

# Biological aspects of implant dentistry: osseointegration

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Osseointegrated oral implants had a clinical breakthrough during the 1980s. In the past regarded as a biological impossibility, a direct bone anchorage of metallic oral implants was first suggested by Bråne-mark et al. (15) and later by other authors (31, 62). The term osseointegration to describe this mode of implant anchorage was coined by Bränemark et al. (16), and Albrektsson et al. (7) presented the first definition of osseointegration. Albrektsson et al. (7) discussed 6 different parameters that needed to be controlled for proper bone anchorage to occur: the biocompatibility, design and surface conditions of the implant, the state of the host bed, the surgical technique and the loading conditions. However, most of the early evidence for a direct bone-to-metallic implant contact was indirect, and first when techniques became available for sectioning of intact bone to metal specimens for microscopical analysis was it possible to demonstrate without reasonable doubt that there was interfacial bone tissue around metal implants (2, 9, 62, 63). The primary reason for the great research interest that remains in the field of direct bone anchorage of implants is a number of clinical reports of superior clinical results with osseointegrated oral implants (1, 12, 64) in comparison to the previously used soft tissue-attached devices that have shown unacceptably poor results over 5 years or more of follow-up (6, 33).

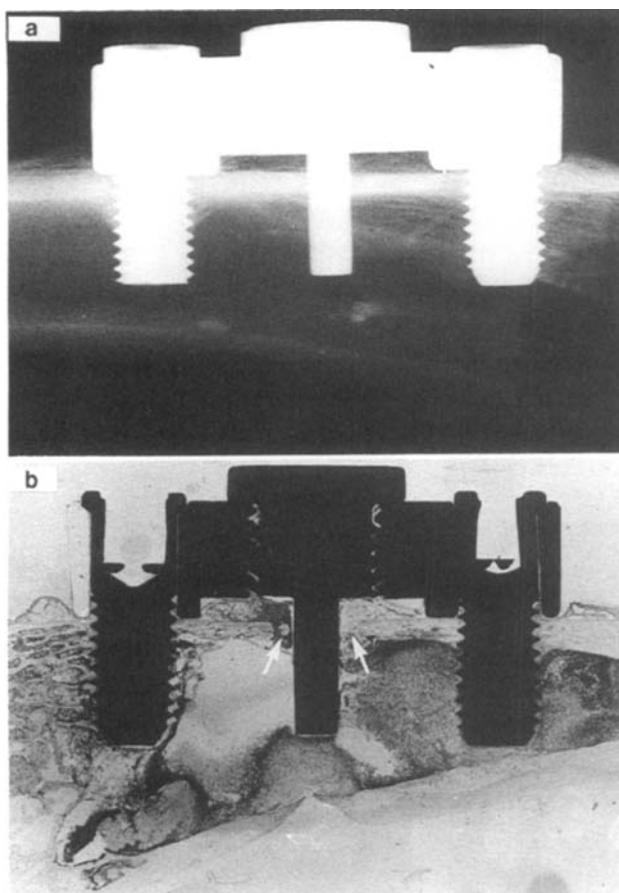
Given the importance of an osseointegrated interface in comparison to a soft tissue one, the problem for the clinician is to differentiate between them in the individual case. Implant mobility tests represent the only way the clinician can judge whether the implant is osseointegrated or not. Finding the implant to be movable with this type of test implies with a high degree of certainty that osseointegration has failed, at least if the mobility test is performed minimally 4–6 months after implant insertion. The chances for later spontaneous stabilization of such a mobile implant are minute. It has been advocated that the stability test should include a torque man-

ometer check with an applied torque of 20 N·cm. However, even if generally quite safe, this torque level could even interrupt a fragile bone anchorage under development in compromised cases, and further studies are needed to investigate whether a torque of 20 N·cm can lead to clinical problems due to the test itself. The Periotest device (54, 65, 73) seems to be more promising for a more objective general evaluation of implant mobility than the manually applied mobility test. In addition, the Periotest probing does not harm the interfacial structures around the implant. However, the considerable range of individual measurements makes the Periotest approach unsuitable as a reliable instrument in individual cases.

The purpose of this chapter is to analyze in some detail current knowledge on the osseointegrated interface. The outcome of different types of interfacial examinations are summarized. Light microscopic and electron microscopic investigations primarily deal with animal experiments. However, the clinical correlates are presented with respect to implant retrieval data. The presentation concludes in a current evaluation of the osseointegration concept as well as presenting the only definition of the term we find acceptable given the level of knowledge of today.

## Radiographic evaluation

The major problem with radiography is that many clinicians are convinced that radiography alone is a suitable approach to demonstrate that an implant is osseointegrated. This is wrong for two reasons. Due to the surrounding bone tissue, an implant may seem to be in direct bone contact even though there is an obvious soft tissue coat in reality (Fig. 1). Furthermore, the maximal resolution level of radiography, under ideal conditions, is 0.1 mm, which is 10 times the size of a soft tissue cell. These statements imply that osseointegration, irrespective of



**Fig. 1.** Radiogram (a) and *in situ* ground specimen (b) of the same commercially pure titanium implant complex. Whereas the radiogram seems to indicate a direct bone-to-implant contact around the central cylinder, the histological section (arrows) shows that there is a soft tissue coating several cell layers thick.

the proper definition of this term (7, 17, 18, 72, 77), does not represent a radiographically based diagnosis of the implant anchorage. Nevertheless, radiography is an important tool in the evaluation of oral implants. Peri-implant radiolucency represents a clinical failure criterion (11). A gradual bone saucerization has been reported with many different kinds of oral implants (24, 27, 35, 44, 46, 51, 56, 58, 61, 71), and this gradual breakdown of the bony anchorage leading to secondary failure of osseointegration may result in definitive long-term failure with several oral implant designs.

## Light microscopic investigations of bone-to-implant interface

Previous sectioning techniques did not allow for cutting through undecalcified bone with inserted metal implants without previous interfacial separation.

However, techniques developed during the 1970s (62) made the study of intact bone-to-metal specimens possible, albeit in relatively thick sections. Many studies of biomaterials are still based on evaluation of sections of a thickness of about 100 µm, which clearly overestimates the true proportion of bone-to-metal contact (37). To enable analysis at the light microscopic level, sections should preferably be 10 µm thick or less, which is possible with the sawing and grinding technique developed by Donath (25). A study of commercially pure titanium implants at varying times of follow-up demonstrated that there was a soft tissue interface at 3–4 weeks after insertion (37). With increasing time, however, more and more bone was formed in the interface that showed a very high degree of bone-to-implant contact at 1 year of follow-up (37). In an investigation of the shear forces carried by the commercially pure titanium-to-bone interface at 12 weeks of follow up, Rubo & Johansson (60) found a mean value of 14.8 N/mm<sup>2</sup>. Most certainly, this value is applicable to the implant design and surface structure of the given example and to the animal model and the time of follow-up only.

Based on a series of reports on commercially pure titanium threaded implants inserted in the rabbit tibial and femoral models (4, 19, 45, 59, 66), we decided to use commercially pure titanium as a control material in a comparative investigation of the bony attachment to other metallic materials and to hydroxyapatite. When the outcome of vitallium implants was investigated with respect to holding power and amount of interfacial bone in such animal models, it was found that there was a significant impairment in the bone response at 12 weeks compared with the situation of the control titanium implant (Fig. 2, 3) (40). The holding power of Duplex stainless screws was likewise significantly less than that of the commercially pure titanium controls at 16 weeks, whereas there was no significant difference with respect to the amount of interfacial bone (Fig. 4) (42). In the comparison with commercially pure niobium metal, there was a significantly stronger bone response to niobium at 3 months but a similar amount of interfacial bone (Fig. 5) (41). Scanning electron microscopic analysis as well as Form talysurf investigations indicated that the observed differences in the tissue behavior to these different metallic materials could depend on surface alterations as well as on true differences in biocompatibility (37). However, no surface topographical differences were observed between commercially pure titanium and Ti<sub>6</sub>Al<sub>4</sub>V, which is why the significantly

