Osseointegration of titanium implants in the tibia

Electron microscopy of biopsies from 4 patients

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We studied the ultrastructure of bone tissue around implants of pure titanium inserted into the tibia in 4 patients with arthrosis or rheumatoid arthritis. Three main appearances of the interface were noted. First, a close contact between titanium and calcified bone with living osteocytes inside the newly-formed bone was observed in all samples. Secondly, a close contact was also seen between the implant and osteoid, the newly formed collagenous matrix being either uncalcified or calcifying. Thirdly, a loose extracellular matrix with fibrillar and nonfibrillar materials was sometimes observed between bone mineral and implant. There was no inflammatory reaction at the interface.

We concluded that the titanium implants were osseointegrated, but the calcification of the bone tissue was not complete even after 20 months. However, mineralization of osteoid and living bone cells revealed the presence of an active tissue.

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With a light microscope, a direct bone-implant contact has been observed in a variety of materials used in orthopedic surgery, such as TiAlV alloy (Lintner et al. 1986), CrCo alloy (Engh et al. 1987), hydroxyapatite-coated implants (Furlong and Osborn 1991, Hardy et al. 1991), and pure titanium (Linder et al. 1988). Methods are now available for transmission electron microscopy (TEM) of the interface between tissue and metallic implants (Linder 1992). However, of the TEM studies performed so far, the vast majority have been done on decalcified bone, and there is today a paucity of knowledge about the distribution of mineral closest to the implant. A TEM study of implants of pure titanium osseointegrated in the jaw has shown mineralization within 400 nm of the implant surface (Sennerby et al. 1991).

We describe the ultrastructure of the bone tissue around osseointegrated implants of pure titanium inserted into the tibia in patients with arthrosis or rheumatoid arthritis.

Patients and methods

In 4 volunteer patients, 2 with arthrosis (1 man and 1 woman) and 2 women with rheumatoid arthritis, screws of pure titanium, 10 mm in length and 3.5 mm in diameter, were inserted into the tibia within 10 mm of the knee joint surface. The implantation technique employed has been described in detail elsewhere (Linder et al. 1988). The implantation time for the arthrosis cases was 11 and 20 months and for the rheumatoid arthritis cases 7 and 14 months.

The implants were retrieved with a surrounding sleeve of bone and immediately fixed in 3% glutaraldehyde in 0.1M sodium cacodylate buffer at 4 °C. Fixation was for about 1 week. The undecalcified specimens were dehydrated in graded ethanols, defatted in xylol and finally embedded in methylmethacrylate. After embedding, the bone tissue was separated from the implant by Linder's method (1985). Then all specimens were re-embedded in Epon B to protect and bury the interface in resin and were finally glued to plastic holders to facilitate further sectioning.

In a first step, 1-µm-thick sections were cut and stained with methylene blue in order to find a level where all the threads of the screw were present and where indisputable bone-implant contacts could be demonstrated. The areas chosen were trimmed down in size to pyramids of approximately 1 × 1 mm. Ultrathin sections were then cut with an Ultracut E (Leica), equipped with a diamond knife. These sections were contrasted with uranyl acetate and lead citrate. Undecalcified sections were finally examined

in a Jeol 1200 EX electron microscope (Centre de Microscopie Electronique et de Pathologie Ultrastructurale, Faculté A. Carrel, Lyon, France).

Results

Light microscopy

The structure of bone tissue was well preserved inside the threads of the screw. When a space was observed between the second embedding medium and bone tissue, it was considered to be an artefact due to shrinkage caused by the double embedding procedure for histology.

In each sample, bone-implant contacts were evident. The amount of bone tissue varied from one thread to another, and within the same thread from one place to another (Figure 1). This bone tissue showed different histologic aspects. Calcified bone could be observed in contact with the titanium interface. Osteoid tissue, i.e., the non-mineralized bone matrix, could also be present between adjacent bone and titanium. Finally, osteoblasts and giant multinucleated cells were observed under the bone, in close contact with the titanium surface (Figure 2). Inside certain threads, Haversian canals were present.

