JOURNAL OF MATERIALS SCIENCE: MATERIALS IN MEDICINE 4 (1993) 494–502

Early tissue response to titanium implants inserted in rabbit cortical bone

Part II *Ultrastructural observations*

L. SENNERBY*, P. THOMSEN, L. E. ERICSON
*Biomaterials Groups, Department of Anatomy and *Department of Handicap Research, University of Gothenburg, Gothenburg, Sweden*

The tissue response to screw-shaped implants of commercially pure titanium was studied 3–180 days after insertion in the rabbit tibia by means of transmission electron microscopy. Red blood cells and scattered macrophages predominated at the implant surface after 3 days. At day 7 and later intervals, multinuclear giant cells were the cell type found at the implant surface protruding into the bone marrow and in areas with no bone–titanium contact. Osteoblasts or mesenchymal cells were rarely seen at the implant surface at any time period. Two modes of mineralization could be distinguished in the interface. Firstly, the typical mineralization of osteoid seams produced by osteoblasts. Secondly, an accumulation of scattered hydroxyapatite crystals in the unmineralized collagen matrix in the interface. Mineralized tissue was observed close to the implants surface from day 14. However, the innermost 2–20 μm were poorly mineralized although scattered hydroxyapatite crystals were present. The collagen fibrils did not reach the implant surface but were separated from it by an amorphous layer, being 0.3–0.5 μm thick which did not decrease in width with time. An electron-dense lamina limitans-like line containing mineral was observed between the amorphous layer and the bone tissue.

1. Introduction

Direct bone–metal contact, as determined in the light microscope, seems to be favourable for the long-term clinical outcome of threaded titanium implants [1]. In two recent studies we have described the bone/titanium interface of clinically retrieved titanium implants [2] and experimental implants inserted in the rabbit tibia [3], using LM and TEM. From these studies it was concluded that the interface morphology varied. In areas with a "direct" bone contact, the implant surface and the mineralized bone was often seen to be separated by a collagen-free amorphous layer, which was about 100–400 nm thick and which did not contain hydroxyapatite crystals. A similar amorphous layer has been described in decalcified specimens by Linder *et al.* [4] who studied the tissue around cylindrical implants of different metals, including titanium, in the rabbit titbia. The amorphous layer may also, at least partially, correspond to the "proteoglycan layer" around plastic implants sputter-coated with titanium and inserted in rabbit tibias, repeatedly described by Albrektsson *et al.* [5–7]. However, the nature of this amorphous layer, and how and when it is formed, is not known at present. In fact, we are not aware of any information concerning the early interface events for threaded titanium implants studied at the cellular and ultrastructural level. In Part I of this article [8] we described the bone healing around titanium implants 3–180 days after insertion in the rabbit tibia using light microscopy. We concluded that bone was not formed primarily on the surface of the implants. The increased bone–titanium contact with time was the result of bone formation at the endosteal surface of the cortex and solitary bone formation in the bone marrow near the implant surface, which approached the implant surface. Moreover, multinuclear giant cells were observed to cover the entire implant surface in areas with no bone–titanium contact.

In the present paper, the light microscopic observations are extended by observations in the electron microscope.

2. Materials and methods

2.1. Animals and surgery

180 screw-shaped implants made of commercially pure titanium (3.75 mm diameter and 4 mm long) were inserted in the tibia and distal femoral condyle of 30 adult New Zealand white rabbits as described in detail in Part I [8]. In the present study only the tibial implants were analysed.

2.2. Implant retrieval, tissue processing and transmission electron microscopy

The implants with surrounding tissue were retrieved 3, 7, 14, 28, 42, 90 and 180 days after insertion after

0957–4530

perfusion fixation with 2.5% glutaraldehyde in 0.05 M cacodylate buffer, pH 7.4, as described elsewhere [8]. The osmificated specimens were dehydrated in a graded series of ethanol and embedded in plastic resin (LR White). After polymerization the specimens were divided in two parts by sawing. One part was used to prepare ground sections according to Donath and Breuner [9]; the remaining implant-tissue blocks were used to prepare thin sections for light and transmission electron microscopy using a fracture technique. By this technique [10], the titanium was separated from the plastic block which then was re-embedded in resin and 1 μm sections were cut for light microscopy using glass knives. Selected areas were further sectioned for TEM using diamond knives and were examined after staining with uranyl and lead citrate. An electropolishing technique was used on the other part of the divided specimens as described elsewhere [11]. This technique has been described to induce a decalcification of the interface tissue [12] but was used to study the organic components in the interface. In brief, the bulk part of the implant was removed by electrochemical dissolution, leaving a layer of titanium in contact with the tissue. After re-embedding in plastic resin, the specimens were sectioned with diamond knives as described above.

Transmission electron microscopy was performed in a Zeiss CEM 902 or in a Philips TEM 400.

3. Results

In the ultrastructural examination, particular attention was paid to the endosteum–implant region as the light microscopic studies showed that the most important early morphological changes took place in this region [8]. In particular, interest was focused on the ultrastructure of the tissue in the interface between calcified tissue and the implant, the morphology of cells close to the implant surface and the ultrastructure of the calcification process around the implants. Most observations were made on specimens prepared by the fracture technique. Selection of appropriate areas for ultrathin sectioning was guided by light microscopic observations in 1 μm thick sections. It should be pointed out that the quality of the tissue preservation often was not optimal which, at least partly, was related to the fact that the tissue was fixed together with the implant which implies long diffusion distances for the fixatives. This restricted the number of specimens available for a more detailed analysis to two to four specimens for each time interval.