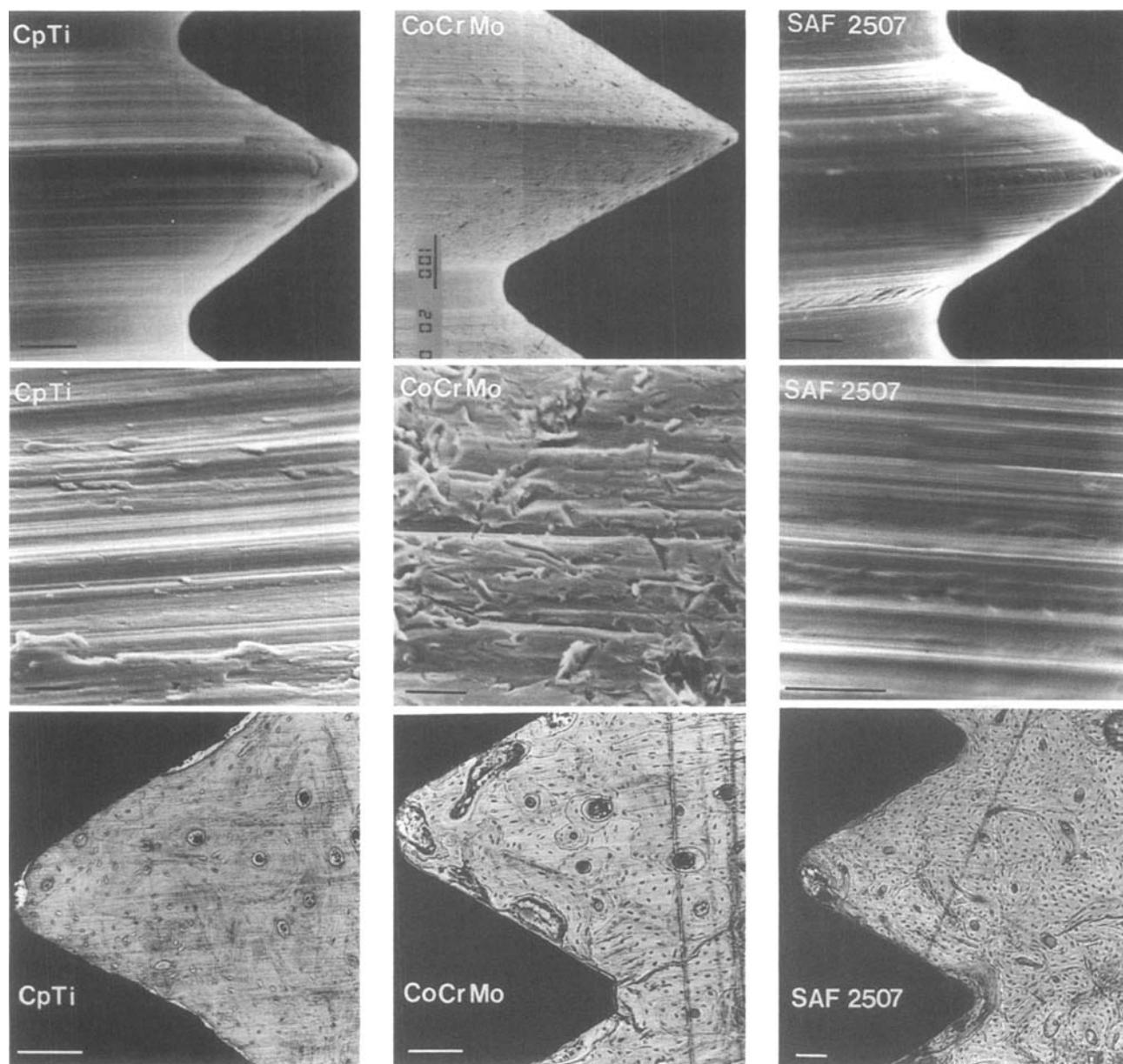


Fig. 2. Scanning electron micrograph in low (bar=100  $\mu\text{m}$ ) and high (bar=10  $\mu\text{m}$ ) power and an *in situ* ground section (bar=10  $\mu\text{m}$ ) of a commercially pure titanium screw-type implant. CpTi=commercially pure titanium.

Fig. 3. Scanning electron micrograph in low (bar=100  $\mu\text{m}$ ) and high (bar=10  $\mu\text{m}$ ) power and *in situ* ground section (bar=100  $\mu\text{m}$ ) of a vitallium screw-shaped implant. Vitallium is a much harder metal than commercially pure titanium, which is why the surface texture of the material differs. There was significantly less bone in the interface

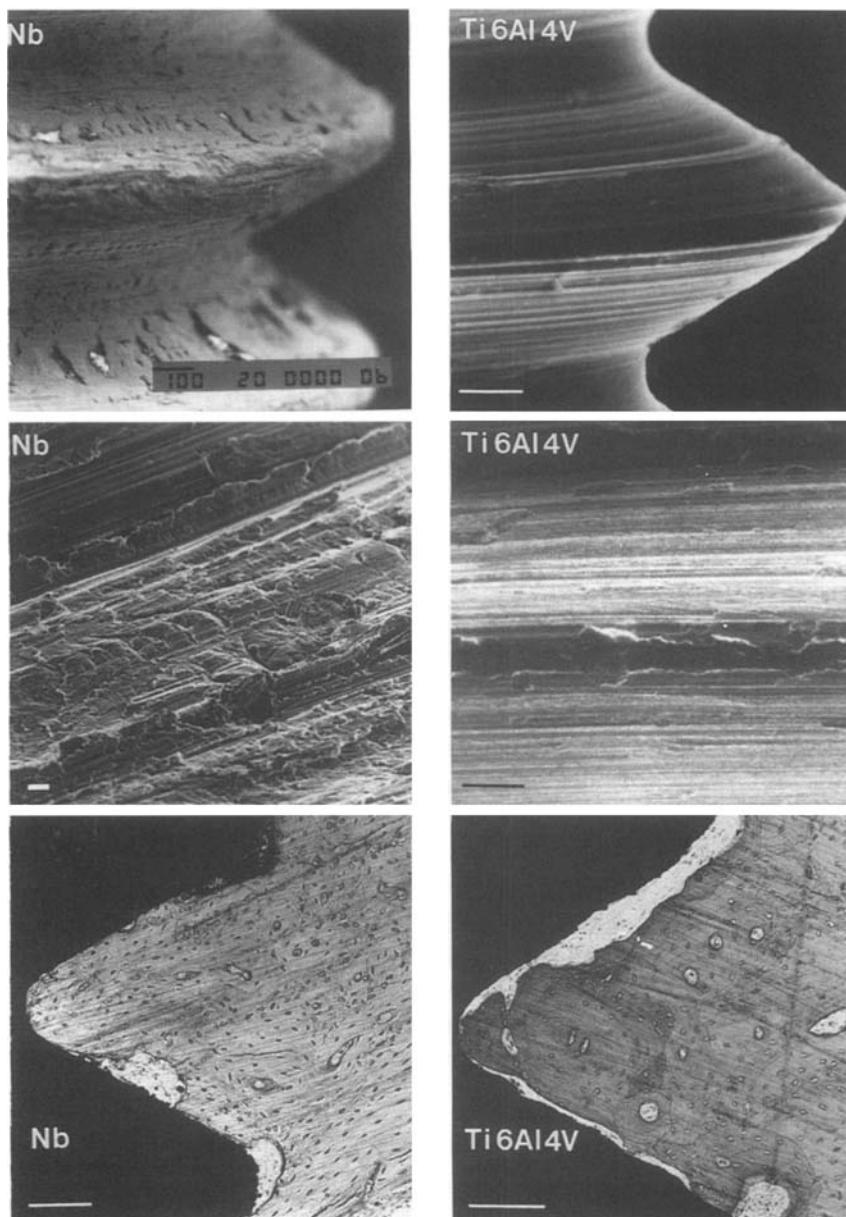
of vitallium screw compared with the control commercially pure titanium. CoCrMo=chromium cobalt molybdenum alloy.

Fig. 4. Scanning electron micrograph in low (bar=100  $\mu\text{m}$ ) and high (bar=10  $\mu\text{m}$ ) power and *in situ* ground section (bar=100  $\mu\text{m}$ ) of a SAF2507 stainless steel implant. The SAF stainless steel implants showed a significantly impaired holding power in the bone compared with the commercially pure titanium at the same times of follow-up.

stronger bone response and holding power of the commercially pure titanium controls must be related to its superior biocompatibility (Fig. 6). One explanation could relate to a demonstrated aluminum leakage from the titanium alloy samples, a leakage that may result in a competition between calcium

and aluminum in the stage of osteogenesis in the alloy-implant interface (39, 43).

One potentially negative aspect of metallic implants is the tendency of ionic leakage from metals that has been reported in the literature (76). In titanium leakage to lung tissue, Woodman et al. (76)



**Fig. 5.** Scanning electron micrograph in low (bar=100 µm) and high (bar=10 µm) power and *in situ* ground section (bar=100 µm) of commercially pure niobium (Nb) screw. The amount of interfacial bone found around commercially pure niobium implants was similar to that seen around commercially pure titanium. The higher removal torque values seen with commercially pure niobium screws could depend on the rougher surface or on a better biocompatibility of commercially pure niobium compared with commercially pure titanium.

