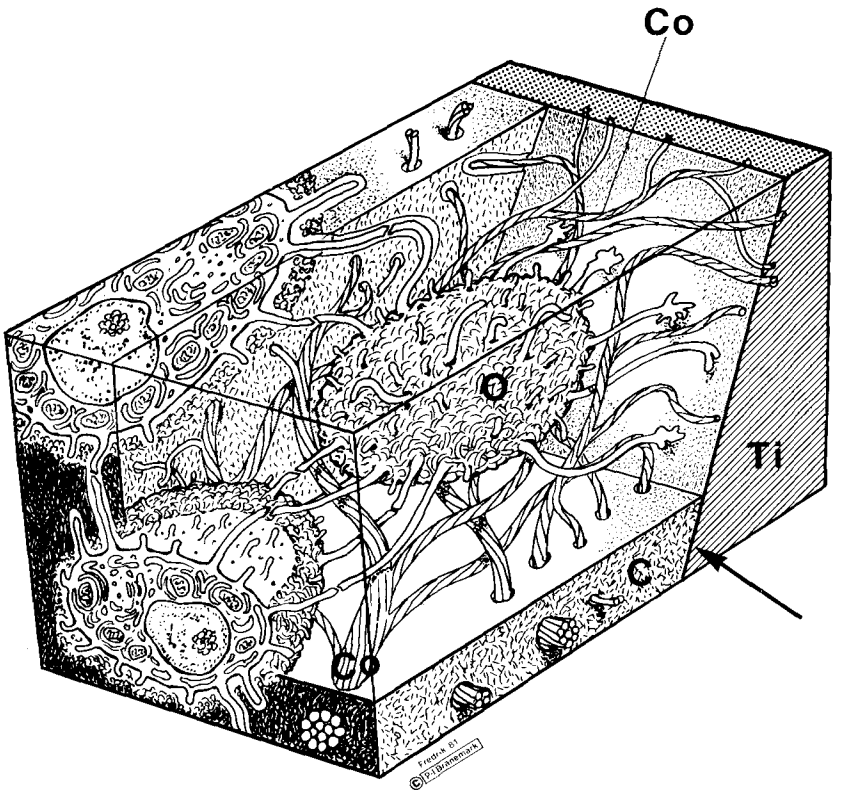


FIGURE 5d. Interface between cortical bone and titanium (Ti). An osteocyte (O) with numerous processes in canaliculi approaches the titanium oxide surface. The calcified ground substance around the osteocyte is removed to show details. A meshwork of collagen (Co) surrounds the bone cell. There is an intimate contact between the ground substance (C) of the Haversian bone and the titanium oxide (arrow).

The above values indicate that the order of magnitude of the stresses in bone at failure are considerably greater than those in titanium at failure. This supports the general conclusion that where there is stress transmitted across the boundary of an interface between bone and titanium, it is usually the bone which is susceptible to failure first. A second conclusion that follows from the above values is that a titanium implant will generally have small values of strain and that the implant may be regarded as a rigid body, approximately, in analyzing the stress and deformation fields in the bone. This is a relevant aspect in the discussion of the detailed transmission of stress via a threaded or rough surface of an implant.

The osseointegrated implants in this study are titanium screws fitted into carefully threaded holes in bone, where osseointegration takes place. The fact that a titanium screw is comparatively rigid suggests also that the stress in the bone will not be dependent on the size or shape of the threads except within a zone of the order of the thread depth in width. The thread effectively distributes the stress in the bone over a much larger area than would have been the case with a smooth implant. As the threads are made finer and finer, the stressed

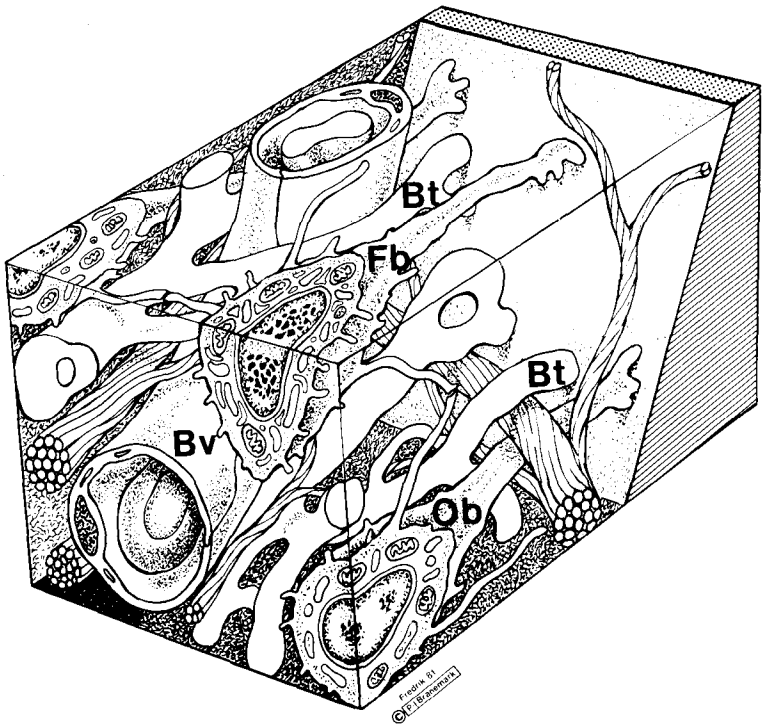


FIGURE 5e. Contact layer between cancellous bone and implant. Note fibroblast (Fb) and osteoblast (Ob) processes approaching the titanium surface. The bone trabeculae (Bt) are seen in close relationship to the implant surface. Bv = blood vessel.

zone becomes narrower. For porous implant surface (pore size of the order of 100 μm) the stresses can be quite localized (if there are no threads) in the vicinity of the pore structure.