3.1. General observations

At 3 days, red blood cells and scattered macrophages, often enmeshed in a fibrin network, predominated at the implant surface. An exception to this was the space between the cut surface of the cortex and the implant which was remarkably acellular (apart from red blood cells) and often contained only proteinaceous material and fragmented bone mineral. At day 7 and later times intervals, the presence of flattened multinuclear giant cells adhering to the implant surface was a predominant feature for the part of the implant protruding into the bone marrow (Fig. 1a and b). They appeared to cover the entire surface area with their bodies and thin cytoplasmic extensions, forming a narrow cytoplasmic rim excluding other cells from contact with the surface (see Fig. 3b). The plasma membrane facing the tissue formed folds, sometimes very extensively (Fig. 1b) but never to the extent as seen in a typical osteoclastic ruffled border (Fig. 1c). The multinuclear giant cells in contact with the implant contained numerous mitochondria and a large amount of free ribosomes (Fig. 1a) but, in general, few endocytic structures, although endocytic vacuoles containing red blood cells, cell debris or bone mineral fragments were present at 3 and 7 days.

At 3 and 7 days, bone fragments torn off by the surgical procedure were present close to the implant surface. These fragments were surrounded by macrophages which also contained chunks of bone mineral in phagocytic vacuoles (Fig. 1d). Osteoclasts were found at the surface of vital bone tissue (Fig. 1c) but not in relation to bone fragments. At day 3 and, more pronounced, at day 7, mesenchymal cells appeared in the endosteum–implant region. These cells, discussed further below, contained varying amounts of endoplasmatic reticulum and a nucleus with a smooth contour which occupied a large fraction of the cell profile. It should be pointed out that such cells were not in contact with the implant surface but were generally separated from it by multinuclear giant cells.

3.2. Formation of bone close to the implant surface

Our light microscopic observations at 7 days [8] showed that bone around the implants was initially formed either as trabecular woven bone from the endosteal surface, or as solitary woven bone in the threads. Sites of solitary bone formation identified in parallel light microscopic sections, consisted initially of mesenchymal cells differentiating into osteoblasts/osteocytes. The cells surrounded themselves with a matrix consisting of collagen bundles running in various directions (Fig. 2a and b). Aggregates of bone mineral were deposited in the bundles in what appeared to be a haphazard way (Fig. 2a), ultimately to fill the entire bundle (Fig. 2c). The rate of mineralization appeared to be different for each individual bundle and almost completely mineralized bundles were located adjacent to bundles with very few mineral aggregates. The mode of solitary bone formation was quite different from orderly morphological arrangements seen at the surface of the bone trabeculae where osteoblasts, orderly arranged, faced a layer of osteoid with a distinct mineralization front (Fig. 3a).

At the implant surface, the general mineralization process appeared to be similar to that observed for solitary bone formation described above. At 7 and 14 days, densely packed mesenchymal cells were present close to the surface (Fig. 3a) and these cells formed a collagenous matrix which was gradually mineralized (Fig. 3c and d). Bone-forming cells did not adhere to