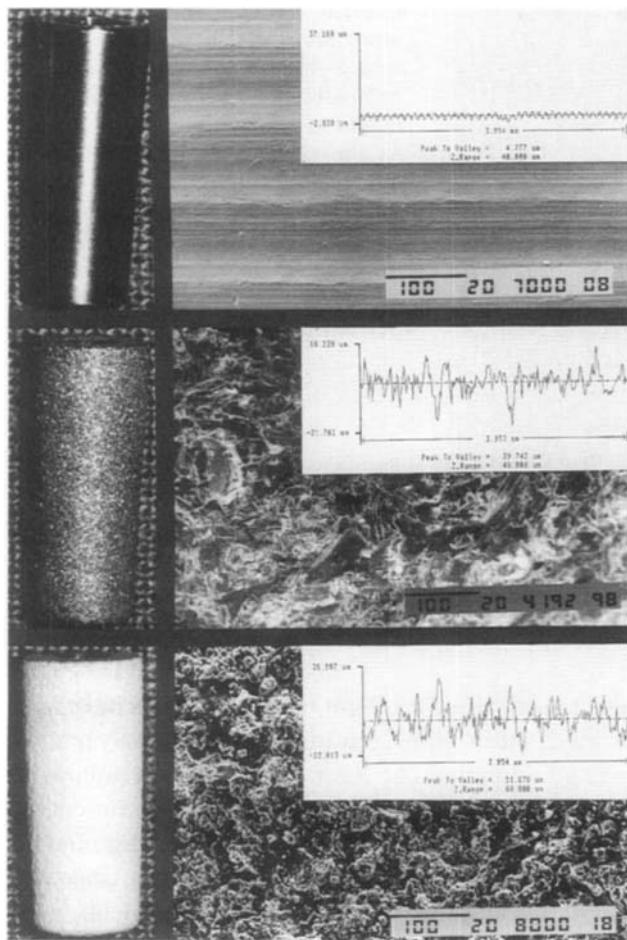
**Fig. 6.** Scanning electron micrograph in low (bar=100 µm) and high (bar=100 µm) power and *in situ* ground section (bar=100 µm) of Ti<sub>6</sub>Al<sub>4</sub>V alloy implant. There was significantly more interfacial bone and a significantly higher removal torque of commercially pure titanium implants in comparison to the Ti<sub>6</sub>Al<sub>4</sub>V. As the surface topography did not differ in scanning electron microscopic or talysurf investigations, the reasons for the observed difference must relate to inferior biocompatibility of Ti<sub>6</sub>Al<sub>4</sub>V alloy, possibly dependent on aluminum leakage.

reported a plateau phase being reached at about 3 years in their experimental study on baboons. This was in contrast to the aluminum leakage from titanium alloy, where no such plateau occurred. The risks of aluminum leakage have been discussed. With respect to titanium, the observation of small leaks of less than 100 ppm does not seem to be very alarming, particularly since no specific side effects have been reported in the literature. However, Osborn et al. (55) found no less than 1600 ppm of titanium outside a plasma-sprayed titanium surface, which the authors claimed could indicate an impairment of osteogenesis. One way to minimize ionic leakage would be coating with hydroxyapatite. There is a substantial bulk of evidence that hydroxyapatite-coated implants likewise show a direct bone anchor-

age (14, 23, 36). Studies at our laboratories have confirmed other data reported with hydroxyapatite-coated implants, at least in short-term follow-up (28). With longer follow-up, hydroxyapatite-coated implants have either showed as much or significantly less interfacial bone than corresponding uncoated titanium controls (28, 30). For cylindrical, non-threaded designs, one animal study demonstrated significantly more bone in the hydroxyapatite-coated compared with the uncoated interface at 6 months of follow-up (29). In a human investigation of cylindrical, non-threaded implants evaluated at 3 and 6 months of implantation, the importance of the surface topography for the outcome of the study was clearly demonstrated. Relatively smooth titanium cylinders showed largely a soft tissue interface, in

contrast to a rough titanium surface that had more interfacial bone on average than the hydroxyapatite-coated specimens, albeit this latter difference was not significant (Fig. 7) (20). Not only general material parameters are of importance when the outcome of hydroxyapatite-coated and uncoated metallic implants is compared but also such factors as the specific type of hydroxyapatite used, implant surface topography and implant size.

In essence, the use of modern cutting and grinding techniques has clearly demonstrated that direct bone-to-implant contact at the light microscopic resolution level is possible with many different metallic and ceramic materials. Some researchers even advocate that a bone encapsulation of a material be seen as a foreign body reaction (26). However, nu-



**Fig. 7.** Three different cylindrical non-threaded implants were inserted in the human tibia, one titanium implant with a smooth surface, one titanium implant with a more rough surface and a rough surface hydroxyapatite-coated implant (as indicated by the Form talysurf graphs). The smooth commercially pure titanium implant showed the poorest bone-to-implant contact, and the rough titanium showed the highest values, although the latter were not significantly different from those of the hydroxyapatite-coated implant.

merical investigations demonstrate significant differences in the amount of interfacial bone around different types of materials. In addition, in loaded clinical cases there are indications that what is referred to as osseointegration depends on the material used as one of several factors being important for implant take (7).

## Ultrastructural investigation of experimental implants

Ultrastructural analysis of the implant-bone interface is technically very difficult, since ultrathin sections are needed for transmission electron microscopy. Ultimately, the undecalcified bone tissue and the implant surface should be present in the sections. However, with conventional histological techniques it is not possible to cut ultrathin sections through a bulk metal implant. Most ultrastructural investigations have therefore been performed in animals using metal-coated polycarbonate implants (32), which permits sectioning, or metal plugs from which the tissue has been separated before sectioning (74). To enhance sectioning further, the bone tissue has been decalcified prior to sectioning in most studies. Moreover, to our knowledge, only one study has presented a detailed description of the ultrastructure between bone and clinically retrieved titanium implants (67).

### The polycarbonate-plug technique

Albrektsson et al. (8) presented a method for ultrastructural analysis of the intact metal-bone interface. A thin layer of titanium or gold was evaporated onto a polycarbonate implant (Fig. 8). The morphology of the titanium-bone interface was described after 3 months in the rabbit tibia. In the light microscope the bone seemed to be in continuum with the titanium. Using transmission electron microscopy, the mineralization was found to be reduced close to the interface. Ordered collagen bundles were observed near the implant surface but the last 100–500 nm consisted of randomly arranged filaments. The last 20–40 nm did not contain any collagen filaments but consisted of partially calcified amorphous substance (Fig. 10). Staining with ruthenium red and lanthanum indicated that proteoglycans and glucosaminoglycans were present in the amorphous substance. Osteocyte processes reached close to the implant but ended about 20 nm from the surface. A layer of

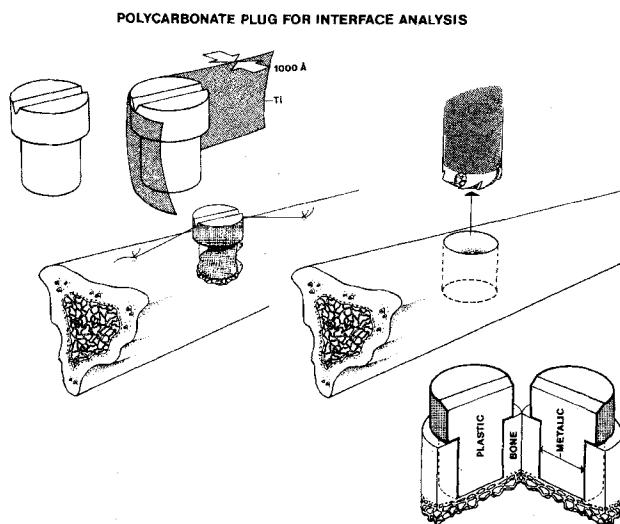


Fig. 8. A schematic drawing of the polycarbonate-plug technique. The implant consists of a soft polycarbonate plastic core coated with a thin layer (100 nm) of the metal to be studied. It proved possible to have sections thin enough for ultrastructural examination with this method. The drawback with the polycarbonate plug is that thin, sputtered or evaporated metal layers may give rise to tissue reactions that differ from those of the bulk material.

amorphous material of about 20 nm also separated cells and tissues from the implant surface in the marrow.