B. Microscopic Properties of the Interface

According to the concepts outlined above, the stresses near a smooth and a threaded implant will be similar, but the details of the manner of transfer of the stress will be different. To discuss this situation further, consider a small area of the thread of a screw and a small section of a smooth implant surface. This is shown in Fig. 6.

The shear stress, τ_0, to be transmitted across a cylindrical surface parallel to the implant axis will be the same in the two cases. The solution for the smooth implant is particularly simple. On every vertical plane, including the interface itself, the shear stress will be exactly τ_0. This means that for such an implant to be successful, the bone must be able to carry the shear and the interface itself must be able to withstand the shear without slip or fracture.

In the case of the screw, the stress distribution is more complex within the bone. On a cylinder of radius equal to the outer face, the stress is again τ_0. But within the threaded region, the stress must be more complicated to meet the

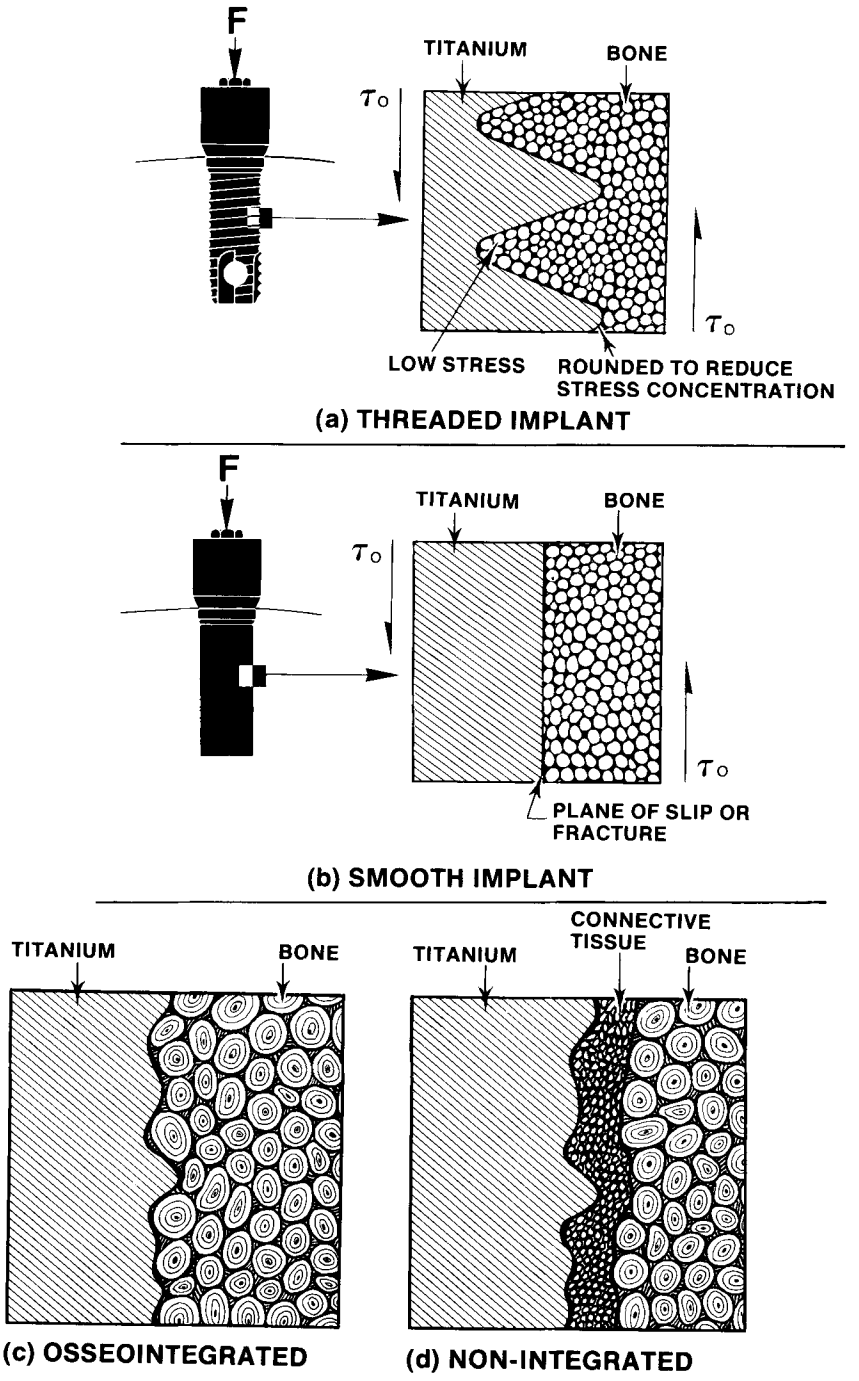


FIGURE 6. Schematic representations of sections of (a) threaded implant and (b) smooth implant. The stressed zones are indicated by the detailed boxes. (c) Microscopic schematic representation of implant surface with close ingrowth of bone (osseointegrated). (d) Implant with nonintegrated bone layer.