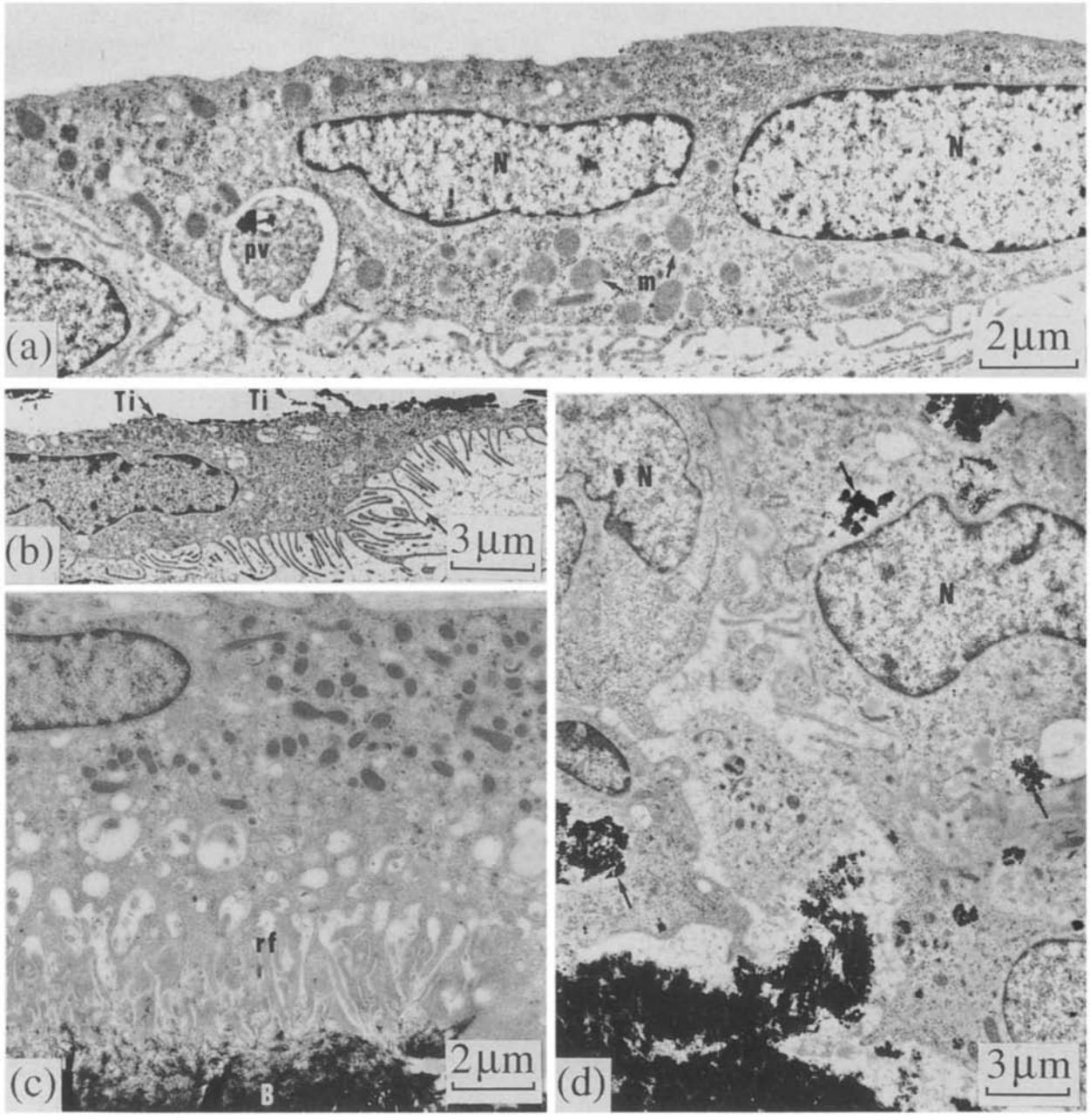

Figure 1 If not otherwise stated all electron micrographs are from specimens prepared by the fracture technique. (a) 28 days. Portion of a multinuclear giant cell at the implant surface; N, nucleus. The cytoplasm contains numerous mitochondria, some of which are indicated (m) and a phagocytic vacuole (pv). (b) 14 days. Electropolished. Multinuclear giant cell at the implant surface; Ti, remaining titanium. The cell membrane facing the tissue is extensively folded. (c) 7 days. Portion of an osteoclast in contact with bone (B) with a typical ruffled border (rb). (d) 3 days. Phagocytosis of bone fragments by macrophages. Dense fragments are located extracellularly and in intracellular phagocytic vacuoles (arrows).

the implant surface and were either separated from the surface by collagenous matrix or by multinuclear cells attached to the implants. Osteoblasts forming an osteoid seam with a mineralization front were sometimes present close to the implant surface but the osteoid-forming surfaces of these osteoblasts were invariably turned away from the implant (Fig. 3c).

3.3. Ultrastructure of the interface between mineralized tissue and the implant

As described above, the contact between bone and implant increased progressively. At early times, 3 and 7 days, such contact areas were few. However, at 14 days, when the amount of newly formed bone in the threads had increased substantially, mineralized tissue was located close to the implant. In most such areas with mineralized bone close to the surface at 14 and 28 days, an unmineralized or poorly mineralized zone, 2–20 μm wide, separated the mineralized bone from the implant (Fig. 3c–f). This interface zone contained collagen, running in various directions but, in general, oriented parallel to the implant surface, as well as osteocyte cell bodies, located at minimum 5 μm (but generally at larger distance) from the surface, and osteocyte processes. The collagen fibrils, which were typically cross-striated, did not reach the implant surface but ended in a less than 0.5 μm wide zone

