Osseointegration of metallic implants

II. Transmission electron microscopy in the rabbit

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In a material of 10 osseointegrated implants of pure titanium, Tivanium®, Vitallium®, and stainless steel, 23 interface areas were studied by transmission electron microscopy. The implant site was the upper tibia of mature rabbits, and the observation time was 11 months.

The absence of a cellular reaction was verified. However, even in cases of apparently uniform osseointegration, electron microscopy revealed an unpredictable variation in interface ultrastructure within 500–1,000 nm of the metal surface, common to all the materials. There was no structural feature that was specific for a particular material.

Commercially pure titanium is claimed to have a unique osseointegrative capacity. Other materials, such as AISI 316L stainless steel and Ti-6Al-4V alloy, consistently provoke an untoward reaction as shown by transmission electron microscopy (Albrektsson and Hansson 1986, Albrektsson and Jacobsson 1987). Albrektsson and Albrektsson (1987) are of the opinion that with time such inconspicuous reactions will lead to loosening of a loaded implant.

Given identical healing conditions, however, bulk implants of pure titanium, titanium alloy, chrome-cobalt alloy, and AISI 316 stainless steel are accepted by rabbit bone with a direct bone-implant contact (osseointegration), without a tissue reaction discernible by high-resolution light microscopy (Linder and Lundskog 1975, Linder 1989). The aim of this study has been to evaluate whether systematic differences in the bone reaction can be defined by transmission electron microscopy.

Materials and methods

The histologic preparation of the tissue embedments has been described in the preceding article (Linder 1989). From the material described in that study, a total of 10 embedments were processed for TEM (2 pure titanium, 3 Tivanium®, 1 Vitallium®, and 4 polished stainless steel – all 11 months' observation time). The inclusion criteria were osseointegration of the implant and a perfectly clean separation of implant and embedment.

The embedments, with an interface area of 5 x 2 mm in most cases, were glued with epoxy to a plastic holder, mounted and sectioned.

As a first step, 1-µm sections were cut and stained with methylene blue. After that, areas with an indisputable bone-implant contact were trimmed down in size to pyramids of approximately 1 x 1 mm. Ultrathin sections were then cut on an Ultrotome V (LKB), using a diamond knife. The sections were contrasted with lead citrate and uranyl acetate and examined in a JEOL 1200 EX electron microscope without knowledge of the materials implanted.

After a sufficient number of ultrathin sections had been taken, the blocks were sectioned anew at 1 µm, exposing deeper levels in the embedment with a direct bone-implant contact. New pyramids could then be prepared and sectioned. In this manner, the 10 embedments yielded a total of 23 interface areas suitable for ultrathin sectioning.

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Results

The interface edge of the embedments was easily found. Although small fragments of this edge were sometimes missing, there was no evidence that the free edge represented a fracture through the entire embedment at some distance from the interface. The fixation of the tissue appeared sufficient in all the cases. There were no remnants of cells, such as membranes or vesicles, at the interface.

In four cases (three stainless steel, one pure titanium), the tissue was separated from the edge of the embedment by a cleft empty of biological material (Figure 1). The tissue, however, had the same appearance as in the other samples. Because in all probability this phenomenon was a shrinkage effect caused by the processing for histology, it was not taken into account in the classification of interface anatomy.

We found three main types of interface structure:

A. More or less regularly arranged fibrils of collagen, approaching the metal surface to within 50 nm. The fibrils showed the longitudinal cross-banding of 68 nm typical of Type-I collagen (Figures 2–5).

B. Type-I collagen fibrils separated from the implant by a zone of indistinct structures, but with some filamentous material, most often about 500 nm in thickness, but sometimes up to 1,000 nm (Figure 6).

C. Type-I collagen fibrils separated from the implant by a 500–600 nm zone of thin filamentous structures, clearly more dense than in *B* (Figure 7).

These types of interface structure were seen around all the materials studied. There was no obvious predilection for a specific material or specific anatomic region: periosteal or endosteal or intracortical.

