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ELECTRON MICROSCOPIC ANALYSIS OF THE BONE – TITANIUM INTERFACE

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Ten cylindrical implants, made of polycarbonate and covered with a 120–250-nm-thick layer of pure titanium, were implanted into each tibial metaphysis of five rabbits. Observation time was 12 weeks. The implants were surrounded by mature, living bone. No soft tissue intervened between bone and implant at any point.

With TEM microscopy the titanium was shown to be bordered by a 20-nm-thick layer of proteoglycans, showing the characteristics of ground substance, and separating the collagen from the implant surface. Cells at the interface were likewise separated from the titanium by such a layer. Hydroxyapatite crystals were observed within the ground substance layer, occasionally seemingly in direct contact with the titanium. Normal mineralization was present 100–500 nm from the implant surface.

While this study aims at defining interface anatomy, it also shows that macroscopically smooth-surfaced titanium can readily heal into bone without a soft tissue envelope. This could be of help for materials' choice and design of permanently fixed implants.

Key words: animal experiment; bone-implant interface; collagen; electron microscopy; implant fixation; proteoglycans; titanium

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The anchorage of endoprostheses to the skeleton has long been one of the major problems in orthopaedic surgery. The introduction of bone cement brought about a dramatic improvement in short- and long-term results. However, as the problems associated with bone cement are becoming recognized, cement-free methods of anchorage are being developed (for review, see Bobyn et al. 1981).

The fixation of implants without cement, be it by means of smooth, porous, metallic or ceramic surfaces, is based on the regeneration of mature, living bone in direct contact with the implant surface, without an intervening soft tissue membrane. Such an interface situation has been termed *osseointegration* by Brånemark et al. (1977), and this term will be used in the following to distinguish the direct bone-implant contact from cases where the implant is bordered by soft tissue.

It should be emphasized that cement-free is *not* synonymous with osseointegration. Several cement-free clinical implant systems, although giving satisfactory results in the 5–10 year

perspective, do not fall within the definition above, as the implants are found to be surrounded by a thin soft tissue membrane (for review, see Albrektsson et al. 1981). The significance in the long term of such a membrane is not known at present.

A direct bone-implant contact has been described at the light microscopic level with various materials as well as in various species, including man (for review, see Albrektsson et al. 1981). Clinical long-term documentation has been made only for pure titanium by Brånemark et al. (1977), who presented a 12-year follow-up of screw-shaped titanium implants in the jaws of edentulous humans; despite years of repeated loads the implants remain osseointegrated and the bone remodels according to the applied loads.

Against this background it is clear that permanent implant anchorage through osseointegration is not a question of academic interest only. The principle represents a new approach to the problem and should be investigated in depth. Obviously, a morphological analysis of the bone-implant interaction is a first step, but so far no ultrastructural analysis of the interface of bone and a metallic implant has been made for technical reasons.

This experimental study aims at describing by high-resolution electron microscopy the anatomy of the intact interface of pure titanium and bone.