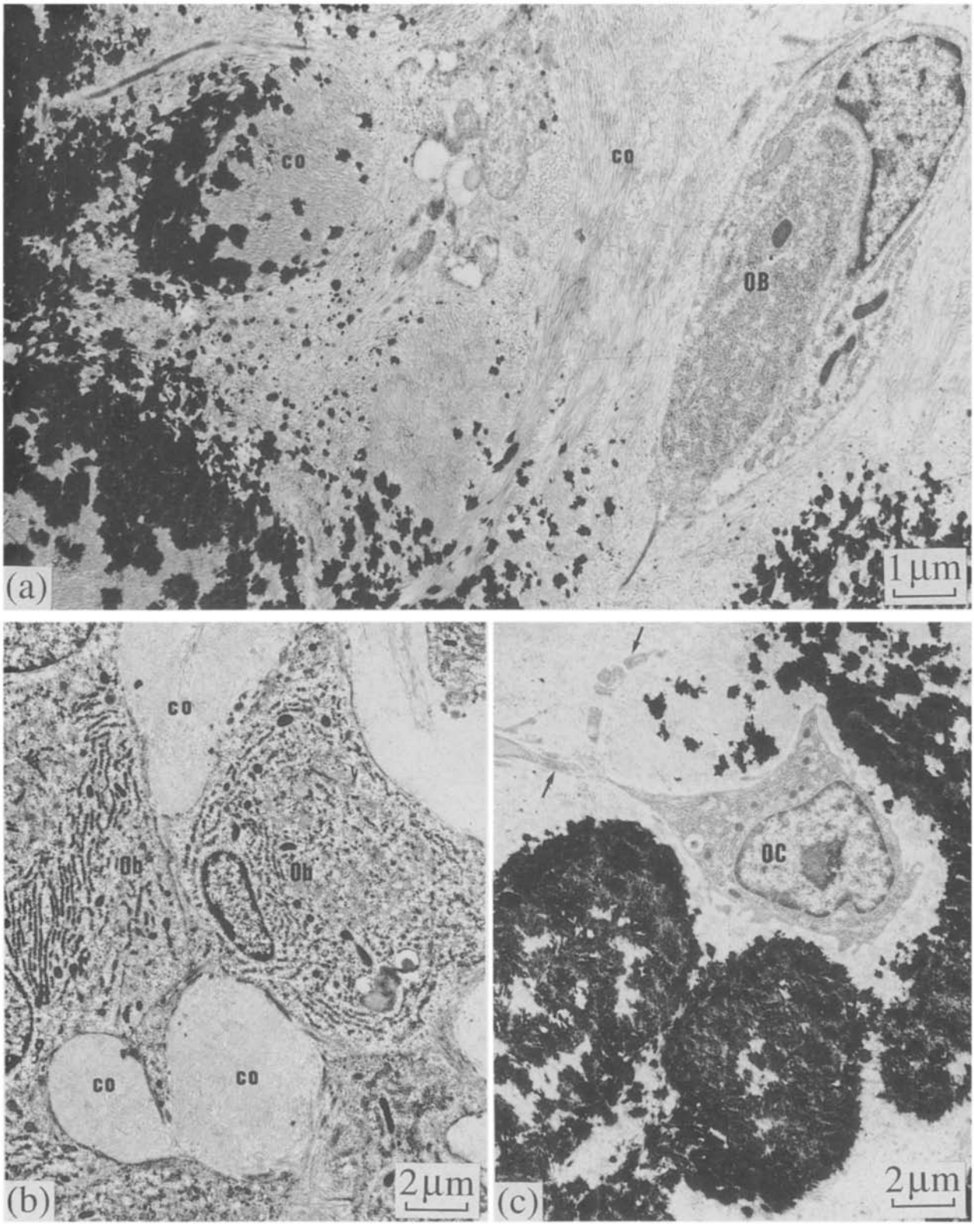

Figure 2 Electron micrographs taken 7 days after insertion. Solitary bone formation. (a) Osteoblast (OB) surrounded by a partially calcified collagen (co) matrix. Note the uneven distribution of aggregates of hydroxyapatite crystals. (b) Osteoblasts partly separated by collagen (co) bundles. No mineralization. (c) Osteocyte (OC) with cytoplasmic extensions (arrows). The degree of mineralization varies in different collagen bundles.

consisting of an amorphous material (Fig. 3f). The structure and electron density of the material was variable, possibly related to differences in the fixation. In certain areas, the layer consisted of a rather dense structureless material of high electron density, whereas in others it was more loosely arranged with a finely granular texture of lower density. At both 14 and 28 days, scattered accumulations of hydroxyapatite crystals were observed close to the implants. These accumulations of hydroxyapatite crystals, always related to collagen, were distributed in a rather haphazard manner and were thus not distributed as a gradient

