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Ultrastructure of the bone–titanium interface in rabbits

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The interface zone between cortical bone and threaded non-alloyed titanium implants inserted in the rabbit tibia for 12 months was examined by light and electron microscopy. The implants were removed *en bloc* with the perfusion-fixed surrounding bone and the undecalcified specimens were, after osmification, dehydrated and embedded in plastic resin (LR White). In ground sections (about 10 μm thick) cortical bone appeared to be in direct contact with the implant surface and the implants were thus "osseointegrated". Sections for light microscopy (1 μm thick) and electron microscopy (40 nm to 0.5 μm) were prepared by using an electropolishing technique by which the bulk part of the metal was electrochemically removed and a fracture technique by which the implant was separated from the embedded tissue before sectioning. In the electropolished specimens an unmineralized zone, 2–10 μm wide, was observed at the interface. The interface zone contained osteoid-like tissue (densely packed collagen fibrils, osteocyte canaliculi) but in general no deposits of calcium mineral. This feature of the interface could not be observed in specimens prepared by the fracture technique, indicating that the electropolishing technique had induced serious artefacts, including decalcification of the interface bone. In sections prepared by the fracture technique, mineralized bone was present very close to the implant surface. No gradient of mineral was observed. A thin layer of amorphous material (100–200 nm wide) was present peripheral to the mineralized bone. An electron dense line about 100 nm wide was formed at the border between the mineralized bone and the amorphous layer. The dense layer had the same characteristics as the lamina limitans observed around osteocyte lacunae and canaliculi or the zone between areas of bone with different degree of mineralization.

Our observations suggest that mineralized bone reached close to the surface of titanium implants inserted in the rabbit tibia for 12 months but that a direct contact is not established.

1. Introduction

Non-alloyed titanium implants are widely used in dental and craniofacial reconstructive surgery. The implants are considered to be "osseointegrated" which seems to imply that mineralized bone is in contact with the implant [1]. However, direct ultrastructural studies on the interface zone between solid metal implants and surrounding undecalcified bone have not been performed due to the lack of techniques for sectioning of the bulk metal together with the adjacent tissue. The concept of "osseointegration" of titanium implants is mainly based on clinical experience and, more recently, on light microsopic (LM) observations made in ground sections. Ultrastructural studies have been performed on decalcified specimens that after *in situ* embedding and subsequent separation have been sectioned for transmission electron microscopy (TEM) [2, 3]. By using this approach ordered collagen, interpreted as previously mineralized bone, has been found close to the supposed implant surface [2, 3]. A problem with this approach is the difficulty of clearly defining the location of the interface. As decalcified specimens were examined, it is also impossible to make any observations on the presence of mineralized tissue and its relation to the surface of the implant. To overcome the problem of defining the interface, Albrektsson *et al.* [4–6], instead of solid implants, used polycarbonate plugs sputtered with a thin film of titanium or other metals, which, after partial decalcification, permitted sectioning for TEM. They found that the titanium surface was covered by a thin layer of amorphous material, called the proteoglycan layer, and that collagen filaments were located at a distance of 20–40 nm from the implant surface [4–6]. However, it is uncertain to what extent the surface and bulk properties of this type of implant represent those of a solid titanium implant and as decalcified specimens were used no conclusions can be drawn about the degree of mineralization in the interface zone.

Recently a method by which the intact interface zone between metal implants and surrounding soft tissue can be studied with LM and TEM was described [7]. The implant and surrounding tissue are embedded *en bloc*. The specimen is then treated by an

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electrochemical procedure (electropolishing) which removes the bulk metal but does not influence the surface oxide layer which is in contact with the tissue. This method appeared to be a promising approach also for studies in other tissues and we therefore used the electropolishing method with the aim to examine the intact interface between titanium implants and bone. Our preliminary results [8] indicated that mineralized bone was absent from a zone within 2–10 μm from the interface surface. However, further evaluation showed that this feature probably is an artefact induced by the electropolishing procedure [9]. In the present study we have combined the information obtained from electropolished specimens with observations using other preparation techniques to study the ultrastructure of the interface between titanium screws and bone.

2. Materials and Methods

2.1. Animals and anaesthesia

Nine adult New Zealand white rabbits fed *ad libitum* were used in the study. During insertion of the implants they were anaesthetized by intramuscular injections of fluanizone (Hypnorm®, Janssen, Brussels, Belgium, 0.7 mg per kilogram body mass (mg kg^{-1})) and diazepam (Stesolid®, Dumex, Copenhagen, Denmark, 1.5 mg kg^{-1}). Additional fluanizone was given when needed.

2.2. Implants

Implants (length 4.0 mm, diameter 3.75 mm) were manufactured from commercially pure titanium by machining. The top of the implant had a slit to fit a screwdriver during insertion (Fig. 1a–c). Before implantation the implants were cleaned ultrasonically in butanol and absolute ethanol for 10 min in each solution. The implants were then sterilized by autoclaving.

2.3. Surgery, implant retrieval and tissue processing

Surgery was performed under sterile conditions. Each tibial metaphysis was exposed by a skin incision and a periosteal flap. Using the standard equipment for the Nobelpharma Implant system (Nobelpharma AB, Gothenburg, Sweden) a hole, 1.8 mm in diameter, was drilled with high speed (2500 revolutions per minute (r.p.m.)) during generous irrigation with saline. The hole was then enlarged to 2.0 mm and finally to 3.0 mm. After tapping, using low speed (16 r.p.m.), the implant was installed by using a screwdriver and placed in level with the cortical bone. Two implants were inserted in each tibia of nine rabbits. The periosteum and fascia were sutured by a resorbable suture and the skin by a silk suture. Postoperatively, the animals were given bensylpencillin (Intencillin®, Leo, Helsingborg, Sweden, 2 250 000 IE (5 ml)$^{-1}$, 0.1 ml kg^{-1}) and analgetics (buprenorphine, Temgesic®, Reckitt and Colman, USA, 0.05 mg kg^{-1}) as single intramuscular injections.

