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Early tissue response to titanium implants inserted in rabbit cortical bone

Part I *Light microscopic observrvations*

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The tissue response to screw-shaped implants of commercially pure titanium was studied by light microscopy 3–180 days after insertion in the rabbit tibia. The implant site in the tibial metaphysis consisted mainly of cortical bone. Three days after implantation, osteoblasts, producing osteoid, were observed at the endosteal surface and elongated mesenchymal cells were present in the injury area. Some macrophages but rather few other inflammatory cells were identified. Multinuclear giant cells were in direct contact with the implant and formed an almost continuous layer along the surface from the 7th day. The number of giant cells decreased with time and with increased bone–titanium contact. Bone formation was never seen direct on the implant surface but was first observed at day 7 as a woven trabecular bone formed at the endosteal surface and extending towards the implant and as a solitary formation of woven bone close to the implant. The solitary bone matrix served as a base for surface osteoblasts which produced osteoid in a lamellar arrangement. With time the two types of newly formed bone fused and more bone filled the threads and became remodelled by bone remodelling units. Light microscopic morphometry in ground sections demonstrated that the bone/titanium contact and bone area in the threads increased with time up to 6 months after implantation

1. Introduction

In spite of the frequent clinical use of 'osseointegrated' titanium implants, only a few studies have described the early events taking place in the tissue during healing of such implants [1–3]. One reason for this is probably the technical problems related to the production of sections of the implant and surrounding tissue thin enough for a meaningful morphological examination. In 1982, Donath and Breuner [4] presented a grinding method which made it possible to obtain approximately 10 μm thick sections of the bone–implant interface for light microscopy. Using this technique, Kirsch *et al.* [1] followed the bone formation around rod-shaped titanium-plasma coated, sand-blasted and smooth titanium implants inserted in rat femurs over the period 1–56 days. Bone formation started at day 5 and the first bone–metal contact was established at the 7th day for plasma-coated implants, at day 11 for sand-blasted and at the 20th day for smooth implants. In a similar study on titanium-plasma coated and smooth titanium rods inserted in the rat femur Donath *et al.* [2] observed that a large number of multinuclear giant cells formed a layer at the implant surface from day 5. The number of cells was higher at the surface of the rough-surfaced plasma-coated implants compared to the smooth implants. The number of giant cells decreased with time in parallel with the increase of bone–implant contact. Roberts *et al.* [3] studied the dynamics of the bone tissue response to threaded titanium implants in the rabbit femur. Using fluorescent bone labels and microradiography of about 100 μm thick sections, they showed that woven bone started to form close to the implant after 3 days. With time bone filled the threads from the endosteal surface of the cortex and became mature within 6 weeks after implantation.

In this study we have followed the tissue response around threaded titanium implants inserted in the rabbit over the period 3–180 days.

2. Materials and methods

2.1. Animals and anaesthesia

Thirty adult New Zealand white rabbits of both sexes were used in the investigation. General anaesthesia was induced by intramuscular injections of fluanison and fentanyl (Hypnorm®, Jenssen, Brussels, Belgium, 0.7 mg/kg body weight) and diazepam (Stesolid®, Dumex, Copenhagen, Denmark, 1.5 mg/kg body weight). Additional Hypnorm® was given when needed. Local anaesthesia at the site of surgery was given

using 1 ml of lidocain/norepinephrine 2% solution (Astra, Södertälje, Sweden).

2.2. Implants

One hundred and eighty screw-shaped implants (3.75 mm in diameter and 4 mm in length) were manufactured by turning from commercially pure titanium. Prior to insertion the implants were cleaned in ultrasonic baths with butanol and ethanol (10 min in each solution) and then sterilized by autoclaving.

2.3. Surgical procedure

The tibial metaphysis and the distal femoral condyle of the knee joint were chosen as the site of implantation. In this study only results from analysis of the tibial implants are reported. Twelve animals were operated on at two occasions, one leg at each time. In six animals two tibial and one femural implants were installed in the right leg 28 days and in the left leg 7 days prior to sacrifice. In another six animals, implants were installed 14 and 3 days prior to sacrifice, in the right and left leg respectively. Eighteen animals, also used in another study [5], were operated on in both legs at one occasion and were followed for 42, 90 and 180 days with 6 animals in each group. The tibial bone surface was exposed via a skin incision and a careful subperiosteal dissection. Two holes, sequentially enlarged in three steps from 1.8 to 3.0 mm, were drilled in each tibial metaphysis during generous cooling by saline. The holes were pre-tapped and the implants installed level with the cortical bone using a screwdriver. The periostal flap and the skin were closed in separate layers. For femoral implants the knee joint was opened by a lateral incision through skin, fascia and capsule. The patella was dislocated medially and one implant was installed through the cartilage and left below the cartilage using the same technique as described above. The joint capsule, fascia and skin were sutured in separate layers. All animals received antiobiotics (Intencillin®, Leo, Helsingborg, Sweden, 2.250.000 IE/5ml, 0.1 ml/kg body weight) and analgetics (Temgesic®, Reckitt and Coleman, USA, 0.05 mg/kg body weight) postoperatively.

2.4. Tissue processing and microscopy

General anaesthesia was induced as described above and the animals killed and fixed by perfusion with 2.5% glutaraldehyde in 0.05 M cacodylate buffer, pH 7.4. The implants were exposed and removed *en bloc* with the surrounding tissue and the specimens were further fixed by immersion in glutaraldehyde for 24 h and post-fixed in 2% osmium tetroxide for 1 h. In this way 12 tibial implants and 6 intra-articular implants were retrieved 3, 7, 14 and 28 days after insertion. In the animals followed for 42, 90 and 180 days, one tibial and one intraarticular implant not included in the present study were unscrewed using a torque meter at the time of sacrifice, as reported elsewhere (5). The remaining implants were processed as described above. As only the tibial implants were analysed 102 implants were included in the present investigation. The 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 [4]. These specimens were sawed and then ground to a thickness of about 10 μm. The specimens were stained with 1% toulidine blue. The implant-tissue blocks that remained after ground sectioning were either used to prepare thin sections for light microscopy (LM) using a fracture technique [6] or were sputter-coated with gold and examined in a JEOL T-300 scanning electron microscope (SEM) equipped with a back-scatter detector. Using the fracture technique [6], the titanium was separated from the plastic block which then was re-embedded in resin and 1 μm sections were cut for light microscopy. In addition, an electropolishing technique was used on the other part of the divided specimens to prepare sections with an intact interface as described elsewhere [7]. By this procedure the bulk of the implant was removed leaving a thin layer of titanium in contact with the tissue. After re-embedding in plastic resin the specimens were sectioned and stained for LM as for the fractured specimens. Tissue blocks from which thin light microscopic sections were cut, were also used to prepare ultrathin sections as reported in separate study [8]. Light microscopic observations were made using a Leitz Orthoplan microscope.