boundary condition at the threads. This condition is that the surface of contact along the threads moves as a rigid body. This may be expected to result in very little stress in the bone at interior points of the thread because the material there is carried along as a rigid body. But it will also result in a stress concentration in the bone at the outer edge of the threads. It may be advisable therefore to round the outer edge of the thread to relieve the stress concentration.

Another qualitative feature of the screw as compared to the smooth implant is that the role of the bond is more important in the latter than in the former. For a smooth implant if the bond of the interface is broken, no stress can be carried except by friction. For a screw, shear stress can still be carried in the absence of bond (as in an ordinary screw–nut system) by compression onto the inclined faces of the threads. This feature is a basic advantage of a screw or any similar set of grooves or roughness. However, this action depends on the very close apposition of the bone and screw, i.e., it depends on the growing bone to be touching the implant prior to loading. Otherwise there may be relative motion under load and the fixture may work loose in time.

The above ideas are also applicable to a discussion of surface roughness and porosity of the implant. Consider cases where the boundary between the implant and bone is irregular. These irregularities are supposed to be large compared to molecular sizes involved but small say compared to the size of the threads and not visible to the naked eye. Note, however, that a close apposition of bone and implant is still assumed. The roughness is originally on the implant and the bone grows into every irregularity perfectly. Under these conditions, the qualitative nature of the results can be readily summarized. In compression the role of roughness is minor, but in shear its role can be very large and beneficial. The small irregularities of the surfaces interlock and aid in transfer of shear stress in the same way that the threads of the screw act, as discussed above. The range of possibilities can go from a fully bonded, interlocked surface which can develop the full strength of the bone itself in shear to a smooth, sliding (unbonded) surface which slips easily under small shear. The roughness is thus an aid to carrying stress provided the bone grows closely into the surface roughness of the implant. The zone of influence of the roughness will be about twice the roughness height, analogous to the case of the screw. If the bone is not in close approximation to the implant, the roughness may be detrimental as the peaks of roughness will then lead to stress concentrations and possible degradation of the bone tissue.

Porosity of the implant in which the pores extend far into the implant may also be helpful in carrying various kinds of stresses across the boundary (27). If this occurs to a sufficient extent, it may be helpful in carrying tensile stresses as well as shear stresses. In compression it will not be needed and will not be beneficial or detrimental.

It should be emphasized that the beneficial effects of roughness and porosity mentioned above occur only if the bone grows closely into the irregularities of the implant surface. If the bone only touches the porosity at a few places, with

nonintegrated material in between, there may be an abrasive effect under shear stress and degradation of the bone (Fig. 6).

III. MICROSTRUCTURE AND BONDING IN THE INTERFACE ZONE

A. Physical Aspects

Having discussed the mechanical properties of titanium implants and having concluded that close contact between the bone and the implant is desirable, it is now appropriate to study the biochemical and biophysical properties of the interface itself. This interface is not to be regarded as a distinct boundary between implant and bone, but rather as a zone which is several hundred Ångström thick and contains a large variety of molecules, and different types of structures containing crystalline and cellular elements. We call this the interface zone. The interface zone is unique in that it contains structures and atomic arrangements that are not found elsewhere, and the question of where the transition from inorganic material to organic tissue takes place becomes delicate and difficult to answer. This unique situation is the result of the chemical and transport processes taking place during osseointegration which leaves no sharp boundary.

In this section we shall discuss the interface zone by approaching it first from the implant side and then from the bone side. Figure 7 is a schematic representation of the interface zone.

The bulk of the implant is pure titanium. According to Avesta Jernverks AB, which is the source of the titanium, its composition is the following:

titanium	Ti	99.75%
iron	Fe	0.05%
oxygen	O	0.10%
nitrogen	N	0.03%
carbon	C	0.05%
hydrogen	H	0.012%

Its structure is polycrystalline, that is, it consists of randomly oriented crystallites whose sizes depend on the material processing. During the manufacturing of the implant and during the cleaning processes (ultrasonic cleaning, autoclaving) an oxide layer develops on the surface of the metallic implant. We shall spend some time discussing this oxide layer since it determines the properties of the implant surface which is of utmost importance for the development of the implant–bone interface zone.

The thickness of the oxide on a fresh implant is of the order of 50 Å, which is ten to twenty times larger than typical atomic dimensions. An important consequence is that a biological molecule approaching the implant from the bone side sees a *metal oxide,* and not a metal surface. Metallic implants which form oxide

layers on their surfaces may, therefore, from a chemical–biological viewpoint, be regarded as ceramics. Thus, in the case of titanium the most relevant chemical properties to consider are those of titanium oxides (which are ceramics) and not the chemical properties of titanium metal. We also want to stress, however, that the composition and morphology of the surface oxide are expected to differ from the corresponding properties of the bulk oxides (see below).