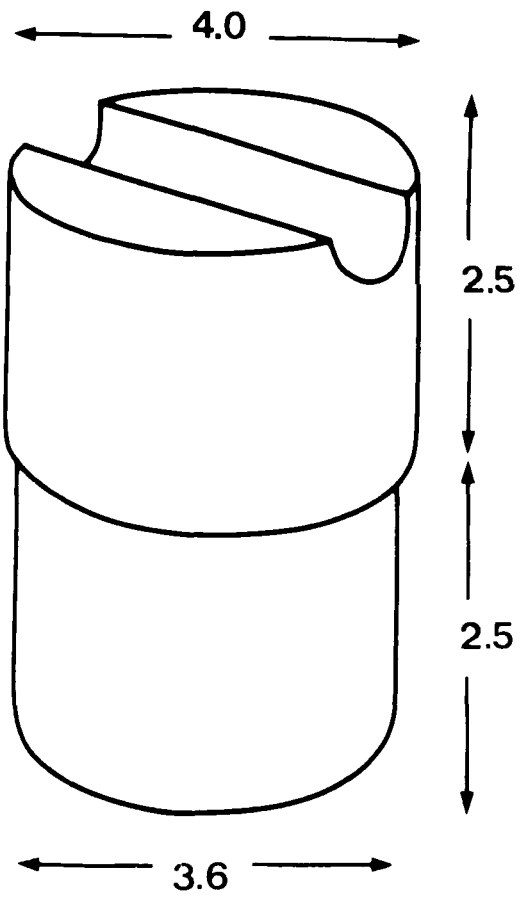

Figure 1. Shape and measurements (in mm) of the implants. Each implant is made of polycarbonate covered with a layer of pure titanium, 120–250 nm in thickness.

MATERIAL AND METHODS

A total of five adult male and female albino rabbits were used, each animal receiving two implants. The experimental method, originally developed by Lundskog (1972), reliably provides a direct bone-implant contact within 3 weeks, thus the limited number of animals. Implants of the same design as those used by Linder (1977) were produced. Instead of being made of bulk titanium, the implants were made of plastic with a thin layer of titanium evaporated onto the surface to allow easy sectioning of the bone with the implant *in situ*.

Implants

Titanium was obtained from Avesta Jernwerk AB, Avesta, Sweden, commercially pure grade ATi 24: 0.05% carbon, 0.03% nitrogen, 0.10% oxygen, 0.012% hydrogen, 0.05% iron, 99.758% titanium.

Rods of polycarbonate were machined into the shape and size shown in Figure 1, cleaned in ethanol in an ultrasonic bath and then dried in nitrogen. The metal was evaporated onto the polycarbonate plugs in an argon atmosphere at a pressure of about 3×10^{-6} Torr at a rate of 0.7 nm/s (7 Ångström/s). The final thickness of the titanium coat was 120–250 nm. The metallic vapor was initially condensed on the plastic at room temperature but gradually the temperature of the implants was determined by the condensing metal.

The titanium-covered implants were left for 1 hour in the evacuation chamber at less than 10^{-6} Torr and then cooled in argon for 2 hours at 1 Torr. The implants were then kept in air-tight boxes until the day of operation. Before implantation the implants were sterilized in 70% ethanol for 2 hours.

Surgery

Each animal received one implant in each tibia. Aseptic precautions were observed. Anaesthesia was induced

and maintained with Hypnorm® (Fluanison and Fentanyl, Leo, Helsingborg, Sweden) in a dose of 0.7 ml/kg body weight.

The skin was opened over the medial aspect of the upper tibial metaphysis. The underlying fascia was excised, laying bare the periosteum. By using microdiathermy all bleeding was avoided. With a circular punch a piece of periosteum of defined size (4.0 mm) was removed without touching any other part of the periosteum. In the centre of this defect a hole was drilled through the cortex, 3.6 mm wide, extending into the medulla. During drilling the bone was cooled by saline irrigation. Bone-cutting tools were made of titanium. The implant was inserted into the hole and fixed in place by a suture running through the slot in the implant to the fascial edges. The stability of the implants was considered adequate in all cases. The skin was closed with interrupted polyamide sutures. Except for surgical tape no external bandage was used.

Preparation for electron microscopy

The animals were sacrificed after 12 weeks. Although titanium implants are bordered by living bone within 3 weeks with the present method (Linder & Lundskog, 1975) the longer time was chosen to allow the bone to mature. The implants were removed from the animals with a trephine, leaving a 2–3-mm-thick cuff of bone around the implant. The specimens were fixed overnight in purified 3% glutaraldehyde in cacodylate buffer.

When examining the specimens in the dissecting microscope it was noticed that the titanium layer detached itself easily from the plastic core whereas it remained strongly adherent to the bone surface. Therefore, the bone in four cases was sectioned after removal of the plastic and in the remaining ones as originally planned with the entire implant *in situ*.