2.5. Morphometry on ground sections

In ground sections from one implant from each of the six animals at each interval, the bone/metal contact and the area of bone were calculated in all five threads on each side of the implant, using a Leitz Orthoplan microscope equipped with a Microvid computer and connected to an IBM PC (Fig. 1).

The distribution of bone tissue in relation to the implant surface at different time intervals was calculated using a grid with five squares (each square was

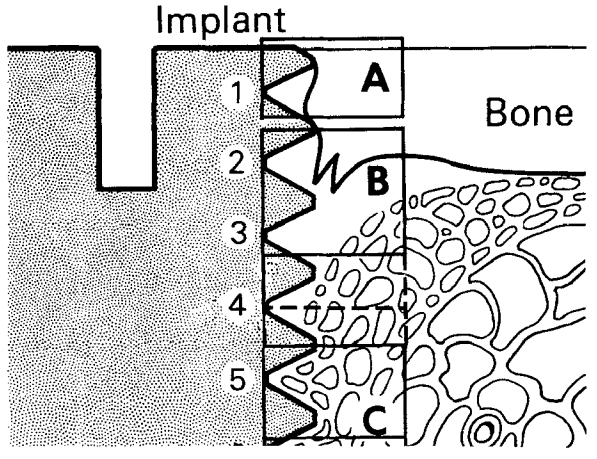


Figure 1 Schematic presentation of the three regions used for description of the tissue response to titanium implants followed from 3 days to 180 days. Region (A), cortical bone including the periosteal surface surrounding the proximal part of the implant; region (B), tissue associated with the endosteal surface of the cortex; region (C), bone marrow.

35 × 35 μm) located 0–175 μm from the surface of the implant. The grid was placed at seven different sites on each side in the most proximal of the subcortical threads (normally the second thread). In each square the amount of bone was estimated to occupy; no (0), 1/3, 1/2, 2/3 or the whole (1) square area. A mean of the 14 readings was calculated for each implant. The distribution was presented as the mean of the means obtained from 6 animals at each time interval (3, 7, 14 and 28 days).

3. Results

3.1. Histology

The implant site consisted of a cortical layer and marrow tissue but no cancellous bone. Consequently, the implants were initially held in position by the first one or two threads in the cortical passage while the major subcortical portion of the implants protruded into the marrow cavity. For description purposes we divided the tissue surrounding the implants in three regions (Fig. 1): (A) cortical bone, including the periosteal surface surrounding the proximal part of the implants (threads 1–2); (B) tissue associated with the endosteal surface (threads 2–3); (C) bone marrow (threads 3–5); this region originally encompassed threads 3–5 but was gradually reduced when bone was growing downwards from the endosteal surface.

3.1.1. Three days

In region (A) (cortical passage) only a patchy bone/metal contact was found (Fig. 2a, b). A mixture of red blood cells and bone fragments filled the space between the cut bone and the implant surface but very few inflammatory cells were present in this location at this (and also later) time (Fig. 2c). Bone fragments were also displaced into the marrow tissue (Fig. 2b). Signs of bone resorption were not observed in any location. In region (B) osteoblasts formed seams at the endosteal surface and in some specimens increased osteoid production in the vinicity of the implant was evident (Fig. 2d). In region (C) red blood cells formed a continuous layer along the entire interface of the implant and very few cells other than red blood cells were in contact with the implant surface (Fig. 2e). Intact bone marrow was rather sharply demarcated from tissue damaged by the surgical procedure and was separated from the red cell layer surface by cells orientated along the cut surface of the bone marrow (Fig. 2f). The majority of the cells had an elongated, fusiform shape and are referred to as mesenchymal cells (Fig. 2e). In addition, macrophages and some multinucleated cells could be identified while polymorphonuclear granulocytes were rare close to the implant.

3.1.2. Seven days

In region (A) neither signs of bone formation at the cut surface of the cortex nor any remodelling of the cortical bone were apparent. The space between the implant and the cortical bone was still occupied by red blood cells, often showing signs of lysis, and by bone fragments (Fig. 3e). In region (B) resorption lacunae with large osteoclasts were observed on the surface of the bone fragments and along the cut surface of the cortex closest to the marrow cavity. The non-mineralized interface tissue in area (B) consisted of a large number of mesenchymal cells with large rounded nuclei, but the exact nature of these cells could not be determined. A few inflammatory cells, mainly macrophages, were identified. Formation of vessels and capillary sprouts were observed. Large, flat multinuclear cells were located at the implant surface, in general forming a continuous layer along the entire unmineralized interface in regions (B) and (C) and also extending into the distal part of the space between the cortex and implant (Fig. 3f, g).

In region (B) two types of bone formation could be distinguished. Firstly, an extensive bone formation was observed at the endosteal surface within 0.5–1 mm from the implant resulting in the formation of a lattice of trabeculated, woven bone which approached the implant (Fig. 3a, b). The trabeculae were covered with typical osteoblast seams with osteoid. Secondly, poorly mineralized (as seen with back-scatter SEM) woven bone appeared closer to the implant surface within threads 2 and 3 (Fig. 3c, d). This newly formed solitary woven bone close to the surface was more intensely stained by toluidine blue than the newly formed trabecular bone. The intensely stained areas were often located in the bottom of the thread and along the implant surface but were only occasionally in close contact with the implant surface indicating that the bone formation did not start at the surface. The solitary woven bone close to the implant could also be distinguished from the trabecular woven bone originating from the endosteal surface by the presence of very large osteoblast/osteocyte lacunae separated by a matrix containing dense granules, representing, as seen in the electron microscope, mineralization foci distributed either discretely or in aggregates. In some specimens occasional points of contacts between the two types of newly formed bone were observed. Osteoblasts were also aligned in rows covering parts of the surface of the solitary bone matrix and these cells produced osteoid seams.

In region (C) red blood cells and large multinuclear cells were covering the implant surface (Fig. 3f, g). Normal marrow tissue with fat cells, haemopoetic cells and sinusoids were present in the periphery but was separated from the implant by elongated mesenchymal cells.

