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Titanium implants in rabbit knee joints

The subchondral bone, cartilage, and synovial tissue adjacent to nonalloyed titanium screws were morphologically evaluated 2, 4, and 6 weeks after insertion in rabbit knee joints. The titanium screws were in direct contact with the surrounding bone. Two weeks postoperatively, bone resorption, as well as formation of new bone on the titanium surface, was evident. The observed inflammatory process was transient, and the amount of bone within the screw threads increased progressively with time. The articular cartilage regenerated and covered the titanium screws. Synovial tissue also regenerated and covered the screws when inserted into a noncartilaginous area of the joint. These findings, together with recent findings in soft tissues, indicate that titanium provides an inert surface for the proliferation of differentiating tissues in vivo. These data, supported by the observation that no inflammation was present in the synovial tissue, suggest that nonalloyed titanium can be used safely intraarticularly.

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Introduction

Several factors, such as the type of metal or metal alloy, the surgical technique, and the postoperative care, seem to influence the anchorage of an implant in bone tissue (Williams 1981, Albrektsson 1985). Brånemark et al. (1969) showed that a direct contact could be established between bone and screws of nonalloyed titanium (osseointegration) when an atraumatic surgical technique and a postoperative period without loading were used. Using this method for the treatment of edentulousness, 89 per cent (upper jaws) and 100 per cent (lower jaws) continuous bridge stability has been achieved in long-term studies (Adell et al. 1981). The observation that screws of pure titanium heal in the jaw bones without an intervening soft-tissue layer and are capable of carrying high loads indicates that titanium might be used also for reconstructive joint surgery.

We are studying the interaction between implanted titanium screws and subchondral bone, cartilage, and synovial tissue in normal and arthritic knee joints of rabbit. We report here that nonalloyed titanium implants inserted in normal joints heal with osseointegration and that cartilage and synovial tissue regenerate and cover the fixtures.

Materials and methods

Adult white rabbits of either sex, weighing 3–3.5 kg and fed on standard chow and water ad libitum, were used. The animals were anesthetized with fluanizone (Hypnorm Vet, Leo, Helsingborg, Sweden) 0.7 ml/kg b.wt. i.m.

The implants were machined titanium screws (>99% pure titanium; Ø3 mm, length 4 mm). They were ultrasonically cleaned in trichloroethylene and ethanol, followed by sterilization by autoclaving before insertion.

Knee-joint surgery. The hind-legs were shaved and washed with 0.1 per cent Jodopax® (Ferrosan, Malmö, Sweden) in ethanol. Surgery was performed under sterile conditions and with a careful technique to minimize trauma. After a lateral parapatellar skin incision, the fascia layers were opened and the lateral part of the joint capsule exposed. In a dissecting microscope, the capsule was opened with a pair of fine scissors and the joint cavity exposed. The knee joint was stretched and the patella was moved medially to expose the joint cavity. The titanium screws were inserted in 30 knee joints (Table 1), either through the cartilage in the patellar femoral groove or through the synovial tissue on the medial femoral condyle.

A dental drill was used to make a hole through the cartilage and bone into the bone marrow. The hole was drilled under generous cold saline irrigation. The screws

Table 1. Titanium implants examined

Observation period (weeks)	No. of rabbits	No. of joints	No. of intraarticular screws through	
			cartilage	synovial tissue
2	6	12	12	
4	6	11	6	5
6	4	7	7	
Total	16	30	25	5

were inserted with their upper surface at the bone-cartilage junction. When screws were inserted through the noncartilaginous areas, the synovial tissue on the medial femoral condyle was removed using a trephine. After insertion, the joint cavity was washed with saline and the joint capsule and skin were closed with sutures. The rabbits were returned to their cages and allowed free motion.

Two, 4, and 6 weeks after surgery, the rabbits were killed with an overdose of pentobarbital i.v., and the knee joints were opened and examined. The gross appearance of the synovial tissue and cartilage was documented with photographs taken in a dissecting microscope.