Sections aimed for transmission electron microscopy were postfixed in osmium tetroxide, dehydrated in a graded series of ethanol and embedded in Epon. For demonstration of glucoproteins and proteoglycans some specimens were immersed in glutaraldehyde containing ruthenium-red (Luft 1971), or immersed for 1 hour in 1% lanthanum chloride in cacodylate buffer, prior to dehydration and subsequent embedding in Spurr's medium (Spurr 1969). For demonstration of the organic matrix of the bone, specimens from several locations were immersed in an EDTA solution prior to osmium tetroxide postfixation and embedding.

Some of the specimens from which the implants had been removed were subjected to treatment with hyaluronidase or chondroitinase (Sigma Chemicals, St Louis, Mo, USA) before staining with ruthenium-red. This is to further characterize the interface; ruthenium-red is an unspecific stain for aldehydes and therefore has affinity for proteoglycans and glycoproteins. Reduced stainability after treatment with glucosaminoglycan-splitting enzymes could therefore help identify individual chemical components present at the metal surface.

Sections for scanning electron microscopy were dehydrated in a grades series of ethanol, dried in a critical-point equipment and coated with gold by sputtering. The specimens were examined in either a JEOL 100 CX TEMSCAN or a JSM 35 electron microscope. Energy-dispersive X-ray analysis was performed with the aid of attached EDAX equipment.

RESULTS

Light microscopy

In all 10 cases light microscopy of the plastic embedded bone revealed an absolute approximation of titanium and bone throughout the entire circumference of the implant. No signs of interposed

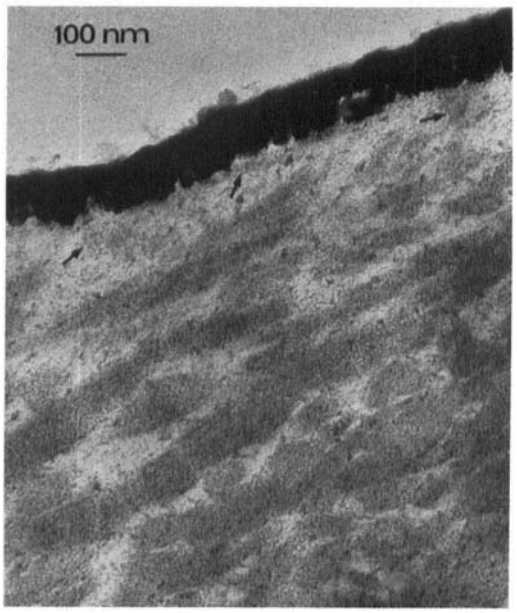

Figure 2. Transmission electron micrograph of the intact interface of bone and titanium. At this magnification the surface of the titanium coating is irregular. Collagen filaments can be seen approaching the titanium. The filaments are not arranged in an orderly manner and are therefore cut longitudinally, obliquely and transversely. The filaments closest to the titanium (arrows) are separated from the metal by a layer of proteoglycans which has the same staining characteristics and width as the ground substance between the collagen filaments within the bone. Although the filaments seem to be very close to the titanium surface, careful goniometry always revealed a distance of 20–50 nm (× 67000).

soft tissue was observed. The newly formed bone was of lamellar type and appeared integrated into the original tibial bone stock. The extraosseous part of the implants was surrounded by loose connective tissue with normal cellularity. The deepest endosteal part including the bottom surface sometimes bordered on normal bone marrow, separated from the marrow cells by only a very delicate soft tissue membrane. No active inflammation, degeneration or remaining necrosis was observed at any site. Scattered macrophages were seen at the interface adjacent to blood vessels and occasionally unrelated to vessels at the deepest part of the implants.

Transmission electron microscopy

Although seemingly in direct contact with the bone in the light microscope, it was evident in the TEM micrographs that the titanium was separated from the nearest collagen filaments by a gap of 20–50 nm (200–500 Å). The width of the zone was variable along the interface, often as narrow as 20 nm (Figure 2) but never less. The zone stained with ruthenium-red and lanthanum, indicating that it contained proteoglycans. After treatment with hyaluronidase or chondroitinase the stainability with ruthenium-red was reduced. Therefore, qualitatively, the zone closest to the titanium contains hyaluronic acid and chondroitine sulphate, the relative proportions of which not having been determined. Generally speaking, the zone reacted in the same way as normal ground substance.