3.1.3. 14 days

In region (A) formation of trabecular bone was for the first time observed at the periosteal surface. An extensive intracortical remodelling of the 'old' bone now occurred within 1 mm of the implant, as indicated by the presence of cutting and filling cones (Fig. 4c). Red blood cells and bone fragments had disappeared from the space between the cut cortical bone surface and the implants (Fig. 4b). In some specimens multinuclear giant cells were present in the part adjacent to the

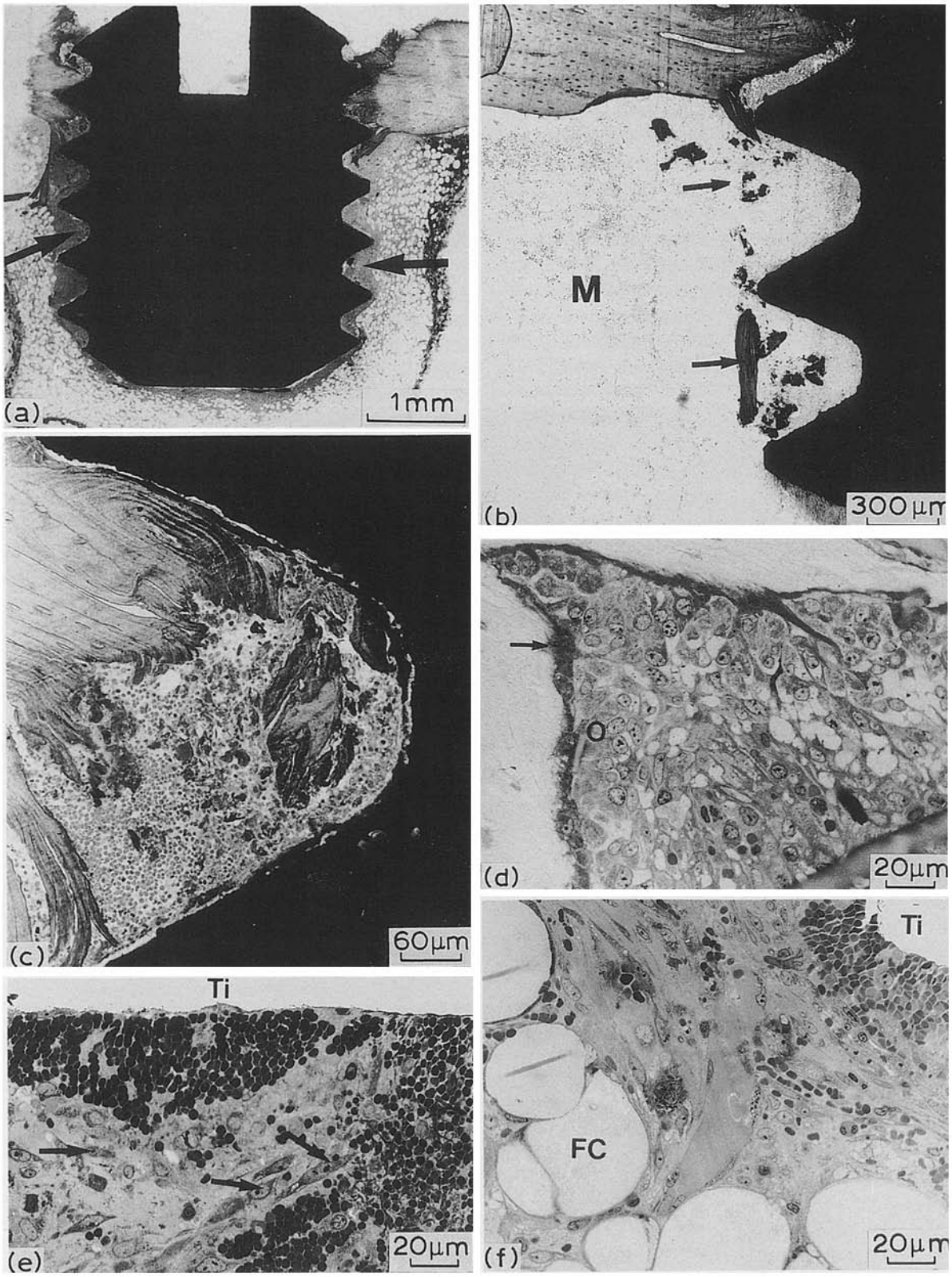
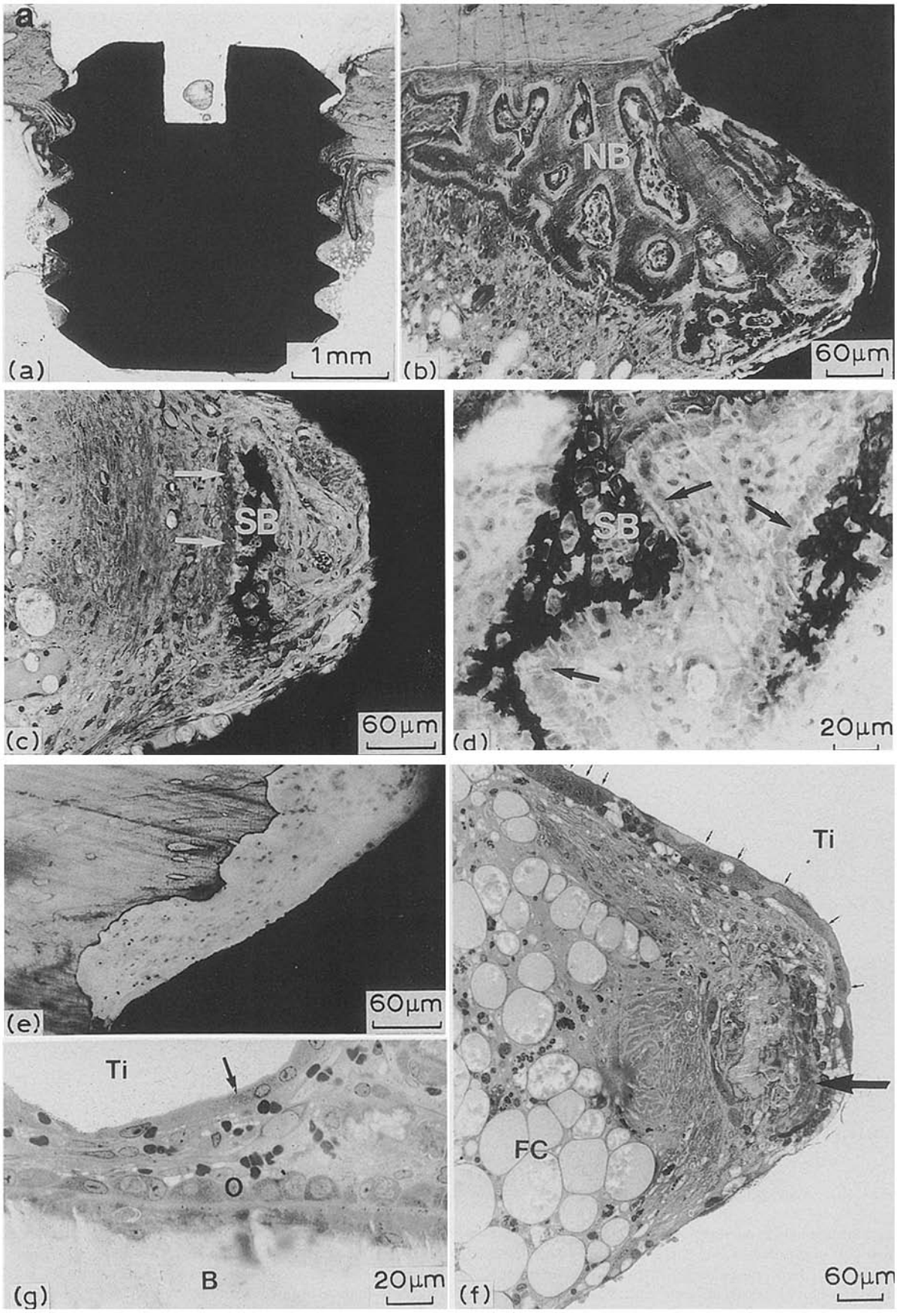