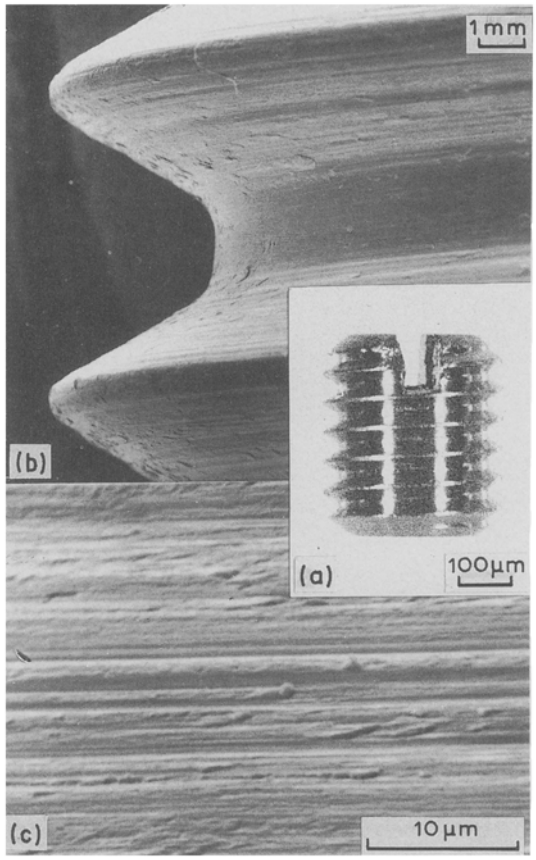

Figure 1 (a) Titanium implant used in the study (diameter 3.75 mm, length 4.0 mm). (b) SEM of a thread of the implant. (c) SEM. The surface seen at higher magnification. Note the typical machining grooves.

After 12 months the animals were anaesthetized as described above. One implant in each rabbit was unscrewed and the removal torque measured with a Tohnichi 15 BTG-S torque gauge manometer (Wareco, Täby, Sweden). To connect the instrument the overgrown bone was carefully removed from the top of the implant by low speed drilling during irrigation with saline. When the rupture between bone and implant occurred the peak force value fell quickly and up to this moment no macroscopical movement of the implant was evident. In five animals the implants were left *in situ* when the rupture occurred to study the morphology of the bone–metal rupture. The animals were given an overdose of pentobarbital intravenously (Mebumal®, ACO Läkemedel AB, Solna, Sweden) and fixed by perfusion with 2.5% glutaraldehyde in 0.05 M cacodylate buffer, pH 7.4 via the left heart ventricle for 5 min. The implants and surrounding tisse were removed *en bloc* and further immersed in glutaraldehyde for 24 h and then postfixed in 2% osmium tetroxide for 1 h. After dehydration in a graded series of ethanol the specimens were embedded in plastic resin (LR White).

2.4. Preparation of sections and specimens

The embedded implants were divided longitudinally by sawing (Exakt cutting and grinding equipment, Exakt Apparatebau, Norderstedt, FRG) (Fig. 2). One

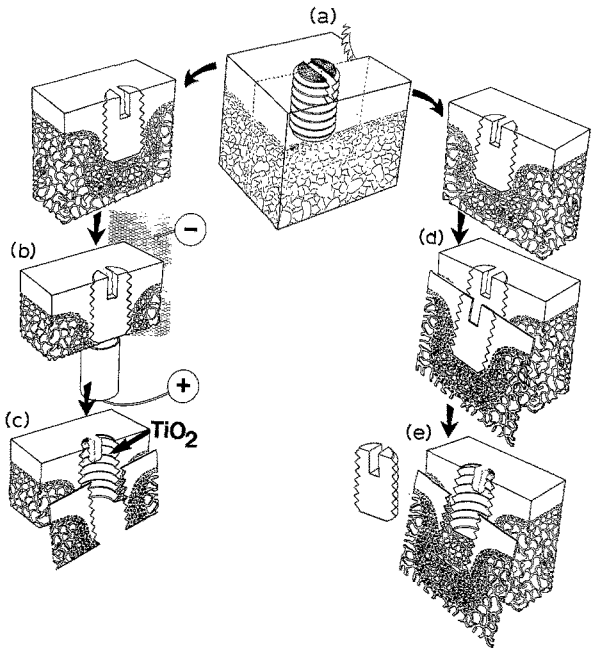

Figure 2 Schematic presentation of the methods. The embedded implant with undecalcified surrounding tissue was divided in two halves (a). One half (b) was used for the electropolishing procedure (see text) and thin sections (c) were prepared for LM and TEM. The other half was used for preparation of ground sections (d) which were observed by LM. The remaining tissue-implant block (d) was used to prepare thin sections for LM and TEM by using the fracture technique (see text).

half was used to prepare ground sections (approximately 10 μm thick) as described by Donath and Breuner [10] (Fig. 2). Sections were viewed unstained or after staining with 1% toluidine blue.

The other half of the tissue-implant bloc was electropolished (Fig. 2) [7]. A hole was drilled in the bottom part of the implant and connected to a sample holder and immersed in an electrolyte in the electropolishing equipment. The sample, serving as an anode, was surrounded by a platinum cathode. The electrolyte, cooled to −30 °C, consisted of a mixture of 5% perchloric acid, 35% *n*-butanol and 60% methanol. The electropolishing was performed at 24 V for about 4 h. The major part of the bulk metal was removed and the major part of the implant surface available for further sectioning. However, metal remained left in the bottom of the cavity formed. After removal of the bulk metal the specimens were re-embedded. Sections for light microscopy (approximately 1 μm thick) were cut with glass knives (Fig. 2). Areas which included one surface of a thread were then selected and cut for TEM. Sections, 50–100 μm thick, were cut for TEM with a diamond knife by using a Reichert Ultracut microtome. Sections were examined unstained or after staining with uranyl acetate and lead citrate.

Sections were also cut for light and electron microscopy from specimens not treated electrochemically (Fig. 2). For this purpose the part of the specimen remaining after the ground section preparation was used. The implant was carefully separated from the plastic embedded tissue (fracture technique). As previously shown [11, 12] this separation, at least in decalcified specimens, takes place close to the implant. The cavity formed after removal of the implant was then filled with plastic resin before sections were cut. Although this method does not allow an exact definition of the tissue-implant border (in some specimens the cavity was sputtered with gold before re-embedment to mark the fractured surface of the specimen) this procedure was undertaken to examine any possible influence of the electrochemical treatment on the morphology and mineralization of the tissue close to the implant.

2.5. Light microscopy, scanning electron microscopy and transmission electron microscopy

The ground sections were examined and photographed in a Nikon Microphot FXA microscope in transillumination and in a Leitz microscope in polarized light.

Ultrastructural analyses of sections were made either in a Philips EM 400 or in a Zeiss CEM 902 equipped with an electron energy loss spectrometer.