A similar titanium-bone interface morphology was observed in a study by Linder et al. (48) using the plastic-plug technique and the rabbit tibia. A layer of ground substance 20–50 nm thick was present between the implant surface and the nearest collagen filaments. The presence of proteoglycans was indicated by staining with ruthenium red and lanthanum. The ground substance layer was bordered by a 100- to 500-nm-thick layer with randomly distributed collagen filaments. Calcium deposits were observed in all layers, and some of these seemed to be in direct contact with the titanium. The bone-titanium interface morphology as found in the studies of Albrektsson et al. (8) and Linder et al. (48) has repeatedly been described in more recent publications comparing titanium to other metals using the same implant model (3, 10, 32, 38).

#### Solid metal plugs

Linder et al. (49) used solid plugs of pure titanium and other metals inserted in the rabbit tibia for 4 and 11 months for light microscopy and ultrastructural analysis of the metal-bone interface in decalcified specimens. Three types of interface structure could be distinguished: the author called them A, B and C.

A: More or less regularly arranged fibrils of collagen approaching the metal surface within 50 nm.

B: Type-I collagen fibrils separated from the implant by a zone of indistinct structures but with some filamentous material, most often about 500 nm in thickness but sometimes up to 1000 nm.

C: Type-I collagen fibrils separated from the implant surface by a 500- to 600-nm zone of thin filamentous structures, clearly more dense than in B.

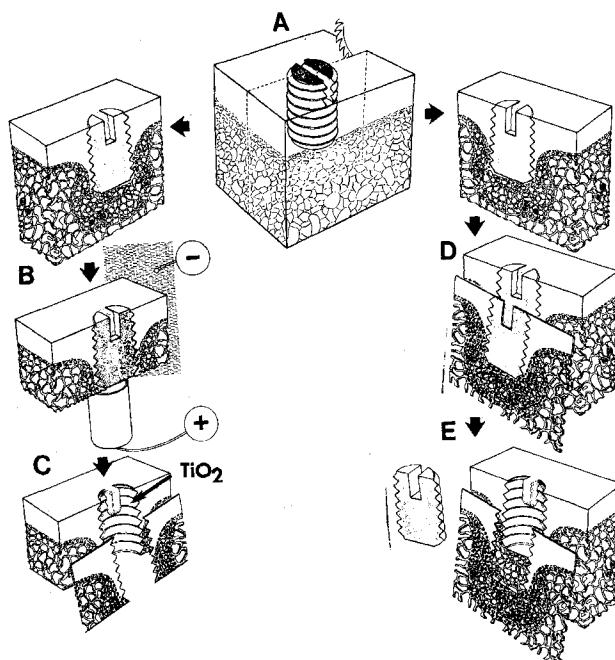
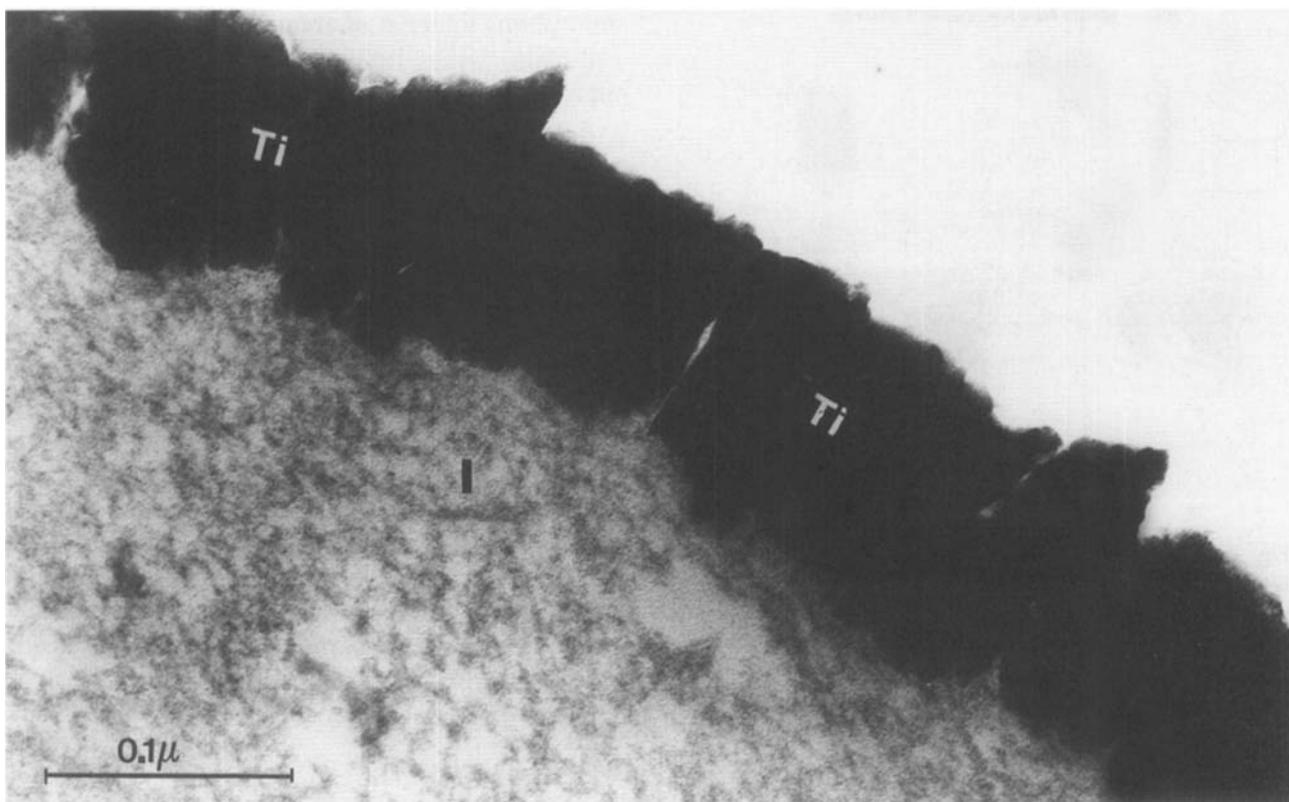


Fig. 9. Schematic drawing of the different methods used for preparing sections using the electropolishing technique (B and C), the Donath technique for ground sections (D) and sections using the fracture technique (E). The implant-tissue bloc remaining after ground sectioning (D) was used for scanning electron microscopy.

#### Screw-shaped commercially pure titanium implants

The ultrastructure of the undecalcified bone-titanium interface was recently studied in rabbits (68–70). The authors used two approaches: a fracture technique and an electropolishing technique (Fig. 9). By the fracture technique, the embedded tissue was separated from the implant surface prior to sectioning. Using the electropolishing technique, the bulk part of the titanium implants was electrochemically dissolved, leaving the surface oxide in the embedded tissue. The electropolishing technique caused de-



**Fig. 10.** The interface between commercially pure titanium (Ti) and bone consisted of a layer of proteoglycans and, a little bit further away from the interface, collagen

filaments. There was a mineral gradient in this zone. Normal bone was seen some 500 nm from the surface of the titanium.

mineralization of the interface and infiltration of titanium ions into the tissue. Nevertheless, it was concluded that the two techniques used in parallel were a valuable tool for analyzing the structure of the interface. Twelve months after insertion in the rabbit tibia, the mineralized bone and the implant were separated by an amorphous unmineralized layer 100–200 nm thick (Fig. 11a). An electron-dense line about 100 nm thick was bordering the amorphous layer, especially in areas with a low degree of mineralization (Fig. 11). Other areas along the interface consisted of a 0.5- to 2-μm-wide zone of nonmineralized collagenous tissue or deeper pockets with osteocytes or vessels. Also, in those locations, a layer of amorphous substance appeared to form the contact with the implant.