Figure 2 Mount of light micrographs taken 3 days after insertion of titanium implants in the rabbit tibia. (a) Ground section. Overview. Only a minor part of the implant is in contact with bone in the cortical passage. A heamatoma (arrows) is present around the entire implant. (b) Ground section. Bone fragments (arrows) are dislocated in to the bone marrow (M) due to the surgical trauma. (c) Ground section. Detail of (a). The thread area is occupied by old bone, bone fragments and red blood cells. Bone resorption or bone formation cannot be observed. (d) Electropolished section showing part of the endosteal surface of the cortex (up) and a dislocated fragment of the endosteal surface (left). A large number of mesenchymal cells are observed. Osteblasts are forming osteoblast seams with osteoid (arrow). (e) Electropolished section. Detail from the bone marrow. Red blood cells are located close to the implant surface (Ti). Elongated mesenchymal cells (arrows) are present in between two areas of red blood cells. (f) Electropolished section. Normal bone marrow with fat cells (FC) is separated from the implant surface (Ti) by red blood cells located close to the surface. A mixture of different cell types, including mesenchymal cells and red cells, are located between the bone marrow and the red blood cells.

Figure 3 Mount of light micrographs taken 7 days after insertion of titanium implants in the rabbit tibia. (a) Ground section. Overview. (b) Ground section. An extensive bone formation is evident from the endosteal surface. A lattice of newly formed trabecular bone (NB) is approaching the implant from the endosteal surface. Typical osteoblast seams are covering the bone trabeculae. (c) Ground section. The



solitary immature woven bone (SB) within the thread is intensely stained with toluidine blue. Note the large osteocyte lacunae. Osteoblast seams are seen at the surface of the solitary bone formation (arrows). (d) Ground section. Solitary woven bone (SB) within a thread. The bone seems to be a compaction of mineralized aggregates and not the result of a mineralization of an osteoid seam. Note osteoblast seams (arrows). (e) Ground section. The edge of the cut cortical bone in the first thread (region (A)). The scalloped contour suggests active bone resorption. However, no osteoclasts can be found. (f) Electropolished section. Overview of a thread in the bone marrow (region (C)). Multinuclear giant cells (small arrows) are forming a continuous layer at the upper part of the implant surface (Ti). Interface bone formation can be observed (large arrow). Bone marrow with fat cells (FC) is present in the periphery. (g) Electropolished section. Bone (B) formation is evident near the implant surface (Ti). Large multinuclear giant cells (arrows) are present at the implant surface.