3. Results

3.1. Removal torque measurements

After 12 months the surface of the implants was covered by bone and the overgrown bone was carefully removed by using a large round burr. The torque force needed to unscrew the nine implants chosen for this purpose was found to be 41.3 ± 11.4 N cm. When the peak value of removal torque force was reached, the torque value fell suddenly to much lower levels. In five animals the implants were not further unscrewed when this point was reached but left *in situ* and perfusion fixation immediately started.

3.2. Morphology

3.2.1. General aspects

During the initial phase of this study we used the electropolishing method extensively as it enabled us to cut ultrathin sections of the bone–implant interface. However, further analysis clearly showed that this preparation method induced two distinct artefacts, namely a dissolution of calcium mineral and an infiltration of titanium in the tissue located close to the implant surface (Figs 3a, b, 5). Therefore, we changed strategy and based our further studies on specimens prepared by the fracture technique. By this technique the implant and resin-embedded tissue are separated from each other before sectioning. A disadvantage with this technique is that the integrity of the interface zone is broken and therefore that the exact location of the implant surface is impossible to define. However, observations in electropolished specimens turned out to be of great importance for the interpretation of the observations made with the fracture technique. In the following we will therefore describe complementary observations based on both of these methods.

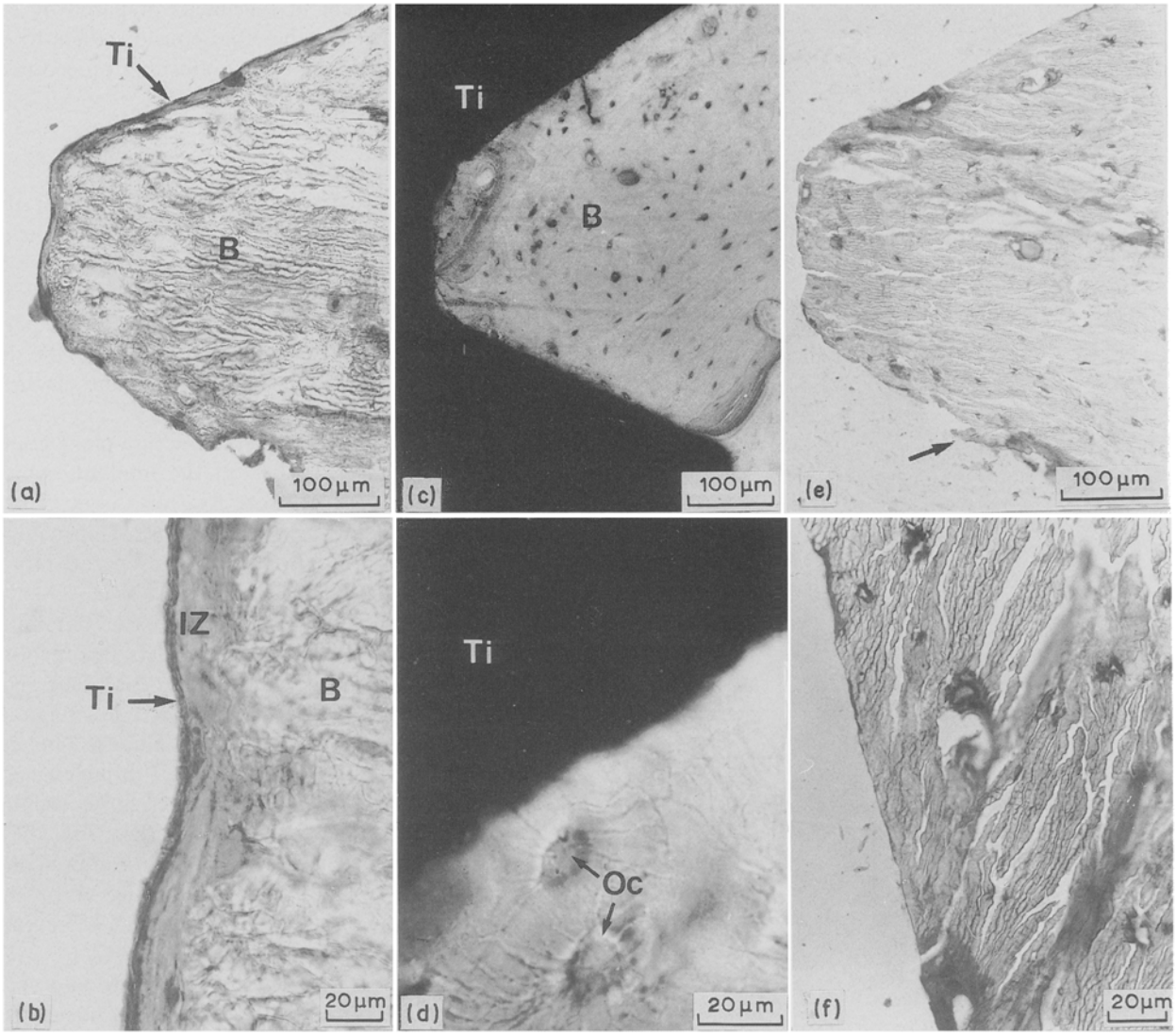

Figure 3 Optical micrographs from the interface between bone and titanium implants inserted for 12 months in the rabbit tibia. (a) Thin (1 µm section of an electropolished undecalcified specimen. The implant surface is indicated by a dark line (Ti). (b) Detail of (e). An interface zone (IZ) is present between the implant surface (Ti) and the mineralized bone (B). (c) Ground section, about 10 µm thick. Mature cortical bone (B) is occupying most of the thread of the implant (Ti). (d) Detail of (a). The bone (B) appears to be in direct contact with the implant surface (Ti). Oc = osteocytes. (e) Thin section (1 µm) of a fractured specimen. The tissue interface has generally a smooth contour, except for one defect (arrow). (f) Detail of (c). The tissue interface is smooth without any defects induced by separation of the implant from the tissue block. The cracks in the bone are sectioning artefacts.

3.2.2. Ground sections

In ground sections (about 10 µm thick) the threads located within the tibial cortical bone were filled with bone, occupying most of the section area (Fig. 3c, d). The bone within the threads was mature and well organized as judged by observation in polarized light. A large fraction of the implant surface appeared to be in direct contact with bone.

In ground sections of implants which were unscrewed before fixation a narrow light gap separated the tissue from the implant along most of its contour, although implant and tissue appeared to be in direct contact in some areas (Fig. 4). No fractures were found in the cortical bone and the bone tissue appeared to be unaffected by the unscrewing procedure (Fig. 4).