The exact thickness of the layer of ground substance separating the collagen from the metal surface was difficult to ascertain. The method of implant-embedment separation (Linder 1985) may theoretically entail a loss of up to 10 nm of plastic from the embedment (Lausmaa and Linder 1988). Because the edge of our tissue sections in many cases was indistinct and dark-staining, a reliable quantification was not possible. Nevertheless, in all the cases showing the Type-A interface, the thickness of the ground substance layer was less than 50 nm, and there was no systematic variation from one material to the next.

Discussion

This is the first TEM study of the response of bone to bulk implants that have the same composition and surface structure as today's clinical implants intended for uncemented use. In the preceding light microscopic study (Linder 1989), these implants were shown to be osseointegrated in a uniform manner. Because this is a study of established osseointegration, obviously no conclusion can be drawn about the speed or ease with which this occurred for the individual materials.

The histologic method used in this study is based on in situ embedding of tissue and implant and subsequent separation of embedment and implant (Linder 1985). The latter procedure has been shown to entail a potential loss of < 10 nm of interface tissue, which should be acceptable even for TEM studies (Lausmaa and Linder 1988). The embedding procedure is less controllable, because all the fluids have to penetrate the tissue before reaching the interface, possibly causing preparation artifacts. However, all the methods using in situ embedding are fraught with this problem, and this makes the difficulties in interpreting the interface anatomy inherently similar (Albrektsson et al. 1983, Linder et al. 1983, Bjursten et al. 1987).

The most striking finding of this experiment was that in cases of established osseointegration, the tissue within 500–1,000 nm of the metal surface showed considerable variation in structure, regardless of material studied. This variation has also been seen adjacent to an osseointegrated implant of pure titanium in the human tibia (Engfeldt and Linder, unpublished data), which suggests that it is not related to species or biocompatibility. The results underline the difficulty in obtaining representative sections for TEM and the danger of relying on TEM in a quantitative analysis. As a consequence of this, we have only divided our findings into broad categories.

Comments on findings

The cleft between tissue and implant (Figure 1) is most likely a shrinkage artifact, corresponding to the clear zone seen in the light microscope (Figure 5 of Linder 1989). Whereas interface type A is a good example of close bone-implant contact, type B is difficult to explain. It could be an artifact, but, in view of the sufficient tissue fixation, the possibility still remains that it might be an in vivo phenomenon. Type C most closely resembles the interface described for pure titanium by Linder et al. (1983).

The degree of mineralization of the interface tissue could not be determined due to the decalcification procedure. The dark-staining line seen in Figures 2 and 3 could represent the border between fully mineralized bone and osteoid or poorly mineralized bone, as previously described in experimental calcification by Boivin et al. (1987). However, Figures 4 and 5 are good examples of bone tissue, because this arrangement of Type-I collagen can hardly be attributed to any other tissue in this location. Indeed, this arrangement can al-

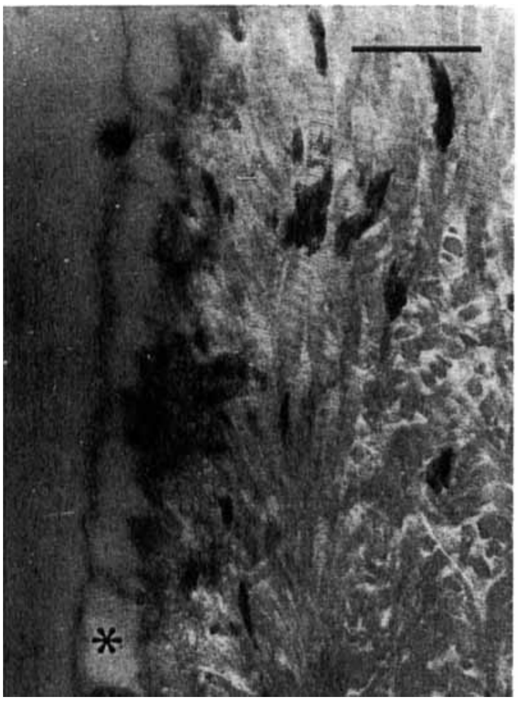

Figure 1. Demonstrating a cleft (*), seemingly devoid of biological material, between tissue and implant surface. The tissue is made up of Type-I collagen. The black specks are remnants of bone mineral. Stainless steel implant. Bar = 500 nm (x35,000).