Specimen processing. The titanium screw with a collar of surrounding tissue (about 1 mm) was excised during irrigation of cold saline using a dental drill with an attached diamond saw blade. The specimen was fixed in 3 per cent glutaraldehyde in 0.1 M sodium cacodylate (pH 7.4). The titanium screw and surrounding tissue was then divided along the axis of the screw with a dental saw, and the tissue was demineralized for 10 days in 10 per cent EDTA. The divided screw could then be carefully removed from the tissue. Thereafter, the tissue was fixed in 2 per cent osmium tetroxide, dehydrated in a graded series of alcohol, and embedded in epoxy resin (Agar Resin 100, Agar Aids, Stansted, Essex, England). One micron-thick sections for light microscopy were stained with 0.5 per cent Azur II and 0.5 per cent methylene blue in 1 per cent disodium tetraborate.

Synovial tissue biopsies were taken from all the joints. They were fixed in 3 per cent glutaraldehyde in 0.1 M sodium cacodylate, postfixed in 2 per cent osmium tetroxide, dehydrated, and embedded as described above.

Results

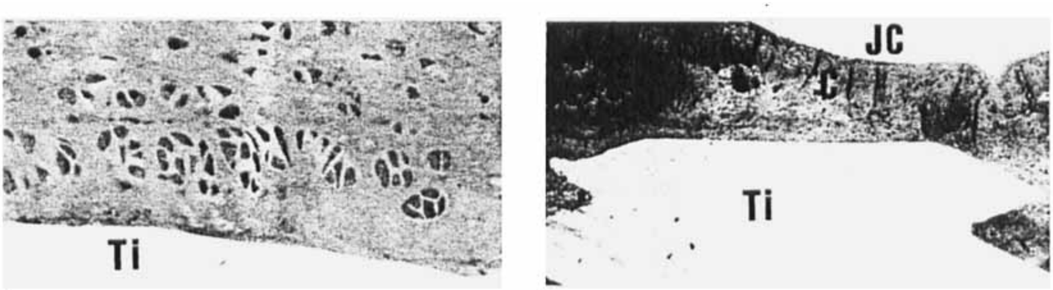
Insertion through cartilage

Two weeks after surgery the titanium screws were covered by a tissue, filling the hole created by drilling through the cartilage. This tissue was whitish, but translucent in some areas, which made the upper part of the screw partly visible. After 4 and 6 weeks, the whitish repair tissue filled the entire hole through the cartilage (Figure 1). We did not see any adverse reactions, such as bleeding, hyperemia, or increased thickness of the synovia.

Figure 1. A titanium screw removed and divided by sawing 4 weeks after insertion through the cartilage. Cartilage covers the screw and creates a smooth articulating surface (AS). The regenerated cartilage is somewhat more whitish than the surrounding articular cartilage (x 7).

The repair tissue covering the screws had a light-microscopic morphology similar to the surrounding hyaline cartilage, with a rather smooth articulating surface and without any vessels (Figure 2). The chondrocytes were often distributed in clusters, most notably at the border between cartilage and bone and close to the titanium surface.

After 2 weeks, light-microscopic examination of the bone between and surrounding the titanium threads revealed marked bone formation and resorption; the demarcation line between old and newly formed bone was clearly visible. Macrophages and osteoclasts were present on the surface of the old bone, as well as around displaced fragments of old bone located between the



A B

Figure 2. A. Light micrograph of newly formed cartilage (C) covering the upper surface of the (removed) titanium (Ti) screw 6 weeks after implantation. JC=joint cavity (x 30). B. Newly formed cartilage in direct contact with the upper surface of the (removed) titanium (Ti) screw. Chondrocytes form clusters (x 220).