3.2.3. Electropolishing technique

Following the electropolishing procedure a metal film covered the walls of the cavity formed in the plastic block. When viewed with scanning electron microscopy the cavity surface appeared as a replica of the surface of the screw with typical machining grooves (not shown). In addition, scattered remnants of the metal could be observed on the surface.

In light microscopic sections, about 1 µm thick, a zone 2–10 µm wide, heavily stained by toluidine blue, outlined the contour of the tissue (Fig. 3a, b). In the electron microscope this zone corresponded to tissue devoid of mineral (Fig. 5). This zone was sharply demarcated from mineralized bone and contained bundles of collagen pierced by osteocyte canaliculi which reached close to the implant surface (Fig. 5a). The implant surface was marked by an often discontinuous layer of titanium of varying thickness (Fig. 5b). Only rarely a thin line of native surface oxide similar to the 8–12 nm thick layer previously observed for titanium implants in soft tissue [7] was present. Such a layer was, however, observed when bone marrow cells were in contact with the implant (not shown).

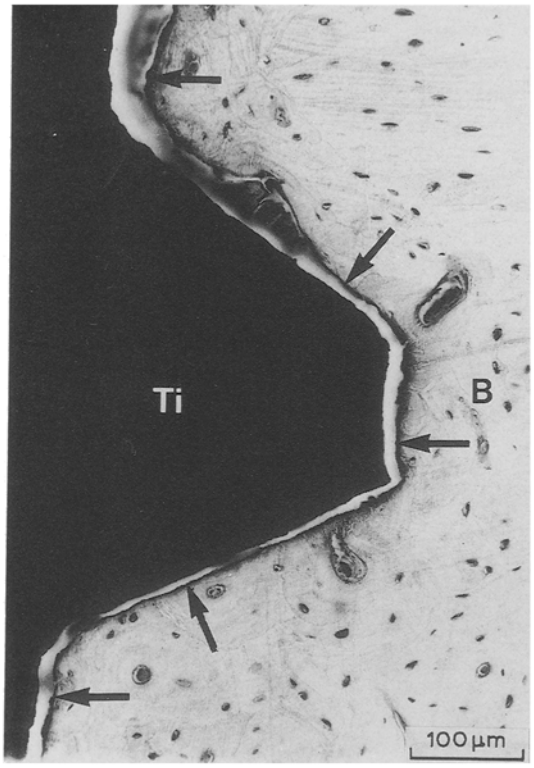

Figure 4 Light micrograph from the interface between bone and implant inserted for 12 months that was unscrewed before perfusion fixation. Ground section, about 10 µm thick. A space (arrows) is seen between the implant (Ti) and the bone tissue (B). No fractures are visible in the bone tissue.

In addition, electropolished fragments of titanium were often found in the plastic resin located further away from the tissue (Fig. 5a).

Another typical feature of the interface region in electropolished specimens was the presence of electron dense material forming a zone, about 1 µm wide, in contact with the titanium (Fig. 5). The dense material surrounded the collagen fibrils and reinforced their typical cross-section (Fig. 5b). Sometimes a narrow zone (about 100 nm) of amorphous material was seen in between the dense material and titanium, but this zone was often difficult to distinguish due to the presence of high electron density of the tissue or by the titanium fragments (Fig. 5b). Element specific imaging using energy loss spectroscopy showed that the dense material contained high amounts of titanium and also calcium [9].

In implants, which had been unscrewed before fixation and then electropolished, the implant surface was in general located at some distance (1–15 µm) from the bone (Fig. 5c, d). The intervening space was either empty or contained proteinaceous material and blood cells. Often a thin layer of amorphous material was seen adhering to the implant surface and a similar type of material was also present on the surface of the tissue (Fig. 5c, d). We did not identify other tissue constituents, for instance collagen fibrils, adhering to the implant indicating that the tissue had been separated from the implant very close to the implant surface during the unscrewing procedure.

As will be further described below neither the poorly mineralized zone nor the dense material containing titanium were present in specimens prepared by the fracture technique. We therefore consider them to be artefacts induced by the electropolishing procedure.

3.2.4. Fracture technique

In light microscopic sections the tissue within the screw threads had a smooth surface contour, indicating that the fracture plane was close to the surface of the implant (Fig. 3e, f). A conspicuous difference to electropolished specimens was the absence of the peripheral heavily stained zone corresponding to the demineralized interface zone observed in the electron microscope.

In the electron microscope the fracture plane could most easily be distinguished in the implants which were unscrewed *in vivo* before fixation. As described above, we found in such electropolished specimens that the implant surface in general was separated from the tissue by a space. In specimens subjected to the fracture technique this space, empty or containing blood elements, could be distinguished, indicating that the plane of fracture did not involve the tissue. The plane of fracture was often indicated by a line separating the original plastic resin from that used for reembedding, and not seldom small fragments of amorphous material but never collagen fibrils or mineralized material adhered to this line (Fig. 7b). These observations on specimens which were unscrewed and then processed either by electropolishing or by the fracture technique indicated that the plane of fracture was very close to the implant surface.

In implants fixed in contact with bone and then processed with the fracture technique the ultrastructural appearance of the interface zone was similar to that observed in unscrewed implants, indicating that also in these specimens the fracture plane was very close to the implant surface.

3.2.5. Ultrastructure of the interface tissue

The most conspicuous morphological feature of the interface zone in specimens prepared with the fracture technique was that mineralized bone was present very close to the implant surface (Fig. 6a–d) along the major part of the interface. No gradient in the mineralization, with lower concentration of bone mineral towards the implant surface, could be distinguished. Peripheral to the mineralized bone a thin layer of amorphous or finely granular material was present. This layer was 100–200 nm wide. When an interface region was followed in a section the amorphous layer was often missing. However, since the same type of amorphous material was also present on the implant surface as seen in electropolished specimens (Fig. 5 b–d), or adhering to the fracture plane in unscrewed, fractured specimens, the partial absence of the amorphous layer was probably due to an artefactual dislocation during the preparation. This was supported by the observation that the layer of amorphous material often was partly detached from the mineralized bone (Fig. 6d).