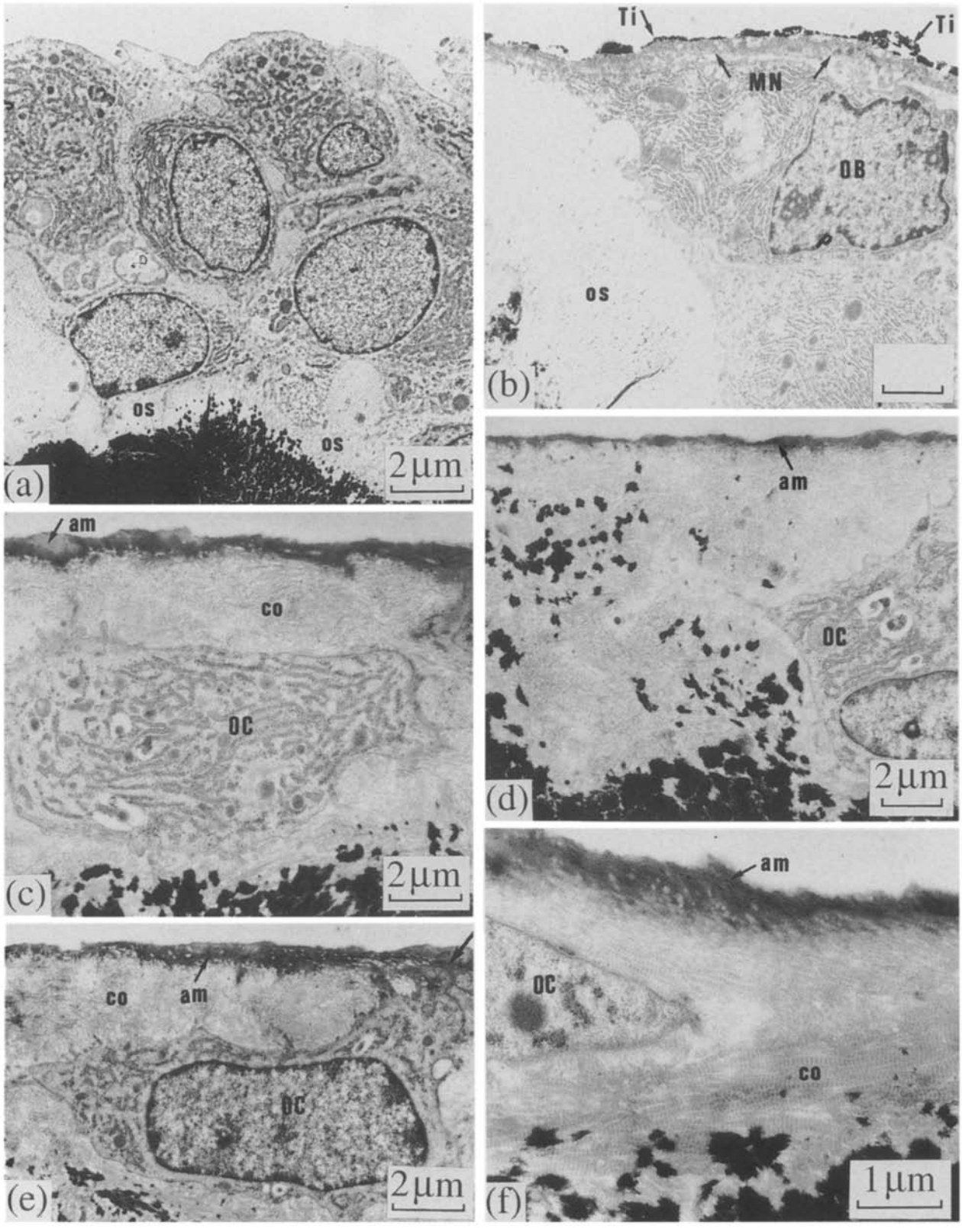

Figure 3 Electron micrographs taken 14 days after insertion. (a) Mesenchymal cells/osteoblasts close to the implant surface. The cell layer(s) close to the surface was removed during preparation. In the lower part of the micrograph an osteoid seam (os) is located adjacent to the osteoblasts. (c) An osteocyte (OC) surrounded by a collagen (co) matrix. No mineralization close to the implant is seen. An amorphous, electron dense layer (am) is located at the implant surface. (d) Aggregates of hydroxyapatite crystals are unevenly distributed in the interface tissue. The aggregates are not concentrated at the implant surface; am, amorphous layer; OC, osteocyte. (e) An osteocyte (OC) with a cytoplasmic extension (arrow) reaching the electron dense amorphous layer (am) at the implant surface; co, collagen. (f) Collagen fibrils extend into the amorphous layer (am); co, collagen; OC, portion of an osteocyte.

towards or from the implant surface (Fig. 3d). The dense amorphous layer closest to the implant did not contain hydroxyapatite crystals (Fig. 3c–f).

Already at 14 days, but gradually more frequently with longer time, apparently fully mineralized bone was seen close to the implant. The mineralized bone ended with a sharp border and was separated from the implant surface by the amorphous layer, generally

0.2–0.4 μm wide (but sometimes wider) apparently identical to that described above (Fig. 3a–d). This is also similar to what was recently found for implants inserted in the rabbit tibia for 12 months [3]. Without further elemental analysis (in progress) we do not know if calcium was present in any form in the amorphous layer. However, it should be pointed out that even after 6 months the interface tissue was not fully mineralized but consisted of collagen fibres ending in the amorphous layer (Fig. 4e and f).

In most areas, the dense hydroxyapatite made any observations on the organic matrix impossible. However, in certain areas where bone mineral was present close to the implant but the general degree of mineral-