One important role of the oxide may be to prevent direct communication between the pure metal and the biological molecules. This does not exclude the possibility that metallic titanium participates in the dynamic growth of the interface zone (see below), but nevertheless a direct contact between metallic titanium and biological molecules is prevented.

From the point of view of chemical inertness the titanium oxides are very attractive since they are chemically among the most stable and corrosion resistant materials (14). Titanium forms several oxides such as TiO_2, TiO, Ti_2O_5, of which TiO_2 is probably the most common. It appears in three different crystalline forms with different physical properties. The anatase and rutile structures are tetragonal, and the brookite structure is orthorhombic. Of special interest is the dielectric constant, ϵ. For anatase it is 48, for brookite 78, and for rutile it is between about 110 and 117 depending on orientation. No other metal oxides have such high dielectric constants. The actual composition of the oxide on the implant is yet unknown, but one would in general predict a rather nonstoichiometric oxide as a result of the low temperature oxidation of the polycrystalline metal. We, therefore, prefer to name the oxide TiO_x with x as a yet undetermined but smaller number. The probable presence of these various oxides means that the material can adapt to variations of redox potential in the environment through variations of the value of x. The titanium oxide probably plays an important role in reducing the rate of corrosion of the implants and the rate of diffusion of metal ions into neighboring biological tissue.

These and other differences between an oxidized metallic titanium implant and a bulk titanium–oxide implant (for instance rutile, TiO_2) may be important for osseointegration. While a bulk oxide implant has a quite uniform stoichiometric composition, the oxide layer on the metal is nonstoichiometric and contains concentration gradients, numerous structural defects, etc., as is illustrated in Fig. 7.

The network in the oxide and metal illustrates the polycrystalline nature of the materials. Where the grain boundaries reach the surface or where there is an excess of metal atoms or oxygen vacancies, sites with specific properties exist, which influence the chemistry of the interface. These sites may very well have a determining influence on the chemical processes that are initiated when the implant is exposed to liquids and to the various biological molecules at the implant location (see also Sec. IV below). A spectrum of defect sites thus represents a range of possibilities regarding, for instance, chemical bond strength and coordination between the implant surface and adjacent biomolecules. The defects could also influence the long term dynamics of the interface zone on a