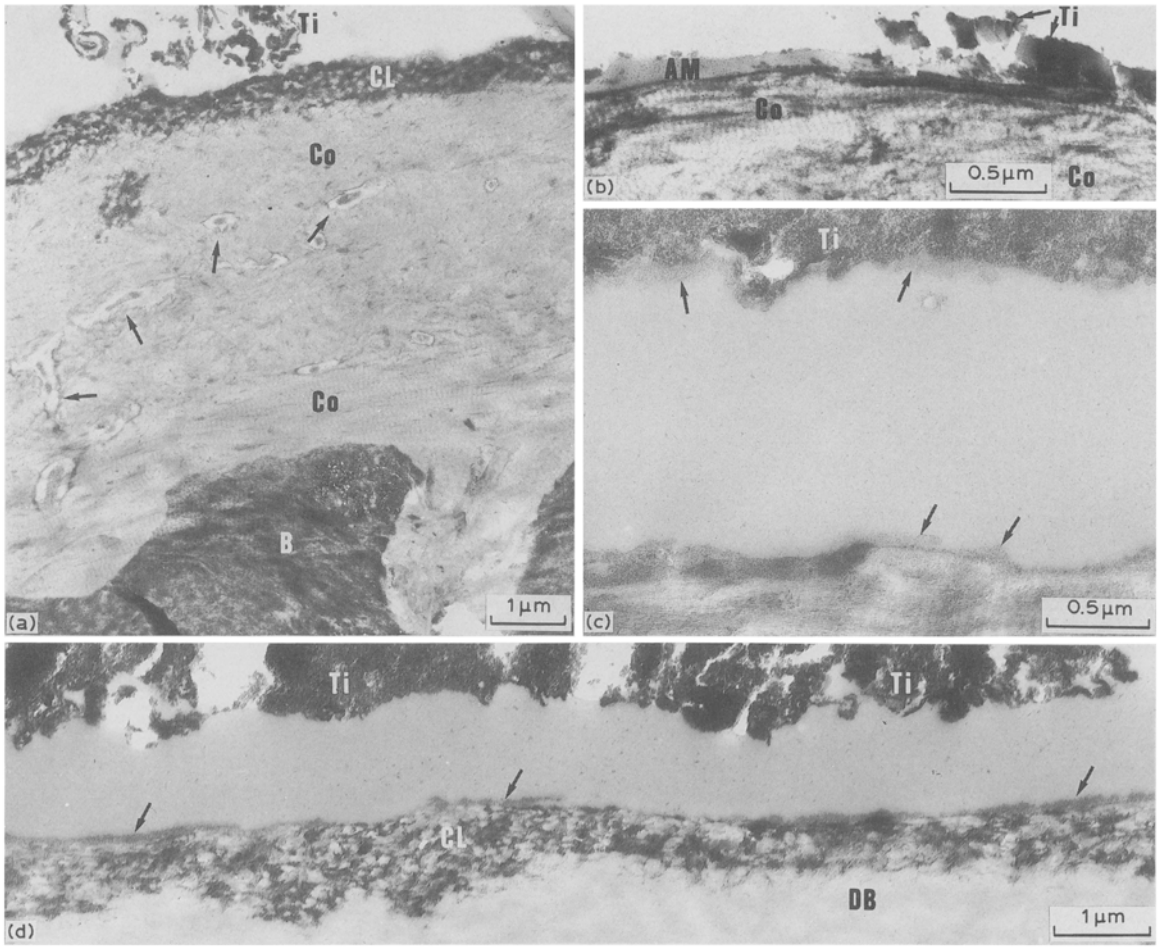

Figure 5 Mount of electron micrographs obtained from the interface zone between cortical bone in rabbit tibia and titanium implants inserted for 12 months. The specimens were prepared by *electropolishing*. (a) The tissue adjacent to the implant is artificially decalcified and consists of collagen (Co) and osteocyte canaliculi (arrows). Titanium fragments (Ti) are located in the plastic resin outside the tissue. The tissue in contact (CL = contact layer) with the implant is infiltrated with a dense material (known to contain titanium and calcium). B = calcified bone. (b) Contact layer. Dense material infiltrates the tissue accentuating the cross-striation of the collagen fibrils (Co). Titanium fragments (Ti) more or less in contact with the tissue. A layer of amorphous material (AM) is in contact with collagen. (c) This implant was unscrewed before fixation and a gap separates the tissue from the uncompletely electropolished titanium implant (Ti). Amorphous material (AM, arrows) is present on the surface of the tissue as well as on the surface of the implant. (d) Unscrewed specimen with a gap separating the implant (Ti) from the contact layer (CL) infiltrated by titanium. Amorphous material (arrows) forms an uncomplete layer along the tissue surface. DB = decalcified bone.

Another prominent feature was that the mineralized bone in immediate contact with the amorphous zone formed an electron dense layer about 100 nm wide. This layer was most readily observed when the mineralization of the bone was comparatively low, probably due to the fact that it was then not obscured by overlying, dense hydroxyapatite (Fig. 6 a–d). The dense layer had the same density and width as the dense layer (lamina limitans) forming the border of mineralized bone observed around osteocyte lacunae and canaliculi (Fig. 6c) or separating bone of different degree of mineralization (Fig. 6d). In fact a direct continuity between the dense layer at the implant surface and lamina limitans could be observed (Fig. 6 c, d).

Although mineralized bone was located close to the implant surface along the major part of the interface this was not always the case. In every interface examined (prepared by the fracture technique) examples could be found where a narrow (0.5–2 µm) zone of non-mineralized, collagen-containing tissue intervened between mineralized bone and implant (Fig. 7b–d). Also in those locations a layer of amorphous material appeared to form the contact with the implant (Fig. 7b, d). In other locations osteocytes (Fig. 7a) or vessels (not shown) surrounded by poorly or non-mineralized tissue were present close to the implant surface.

4. Discussion

In ground sections we found a close contact between the titanium screws and bone without any intervening connective tissue in all nine specimens examined. Thus, the titanium screws were "osseointegrated" [1]. This was further supported by the torque force values required to unscrew the implants. These values were in the same range as previously found for clinical [13] and experimental [14] screws considered to be "osseointegrated".