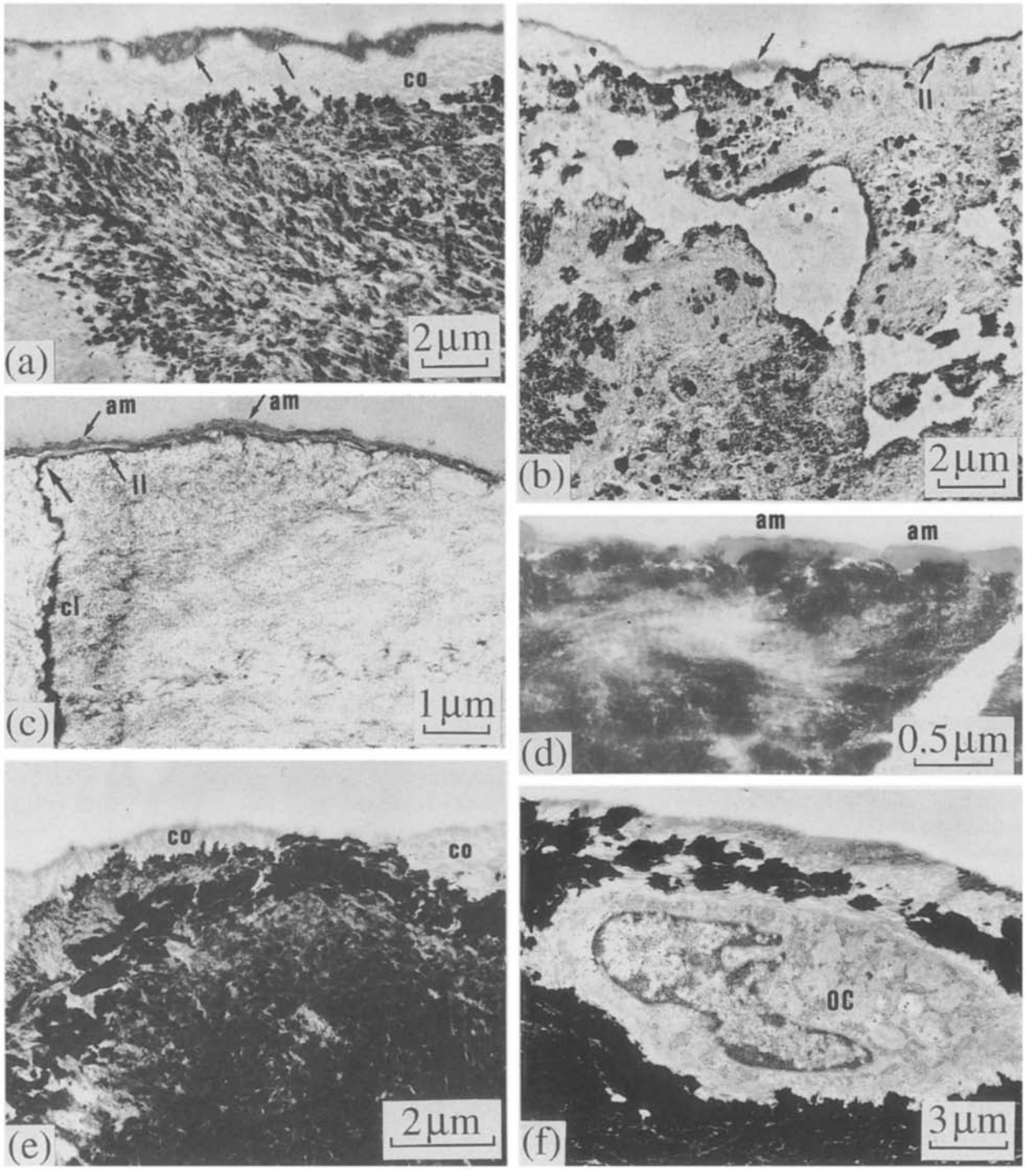

Figure 4 Electron micrographs of the interface tissue taken 42–180 days after insertion. (a) 42 days. An electron-dense layer (am) of varying thickness is present at the implant surface. Mineralized bone is separated from the surface by a non-mineralized zone containing collagen (co). (b) The degree of mineralization varies in the interface. The bone forms a dense lamina limitans (ll) at the implant surface. An amorphous layer (arrow) is present where non-mineralizing tissue contacts the implant, but is mainly absent (artefact) along lamina limitans. (c) 90 days. Poorly mineralized bone in contact with the implant. A lamina limitans (ll) at the implant surface is in continuity (arrow) with a cementing line (cl). An amorphous layer (am) is located close to the implant. (d) 90 days. An amorphous layer (am) separates mineralized bone from the implant. (e) 180 days. Although mineralized bone reaches the implant along the major part of the interface, narrow rims of non-mineralized collagen (co) matrix, certainly not identified in the light microscope, are common. (f) 180 days. Another example of partly mineralized interface with an osteocyte (OC) close (about 3 μm) to the surface.

ization was low, we observed an electron-dense line at the border of the calcified tissue and the amorphous layer. This electron-dense line was about 100 nm wide and was in continuity with the osmiophilic line bordering osteocyte canaliculi or osmiophilic lines (resting lines) in the bone tissue (Fig. 4c). Morphologically, the dense line adjacent to the amorphous layer was similar to lamina limitans, as described in demineralized bone [13–16]. As we studied undecalcified tissue, we could not distinguish any further ultrastructural features of the lamina limitans-like line. However, sometimes the lamina limitans-like line deviated from the implant surface and was separated from the amorphous layer by an unmineralized collagenous matrix. In such situations, collagen fibrils appeared to continue into the mineralized matrix through the lamina limitans-like line, which suggests that collagen is a component of the line. The organization of the tissue close to the implant surface implies that there is a direct continuity between the amorphous layer and the osteocyte canaliculi, which thus form a compartment delineated from the mineralized bone by a lamina limitans. We found no evidence that osteocyte processes actually reached the surface, but cannot exclude this possibility.

5. Discussion

In the accompanying light microscopic study [8] we found that an intense formation of new trabecular bone was initiated at the endosteal surface within 1 week after insertion of the threaded titanium implants. The newly formed bone trabeculae extended towards the subcortical portion of the implant. In this early phase no signs of bone formation were apparent around the portion of the implant located in the cortical bone. However, newly formed bone, with a morphology different from that of trabecular bone, also appeared as islets in the subcortical threads. This solitary bone then fused with the laterally coming bone trabeculae to form a bone collar around the subcortical portion of the implant. The areas of solitary bone formation were easily identified in the electron microscope. In these areas osteoblasts/osteocytes surrounded themselves by a collagenous matrix consisting of distinct collagen bundles running in various directions. These bundles were then mineralized and aggregates of hydroxyapatite crystals were distributed within each bundle in an apparently haphazard manner. These mineral aggregates probably correspond to the dark granules located between the cells as found in the light microscope.

Our light microscopic observations, supported by morphometry, suggested that bone formation was not initiated at the implant surface but that newly formed bone grew towards rather than from the implant surface. The ultrastructural findings support this observation. A possible theoretical scenario for bone formation around the implants is that bone and bone cells are closely related, or even attached to, the implant surface and from this position start to deposit osteoid on the implant surface much in the same way as osteoblasts attach and align to the bone surface at the reversal phase during cortical bone remodelling when a filling cone starts to form from a cutting cone produced by osteoclasts. However, we never observed osteoblasts aligned along the implant surface forming a gradually mineralized osteoid seam; when an osteoid seam was present in the vicinity of the implant surface the osteoblasts invariably had their bone-forming surface away from the implant. Instead the bone formation close to the implant occurred in the same manner as observed in sites of "solitary bone formation" with scattered osteoblasts surrounding themselves with a collagenous matrix which was gradually, and compared to other areas around the implant, rather slowly mineralized. In fact, the interface zone appeared to be the last part of the bone surrounding the implant to be mineralized and a narrow rim, a few micrometres wide, of unmineralized collagenous matrix was not seldom found after 6 and even 12 months [3] after implantation.