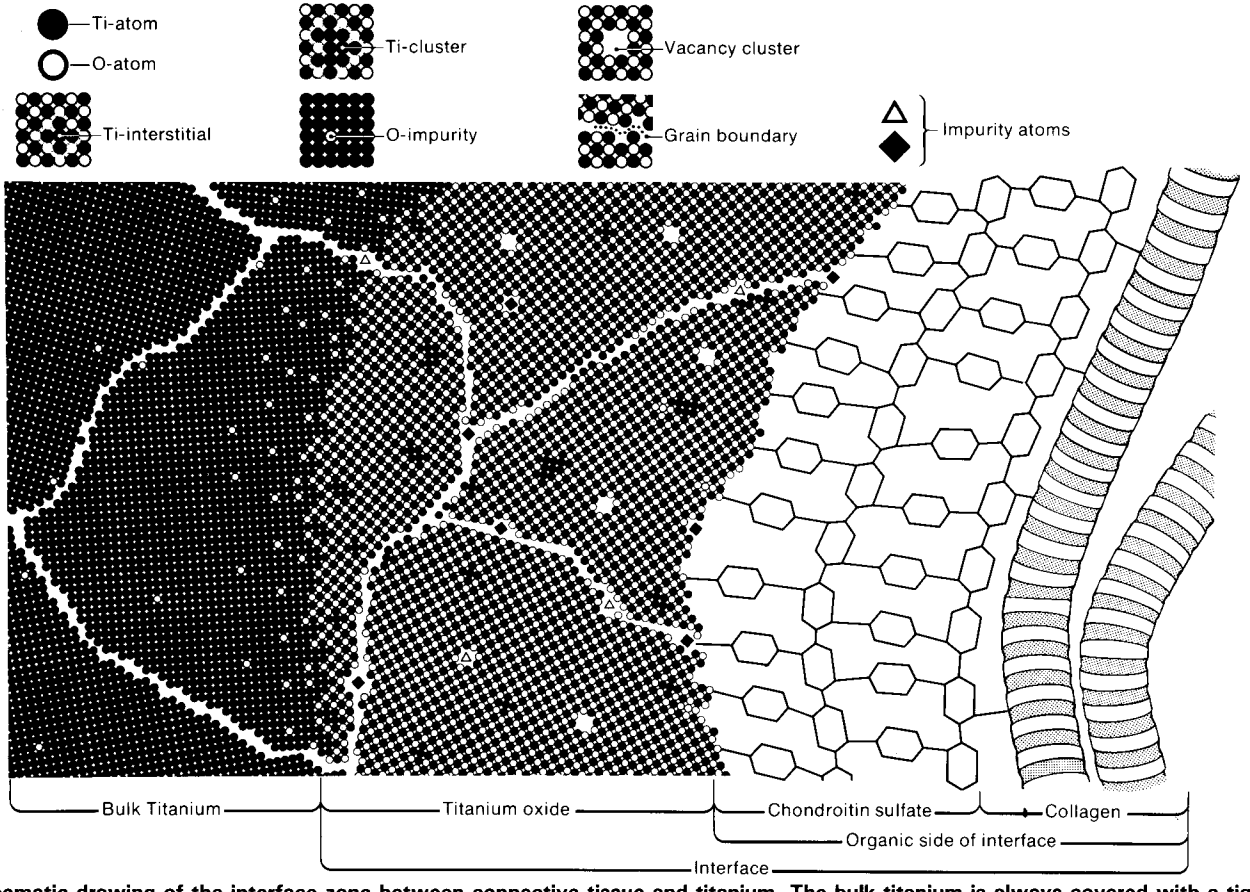


FIGURE 7. Schematic drawing of the interface zone between connective tissue and titanium. The bulk titanium is always covered with a tightly adherent oxide layer of at least 50 Å thickness, formed during the mechanical processing and cleaning and autoclaving treatments of the implants. Therefore, the most relevant interface in the osseointegration process is the one between the titanium oxide and the biomolecules. The tissue ground substance (not to scale!) acts as a glue between the collagen filaments and the implant. The drawing indicates several structural properties and defects of the implant that may be important for the chemistry of the interface, such as grain boundaries, vacancies, interstitials and impurity atoms.

molecular level via their catalytic activity, that is, the implant may be a much more active component than is usually assumed.

The investigation of the role of the microstructure of the implant surface on an atomic scale is therefore of utmost importance in future research on this subject. Such studies require methods of controlling the nature and density of defects on the implant surface, and their chemical properties.

In the discussion above, a local molecular bonding of biomolecules to the implant surface was implicitly assumed. The alternative is that bonding is achieved by long range, dipolar or electrostatic forces. In such a case the atomic defect structure may be less important. Due to their high dielectric constants, near that of water, titanium oxides may exhibit more "natural" interactions with biomolecules than other oxides, for instance aluminum oxides. The most likely situation is that the bonding at the interface is a combination of local chemical bonds, preferably at defect sites, and long range dipolar or electrostatic interaction.

So far we have neglected the small amount of metallic trace element in the "pure" titanium implants. In spite of their small quantity their possible importance cannot be excluded, particularly as it is well known from surface physics and metallurgy that enormous accumulations of low concentration alloy constituents may form at surfaces and interfaces. Before experimental results are available even minor changes in the composition of the implant material should be avoided.

B. Biological Aspects

The structure and biophysical properties of the biological tissue surrounding the implants are of great importance. Bone and collagenous fibres transfer the load exerted on the implant into its surrounding anchoring tissue. The main load directions may vary, as well as the forces exerted, as discussed above.

The oxide surfaces of osseointegrated titanium implants are covered by a very thin layer of ground substance, that is proteoglycans and glucosaminoglycans (Fig. 8). Collagen filaments from the surrounding bone tissue approach the titanium, but are rarely observed closer than 200 Å to the surface (24). The collagenous filaments are usually arranged as a three-dimensional lattice surrounding the implant at a distance of from about 200 Å to 1 μm. Gradually, the fine filamentous network is replaced by bundles of collagenous fibrils and fibres, which are continuous with those of the surrounding bone. Processes from osteocytes also approach the titanium oxide surface, although they are always separated by a layer of ground substance at least 200 Å thick (Fig. 9). In sections which are not decalcified, calcium deposits can be observed very close to the oxide surface, lacking distinct demarcation from the implant. The calcification may sometimes be reduced at the distance of a few μm from the oxide surface. However, it should be stressed that in most areas no gradient in the calcification is observed.

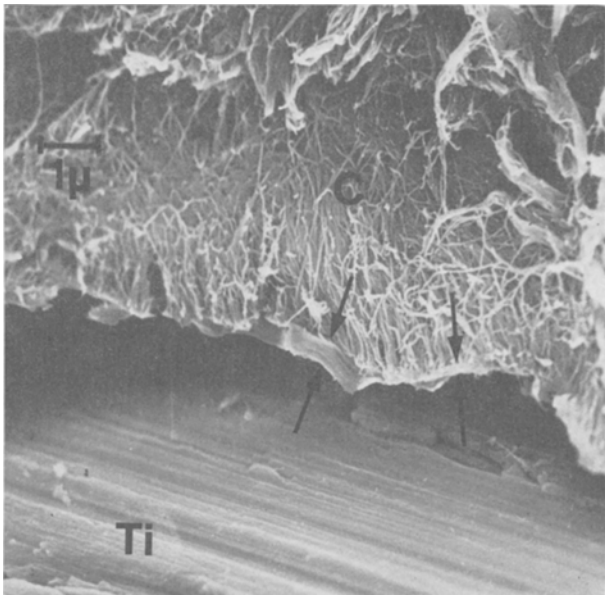


FIGURE 8. Scanning Electron Micrograph of a decalcified specimen originating from the tissue-to-bone interface of a titanium implant which had been load-bearing for 5 years in a female patient. The irregular titanium surface (Ti) is seen in the lower part of the picture. The collagenous network (C) is regularly arranged but is separated from the implant due to the decalcification procedure. However, between arrows, a thin proteoglycan layer is found in the interface zone.