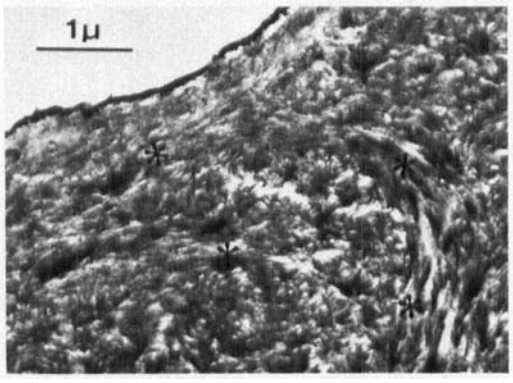

Figure 3. Low-power TEM image of the interface region. Regularly structured collagen bundles (*) approach the interface in an arch-like manner. Some 100 nm from the titanium surface the collagen is disarranged with filaments running in a random fashion (×12200).

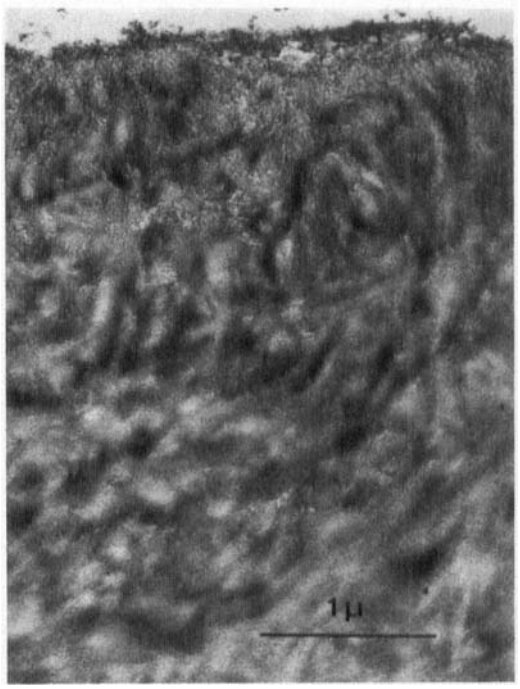

Figure 4. Partly decalcified specimen showing the irregular arrangement of collagen filaments at the surface and the more regularly arranged, coarse collagen bundles in deeper parts. Here, the cross-striation is somewhat masked. In this specimen the titanium was artifactually lost during sectioning, but minute titanium deposits can be discerned at the bone surface (×25800).

This layer of ground substance was bordered by a layer of collagen filaments which were randomly distributed. This collagen layer was 100–500 nm thick, peripheral to which was collagen arranged in orderly bundles, lined up at an acute angle to those nearby (Figures 3 and 4). Calcified deposit were present in each of these three layers but there was a gradual decrease in density towards the interface. Thus in the layer of structured collagen the density was in approximately the same range as that of the original tibial bone and in the layer of collagen filaments there was a gradual fall so that in the layer of ground substance there were only scattered calcified deposits to be seen (cf. Figure 6). Some of these, however, seemed to be in direct contact with the

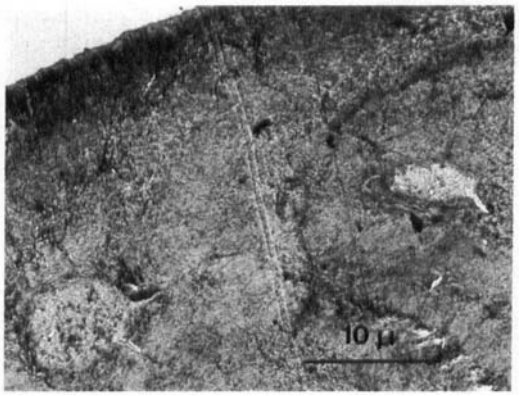

Figure 5. The interface region showing two osteocytes with slender processes. In this case, too, the titanium was detached during sectioning, remnants appearing within the section (× 1700).

titanium surface, as far as can be determined by the present level of resolution (4 nm).