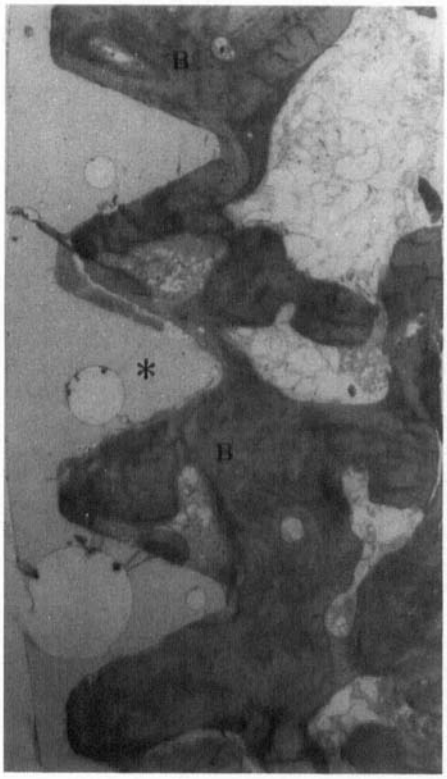

Figure 1. Low-power magnification showing the general aspect of bone-tissue (B) close to the embedding medium (*) replacing the titanium implant, ×21.

Electron microscopy

The observations confirmed the light microscope findings, and 3 main appearances of the interface structure could be distinguished:

a) A close contact between calcified bone tissue and titanium was observed in all the samples studied (Figure 3), and inside the newly-formed bone, osteocytes with a well preserved structure were present.

b) A close contact was seen between the biomaterial and osteoid, the newly formed collagenous matrix. This osteoid seam had a variable thickness, from 3500 to 9000 nm, and contained Type I collagen fibrils showing the characteristic periodic striation. The fibrils could be regularly arranged in bundles with the same orientation or irregularly disposed in the space separating bone and implant. This matrix was either uncalcified (Figure 3) or calcifying (Figure 4). In the area of calcification, electron-dense needle-like crystals were packed in nodules of various sizes (Figure 4).

c) A loose extracellular matrix with fibrillar and non-fibrillar materials was also sometimes observed between bone mineral and implant (Figure 5).

The bone cell structure was relatively well preserved in all samples. Within the matrix, referred to as the Type b interface, cells in contact with bone or

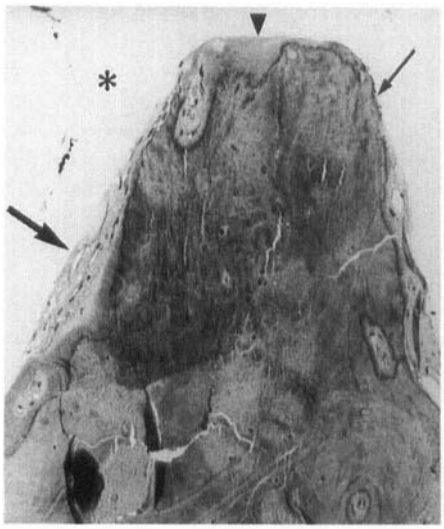

Figure 2. Semi-thin section illustrating the different aspects of the interface between embedding medium (*) replacing the titanium implant and bone tissue (B). Fully calcified bone (small arrow), osteoid tissue (arrow head) or bone cells (large arrow) can be seen, ×64.