The titanium oxide surface is thus covered by ground substance, i.e., macromolecules consisting of proteoglycans and glucosaminoglycans attached to a backbone of hyaluronic acid. The proteoglycans form the biological "glue" responsible for adhesion between cells, fibrils and other structures. The collagen filaments are easily recognized due to their cross-striation, reflecting their strictly repetitive chemical composition. The individual filaments are arranged in bundles which may reach considerable dimensions. The ground substance forms the cementing matrix.

The glucosaminoglycans of the ground substance, observed to be as close to the titanium oxide as could be resolved in the electron microscope, consist of monosacharides, including hexosamines, interconnected by glucosidic bonds. One of the most common glucosaminoglycans in bone is chondroitinic sulphate [glucoronic acid–*N*(acetylgalactosamine sulphate)]. The number N of such units varies, but is usually in the range of 30 to 60. The other glucosaminoglycans present are simpler in their chemical structure, but vary greatly with regard to molecular weight, charge properties and tendency to form complexes with other substances.

Studies have been published indicating that the hydroxyl groups of the ground substance are important sites for the calcification processes (30). It is possible that the titanium oxide forms hydrogen bonds to the hydroxyl groups of the glucosaminoglycans as well. This interpretation is in agreement with the results obtained in the electron microscope studies of the structure of the interface between titanium implants and bone. In experiments using titanium-

FIGURE 9. Decalcified bone tissue from the interface (arrows) zone of a titanium implant after 5 years of clinical function. An osteocyte (O) with processes (*) penetrating in the direction of the interface. The collagen in a two micron wide zone (**) close to the implant is less regularly organized in comparison to the deeper parts of the tissue.

covered plastic implants (5), an ultrastructural analysis of the intact interface zone between metal and bone has been possible. The implants were inserted into the proximal tibial metaphysis of the rabbit and left *in situ* for three months. The animals were allowed full weight-bearing. The implants were then removed using a trephine and it was possible to cut through the intact bone-to-titanium interface and perform TEM-analysis (Fig. 10). In some places calcified tissue was seen in direct contact with the titanium within the resolution level of the equipment (30–50 Å). There was a ground substance layer with a typical thickness of about 200 Å observed in certain places. Staining reactions indicated that this ground substance consisted of proteoglycans. The absence of inflammatory reactions implies the acceptance of the titanium implant by the tissue.

Comparative studies have been performed using plastic implants covered by a thin layer of gold of 99.6% purity. These implants were inserted into the other tibia of the same animals that received titanium implants described above. The interface zone between gold and bone was characterized by a larger distance between the metallic surface and the collagen filaments. This distance was never less than 400–600 Å. Cells were frequently observed separating the bone

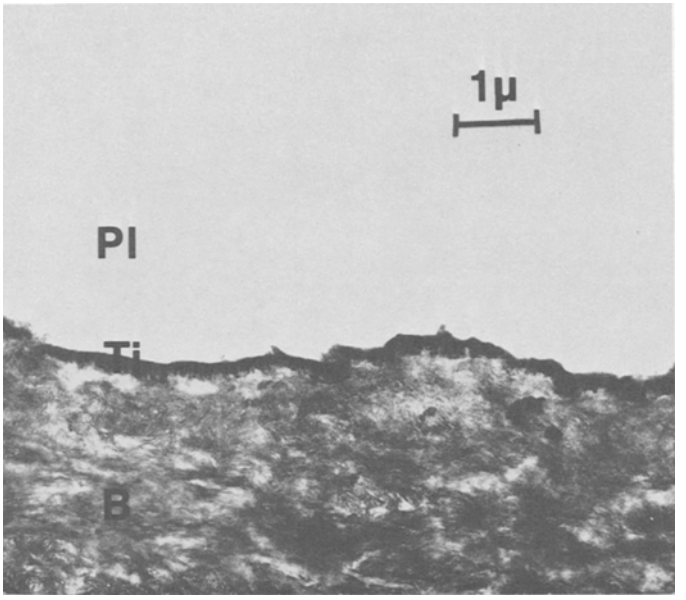


FIGURE 10a. Experimental titanium implant after 10 weeks of loading in the rabbit tibia. In this case a thin titanium (Ti) layer had been sprayed onto a plastic implant (Pl) to allow for later sectioning for TEM. The hydroxiappatite crystals are in immediate contact with the titanium oxide surface. Note the absence of interposed connective tissue. B = Bone tissue.

tissue from the implant. This indicates that gold is not accepted by biological tissues as well as titanium.

Interface analyses have also been performed on titanium alloy (Ti-6A-4V) implants. A fairly thick layer of ground substance was interposed between the collagen fibers of the bone and the oxide surface. In many places there was a cellular covering separating the oxide surface of the alloy from the surrounding bone. Thus, it has not been possible to demonstrate the same close contact between titanium alloys and bone as with pure titanium and bone. More studies are necessary before definite conclusions can be drawn concerning titanium alloys. However, it is clear that results indicating excellent tissue compatibility for pure titanium are not to be regarded as automatically valid for titanium alloys.