Osteocytes in the interface region contained normal organelles and there were no signs of degenerative changes in their cytoplasm (Figures 5 and 6). The slender processes sometimes extended in narrow canals towards the titanium surface, but the processes never came in contact with the titanium and were separated from it by a layer of proteoglycans of about 20 nm. Sometimes osteoblasts bordered the titanium surface without intervening collagen filaments but the layer of proteoglycans was always present.

The extraosseous part of each implant was co-

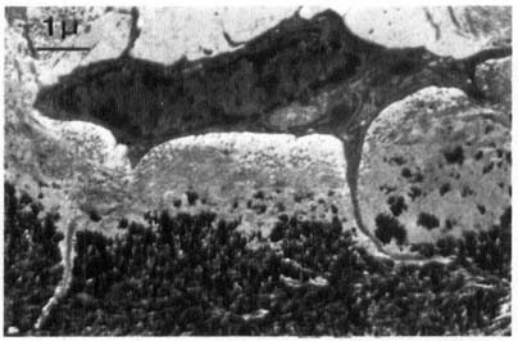

Figure 6. High-power view of an osteocyte close to the titanium surface. The specimen is not decalcified. The cell is surrounded by ground substance and unmineralized collagen filaments. Although scattered mineral deposits (black) can be seen in this nonmineralized zone, mineralization is of normal density only at a distance from the cell surface. This is analogous with the situation at the interface, where there is also a gradient in the density of mineral deposits. The cell in this case has a normal appearance. Two processes can be seen emerging from the surface, penetrating the bone matrix (× 6000).

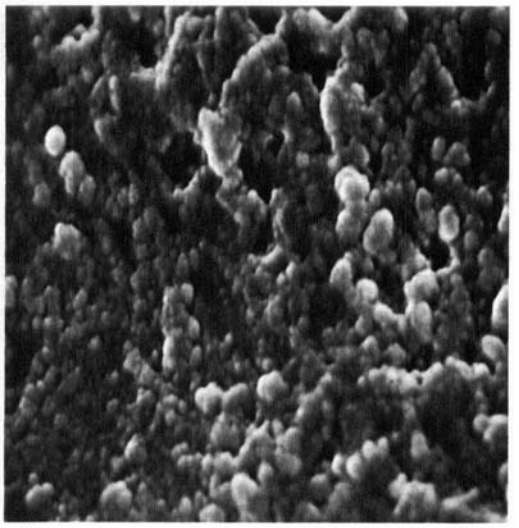

Figure 7. Scanning electron micrograph of the surface of the bone bordering the titanium. There is a granular substance masking the underlying structures, corresponding to the proteoglycan layer seen in the TEM micrographs (× 21 100).

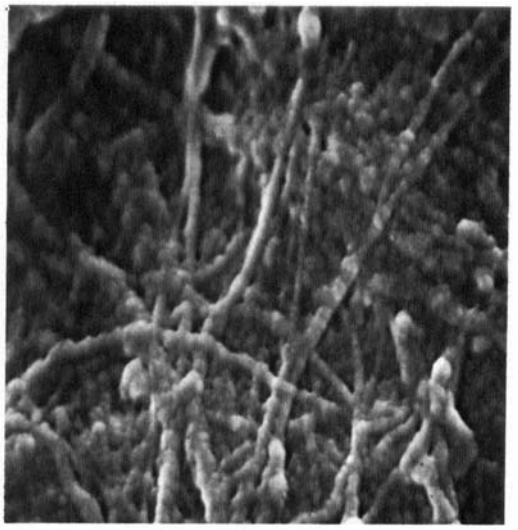

Figure 8. In places the proteoglycan layer is thinner, revealing the fibre structure underneath. Observe the random distribution of filaments (× 13 100).