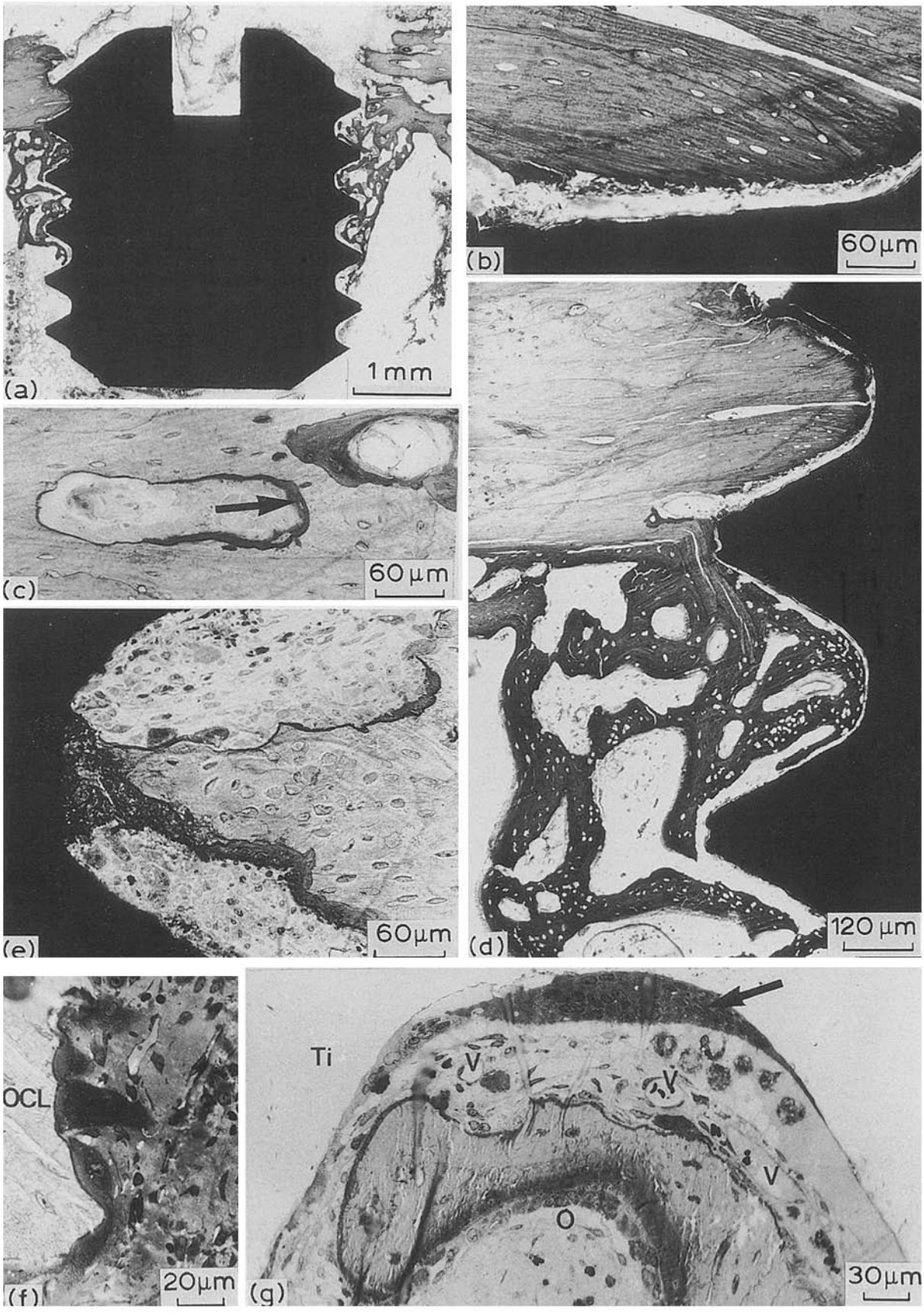


Figure 4 Mount of light micrographs taken 14 days after insertion of titanium implants in the rabbit tibia. (a) Ground section. Overview. Newly formed trabecular woven bone is now reaching the more distal threads. (b) Ground section. Detail of (a). The first thread does not show any bone formation or resorption. Few cells can be found between the bone and the implant. (c) Ground section. Intracortical remodelling is evident by the presence of cutting and filling cones. Bone resorption is seen in one end of the cone and osteoid production (arrow) in the other end. (d) Ground section. Detail of (a). A fusion of trabecular woven bone from the endosteal surface and the solitary woven bone formed close to the implant has probably occurred in the second thread. The trabecular bone has a lamellar appearance and the interface bone large scattered osteocyte lacunae. (e) Ground section. Showing a fusion of trabecular woven bone and the more intensely stained solitary woven bone which seems to be in direct contact with the implant surface. Bone resorption is evident at the upper border of the trabecular bone while osteoid is seen at the lower border. (f) Electropolished specimen. A number of osteoclasts in typical Howships lacuanae are present at the edge

endosteal surface. In other specimens newly formed woven bone was present in the space, especially in the part nearest to the endosteal surface, whereas in other specimens bone resorption predominated at the cut cortical surface (Fig. 4f). In many areas of bone resorption a cellular, fibrous tissue was interpositioned between the implant and the bone. This fibrous tissue was mainly found in the most proximal part of the implant and appeared to be formed by down growth from the periosteal surface.

In region (B) characteristic features after 14 days were an increased amount of newly formed bone filling the threads as well as a compaction of this bone (Fig. 4a, d). It was now not possible to unequivocally distinguish between trabecular bone originating from the endosteal surface and bone formed locally close to the implant. The trabecular type predominated and had apparently assimilated the locally formed bone as well as fragments of old bone (Fig. 4e). Trabeculae reached the implant surface where they appeared to follow the contour of the surface. Trabecular bone now also appeared in more distal threads (mainly threads 3–4). As also observed at 3 and 7 days, flat multinuclear cells formed an almost continuous layer along the implant surface, the exception being those areas in which mineralized bone was reaching the implant surface (Fig. 4g).

Osteoblasts forming typical seams covered most parts of the trabecular bone surface but could not be identified at the implant surface. Most of the tissue located close to the surface, but separated from it by multinuclear giant cells, was not mineralized and consisted of numerous, densely packed mesenchymal cells and numerous vessels.

In region (C), normal marrow tissue with haemopoetic cells, sinusoids and fat cells was now located close to the implant surface and red blood cells had largely disappeared. However, multinuclear giant cells were still present in direct contact with the implant surface, although their number was decreased. In addition a narrow zone of loose connective tissue separated the implant from the marrow tissue.

3.1.4. 28 days

In region (A) typical findings at this time interval was, as at day 14, extensive bone formation at the periosteal surface and remodelling of the cortical bone (Fig. 5a, b). In most specimens trabeculated bone was growing over the entire upper surface of the implant. The amount of fibrous tissue in contact with the first thread was reduced and replaced by bone and consequently, due to replacement of fibrous tissue and remodelling, in the space between the implant and surrounding bone seen at previous intervals were now to a large extent filled by dense woven bone in contact with the implant.

In region (B) bone filled a larger part of the threads (Fig. 5d). The bone was still immature with large osteocyte lacunae. Formation of osteons and lammellation of the bone trabeculae was observed and the area of direct contact between bone and implant had increased. The number of multinuclear giant cells at the implant surface was correspondingly decreased but they were still present in areas containing mesenchymal cells located close to the implant surface (Fig. 5c).

The repair of the marrow tissue was almost completed. Multinuclear giant cells were still observed along the implant surface, although in reduced number (Fig. 5e, f).

3.1.5. 42 days

In region (A) remodelling of the cortical bone was still observed and the cutting and filling cones also involved the newly formed bone close to the implant (Fig. 6a). In region (B) the newly formed bone had now a dense rather than a trabecular appearance. However, it was still possible to distinguish between the original bone and the newly formed due to the interupted lamella. Multinuclear giant cells were rarely observed in threads with bone. In region (C), normal marrow with blood cells containing sinusoids, fat cells and reticular connective tissue occupied a great part of the threads. Most often a fibrous capsule had formed between the normal marrow and the implant surface. Multinuclear giant cells were the most common cell type seen in direct contact with the implant surface.