IV. INTERACTIONS BETWEEN BIOLOGICAL AND IMPLANT MATERIALS

There is probably no single parameter which alone determines the biocompatibility of a surface or specifically the osseointegration and long term stability of the connection between living tissues and titanium. Several different properties of the implant material have therefore to be taken into account in order to obtain optimal conditions of interaction with the biological surroundings. An obvious requirement is that the surface is not thrombogenic. For a recent review of the materials considerations see Hench (19).

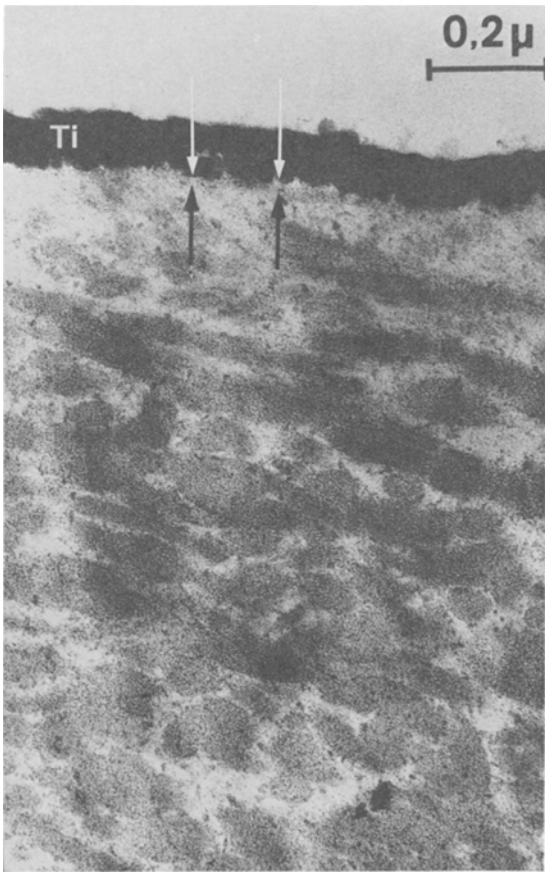


FIGURE 10b. Detail of a decalcified specimen from an experimental tibial implant. The collagen filaments are approaching the titanium (Ti) but remain separated from the Ti by a several hundred angstrom thick proteoglycane layer (between arrows). No cell processes are seen in the interface region.

Consider what happens when the implant surface comes into contact with the relevant biological environment. The first contact is with the blood of the patient. A number of phenomena will take place on a time scale from fractions of milliseconds and upwards. Ions and small molecules are quickly adsorbed on the surface and determine to a large extent the chemical properties of the surface exposed to surface active components such as proteins and lipids. Proteins have a strong tendency to adsorb in order to reduce their free energy, which takes place in several steps. Although the lipids are more surface active and therefore capable of squeezing out adsorbed proteins from the interface, they occur in blood in such a physical state that they are not likely to compete with the proteins. The initial steps, requiring milliseconds, are diffusion controlled and the smaller proteins and lipids willl therefore reach the surface first. If they are not irreversibly adsorbed, these proteins on the surface can be exchanged by others later on. Under all circumstances there may be structural and conformational changes in the adsorbed protein layer. This probably requires seconds or up to minutes of time. At this stage, diffusion into the implant surface may also

take place. In the next stage cells start to interact with the surface and its protein coat. If the conditions are favorable bone starts to be formed resulting in osseointegration of the implant and its biological surroundings, requiring at least days and perhaps up to a few months for completion.

Several links in such a complicated chain of events can be crucial and determine the future behavior of the implant. It is very important that the electrochemical properties of an implant are such that certain ions do not leak out and poison the surroundings. In that respect titanium appears to be very favorable due to the stable oxide surface. The electrochemical properties are determined not only by the material but depend also on (surface) defects and impurities in the material, on local pH, ionic strength, redox potential and so on. External parameters may thus influence the initial conditioning of the implant surface. Divalent ions like Ca^{2+} are believed to be important at an oxide surface which is negatively charged at physiological pH.

The proteins which finally adsorb irreversibly on the surface have to fulfill one critical requirement. They should adsorb in such a way that they still have their native structure mainly unchanged. Glucoproteins like fibrinogen and fibronecetin are probably involved in establishing the close contact between cells and foreign surface.

An important property of a surface is its surface energy. It is known that most proteins denature on high energy surfaces, such as most metals. Baier (6) has shown that at values of the surface energy in the range 30–40 dyne/cm there is a minimum in adhesiveness, and surface energy can thus be used as one indication of biocompatibility. However, it is not possible to use the surface energy of the material alone as a parameter.

An intriguing question is what role the oxide which grows on titanium plays during osseointegration. Our investigations so far suggest that the oxide incorporates both organic and inorganic material during its growth *in vivo* (26). Especially after a few years, the oxide of a successful titanium implant appears to provide a transition region between the "pure" implant (titanium metal) and the organic material (bone), that is, a diffuse transition zone from inorganic to living matter.