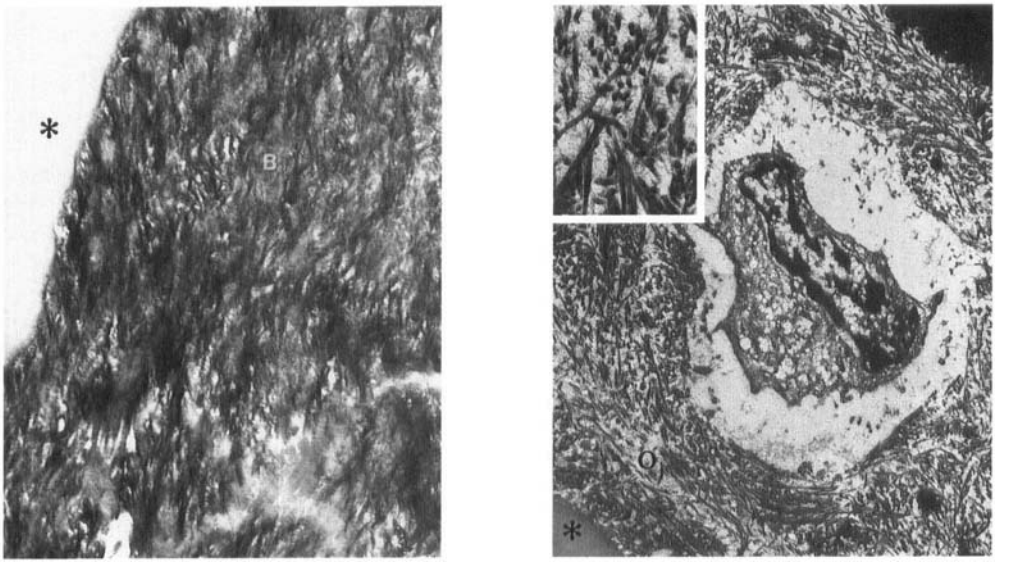

Figure 3. Electron micrographs showing either a fully calcified bone matrix (B) or osteoid tissue (O) in direct contact with the embedding medium (*) replacing the titanium implant, ×17100 and ×6000, respectively. Inset shows detail of osteoid tissue, ×16000.

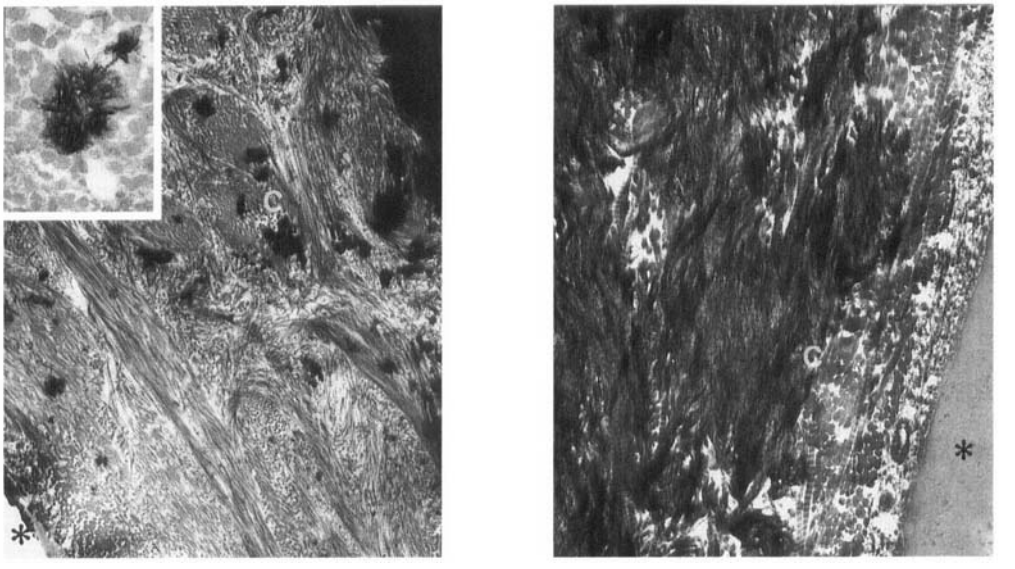

Figure 4. Two different aspects of the calcifying matrix (C) can be observed in contact with the embedding medium (*) replacing the titanium implant, ×16000 and ×7000, respectively. Inset shows that mineral substance is constituted of electron-dense needle-like crystals, ×27700.

osteoid could be identified as osteoblasts or lining cells according to their shape and structure. Osteoblast-precursors were also present in the newly formed extracellular matrix, either regularly disposed or scattered throughout the collagenic matrix. Osteocytes, inside bone and osteoid (Figure 6), appeared active with a well-developed endoplasmic reticulum. Multinucleated giant cells in contact with the surface of the biomaterial were mainly observed in the matrix of the Type c interface (Figure 6). Sometimes they were observed in contact with the calcified material. These cells were very often poorly preserved. Their nuclei were numerous, in some cases associated with a Golgi apparatus. The cyto-

Figure 5. In places, a loose extracellular matrix constituted of fibrillar and non-fibrillar materials is located between the calcifying bone (C) and the embedding medium (*) replacing the titanium implant, ×8200.

plasm contained numerous small and dark mitochondria and prominent vacuoles. Sometimes, a clear ruffled border was visible. Thus, these cells could be identified as osteoclasts.