The healing process around screw-shaped implants of commercially pure titanium was studied 3–180 days after insertion by Sennarby et al. (69, 70). The healing process started with a migration of mesenchymal cells and macrophages from the marrow into a hemorrhage that occupied the entire bone-titanium interface, as observed 3 days after insertion in rabbit cortical bone (Fig. 12). Bone formation was

first observed on day 7 at the endosteal surface of the original cortex as a lattice of trabecular woven bone approaching the implant (Fig. 13a) and as solitary woven bone formation near the implant surface (Fig. 13b, c, 14). The latter type of bone served as a base for surface osteoblasts producing osteoid seams (Fig. 13c). With time, the two woven bone types fused and filled an increased area of the threads (Fig. 15). Consequently, the bone-titanium contact increased with time. Multinuclear giant cells form a nearly continuous layer at the implant surface in areas with no bone-titanium contact and can be observed from day 7 to 180 (Fig. 16). The number of multinuclear giant cells decreases with time in parallel with an increased bone-titanium contact. Two models of calcification of the interface tissue were distinguished with transmission electron microscopy. First, there was a calcification of typical osteoid seams, although it did not reach the implant surface (Fig. 17a). Osteoblasts were never seen producing osteoid directly on the titanium surface. Second, an accumulation of scattered hydroxyapatite crystals was seen in the collagen matrix (Fig. 17a). Collagen fibrils did not reach the implant surface but

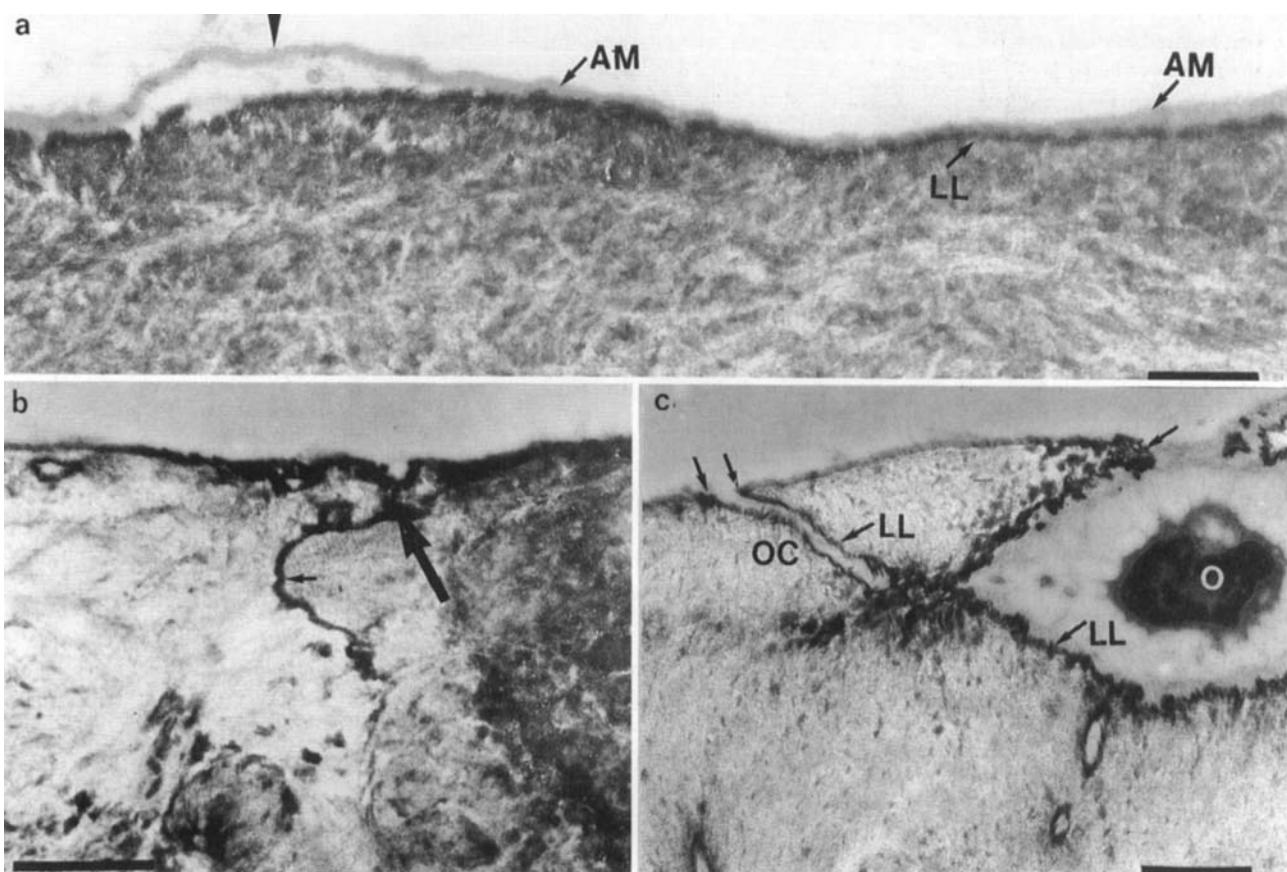


Fig. 11. Mount of transmission electron micrographs from Sennerby et al. (68). a. A layer of amorphous material (AM) is visible on top of the mineralized bone. An electron-dense lamina limitans-like line (LL) is located at the edge of the bone beneath the amorphous layer. Bar=1  $\mu\text{m}$ . b. The lamina limitans-like line is in continuity (large

arrow) with a cement line (small arrow) that separates bone of different ages. Bar=2  $\mu\text{m}$ . c. The lamina limitans-like line (arrows) is in continuity with the lamina limitans (LL) that lines the osteocyte (O) lacunae and the osteocyte canaliculi (OC). Bar=2  $\mu\text{m}$ .

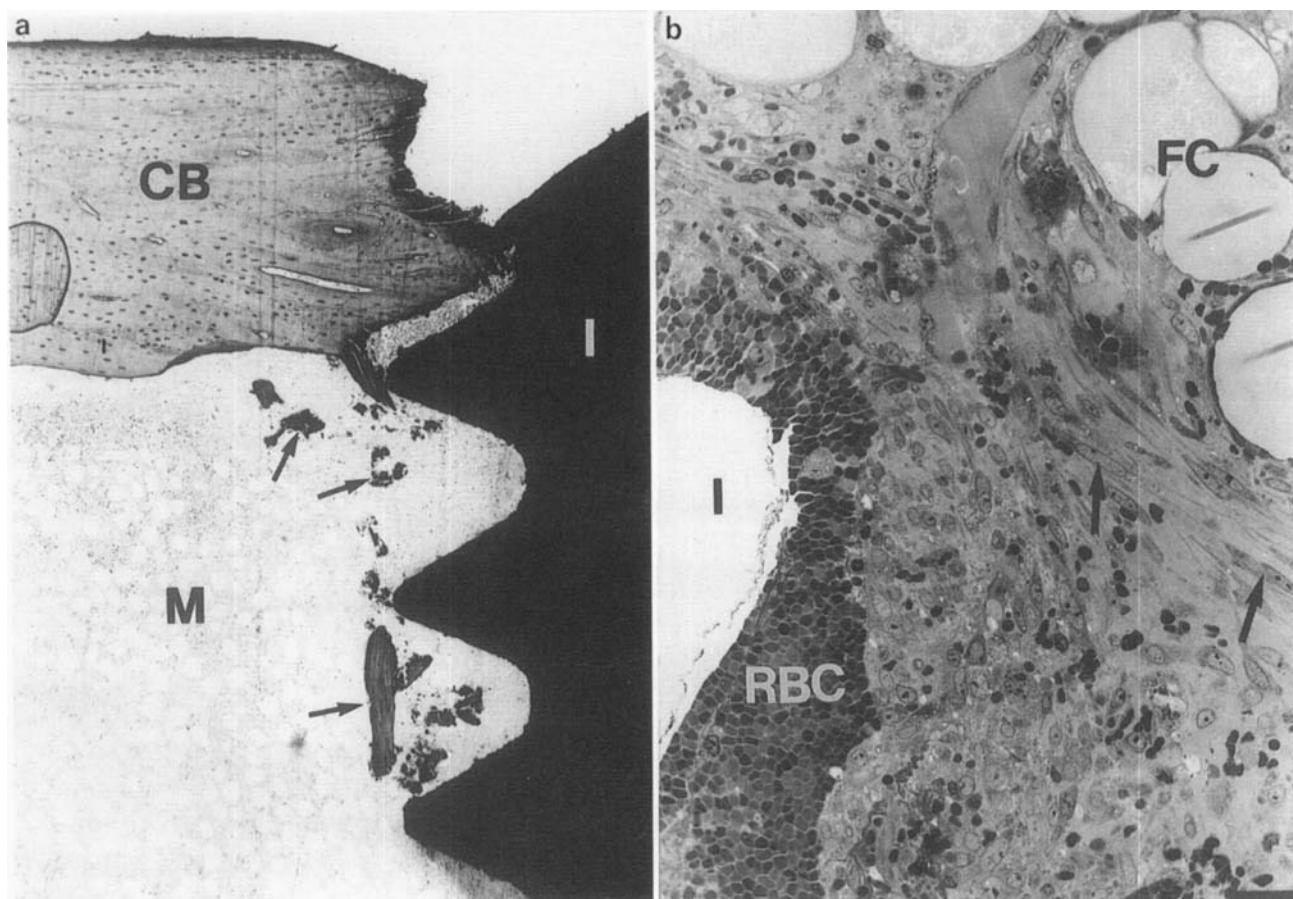
ended in a zone less than 0.5  $\mu\text{m}$  wide consisting of a dense amorphous substance as described above. In addition, an electron-dense mineral containing a lamina limitans-like line was bordering the amorphous layer (Fig. 17b).