Osteoblasts were very rarely observed in direct contact with the implant surface and in this respect the situation *in vivo* is quite different from that created *in vitro* when bone-forming cells are initially attached to the culture plate. Instead, the implant surfaces were covered by flat multinuclear giant cells which excluded other cells such as osteoblasts from direct contact with the surface. The nature of these cells, whether being a foreign-body type of multinuclear giant cell or related to osteoclasts, is at present unknown.

Multinuclear giant cells covering the surface are not seen at titanium implants in other locations than bone (although such cells may occur) indicating that their presence at implants in bone/bone marrow is related to the particular conditions in these tissues rather than to a specific property of the titanium implants. As mentioned, the possibility exists that these cells belong to the osteoclastic lineage and it appears quite possible that osteoclastic progenitor cells are recruited to the implant from the bone marrow surrounding the implant. If so, they may play a role in preparing the implant surface for the ensuing formation of bone in analogy to the events in a resorption lacunae during the reversal phase in which the naked bone surface is cleaned and conditioned for new bone formation by osteoclasts and macrophages [17]. These cells thus deposit a non-collagenous cement line which appears to be important for the initiation of osteoblast activity. As will be further discussed below, cement lines are in some respects morphologically similar to the amorphous layer at the implant surface and it is thus possible that, although we do not have any direct evidence supporting this possibility, that the amorphous layer is produced by the multinuclear giant cells attached to the implant.

In spite of the widespread use of titanium implants in clinical applications for more than 25 years, the accompanying light microscopic [8] and the present ultrastructural study seem to represent the first attempt to study the cellular events close to the surface of titanium implants. At present we do not know if our observations can be generalized also to other non-bonding biomaterials, such as, for instance, other

metals and metal alloys. Neither do we know if the behaviour of cells around biomaterials considered as bone-bonding, as for instance hydroxyapatite, are different, because this has never been examined *in vivo*. A general impression is that bone formation around hydroxyapatite-coated implants is more rapid [18]. One reason for this difference in the healing response might, for instance, be that hydroxyapatite and other bone-bonding biomaterials provide a surface promoting the attachment and activity of osteoblast rather than the attachment of multinuclear giant cells, which we found predominated at the uncoated titanium surface.

In the present study we also studied the ultrastructure of the tissue located close to the implant in the interface zone. The observations in this zone are hampered by technical shortcomings in the tissue preparation with the possibility of induction of artefacts. We have previously introduced electropolishing as a suitable preparation method to study the tissue in the interface zone at the ultrastructural level. By this method the bulk of the metal implant is removed by the electropolishing procedure, leaving the surface oxide in contact with the tissue. This technique has been successfully applied to soft tissue implants [11], but it induces in bone serious artefacts consisting of demineralization of the tissue and impregnation with titanium [12]. Until a modified electropolishing procedure has been worked out, we therefore have to rely on the fracture technique in which the implant and tissue are separated from each other after embedment. We used this technique in previous studies on the interface zone around machined, threaded titanium implants inserted in rabbit tibias for 12 months, and concluded in that study that the fracture plane generally was very close to the implant surface. However, it is obvious that the fracture plane does not always follow the rather variable topography of the machined surface and that it is therefore not possible to make any reliable quantifications of, for instance, the variability of the thickness of the amorphous layer.

The morphology of the interface tissue as found in the present study was characterized by two main features: the presence of an amorphous zone in contact with the implant, and an osmiophilic, lamina limitans-like line, mainly the latter being observed in poorly mineralized bone. Both these structures, clearly distinct from each other, were also found in our previous studies on titanium implants inserted in the rabbit tibia for 12 months [3] and on retrieved clinical implants inserted in human jaw bone up to 12 years [2].

The amorphous layer varied in width but was usually 100–400 nm wide and had a morphology varying from dense, structureless to a less dense, finely granular to fibrillar texture. Collagen fibrils appeared to be attached to the layer, but the major part of the layer did not contain typical collagen fibrils. The varying morphology might be due to variations in fixation quality and we observed that part of the amorphous layer was dense, structureless while another part had a more granular texture. We did not observe any hydroxyapatite crystals in the amorphous layer but we cannot at present exclude that calcium may be present in the layer in any other form, or that hydroxyapatite has been dissolved from it during the preparation procedure. The amorphous layer as observed in the present study, appears to be similar to that described in decalcified tissue surrounding implants of different metals [4] and around implants of polycrystalline alumina and hydroxyapatite [19]. Also, Albrektsson *et al.* has, in a number of studies using plastic plugs covered with films of different metals described a collagen-free zone, in general much narrower (20–40 nm) than found by ourselves [5–7]. Davies *et al.* [13] described the afibrillar layer in contact with the implant surfaces as cement-like, inferring similarities in structure and composition with cement lines which is the first laid-down matrix when calcification starts. In human bone the cement line (or reversal line) contains calcium and phosphorus and significantly more sulphur than the surrounding bone matrix than in collagen [20]. A cement-like calcified extracellular matrix was also found on the substrate surface of different types, including titanium, in bone cell cultures [21, 22]. In culture, calcification of the cement-like substance was initiated by the deposition of globules of hydroxyapatite in relation to osteocyte processes [21, 22]. As mentioned above, we cannot at present exclude the presence of calcium in the amorphous layer but we can definitely state that we did not observe any globular, mineralized accretions in the amorphous layer, or gained the general impression that calcification started in this layer. On the contrary, our general impression was that the tissue close to the implant was the last to be mineralized.