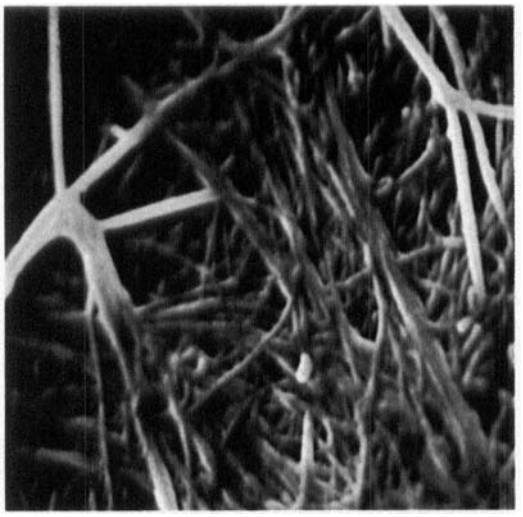

Figure 9. Due to the preparation method the collagen filaments close to cellular elements are sometimes completely demasked. Filaments of varying width and direction of orientation are seen (× 12 100).

vered by connective tissue proper, which was fairly cell rich but without obvious aggregates of inflammatory cells. Even in this area the collagen fibres and the surface cells were separated from the titanium by a layer and proteoglycans of 20–50 nm. There were no signs of a basement membrane or a basement membrane-like structure along the interface. In the medulla there were occasional macrophages at the interface but the cells had the appearance of normal soft tissue macrophages without signs of resorptive activity. The blood cells in the vicinity of the titanium surface, both of erythropoietic and myelopoietic origin, appeared normal.

Analysis with X-ray dispersive equipment did not indicate detectable amounts of titanium in the tissues surrounding the implants.

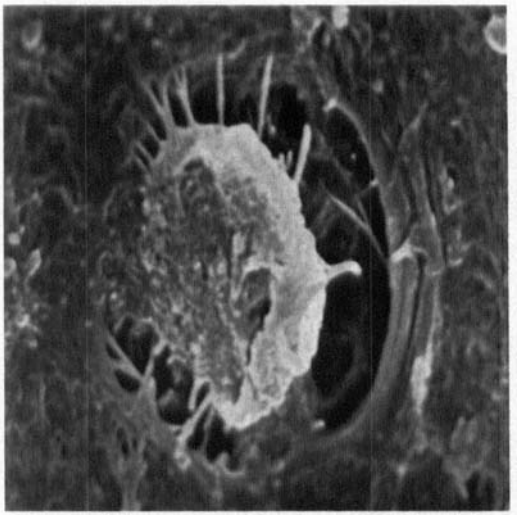

Figure 10. Osteoblast bordering directly on the titanium. Here, the cover of proteoglycans has been removed during preparation. The cellular processes extending into the bone matrix are evident (× 4500).

Scanning electron microscopy

Some of the bone specimens in which the titanium had separated from the plastic core of the implant were examined with SEM. In minute areas there were defects in the titanium coating, revealing the surface of the biological tissue facing the titanium. Collagen filaments were seen in such areas, random in distribution, covered by an amorphous granular substance (Figures 7, 8 and 9). Contours of osteoblasts and their processes could also be identified (Figure 10).

DISCUSSION

The permanent fixation of implants to the skeleton can be used not only for replacement of degenerated joints but also for segmental replacement of skeletal defects (Andersson et al. 1978), ligament and tendon anchorage (Murray & Semple 1981), and as fixation points for bone transplants and epistheses (Brånemark et al. 1975, Tjellström et al. 1981). It is of primary concern that such implants do not loosen with time, especially as load is applied.

It has been shown that the strength of the bond between an osseointegrated implant and the bone increases with time, regardless of whether the implant is a macroscopically smooth-surfaced cylinder (Linder & Lundskog 1975) or a porous-surfaced plug (Cameron et al. 1972). It is also known from clinical practice that the bond does not weaken even if the implant is functionally loaded for years (Brånemark et al. 1977). This seems to be in contrast to the cemented implant,

which is never as secure in the bone as when first inserted (Willert 1972). Instead of deforming the interface itself, loads on an osseointegrated implant deform the bone surrounding the implant, at the same time remodelling it according to Wolff's law to meet the demands of the loading condition. This process requires living bone, which may also diminish the risk of fatigue phenomena in the bone.