One of several factors which determine the adsorption kinetics of proteins on surfaces is the electrical nature of the oxide, especially with regard to electrostatic forces. For materials such as titanium oxide the electrostatic part of the energy needed to move a charged particle from the (highly conducting) aqueous medium into the oxide is inversely proportional to the dielectric constant, which is uniquely high for titanium dioxide, as discussed earlier. Similarly, denaturation effects are probably more likely on surfaces with low dielectric constants, such as aluminum oxides, than on titanium dioxide, all other parameters being equal. Only the basic outline of a kinetic theory of adsorption of biological molecules on oxide surfaces exists today, so this is an area of great interest from both practical and theoretical viewpoints.

Some limited experimental evidence for the conjectures above has been obtained recently. The results of electron microscopic studies have already been

discussed under Sec. III. Model experiments on protein adsorption and exchange on various metal surfaces have been carried out using ellipsometry, Galvani potential and surface capacitance (20,21). These *in vitro* experiments showed that human fibrinogen can replace human serum albumin adsorbed on titanium samples at physiological concentrations. This requires seconds and minutes to accomplish.

Auger electron spectroscopic studies have shown that the oxide thickness on osseointegrated titanium implants increases continually during implantation. Further, both calcium and phosphorous were found in the entire thickness of the oxides (26). Preliminary X-ray photoelectron spectroscopy (ESCA) results indicate that the oxide on the implant before implantation is TiO_2-like.

These experimental results are clearly not sufficient to describe all details of the osseointegration process. Instead a number of questions are raised by these limited results. We conclude by discussing some of the more interesting questions.

Exactly which proteins, polysaccharides and other biomolecules bind to the oxide, and what is the binding mechanism? Are the bonds specific (local bond picture) with specific types of oxide defects playing an important role, or are electrostatic forces of greater importance? What is the role of protein denaturation here? Do van der Vaal's forces play any role?

On a larger scale, which cells are most important in the bonding process, and how do they control their activities in relation to the implant surface? What are the optimum conditions for such cell adhesion (pH, hormones, calcium and phosphate concentrations, etc.)? Are certain vitamins, hormones or locally formed growth factors essential for proper growth adjacent to an implant surface?

What are the important physical properties of the implant surface, and how are they affected by fabrication techniques, heat treatment, etc.? Can the titanium oxide be viewed as essentially homogenous, or are defects, cracks, impurities, grain boundaries and other inhomogeneties important? What is the role of small concentrations of impurity elements in the essentially pure titanium implants? Does the implant surface actively participate in osseointegration, acting as a catalyst?

The above questions relate mainly to steady-state situations, but are also coupled to the dynamic build up of osseointegration. For instance, what is the role of blood clotting? What are the relevant transport mechanisms which bring raw materials (ions, hormones, biomolecules) to the implant site and remove other materials (initially adsorbed proteins, blood clot components, etc.)? Which transport properties of titanium oxide if any are important? Can molecules larger than calcium and phosphate ions penetrate titanium oxide? On what time scale can these events take place? What is the role of blood flow to the region of the implant?

Experiments aimed at providing answers to some of these questions are under way, and others are planned. Through them we hope to increase our understanding of the interface on submolecular, molecular, and cellular levels.

When the answers are available they are likely to lead to improved implants and implantation techniques.

The experimental methods necessary to approach the problems and questions formulated above can be divided conveniently into three groups, one aimed at characterization of the implants, another at studies of the adsorption of biomolecules on the implant surface, and the third devoted to cellular response, tissue growth and vascular studies.

In the first group, the modern methods for characterizing surfaces at high resolution are particularly important. The electron microscopic methods (SEM, TEM) give structural information at the level of 10–100 Å. In the analysis of chemical composition modern surface spectroscopic techniques can be used to obtain concentration profiles and elemental composition at the 0.1% level or sometimes even better. The three most important methods are Auger Electron Spectroscopy (AES), X-ray Photoemission Spectroscopy (XPS or ESCA) and Secondary Ion Mass Spectroscopy (SIMS). Vibration spectroscopic techniques (IR and EELS) may also be important.

Model experiments are very useful for studies of adsorption of biomolecules on implant surfaces. We have concentrated on ellipsometry in combination with measurements of electrical parameters such as electrode capacitance and Galvani potential. Another method of potential interest is photoacoustic spectroscopy, which can be effective on somewhat thicker samples that those appropriate for ellipsometry, and which allows spectroscopic analysis of the surfaces. Other methods of interest are photoelectron emission spectroscopy and electron reflectance studies which have not yet been used in this context to any significant extent.

Of course studies of tissue reaction to various stimuli are important. In addition to the well known techniques (light and electron microscopy), a useful technique is the vital microscopy method in which a titanium chamber is inserted into living bone and is so constructed that living tissue can be studied microscopically without causing disturbances. Other methods include growing cell cultures on implant surfaces as model experiments. Blood flow can be studied in the titanium chamber and by manipulation of blood volumes, pressures and compositions.

A combination of methods using the various physical and biological techniques should ultimately allow precise definition of significant factors which in turn will allow rational and reliable design of successful osseointegrated implants.

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