4.1. Specimen preparation

A puzzling observation was that when using the electropolishing method we were in general unable to

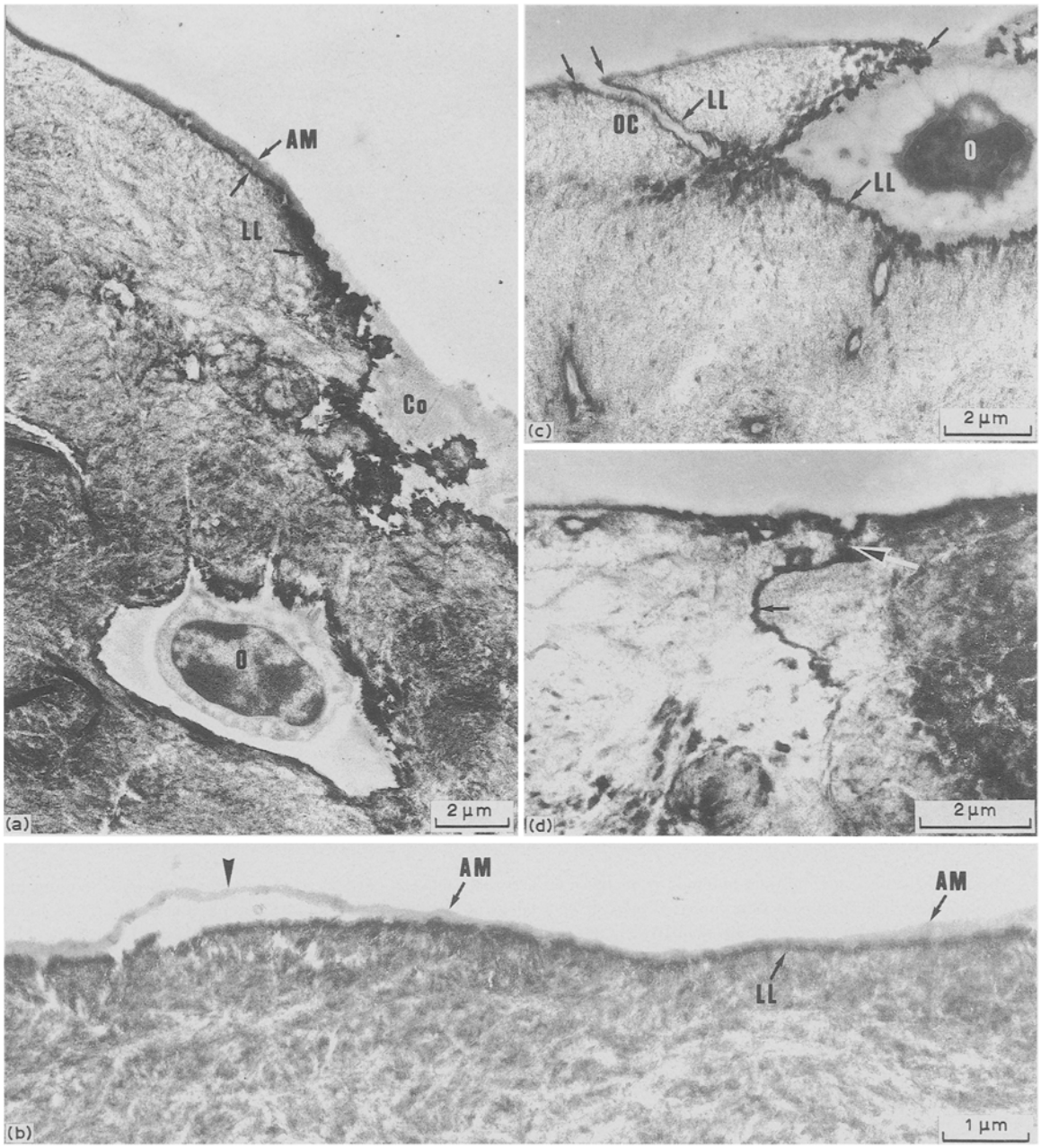

Figure 6 Mount of electron micrographs of the interface zone prepared by the *fracture technique*. Titanium implants retrieved from rabbit tibia after 12 months. (a) The implant was unscrewed before perfusion fixation. Mineralized bone reaches close to the (removed) implant surface where it ends with an osmiophilic lamina-limitans-like line (LL). A layer of amorphous material (AM) is located along this line. Along a part of the interface the tissue, consisting of collagen fibrils (Co), is not mineralized. An osteocyte (O) is located close to the implant surface. (b) A layer of amorphous material (AM) is present along the entire interface. The amorphous layer is artefactually detached from the underlying bone in a part of the interface. A lamina-limitans-like osmiophilic line (LL) is clearly visible due to the rather low mineralization of the bone. (c) A poorly preserved osteocyte (O) is located close to the implant surface. The lamina-limitans-like line along the interface is in direct continuity (arrows) with the laminae limitantes (LL) lining the osteocyte lacuna as well as an osteocyte canaliculus (OC) reaching the implant surface. Osteocyte canaliculi are also located in deeper part of the bone tissue. (d) The osmiophilic lamina-limitans-like line along the interface is in continuity (arrow) with a lamina limitans (LL) (or cement line) separating bone tissue of different ages. In this section an amorphous layer along the interface cannot be distinguished.

distinguish the distinct oxide layer similar to the one previously observed with similarly machined pure titanium inserted in soft tissues. Instead large amounts of titanium chunks were present. This might partly be due to the size and geometry of the screws resulting in an incomplete removal of metal during the electropolishing process. However, when screws without prior contact with tissue were resin embedded directly and then electropolished a distinct oxide line was present (unpublished observations). Furthermore, in the electropolished screws retrieved from animals a distinct oxide layer was present in areas in contact with the bone marrow but not in areas in contact with bone. These observations suggest that the implant surface oxide in contact with bone might have been chemically altered due to contact with bone. The nature of this alteration is not known. One possibility is that the surface oxide (TiO_2) had increased in thickness. Another possibility is that a modification of the physicochemical properties had occurred via an incorporation of ions from the biological environment. A change in the composition of the surface oxide might in turn