3.1.6. 90 days and 180 days

The morphology was similar at these two times (Fig. 6b, c). Remodelling of the cortical bone was not apparent. The bone close to the implant had the same mature appearance with about the same degree of mineralization as the original bone. In some specimens bone was seen in all threads. The bone in the distal threads often appeared as rounded islets situated in the bottom of the threads and were probably cross-sections of extensions of bone trabeculae coming from above or possibly connected to the opposite endosteal surface. In region (C) multinuclear giant cells and a fibrous capsule were commonly observed at the implant surface also at these observation intervals (Fig. 6d).

3.2. Morphometry

The bone area and bone contact measurements showed an increase with time (Fig. 7a, b). The distribution calculations showed that the bone was formed at a distance from the implant surface rather than at the surface (Fig. 8).

of the cut cortical bone facing the implant. (g) Electropolished specimen. Region (B). A large multinucleated giant cell (arrow) is located at the surface of the implant (Ti). A bone trabeculae is located in the thread. Bone resorption is seen at the part facing the implant while bone formation and osteablast seams (O) are present at the part facing the bone marrow. Newly formed vessels (V) are seen in the unmineralized interface tissue.

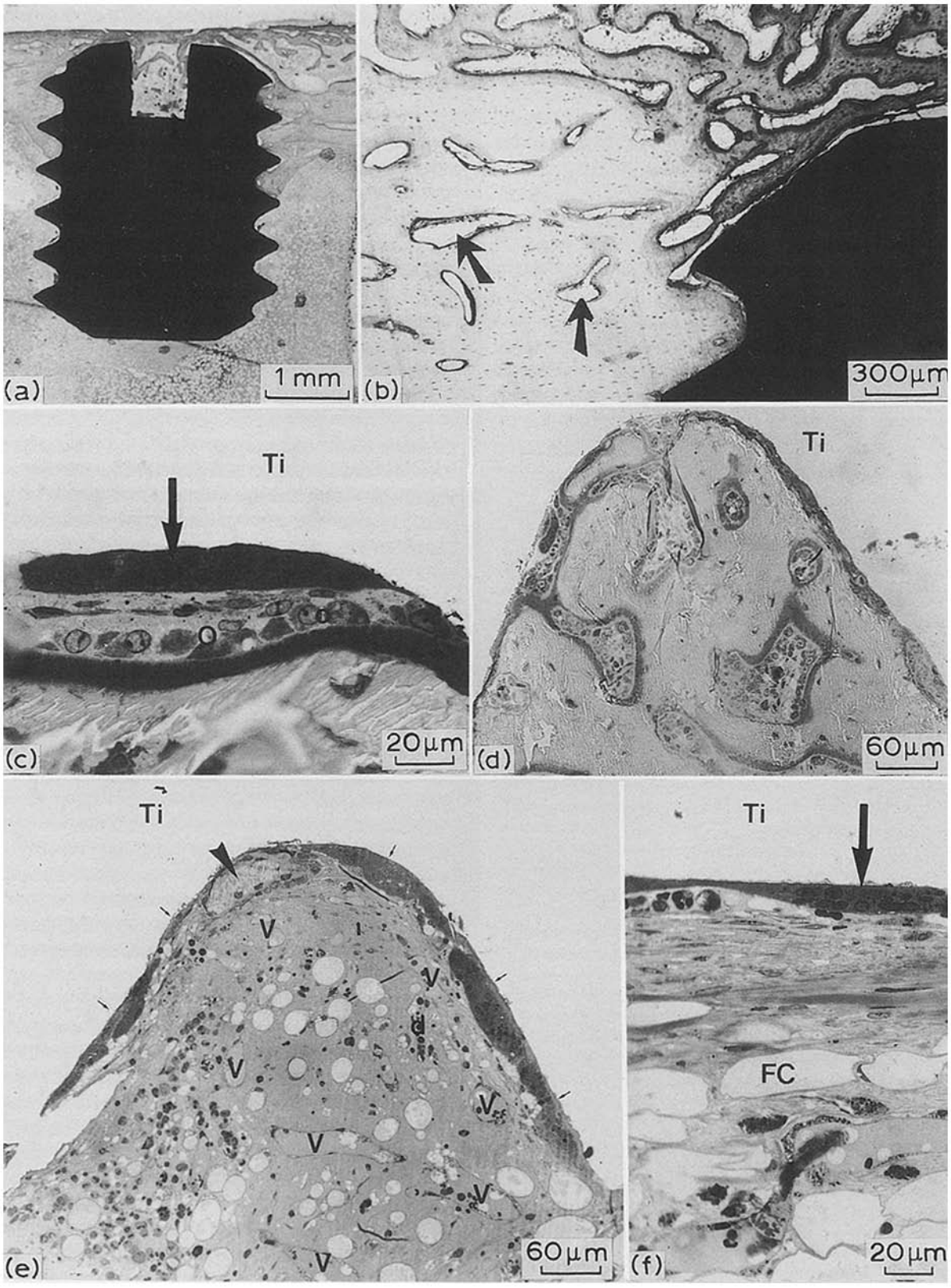


Figure 5 Mount of light micrographs taken 28 days after insertion of titanium implants in the rabbit tibia. (a) Ground section. Overview. Periosteal bone formation which is covering the upper implant surface is seen. (b) Ground section. The periosteal bone formation has a similar appearance as endosteal bone formation observed 14 days after insertion. Intracortical bone remodelling is evident (arrows). (c) Electropolished section. Region (B). Large multinuclear giant cell (arrow) is present at the implant surface (Ti). The bone is coming close to the implant surface to the right. (d) Electropolished section. Overview of a thread showing that the major part of the thread is occupied by bone. Active bone formation is evident by the typical osteoblast seams at the surface of the bone. The unmineralized zone in the interface where bone is reaching close to the surface of the implant (Ti) is, at least partially, an artifact induced by the electropolishing procedure. (e) Electropolished section. Overview of a thread located in the marrow. Multinuclear giant cells (arrows) are following the implant surface contour (Ti). A small area of bone is seen to the left in the bottom of the thread. This bone is not intensely stained by toluidine blue and is probably a cross-section of a bone trabeculae coming from above or beneath. Numerous of vessels (V) are located in the bone marrow. (f) Detail of a typical bone marrow/implant interface. A thin fibrous capsule (F) with elongated fibroblasts and some macrophages is intervening a normal bone marrow, with fat cells (FC) and haemopoetic cells, and a multinuclear giant cell (arrow) which is located at the implant surface (Ti).