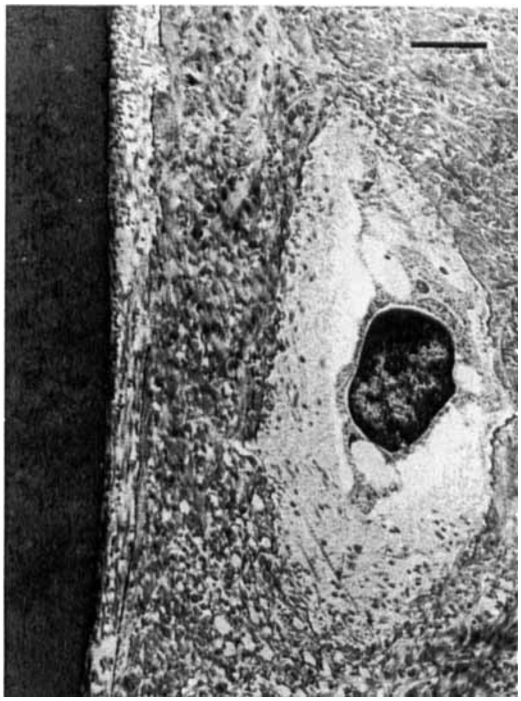

Figure 2. Low-power electron micrograph of the tissue adjacent to a steel implant. The cell is an osteocyte. The interface is classified as type A. Bar = 1 micron (x9,000).

Figure 3. Magnification of the interface in Figure 2. The predominantly cross-sectioned collagen fibrils are within 50 nm of the implant surface. The tissue closest to the implant (*) may be osteoid or poorly mineralized bone, whereas the tissue peripheral to the black borderline is mature bone. Bar = 500 nm (x15,000).

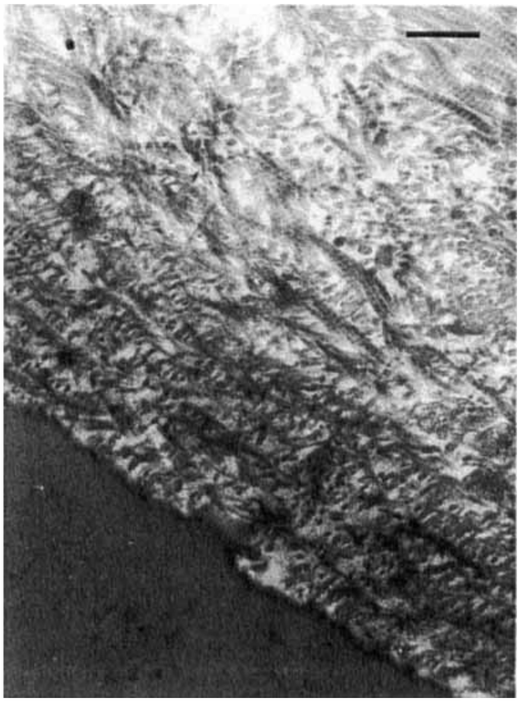

Figure 4. Example of mature bone in close contact with the implant surface. The ground substance layer separating the collagen from the implant is less than 50 nm. Interface type A. Implant of pure titanium. Bar = 500 nm (x19,000).

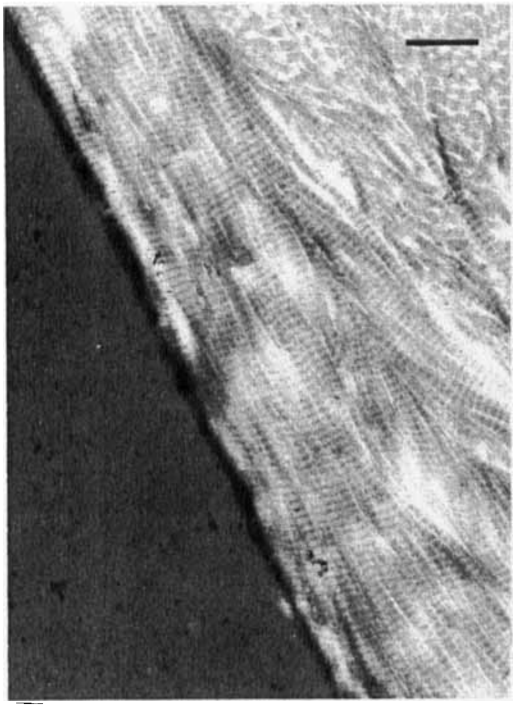

Figure 5. Longitudinally sectioned Type-I collagen fibrils adjacent to a stainless steel implant. Interface type A. Bar = 500 nm (x19,000).