Discussion

The integrity of the interface depends on the quality of the embedding procedure described. The method is now well established, since Auger electron microscopy (AES) as well as scanning electron microscopy (SEM) have shown no contamination of the interface with titanium (Lausmaa and Linder 1988). However, it is always possible that a thin layer of tissue (< 10 nm) could remain on the implant.

The material used in this study was totally embedded without prior decalcification. This technique is unique in allowing demonstration of a contact between the implant and a more or less mineralized matrix. In a previous study (Linder et al. 1989), osseointegration of metallic implants was demonstrated in decalcified samples, but in these samples it was impossible to distinguish osteoid from poorly mineralized matrix. The decalcification of the bone matrix performed by acid solutions affects the fine structure of the tissue, especially the cell structure. Decalcification could also be responsible for swelling or material dissolution, affecting the interpretation of the reaction to an implanted material.

Three important observations were made: 1) there was no inflammatory reaction at the interface, 2) the bone within the screw threads had the ultrastructure of normal bone tissue (Boivin et al. 1990) and the newly formed bone was living, since osteocytes were clearly present in periosteocytic lacunae, 3) the TEM

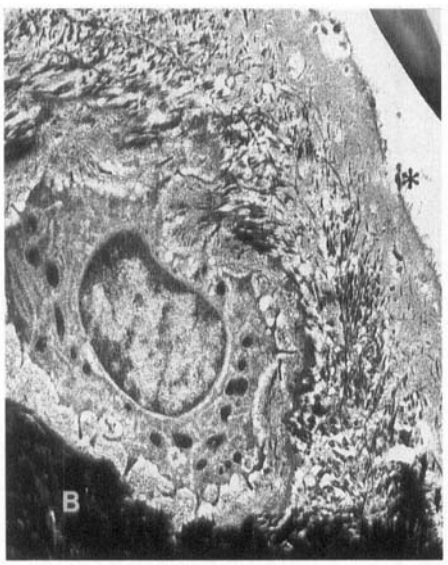

Figure 6. Osteoid osteocyte and multinucleated giant cells (M in right figure) can be observed along the interface between the embedding medium (*) replacing the titanium implant and bone tissue (B), ×8700 and ×1600, respectively.

appearance of the interface was the same in patients with rheumatoid arthritis and arthrosis.

The detailed ultrastructural findings revealed that calcified bone could be found in direct contact with the titanium surface. However, an unmineralized zone, 3–9 μm in thickness, could also be seen within the same sections and the interface therefore varied somewhat. Mineral substance, calcifying organic matrix and osteoid tissue were the three main appearances of the bone/titanium interface. They were considered as three steps in the same process leading from production of organic matrix to its complete calcification.

When mineral substance was not seen in contact with the epoxy resin, empty spaces or a very loose tissue have sometimes been observed. Empty spaces might be explained by a pulling out of the interface tissue with the implant and could be artefacts, but the loose extracellular matrix is rather a sign of an incomplete mineralization up to the implant. As in our previous study on decalcified material (Linder et al. 1989), but in contrast to Sennerby et al. (1991), we did not observe a 100–400 nm layer of amorphous material at the interface level.

The decreasing mineralization within the 500 nm closest to the metal, as described in earlier studies in the rabbit (Linder et al. 1983, Albrektsson 1984), was not typical in these cases. However, the heterogeneity observed in close contact with titanium implants in the human jaw (Sennerby et al. 1991) and in rabbit tibia (Linder et al. 1989, Sennerby et al. 1992) was confirmed in our present study. These authors reported that parts of the implant surfaces were in contact with mineralized bone, while other parts were in contact with unmineralized bone tissue and bone marrow.

Our findings provide a baseline for future comparative TEM studies on the bone/titanium interface. Load-bearing implants in the jaw have been used for decades without signs of loosening (Branemark et al. 1985). It is therefore quite possible that the variability of the zone closest to the implant surface has little bearing on the long-term function of the implant (Linder 1992).

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