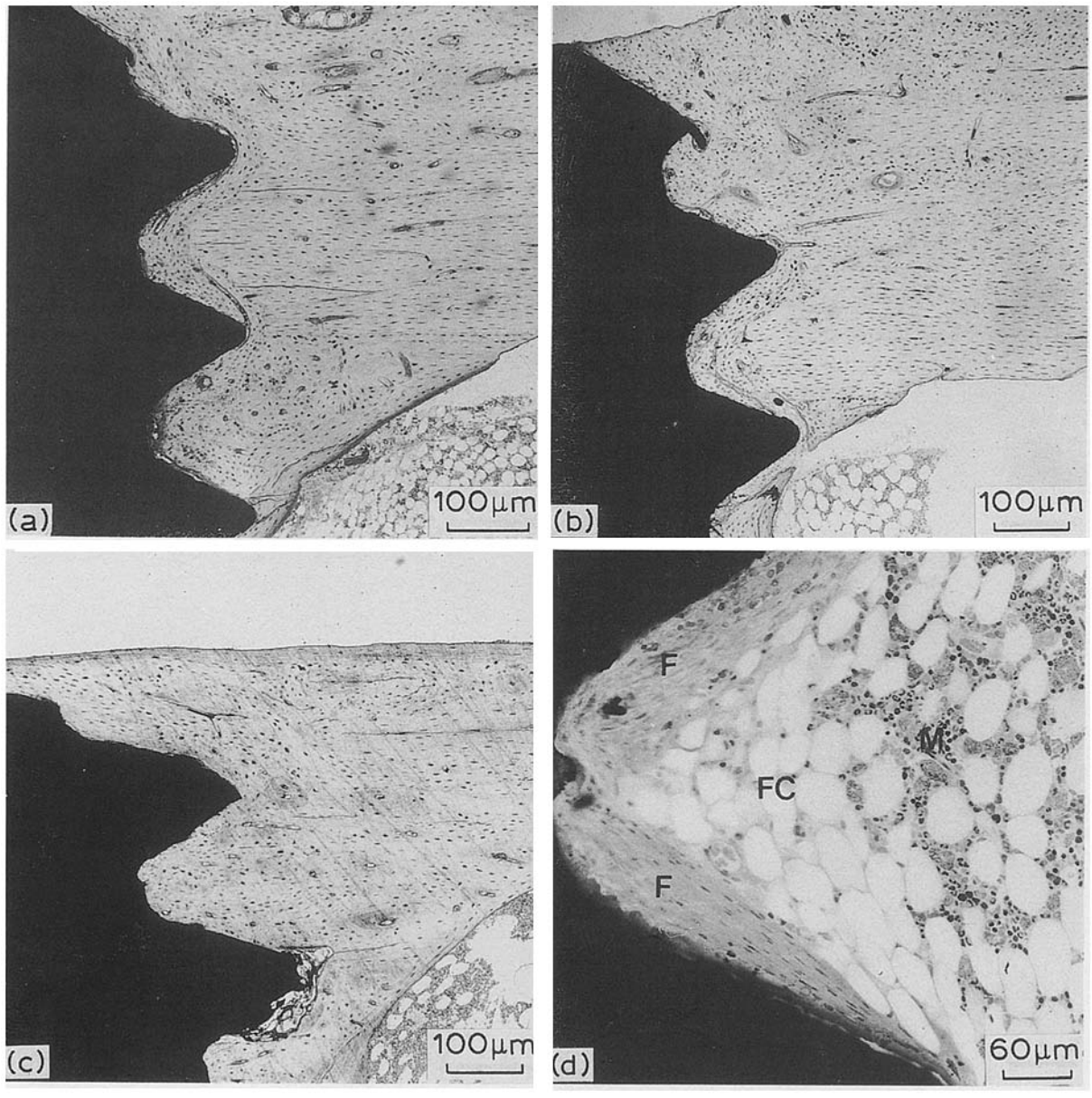


Figure 6 Mount of light micrographs from ground sections. (a) 42 days after insertion. A large portion of the implant surface is in direct contact with mineralized bone. In this specimen there are only minor signs of remodelling. (b) 90 days after insertion. The bone remodelling seems to be completed. (c) 180 days after insertion. (d) Showing the bone marrow/implant interface 90 days after insertion. A fibrous capsule is present in the interface. Fat cells (FC) and a normal marrow (M) with haemopoetic cells is observed.

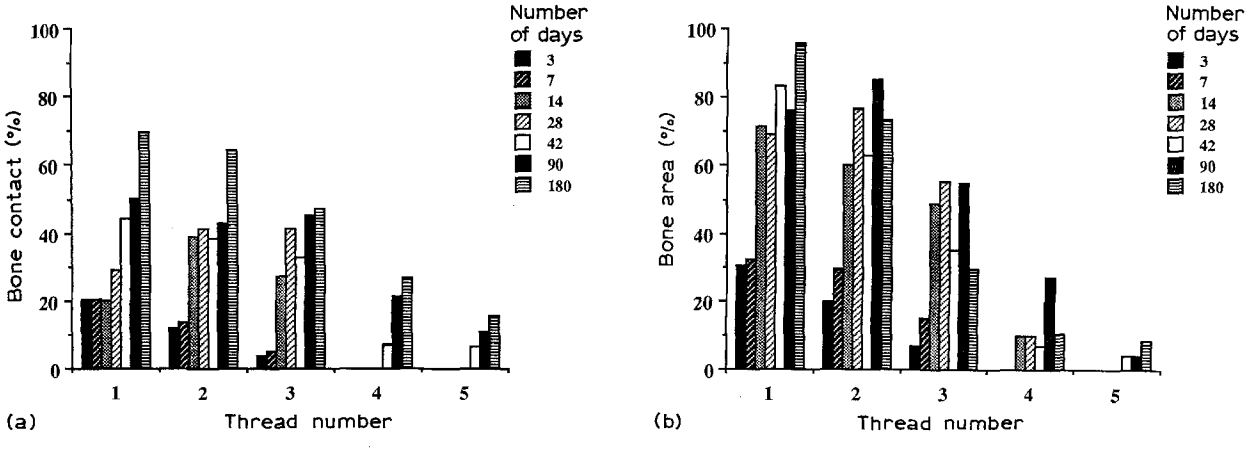


Figure 7 Mineralized bone/implant contact (a) and bone surface area (b) measurements in one thread of titanium implants inserted in rabbit bone for 3–180 days (see text).