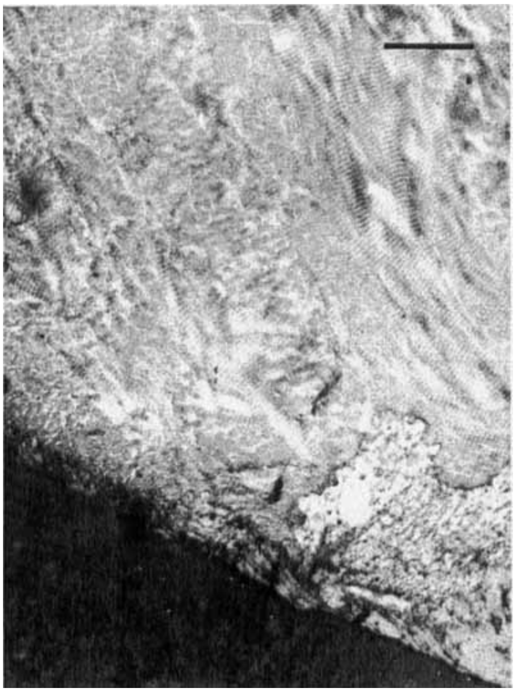

Figure 7. Interface type C, stainless steel implant. The Type-I collagen fibrils do not reach the implant surface, but are replaced by a 500–600-nm zone of fine filaments. Bar = micron (x12,000).

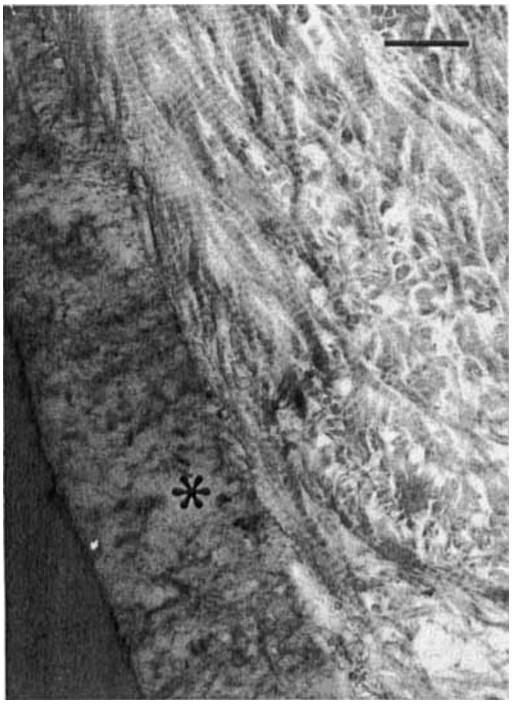

Figure 6. Interface type B, Tivanium® implant. The collagen and implant are separated by an indistinct zone with some filamentous material (*). It is unclear whether this zone represents a histologic artifact or is an in vivo event. This zone was seen adjacent to all the materials in the study. Bar = 500 nm (x24,000).

so be seen in ligaments and tendons, but not in newly formed connective tissue proper, which would be the alternative to bone at the interface.

This study thus confirms earlier observations that mature bone can be found in direct contact with pure titanium, and that the ground substance layer separating the collagen from the implant may well be of the order of 20–40 nm (Linder et al. 1983, Albrektsson and Jacobsson 1987). However, the same tissue organization could be seen adjacent to Tivanium®, Vitallium®, and stainless steel; and this uniform tissue response agrees with findings in soft tissue (Brunet et al. 1986).