This study also indicates that a titanium implant does not have to be porous, screw-shaped or rough-surfaced to become osseointegrated, nor does it have to be covered with "bone-attracting" coatings. The present results thus agree with those of Bobyn et al. (1981) that the macrostructure of the titanium implant has little influence on the tissue response. However, in the clinical situation it may be advantageous to choose an implant with a certain surface design in order to guarantee stability during the healing period, one of the denominators for successful osseointegration (Albrektsson et al. 1981).

Clark et al. (1976) have shown that the response of bone to bioglass can be modified by alteration of the chemical composition of the implant surface, thus influencing, among other things, the surface energy of the implant. A titanium implant is always covered by an oxide layer, consisting of variety of oxides (Albrektsson et al. 1981). In the present study the titanium has been evaporated onto the implant in a homogeneous layer. This deposition method offers the possibility to change and characterize the microstructure of the titanium layer over a wide range with careful control of the evaporation parameters (Jacobson 1980). This study may serve as a basis for further investigation of the importance of the surface microstructure for the tissue reaction.

It is not known whether the oxide film on our implants is of the same composition as that of an implant made of bulk titanium, and whether the biological response is thereby altered. However, previous experimental work with titanium implants of various models of manufacture have all uniformly shown the absence of any untoward effect of the implant (Andersson et al. 1978, Bobyn et al. 1981, Brånemark et al. 1977, Linder & Lundskog 1975, Lundskog 1972, Schroeder et al. 1981). Indeed, Albrektsson et al. (1981) have shown an intimate contact between collagen and bulk titanium in man in electron micrographs of up to 4000 × magnification.

The oxide layer on the implants used in this study has been measured to be 5–10 nm. It has been shown by McQueen et al. (1982) that after a number of years of implantation this oxide layer increases in thickness to some 200 nm and at the same time there seems to be an exchange of ions between the biological tissue and the oxide, so that calcium, phosphorus and sulphur may be found in the outer parts of the oxide.

Apart from such chemical interaction there also seems to be a mechanical interaction of the tissue and implant, evidenced by the strong adherence of the titanium to the bone rather than to the plastic core of the implant, as found by Schroeder et al. (1981) as well. The nature of those forces are unknown. The morphological substrate for the attachment is the thin layer of proteoglycans interposed between the titanium surface and the most superficial collagen filaments, which in turn are interconnected with the regularly arranged fibre bundles of the lamellar bone. It seems likely that the ground substance in this special case acts as a glue, connecting the stress-loaded collagen with the implant surface. There also seems to be an inverse relationship between the thickness of the ground substance layer and the force of attachment, as gold-covered implants of identical design as those used in this study were separated from the collagen by at least 50 nm and were readily detached from the bone surface (Albrektsson et al. 1982). Whether the optimal thickness of the biological glue is 20 nm, as for the titanium in this study, is a matter for further investigation.

That cells are farther away from a gold surface than from a titanium surface has also been shown in tissue cultures (Kawahara et al. 1979). It would therefore seem that the ultrastructural appearance of the interface may differ for different implant materials, even though there seems to be a direct implant-bone contact by light microscopy. It is perhaps only at this level of resolution that differences in biocompatibility can be distinguished.

Hicks (1958) divided the response of bone to

an implant into three categories according to the compatibility of the implant material: Inflammation, fibrotic reaction and zero reaction, the latter denoting a direct connection of implant and bone. Philosophically, the ideal implant material ought not to be recognized by the bone as a foreign substance. It is interesting to note that in this study the thickness of the proteoglycan layer is of the same degree as the distance between two collagen filaments in the same collagen fibre and between the cell membrane and the nearest collagen fibril (Figure 2). Even though further comparative studies are necessary, the findings in the present investigation indicate that there is no inherent antagonism between titanium and biological tissue. They also lead "zero reaction" a new and intriguing dimension.

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