Another distinct feature of the interface tissue was the presence of an osmiophilic lamina limitans-like line adjacent to the amorphous layer. This line was observed when the general level of mineralization was low, indicating that it was mineralized, but its presence obscured by the dense bone mineral. The osmiophilic lamina limitans-like line, separated from the implant surface by the amorphous layer, was in continuity with the osmiophilic line surrounding osteocyte canaliculi (the matrix of which was in continuity with the amorphous layer) and also with osmiophilic lines (which might correspond to resting lines) extending into the bone matrix. Lamina limitans was originally described under various conditions in mouse embryonic bone, studied *in vitro* [13, 14], and has been used to denote the osmiophilic structure located at the junction of mineralized and non-mineralized matrix, for instance at the endosteal surface and lining osteocyte lacunae and canaliculi [15, 16], although it also denotes osmiophilic lines appearing when calcification is only temporarily arrested (resting line) [14]. The osmiophilic character, continuity with the osmiophilic lines bordering osteocyte canaliculi, its dimension and the fact that it probably is mineralized, are factors which warrant the use of the term "lamina limitans-like". However, it is quite obvious that this is only an operational nomenclature (also true for "cement-like" substance, and "amorphous layer") useful until its chemical composition can be described.

Acknowledgements

Mrs Lena Emanuelsson and Gunnel Bokhede are thanked for their skilful technical assistance. The financial support from the King Gustaf V 80-year Fund, The Swedish National Association Against Rheumatism, The Faculties of Medicine and Odontology, University of Gothenburg, The Gothenburg Medical Society, The Swedish National Board for Technical Development (90-00536 and 90-01480), the Trygg-Hansa Research Fund and The Swedish Medical Research Council (B91-16X-09495-01A, K89-24P-07676-01-04) is gratefully acknowledged.

References

1. T. ALBREKTSSON and L. SENNERBY, *Int. J. Prosth.* **3** (1990) 30.
2. L. SENNERBY, L.-E. ERICSON, P. THOMSEN, U. LEKHOLM and P. ÅSTRAND, *Clin. Oral Impl. Res.* **2** (1991) 103.
3. L. SENNERBY, P. THOMSEN and L.-E. ERICSON, *J. Mater. Sci. Mater. Med.* **3** (1992) 267.
4. L. LINDER, K. OBRANT and G. BOIVIN, *Acta Orthop. Scand.* **60** (1989) 135.
5. T. ALBREKTSSON, P.-I. BRÅNEMARK, H.-A. HANSSON, B. IVARSSON and U. JÖNSSON, *Adv. Biomater.* **4** (1982) 167.
6. T. ALBREKTSSON, H.-A. HANSSON and B. IVARSSON, *Biomaterials* **6** (1985) 97.
7. T. ALBREKTSSON and H.-A. HANSSON, *ibid.* **7** (1986) 201.
8. L. SENNERBY, P. THOMSEN and L.-E. ERICSON, *J. Mater. Sci. Mater. Med.* **4** (1993) 240.
9. K. DONATH and G. A. BREUNER, *J. Oral Pathol.* **11** (1982) 318.
10. P. THOMSEN and L.-E. ERICSON, *Biomaterials* **6** (1985) 421.
11. L.-M. BJUSTEN, L. EMANUELSSON, L.-E. ERICSON, P. THOMSEN, J. LAUSMAA, L. MATTSON, U. ROLANDER and B. KASEMO, *ibid.* **11** (1990) 596.
12. L. E. ERICSON, B. R. JOHANSSON, A. ROSENGREN, L. SENNERBY and P. THOMSEN, in "The bone-biomaterial interface", edited by J. Davies (Toronto University Press, 1991) p. 425.
13. J. B. SCHERFT, *J. Ultrastruct. Res.* **38** (1972) 318.
14. *Idem, ibid.* **64** (1978) 173.
15. S. C. LUK, C. NOPAJAROOSZI and G. T. SIMON, *ibid.* **46** (1974) 165.
16. *Idem, ibid.* **46** (1974) 184.
17. P. TRAN VAN, A. VIGNERY and R. BARON, *Cell Tissue Res.* **225** (1982) 283.
18. M. GOTTLANDER and T. ALBREKTSSON, *Int. J. Oral Max. Impl.* **6** (1991) 399.
19. J. DAVIES, N. NAGAI, N. TAKESHITA and D. C. SMITH, in "The bone-biomaterial interface", edited by J. Davies (Toronto University Press, 1991) p. 285.
20. M. B. SCHAFFLER, D. B. BURR and R. G. FREDRIKSSON, *Anat. Rec.* **217** (1987) 223.
21. J. E. DAVIES, B. LOWENBERG and A. SHIGA, *J. Biomed. Mater. Res.* **24** (1990) 1289.
22. J. E. DAVIES, P. OTTENSMEYER, X. SHEN, M. HASHIMOTO and S. A. F. PEEL, in "The bone-biomaterial interface", edited by J. Davies (Toronto University Press, 1991) p. 214.

Received 29 July
and accepted 14 September 1992