Stainless steel was included in the study partly because of its wide use, but in particular because of its allegedly inferior biocompatibility (Albrektsson and Albrektsson 1987). It is noteworthy that in the light microscopic study on which this material is based (Linder 1989) 17/18 steel implants were solidly integrated, including 8/9 with a polished surface, which should have given the implants a weak primary interlock with the tissue. These results clearly contradict the findings of Albrektsson and Hansson (1986). These authors, using the same experimental model as in this investigation, but using plastic plugs covered with "stainless steel" by magnetron sputtering, showed that such experimental implants consistently caused an inflammatory reaction in the bone. As indicated by the authors themselves, the most likely explanation for their results is that the sputtered surface is not representative of a clinically used stainless steel implant.

Clinical implications

Although the implants in this study were made from different materials, it is the surface oxides and the adsorbed coats of biomolecules that interact with the tissue (Kasemo and Lausmaa 1986). In all probability, there are differences in the biological response to these implants at some level of resolution, but this investigation questions the feasibility of determining the quality of osseointegration on morphologic grounds alone. Nevertheless, the results support the rather cautious suggestion that osseointegration is the result of the basic healing capacity of bone, and this view has a bearing on implant surgery in clinical practice.

The problem today is whether osseointegration may be achieved at all. Our study strongly supports the view (Ling 1986) that this problem can be overcome primarily by improved tissue handling and by control of the interface mechanics, even with current implant materials. With such osseocompatible surgery, it will eventually become possible to evaluate the long-term merits of osseointegration as such and the submicroscopic events taking place on the surface of a biomaterial.

References

Albrektsson T, Brånemark P I, Hansson H A, Kasemo B, Larsson K, Lundström I, McQueen D H, Skalak R. The interface zone of inorganic implants in vivo: Titanium implants in bone. Ann Biomed Eng 1983;11(1):1–28.

Albrektsson T, Hansson H A. An ultrastructural characterization of the interface between bone and sputtered titanium or stainless steel surfaces. Biomaterials 1986;7(3):201–5.

Albrektsson T, Albrektsson B. Osseointegration of bone implants. A review of an alternative mode of fixation. Acta Orthop Scand 1987;58(5):567–77.

Albrektsson T, Jacobsson M. Bone metal interface in osseointegration. J Prosthet Dent 1987;57(5):597–607.

Bjursten L M, Ericson L E, Lausmaa J, Mattsson L, Rolander U, Rosander L, Thomsen P, Kasemo B. A new method for ultrastructural studies of the intact tissue titanium interface. Read at the 13th Annual Meeting of the Society for Biomaterials, New York 1987.

Boivin G, Walzer C, Baud C A. Ultrastructural study of the long term development of two experimental cutaneous calcinoses (topical calciphylaxis and topical calcergy) in the rat. Cell Tissue Res 1987;247(3):525–32.

Brunet J A, Sarkar K, Uhthoff H K. Ultrastructure of the fibrous tissue surrounding internal fixation devices. Clin Orthop 1986;(208):84–94.

Kasemo B, Lausmaa J. Surface science aspects on inorganic biomaterials. Crit Rev Biocompat 1986;2(4):335–80 .

Lausmaa J, Linder L. Surface spectroscopic characterization of titanium implants after separation from plastic embedded tissue. Biomaterials 1988;9(3):277–80.

Linder L. High resolution microscopy of the implant tissue interface. Acta Orthop Scand 1985;56(3):269–72.

Linder L. Osseointegration of metallic implants: I. Light microscopy in the rabbit. Acta Orthop Scand 1989;60(2): 129–134.

Linder L, Lundskog J. Incorporation of stainless steel, titanium and vitallium in bone. Injury 1975;6(4):277–85.

Linder L, Albrektsson T, Brånemark P I, Hansson H A, Ivarsson B, Jönsson U, Lundström I. Electron microscopic analysis of the bone titanium interface. Acta Orthop Scand 1983;54(1):45–52.

Ling R S M. Observations on the fixation of implants to the bony skeleton. Clin Orthop 1986;(210):80–96.

Acknowledgements

Financial support was given by the Swedish Medical Research Council (Project B86-17x-07502-01), Riksförbundet mot Reumatism, Herman Järnhardts Stiftelse, Alfred Österlunds Stiftelse, and the Faculty of Medicine, University of Lund, Sweden.