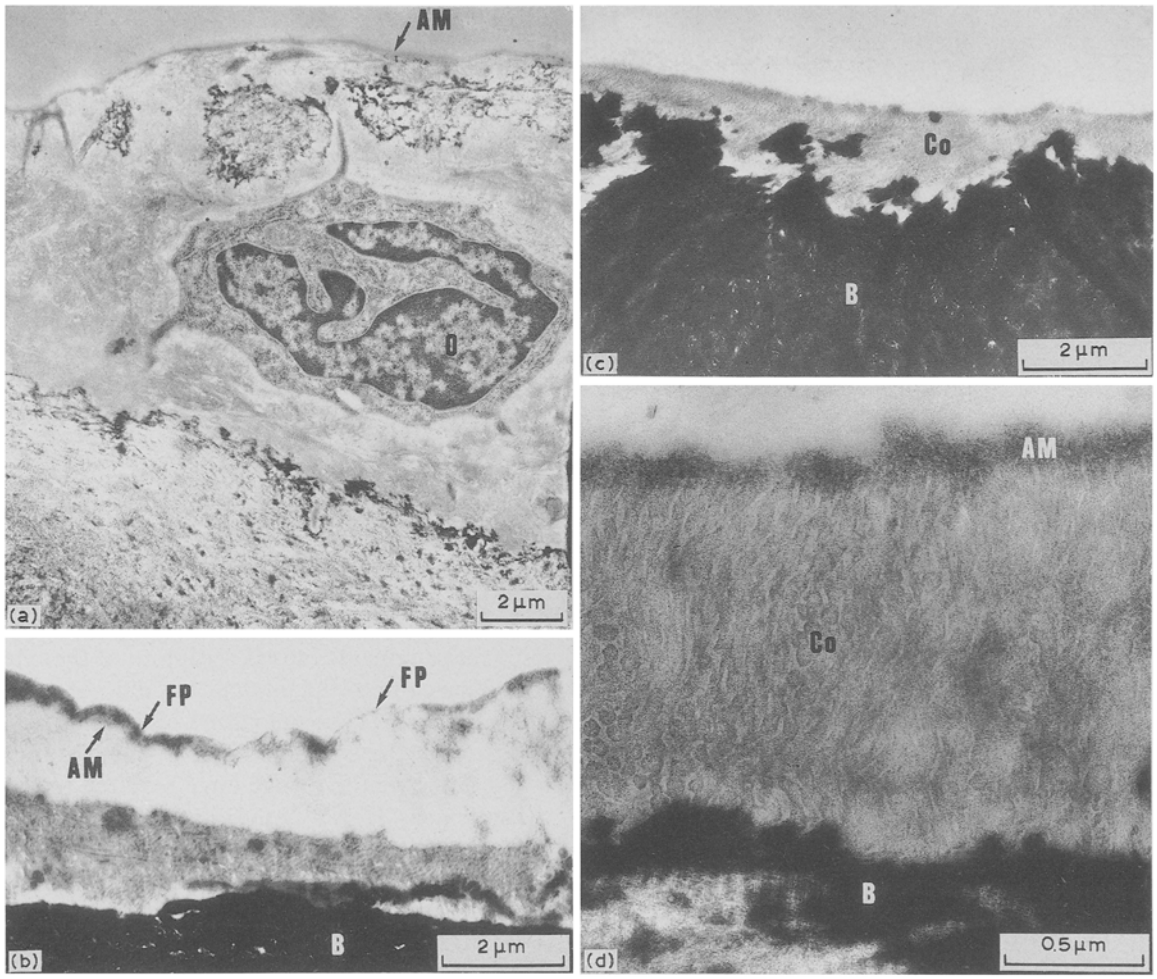

Figure 7 Examples of titanium–bone interfaces where mineralized bone does not reach the implant surface. The *fracture technique* was used in all specimens. (a) An osteocyte (O) located close to the interface is surrounded by an unmineralized or poorly mineralized collagenous matrix. A layer of amorphous material (AM) is located along the interface. (b) Unscrewed implant. The fracture plane (FP) forms an undulating line. Amorphous material (AM) which probably has detached from the tissue during the unscrewing procedure is associated with the fracture plane (and probably with the implant surface). Mineralized bone (B) is separated from the implant by a narrow zone of unmineralized tissue containing collagen. (c) Another interface where the interface tissue consists of only partly calcified collagenous matrix (CO). B = mineralized bone. (d) In this interface amorphous material (AM) is located closest to the implant. Densely packed collagen fibrils (CO) form the predominant structural component of the unmineralized interface tissue zone. B = mineralized bone.

have caused a loss of its barrier function between the bulk and the tissue, a condition which is reflected in the movement of calcium and titanium out and in of the tissue block during the electropolishing process. In soft tissue in which the oxide layer is thin and morphologically intact we have not found any evidence by using imaging electron energy loss spectroscopy of titanium infiltration in the interface tissue indicating that in this case the surface oxide functions as an effective barrier [9].

Our observations thus clearly demonstrate that the electropolishing methods used in this study are not suitable for preparation of bone. However, the electropolishing procedure is by itself of great potential value in studies of the interface, so it is certainly of great importance to explore the use of other electrochemical procedures, as for instance the one recently applied by Christel *et al.* on metal implants in soft tissue [15]. However, we want to underline the fact that the electrochemical procedure we have used in this study is very useful in studies of soft tissue implants without any evidence of titanium redistribution from the bulk into the implant. Recently we have, in soft tissue, combined the electropolishing procedure with mild chemical fixation followed by freeze-substitution which enables us to study the chemical composition of intact interface zone by using light and electron microscopic immunocytochemistry [16] and application of a similar procedure, by using a modified electrochemical procedure, on bone should be a very useful tool in dissection of the molecular anatomy of the bone–metal interface.

4.2. The ultrastructure of titanium–bone interface

For the evaluation of the interface ultrastructure we had to rely on the fracture technique [11, 12]. The apparent drawback with this method is that it is not possible to exactly define the fracture plane and the exact location of the implant surface. However, surface analysis of implants removed from the plastic-embedded decalcified bone indicate that the fracture plane in general is within 10 nm from the implant

surface [11]. In the present study SEM observation of the implant surface after fracture from the undecalcified bone showed that large areas displayed the similar surface texture with machining grooves typical for the native implant. These findings together with the ultrastructural observations of the interface gathered by a correlative use of fractured and electropolished specimens indicate that the fracture plane in general, although not always, is close to the implant surface.

A characteristic feature of the interface was that calcified bone reached very close to the implant surface along its major part. Judging from the density of the tissue, there was no apparent gradient in the degree of mineralization. Such a gradient has been reported in partly decalcified bone surrounding titanium-coated plastic plugs inserted in the rabbit tibia for three to six months [4–6]. In the present study a typical feature of the interface was the presence of an electron dense layer forming the border of mineralized bone towards the implant. This layer appeared to be of the same dimension and structure as lamina limitans [17] found around osteocyte lacunae and canaliculi and in between bone tissue with different degree of mineralization (here also called cement line). In fact, we frequently observed a direct continuity between the lamina-limitans-like layer at the implant surface with that in other locations. The osmiophilic lamina limitans, present also in decalcified specimens, is considered to be located where the calcification has started or ended and to be caused by the accretion of organic material [17]. In previous studies on the ultrastructure of titanium–bone interface using decalcified specimens a lamina-limitans-like structure has not been described. However, it is present at the surface of hydroxyapatite [18], bioglass [19], and polyactive polymer [20] surfaces. In the present study we were unable to examine any fine details of this layer due to the presence of calcium mineral.