#### *In vitro* investigations

Davies et al. (21) studied the bone-like tissue-titanium interface *in vitro* using bone marrow cells that were incubated on titanium discs for 2–3 weeks. The cells produced an extracellular matrix that became mineralized. Globular masses of calcified material were observed in association with collagen fibers in the matrix. Between the titanium surface and the mineralized extracellular matrix, an interface zone was detected by transmission electron microscopy. Two layers could be distinguished in the interface zone: a bonding zone containing few collagen fibers and a zone that was positively stained

by ruthenium red, which indicated the presence of proteoglycans, and that contained densely packed collagen fibers. The authors concluded that the interface morphology observed *in vitro* was similar to that described *in vivo* by Albrektsson et al. (9).

Using the same *in vitro* model, the bone-like tissue-titanium interface was further investigated in a recent publication from the same group (50). The same morphology as described by Davies et al. (21) was found. A calcium- and phosphorous-containing afibrillar layer was found on the titanium substratum. This layer was evolved by the fusion of single calcified globules less than 1  $\mu\text{m}$  in diameter formed by the osteoblasts. Collagen fibers produced by the cells attached to this initial layer. The collagen matrix became mineralized with time. However, a zone could be distinguished close to the initial afibrillar layer that was stained by ruthenium red. The interfacial zone, including the collagenous and the afibrillar zone, was about 0.5 to 1  $\mu\text{m}$  thick.



**Fig. 12.** a. Light microscopy of a ground section showing a titanium implant 3 days after insertion in the rabbit tibia. The implant (I) is stabilized by a patchy contact with the cortical bone (CB). Bone fragments (arrows) from the surgical preparation are seen in the bone marrow (BM). No signs of bone formation at this stage. Bar=240  $\mu$ m.

b. Light microscopy of an ectropolished thin section 3 days after insertion. The entire interface (I) is occupied by red blood cells (RBC). Mesenchymal cells (arrows) are migrating into the wound area from the bone marrow. FC=fat cells. Bar=20  $\mu$ m.

## Comments on published ultrastructural investigations

The transmission electron microscopy analysis of screw-shaped titanium implants from rabbits as well as of clinically retrieved specimens made by Sennerby et al. (67, 68), revealed that mineralized bone never came in true direct contact with the implant surface. There was always a 100- to 500-nm-thick interposed layer with a dense amorphous substance, irrespective of time after implantation. This layer has been observed 14 days after implantation in rabbits (70) and was associated with both nonmineralized and mineralized bone tissue, but it was never seen in areas with fibrous soft tissue. Thus, it seems as if the amorphous layer, at least partly, originated from the organic matrix of bone. The content of the layer is not known at present, and single molecules cannot be identified with the morphological techniques

described here; other approaches are required for this purpose, such as element analysis and immunological techniques. A similar amorphous layer, but with smaller dimensions (20–50 nm), was described at the surface of titanium-coated plastic plugs inserted in the rabbit tibia for 3 months (5, 8, 10, 32, 39, 48). These studies found that the layer contained proteoglycans due to the stainability with ruthenium red and lanthanum. In this proteoglycan layer there were calcium deposits, of which some were in direct contact with the titanium. There was also a gradual decrease of the mineral content of the innermost interfacial bone the closer the distance to the titanium oxide surface, which was different from the findings of Sennerby (66). These differences could certainly be due to the use of different types of implants. The team behind the plastic plug implants have pointed out that their findings are relevant only to the type of sputtered or evaporated metal layers studied by

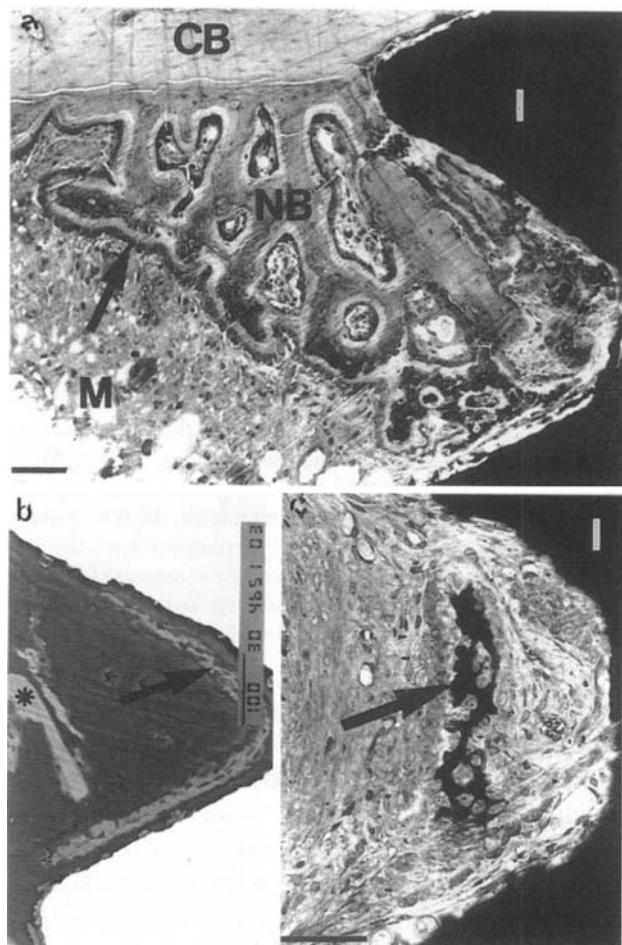


Fig. 13. a. Light microscopy of a ground section 7 days after insertion in the rabbit tibia. Trabecular woven bone (NB) is formed at the endosteal surface of the cortical bone (CB) and approaches the implant (I). M=bone marrow. Bar=60  $\mu$ m. b. Back-scattered scanning electron micrograph of a ground section showing solitary woven bone formation (arrow) near the implant surface. This bone is less mineralized than the original mature bone (star). Bar=100  $\mu$ m. c. Ground section of solitary woven bone formation. The bone consists of large osteoblast/osteocyte lacunae. The surface of the solitary bone served as a base for osteoblast seams (arrows). Bar=60  $\mu$ m.

them and are not necessarily applicable to bulk metallic implants (3, 39). Indeed, not only may the evaporated metal layer react differently from a bulk version but there are also differing moduli of elasticity for a metal-coated plastic plug and a bulk metallic implant that may affect the interfacial reactions too. If (as would seem likely) there are proteoglycans in the interface, these constituents may only represent some of several other possibly present molecules. Nevertheless, the amorphous interfacial zone described by Sennerby (66) may be analogous to the previously described proteoglycan layer. A similar layer with varying thickness was also described by Linder et al. (49).

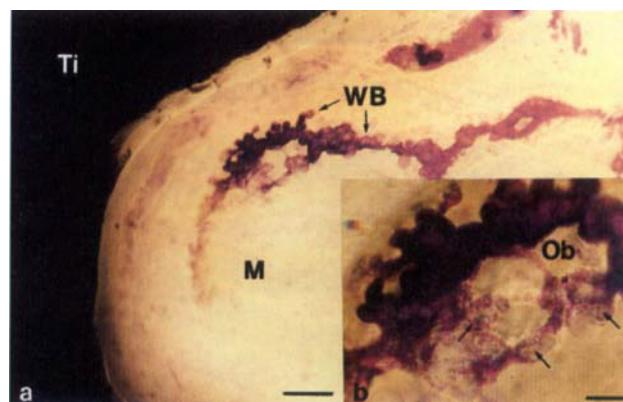


Fig. 14. Light microscopy of solitary woven bone formation (WB) near the implant surface (Ti). M=nonmineralized matrix. Bar=30  $\mu$ m. B. Close-up showing osteoblasts (Ob)/osteocytes surrounded by darkly stained mineralized aggregates that seem to be formed by compaction or fusion of small granules (arrows). Bar=10  $\mu$ m.