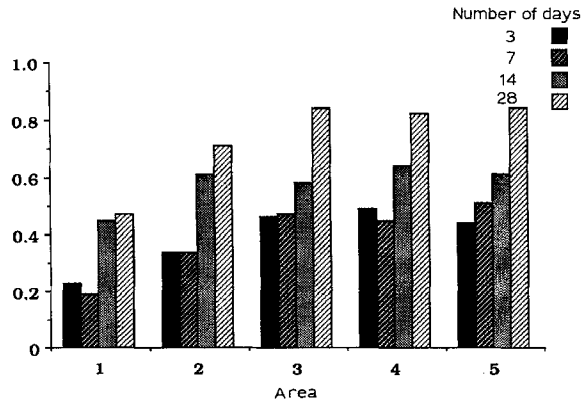


Figure 8 Distribution of mineralized bone in one thread of titanium implants inserted in rabbit bone for 3–28 days implant (see text).

4. Discussion

The insertion of a titanium implant into the rabbit tibia induced an early cellular response. This was seen as a migration of mesenchymal cells from the bone marrow into the defect and formation of osteoblast seams at the endosteal surface of the cortex at the 3rd day. However, although osteoid production was seen, no newly mineralized bone could be detected at this time, which is in contrast to the observations made by Roberts *et al.* [4]. Using tetracyclin derivates as markers for bone formation, they described an extensive formation of bone 3 days after insertion of threaded acid-etched titanium implants into the femur of rabbits. We observed the similar morphological picture first at the 7th day after implant surgery. One reason for the differences may be due to a slow release of the intramuscularly injected tetracyclin, i.e. the tetracyclin may be labelled by the bone not only at day 3 but also a few days after. Another reason might be that the surface properties and geometry of our implants and those used by Roberts *et al.* are differently influencing the tissue response. In our experiments the threads located at the level of the endosteal surface were rapidly filled with immature woven bone from the 7th day and this bone was then remodelled to a lamellar bone, which was completed 6 weeks to 3 months after insertion. A similar kinetics of bone formation and remodelling was also noted by Roberts *et al.* [3], who concluded that the bone around the implants was mature after 6 weeks as indicated by histology and biomechanical testing. This is in agreement with our previous findings using the same type of implants inserted in the same location as in the present study [5]. In the study mentioned, we found that the torque required to unscrew the implants was the same 6 weeks, 3 months and 6 months after insertion, indicating a roughly similar degree of maturation of the bone around the implants.

A striking finding from day 7 was the presence of multinucleated giant cells at the implant surface. These cells formed an almost continuous layer along the entire implant surface and decreased in number with time in parallel with an increased bone–metal contact. Our findings are in line with Donath *et al.* [2] and Linder *et al.* [9]. Donath *et al.* [3] found multinuclear giant cells at the surface of titanium rods inserted in the distal femur of rats after 5 days. Interestingly, they observed a larger number of these cells at the surface of rough titanium implants compared to smooth implants. Linder *et al.* [9] observed numerous multinuclear giant cells on the surface of titanium screws inserted in the tibia of patients. At present we do not know the significance of the presence of giant cells on the surface of the implants. Multinuclear giant cells on the surface is not an obligatory finding for titanium implants. For instance, machined titanium implants inserted in the rat abdominal wall are in contact with macrophages but only rarely with multinuclear giant cells [10]. This indicates that the presence of multinuclear giant cells at the titanium implants in bone, as found in this and previous studies, is a phenomenon related to properties of the host tissue (bone, bone marrow). A possibility is that these cells are related to osteoclasts. They may also be foreign body type giant cells rather than osteoclasts, as suggested by Donath *et al.* [2]. However, it is not possible to safely distinguish between these two cell types with morphological techniques and further studies, for instance immunocytochemical techniques, are required. Giant cells disappeared gradually when the interface tissue developed into bone and at later time intervals they were only present at those parts of the implant which were in contact with bone marrow. It has been suggested that osteoclasts and macrophages at bone surfaces may have a priming function for the deposition of osteoid by osteoblasts [11]. A possibility is that the giant cells on the implant surface may have a similar function, although our morphological observations indicate that formation of bone at the implant surface proceed by a mechanism different from that observed at bone surfaces.

Another interesting finding was the formation of bone in the threads first seen 7 days after implantation. This bone had other morphological characteristics than trabecular bone originating from the endosteal surface and was characterized by large osteocyte lacunae and by a more intense staining by toluidine blue, which probably reflected differences in the ground substance [12]. The morphological appearance of the two bone types are very similar to those described by Shapiro [13] who studied the healing of bone defects in the tibia and femur of rabbits. A woven trabecular bone was formed at the endosteal surface in the periphery of the defects in parallel with a solitary bone formation in the centre of the defect. As also found in the present study, the solitary bone matrix served as a base for surface osteoblasts which produced osteoid seams. The solitary bone formation also has morphological similarities to callus during fracture healing and provides a provisional scaffold until it is replaced by lamellar bone [12]. However, no cartilaginous callus was observed by us or Shapiro [3].

The major part of the new bone formed around the titanium implants was bone formed from the endosteal surface growing towards and gradually enclosing the implant. The bone formation around the proximal part of the implant located in the cortex, proceeded by a different mechanism. In this location

formation of new bone filling the gap between the implant and bone surface was related to remodelling of the surrounding cortical bone. It is notable that the kinetics and mechanisms of this bone formation was different from that seen around the part of the implant protruding into the marrow cavity. In the cortical part signs of new bone formation appeared after 14 days while in the marrow bone formation was intense already after 7 days. These differences in kinetics and mechanism of bone formation should be borne in mind, for instance in quantitative studies of bone apposition to implants. In the present study the implantation site chosen consisted almost entirely of cortical bone. It appears quite possible that bone formation around implants in cancellous bone is different from that observed in this study around the portion of the implants located in cortical bone and in the marrow cavity. These considerations also suggest that quantitative data, for instance of bone apposition, for implants in different locations cannot be directly compared and that the mechanism and kinetics of new bone formation may be profoundly influenced by the organization of the bone at the implant site.

We conclude that the insertion of a threaded titanium implant in the rabbit cortical bone initiated a rapid and organized bone formation. Bone formation was not observed directly on the implant surface; the increased bone/titanium contact with time was rather a result of condensation of woven trabecular bone, which grew towards the implant's surface. The bone around the implant showed a mature morphology after 6 weeks.

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