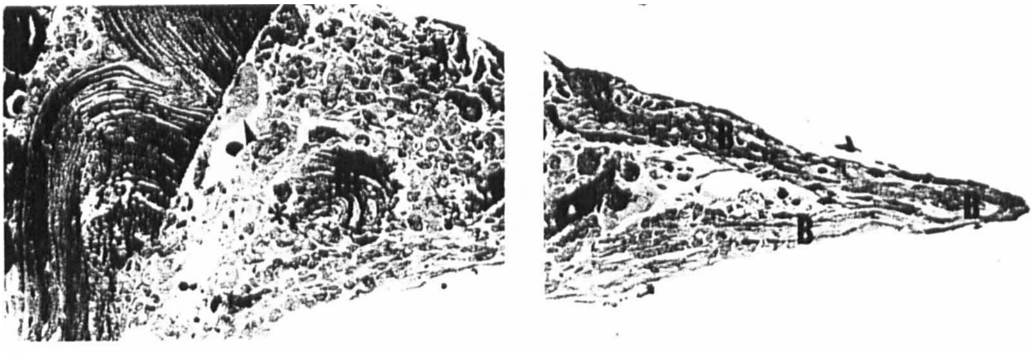


Figure 3. A. Light micrograph of tissue between two threads 2 weeks after implant insertion. The demarcation between bone and newly formed tissue is distinct (arrow). An osteoclast (arrowhead) is located on the surface of the cut bone. A bone fragment (BF) is surrounded by inflammatory cells (*) (x 150). B. Light micrograph 2 weeks after surgery. Newly formed bone tissue (B) is found close to the implant surface (x 150).

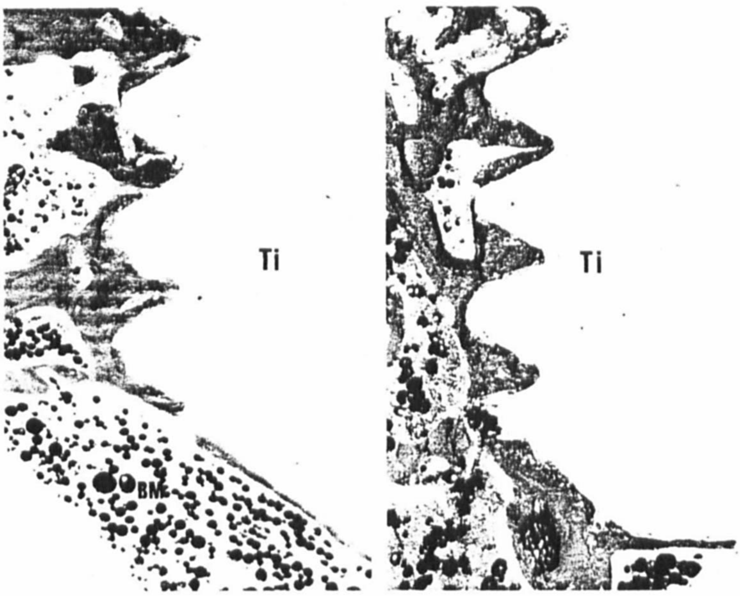


Figure 4. A. Survey light micrograph of the tissue surrounding the lower part of a (removed) titanium (Ti) screw 4 weeks after insertion. The areas between the threads are almost completely filled with bone tissue (BM=bone marrow (x 30). B. Tissue surrounding a screw 6 weeks after insertion. The areas between threads are almost entirely filled with bone tissue (x 30).

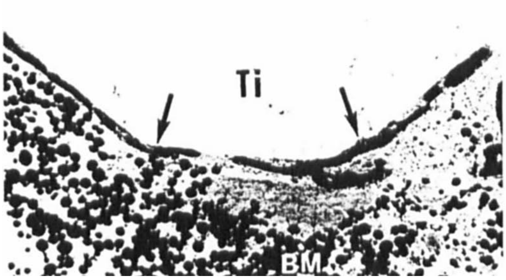


Figure 5. Four weeks after implantation. A thin rim of bone (arrows) covers the distal part of the (removed) screw (Ti) projecting into the bone marrow (BM) (x 30).

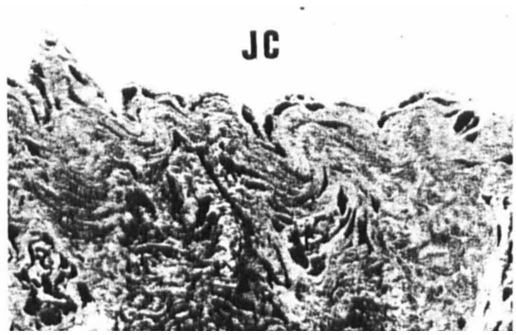


Figure 6. Regenerated synovial tissue covering the implant surface 4 weeks after surgery. A synovial lining layer separated the joint cavity (JC) from the dense connective tissue (x 250).

threads (Figure 3). A prominent finding at 2 weeks was the presence of newly formed bone between the threads. The bone was mainly present at the surface of the implant with the largest amounts in the inner corner between the threads.