In their *in vitro* description of the interface, Davies et al. (21) and Lowenberg et al. (50) found an interfacial zone between the titanium substratum and the mineralized matrix that consisted of two layers. The innermost layer, the bonding zone, contained calcium and phosphorous but few collagen fibers; the outer layer was densely packed with collagen and positively stained with ruthenium red, indicating the presence of proteoglycans. The results of Davies et al. (21) and Lowenberg et al. (50) were based on *in vitro* findings that may be different from the *in vivo* situation. The authors pointed this out, referring to the relative similarity between their observations and the *in vivo* ones by Albrektsson et al. (9) as support for the relevance of their *in vitro* findings. However, there is one major difference between *in vitro* and *in vivo* findings. The interface layer described by Davies et al. (21) and Lowenberg et al. (50) was formed by fusion of calcified globules that were laid down directly at the surface of the titanium substratum by the cultured osteoblasts. In an *in vivo* study by Sennerby et al. (69, 70) using threaded titanium implants inserted in rabbits and followed for 3–180 days, osteoblasts were rarely seen on the surface of the implant and mineral was never observed being laid down on the surface by osteoblasts. Furthermore, with few exceptions, the only cells in direct contact with the implant surface were red blood cells and macrophages, during the early healing phase, and later, multinuclear giant cells. The innermost interface was the last area to be mineralized, and this process seemed to be acellular. Moreover, the amorphous layer, and in some instances the electron-dense layer, could be distinguished before

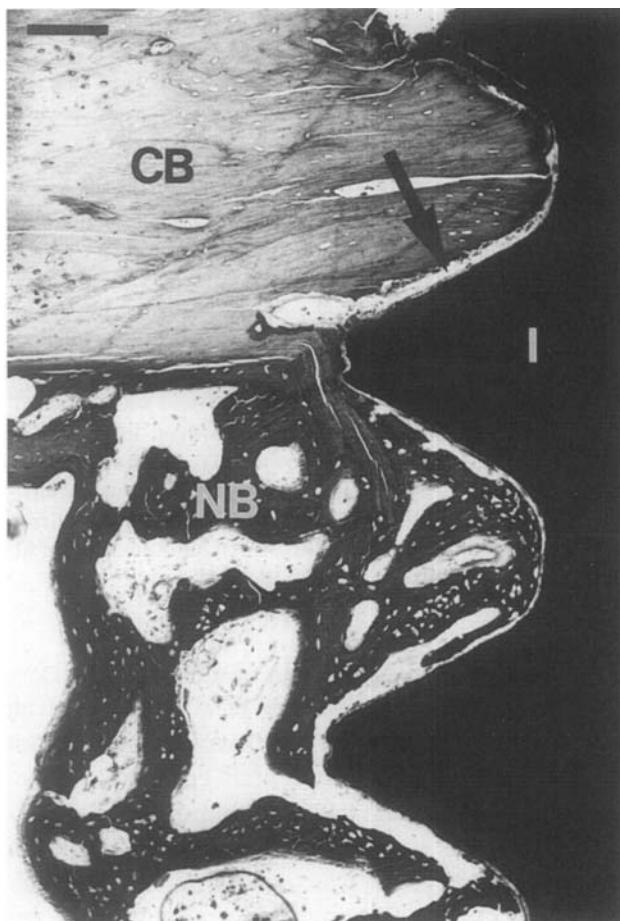


Fig. 15. Light microscopy of a ground section 14 days after insertion in the rabbit tibia. A fusion of the trabecular woven bone (NB) from the endosteum and the solitary woven bone formations has occurred in the second thread. The trabecular woven bone has a lamellar appearance, and scattered large osteocyte lacunae is typical for the solitary woven bone. No bone formation or resorption can be observed of the cut cortical bone (CB) (arrow) adjacent to the implant surface. Bar=120  $\mu\text{m}$ .

the extracellular matrix became mineralized. Since the *in vivo* interface scenario seems to be completely different from that observed *in vitro*, the relevance of the latter approach as an indication of true interface morphology could be questioned.

### The commercially pure titanium-bone interface in relation to other interfaces

One feature described by Sennerby et al. (68–70) was the lamina limitans-like line (50–100 nm thick) that separated the mineralized bone from the amorphous layer. This line was most often in continuity with that lining osteocyte lacunae and canaliculae and it

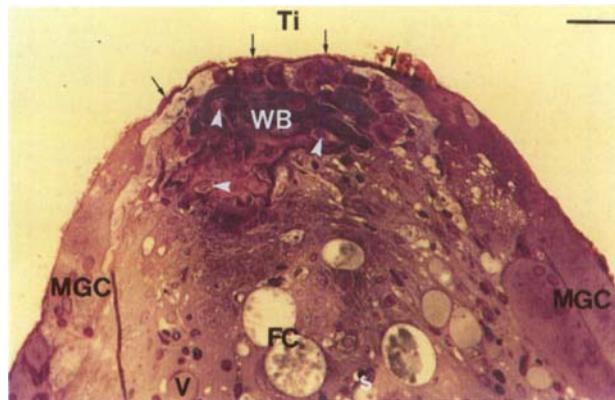


Fig. 16. Light microscopy of a thread in the bone marrow 7 days after insertion in the rabbit tibia. Multinuclear giant cells (MGC) are observed along the entire implant surface. Formation of solitary woven bone (WB) is evident in the bottom of the thread but is separated from the implant surface by cytoplasmic extensions (arrows) from the giant cells. Note the scattered and large osteocyte lacunae (arrow heads). V=vessel, FC=fat cell. Bar=30  $\mu\text{m}$ .

was similar to reversal lines. A similar structure has been observed *in vivo* around implants of hydroxyapatite, bioglass and polyactive polymer (36, 47, 57, 75). The nature of this structure is not known, but the natural lamina limitans is associated with the calcification front of collagen matrix. The natural lamina limitans contains collagen fibrils, in contrast to cement lines, which are afibrillar structures. Davies et al. (22) suggested that the afibrillar cement-like layer observed *in vitro* on the surface of various non-bonding materials (aluminum, titanium and titanium alloy) was similar to the afibrillar cement lines observed *in vivo* and a general morphological appearance for non-bonding materials. In addition, it was suggested that the difference between a bone-bonding material (for instance, hydroxyapatite or bioglass) and a non-bonding material (such as metals) is the presence of collagen interdigitating with the implant surface in the case of bone-bonding (22). However, an afibrillar calcified layer has repeatedly been described *in vivo* at the interface of dense hydroxyapatite (36, 47, 57, 75) and macroporous hydroxyapatite (21, 53), a biomaterial that is considered to form a biological bond with the bone. Collagen interdigitation has been shown to occur with bioglass *in vivo* (34) and *in vitro* (52). However, Matsuda & Davies (52) demonstrated areas of the interface that were not interdigitated with collagen. Thus, the interface descriptions found in the literature point to a variability of the interface morphology both for bone-bonding and non-bone-bonding materials, which is why this type of classi-

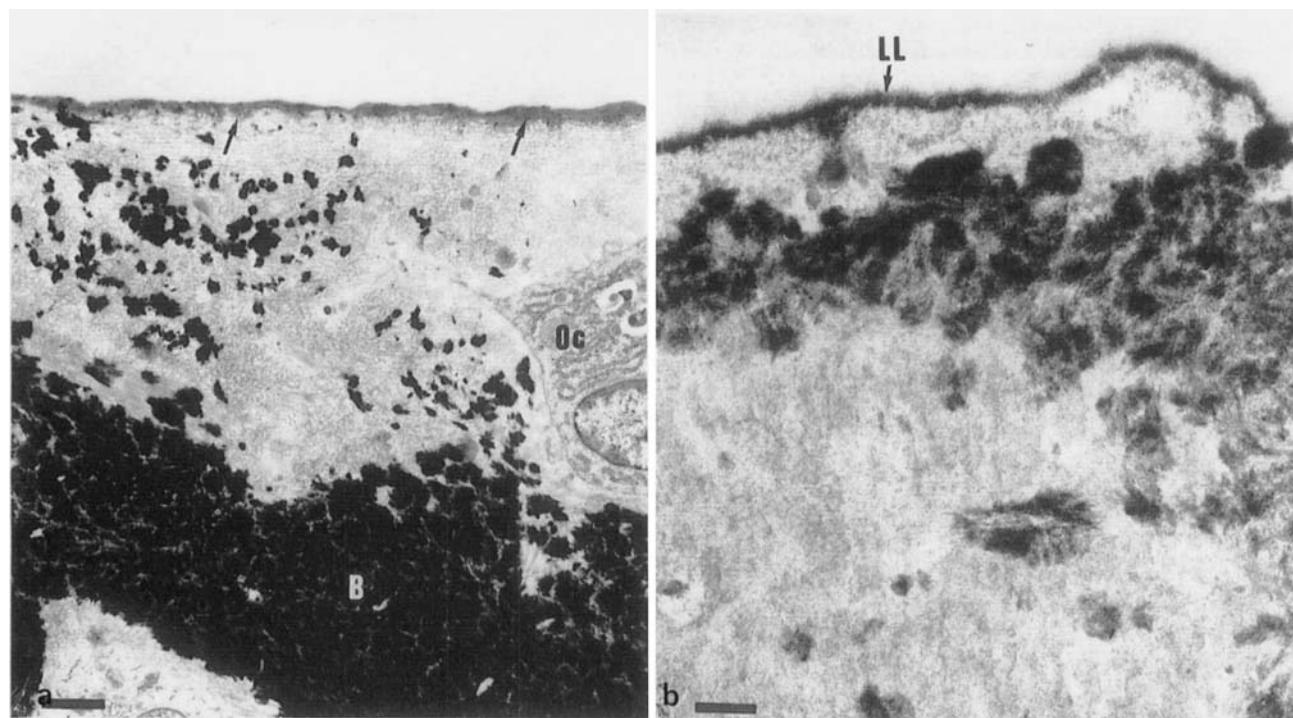


Fig. 17. Electron micrographs. a. Fourteen days after insertion in the rabbit tibia. Scattered amounts of hydroxyapatite crystals are seen between the mineralized bone (B) and the implant surface. An amorphous layer (arrows) is separating the matrix from the implant surface. However, a lamina limitans-like structure cannot be distinguished in this section. Oc=osteocyte. Bar=2 µm. b. Six weeks

after insertion. The mineral content in the interface zone is varying. The matrix adjacent to the implant forms a dense lamina limitans-like line (LL). In this part of the section the amorphous layer is missing, probably due to artifactual separation during the preparation procedure. Bar=0.5 µm.

fication could be criticized, at least given the present state of knowledge.