Another feature was the presence of amorphous material with a width of 100–200 nm peripheral to the lamina limitans. Such a layer was also observed between titanium and collagen fibrils in electropolished specimens. However, the layer was not uniformly present and could be absent from portions of the interface both in unscrewed specimens and in specimens fixed *in situ* and fractured. At present we do not know for certain whether the varying appearance of the amorphous layer reflects a biological variation or is an artefact caused by the fracture technique. Neither is the nature of this amorphous layer knwon. A similar layer has been described by Linder *et al.* [3] studying decalcified specimens prepared by the fracture technique to be typical to parts of the interface between bone and several types of metal implants. Albrektsson *et al.* [4–6], however, have repeatedly described that the typical feature of the (titanium-coated plastic plugs)–bone interface is a 20–40 nm thin amorphous proteoglycan layer located between the metal and collagen fibrils, which is five times less than the width of the amorphous layer observed by us. Possibly this difference is related to differences in bulk and surface properties between the cylindrical, metal-coated polymer and the solid, threaded implants used in the present study. In fact, the thickness of amorphous layer around metal-coated implants has been found to vary depending on the implant material used [4–6, 21]. We have recently [22] found an amorphous layer between mineralized bone and threaded dental titanium implants retrieved from patients indicating that the presence of an amorphous layer is a common feature for threaded titanium implants. Obviously, very little can be said about the molecular composition of this layer, without further studies using immunocytochemistry and element analysis at the ultrastructural level.

5. Conclusions

In the present study we examined titanium implants fixed *in situ* when in contact with tissue as well as implants which were unscrewed before fixation. In both situations the ultrastructure of the tissue was very similar indicating that unscrewing (and torque force measurement) caused a rupture of the interface zone very close to the implant but did not cause any other fractures. Thus the nature of the interface zone and thereby the bonding of the implant to the bone might influence the torque force required to unscrew the specimens. However, the relative importance of the interface for the fixation of titanium screws is unknown. As we recently have discussed in more detail [23] also the structure of the bone filling the threads seems to influence the torque force values measured.

To conclude, our observations in the present study show that calcified bone reaches close to the surface of titanium implants inserted in the rabbit tibia for 12 months. Our observations suggest that a direct contact between bone mineral and the implant is not established.

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References

1. P. I. BRÅNEMARK, G. A. ZARB and T. ALBREKTSSON (eds), "Tissue-integrated prostheses: osseointegration in clinical dentistry" (Quintessence, Chicago, 1985).
2. T. ALBREKTSSON, P. -I. BRÅNEMARK, H.-A. HANSSON and J. LINDSTRÖM, *Acta. Orthop. Scand.* **52** (1981) 155.
3. L. LINDER. K. OBRANT and G. BOIVIN, *ibid.* **2** (1989) 135.
4. T. ALBREKTSSON, P. I. BRÅNEMARK, H. A. HANSSON, B. IVARSSON and U. JÖNSSON, in "Clinical applications of biomaterials", edited by T. Albrektsson and P. I. Brånemark (John Wiley & Sons Ltd, 1982).

5. T. ALBREKTSSON, H. A. HANSSON and B. IVARSSON, *Biomaterials* **6** (1985) 97.
6. T. ALBREKTSSON and H. A. HANSSON, *ibid.* **7** (1986) 201.
7. L. M. BJURSTEN, L. EMANUELSSON, L. E. ERICSON, P. THOMSEN, J. LAUSMAA, L. MATTSON, U. ROLANDER and B. KASEMO, *ibid.* **11** (1990) 596.
8. L.-E. ERICSON, L. SENNERBY, G. BOKHEDE, L. EMANUELSSON and P. THOMSEN, *Adv. Biomater.* **9** (1990) 333.
9. L.-E. ERICSON, B. R. JOHANSSON, A. ROSENGREN, L. SENNERBY and P. THOMSEN, in "The bone–biomaterial interface", edited by J. Davis (Toronto University Press), in press.
10. K. DONATH and G. A. BREUNER, *J. Oral Pathol.* **11** (1982) 318.
11. J. LAUSMAA and L. LINDER, *Biomaterials* **9** (1988) 277.
12. P. THOMSEN and L. E. ERICSON, *ibid.* **6** (1985) 421.
13. A. TJELLSTRÖM, M. JACOBSSON and T. ALBREKTSSON, *Int. J. Oral Maxillofac. Implants* **3** (1988) 287.
14. C. JOHANSSON and T. ALBREKTSSON, *ibid.* **2** (1987) 69.
15. P. CHRISTEL, A. MEUNIER and M. THERIN, *J. Appl. Biomater.* **1** (1990) 205.
16. A. ROSENGREN, B. JOHANSSON and L.-E. ERICSON, in Transactions 16th Annual Meeting of the Society for Biomaterials (1990) p. 222.
17. J. SCHERFT, *J. Ultrastruct. Res.* **64** (1978) 173.
18. C. A. VAN BLITTERSWIJK, J. J. GROTE, W. KUYPERS, C. J. G. BLOEK-VAN HOEK and W. T. H. DAEMS, *Biomaterials* **6** (1985) 243.
19. A. E. CLARK, L. L. HENCH and H. A. PASCHALL, *J. Biomed. Mater. Res.* **10** (1976) 161.
20. C. A. VAN BLITTERSWIJK, S. C. HESSELING, J. VAN DER BRINK, H. LEENDERS and D. BAKKER, in "The bone–biomaterial interface", edited by J. Davis (Toronto University Press), in press.
21. J. JOHANSSON, J. LAUSMAA, M. ASK, H.-A. HANSSON and T. ALBREKTSSON, *J. Biomed. Engng* **11** (1989) 3.
22. L. SENNERBY, L. E. ERICSON, P. THOMSEN, V. TEKHOLM and P. ASTRANC, *Clin. Oral Iumpl Res.* **2** (1991) 103
23. L. SENNERBY, P. THOMSEN and L. E. ERICSON, *Int. J. Oral. Maxillofac Implants* **7** (1992) 62.

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