Four weeks after surgery, the amount of newly formed bone between the threads had increased (Figure 4). At this time interval, signs of bone resorption or inflammation were rare. After 6 weeks, the amount of newly formed bone was further increased. After 4 and 6 weeks, a layer of newly formed bone was also covering the part of the screw, including the distal threads and distal surface, protruding into the marrow cavity (Figure 5).

Insertion through synovial tissue

A close contact between bone and implant surface, similar to that observed for screws inserted through the articular surface, was also seen after 4 weeks for the titanium screws inserted through the synovial membrane into the medial femoral condyles. The defect in the synovial membrane was filled with collagenous connective tissue covered by an apparently normal lining layer (Figure 6).

Synovial tissue morphology

When the rabbits were killed, biopsies were obtained from the joints and studied with respect to inflammatory changes. No such adverse effects, as judged with light microscopy, were found in any of the biopsies examined.

Discussion

Brånemark et al. (1977) have shown that a careful surgical technique, the use of threaded nonalloyed titanium, and a postoperative period without loading are important factors for successful long-term clinical results with bone-anchored dental prostheses. However, as reviewed by Albrektsson (1985), little is known about the morphology of the interfacial tissue and the mechanisms behind the tissue differentiation during the healing-in of titanium implants.

Our study shows that titanium implants which have been inserted in rabbit knee joints become anchored in the subchondral bone without any intervening fibrous tissue as judged with light microscopy. This was the case, independently of access route to the bone, via drilling through the cartilage or the synovial tissue. Although this study mainly focused on qualitative changes, it was apparent that the amount of newly formed bone tissue between the threads increased progressively with time. Preliminary observations indicate that the amount of newly formed tissue between threads at different distances from the bone surface can be quantified by morphometry and that our implantation method is useful for quantitative comparison of the response in bone tissue to different types of implant material.

Bone formation close to the implant surface was observed at 2 weeks. At the same time, an inflammatory response, with resorption of old bone by phagocytic cells, was evident. Most likely, the observed inflammation was due to the tissue injury caused by the insertion of the implant and was not caused by the implant itself. This is supported by the finding that the phagocytic cells were not preferentially located at the implant surface, but rather to injured bone tissue and also by the observation that the process was transient, because no signs of inflammation in bone and in synovial tissue ware detected at later time intervals. This conclusion is also supported by recent experimental studies in soft tissues (Thomsen et al. 1986). In contrast to the mild and transient inflammatory response induced by the surgical trauma when titanium implants were inserted, polymers (Delrin, Teflon) induced a greater and persistent accumulation of phagocytic cells in the interface tissue, followed by a fibrous encapsulation of the implants. Taken together, these experimental studies indicate the importance of the early material surface-cell interaction for the healing in bone and soft tissues and suggest that the outcome of this early interaction could determine the interfacial tissue composition and the subsequent long-term fate of endoprostheses.

Our study indicates that titanium might serve as a supporting surface for the proliferation of bone cells. At 2 weeks, osteoblasts were located at the implant surface and formation of new bone was most evident in the inner corner between the threads where the surface/volume ratio is largest.

Another observation indicating a role of titanium as a substrate surface for bone-forming cells was the appearance of newly formed bone on the distal part of the implant projecting into the bone marrow cavity.

The capacity to repair cartilage defects is controversial (Radin & Burr 1984). Superficial cartilage defects do not appear to heal (Meachim 1963, Fuller & Ghadially 1972, Salter et al. 1980). In contrast, full-thickness defects heal, possibly by proliferation of undifferentiated subchondral marrow cells (De Palma et al. 1966, Meachim & Roberts 1971, Mitchell & Shepard 1976). We found that cartilage was formed covering the head of the titanium screw. This suggests that titanium can serve as an artificial substrate surface for the formation of new cartilage, replacing a continuous subchondral bone plate otherwise required for the healing of an articular cartilage defect (Meachim & Roberts 1971).

We conclude that 1) intraarticularly inserted titanium screws become osseointegrated; 2) cartilage has the capacity to regenerate above osseointegrated titanium screws; 3) new synovial tissue forms rapidly over implants inserted in noncartilaginous areas; and 4) no adverse reactions, such as inflammation, occurs in the synovial tissue of joints with implanted titanium screws. Together, these findings suggest that pure titanium can be used intraarticularly.

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