## The ultrastructure of the bone-titanium interface

In essence, it seems as if all authors who have tried to describe the bone-titanium interface at the ultrastructural level have come to the same conclusion: there is one type of amorphous layer in the bone-to-metal interface, even if the width and the content (mineral, collagen and proteoglycans) has been debated (the different bone-implant interfaces are summarized in Fig. 18). This relative agreement with respect to the correct interfacial description is well summarized by Linder et al. (49). Linder et al. (49), who used solid metal plugs inserted in the rabbit tibia and a fracture technique similar to that used by Sennerby et al. (66, 68–70), came to conclusions similar to Sennerby et al. (66, 67) with respect to the variability of the thickness of the amorphous zone. At the same time, Sennerby et al. (66, 67) concluded

that, at certain locations, the ground substance layer separating the collagen from the implant may well be of the order of 20–40 nm, as had previously been described by Linder et al. (48) and Albrektsson & Jacobsson (5).

## Evaluation of retrieved oral implants removed despite a remaining anchorage

Albrektsson et al. (7) analyzed 38 screw-shaped titanium implants inserted in the mandible, maxilla, temporal bone, tibia and iliac crest of 18 patients and removed after 5–90 months for various reasons. The implants with surrounding bone were analyzed with light microscopy and scanning and transmission electron microscopy. An intact titanium-bone interface was studied with transmission electron microscopy using an oblique cutting technique. No detailed description of the ultrastructure of the interface was given, but it was concluded that bone was in direct contact with the implant surface. The

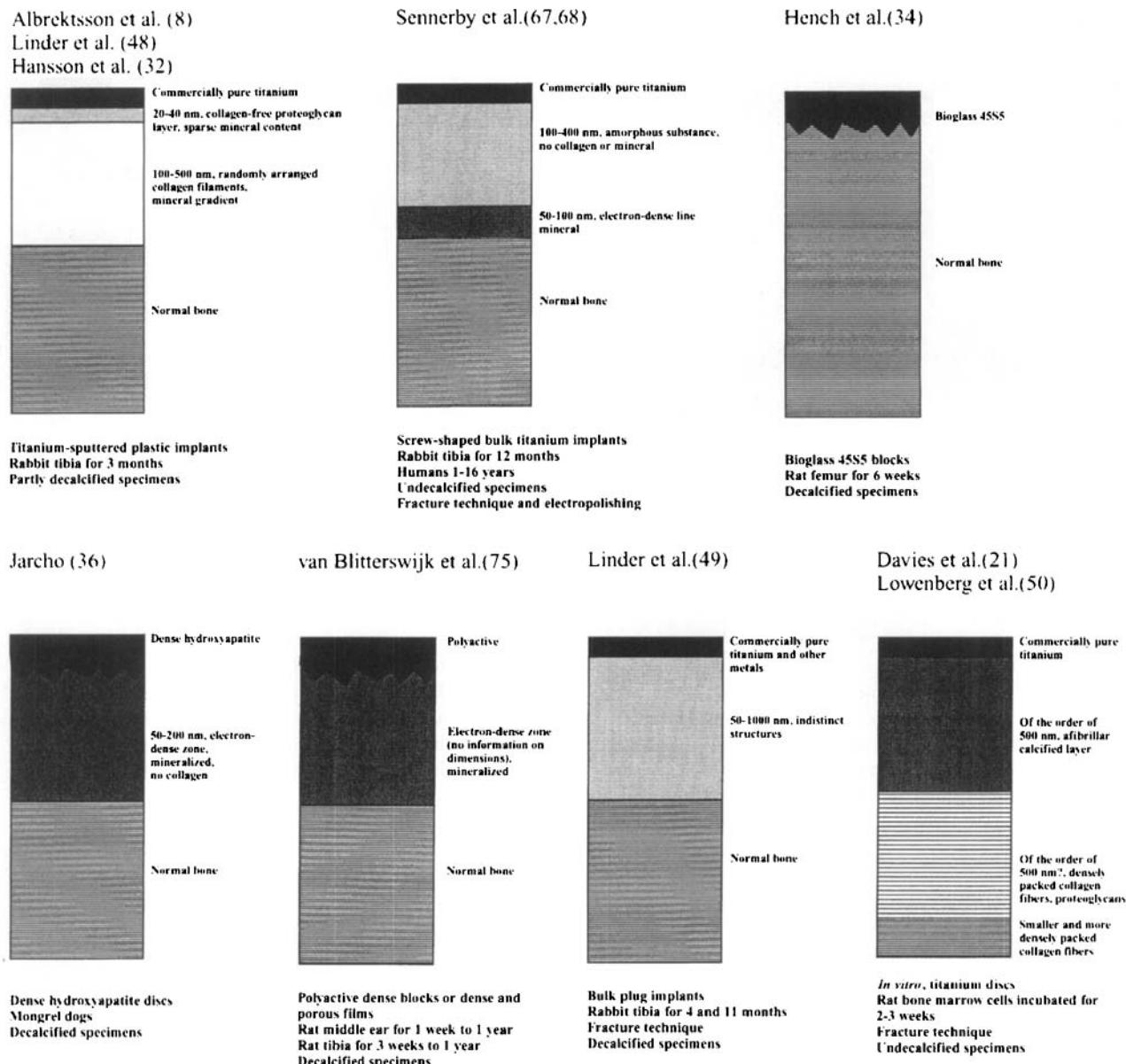


Fig. 18. Schematic representations of bone-implant interface morphology according to authors cited in the text

observations made on clinically retrieved titanium implants were presented by Hansson et al. (32) and Albrektsson et al. (9). Using scanning and transmission electron microscopy, bundles of collagen were seen at a distance of 1–3 µm from the implant surface and were then replaced by collagenous filaments arranged perpendicularly to the interface. However, the filaments were separated from the implant surface by a layer of ground substance about 20 nm thick.

The ultrastructure of the bone-titanium interface in clinically retrieved Nobelpharma® system implants was studied by Sennerby et al. (66). These implants had been loaded for 1–16 years. The mor-

phology was very similar to that seen in rabbits (68). In general, a nonmineralized amorphous layer 200–400 nm wide was bordering the mineralized bone with an electron-dense lamina limitans-like line (50 nm thick). Areas with nonmineralized tissue containing collagen and sometimes osteocytes or vessels were present along the interface.

In a light microscopic investigation on 33 retrieved Nobelpharma® implants after a time of function between 1 and 16 years, the authors (13) reported a relatively consistent degree of direct bone-to-implant contact between 60% and 99% with no tendency to increase with a longer time of insertion. The proportion of bone in the threads of the implant

was likewise more than 80% on average (13). These figures relate to the 3 best consecutive threads on both sides of the section, as sometimes the implants had been cut obliquely and there was no more interfacial bone available for analysis. In the implants retrieved as postmortem specimens, the entire surfaces were available for inspection, revealing about 10% lower figures than those presented above. The reasons for implant removal in the studies cited above included a mental disorder of the patient, implant fracture, unexplained proximal bone resorption and postmortem cases. All implants included in these analyses were clinically stable at the time of retrieval.

## Conclusion

There have been repeated attempts to define osseointegration with respect to histological (7, 17, 18) or biomechanical references (72). However, too little is known to justify the usage of the term osseointegration with any of these definitions. As we see it, the only acceptable mode of defining osseointegration is based on clinical examination finding stable implants: a process in which clinically asymptomatic rigid fixation of alloplastic materials is achieved and maintained in bone during functional loading (77).

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