Osseointegration of metallic implants
I. Light microscopy in the rabbit

Lars Linder

Thirty-eight adult albino rabbits received one implant of pure titanium and one implant of another, test, material in each tibia. The test materials were titanium-aluminum-vanadium alloy, chrome-cobalt alloy, and stainless steel. Observation times were 4 months and 11 months.

Light microscopy of the interface revealed a direct contact between bone and implant surface (osseointegration) in 73 of the 76 cases. The exceptions were two implants of pure titanium and one of stainless steel. Thus, given identical healing conditions, the modern implant metals were accepted by the bone in the same way.

It is suggested that osseointegration should be regarded not as an exclusive reaction to a specific implant material, but as the expression of a nonspecific and basic healing potential in bone.

Osseointegration, the apposition of viable bone on an implant surface, is considered the most stable long-term fixation of an implant to the skeleton (Brånemark et al. 1977, Ling 1986). Most of the cement-free implant systems in use today are aimed at a regeneration of bone into a structured surface. Although a direct bone-implant contact has been documented in the human femur (Linter et al. 1986, Engh et al. 1987), the dominant finding, especially in cancellous bone, is a fibrous membrane between the implant and the surrounding bone (Thomas et al. 1987). However, Linder et al. (1988) have shown in man with implants of pure titanium that the biological requirements for osseointegration are indeed present adjacent to diseased joints, even in cancellous bone and in pronounced osteopenia.

Against this background, it seems that the discrepancy between the biological potential of bone and the usual clinical outcome of prosthetic implantation must be related to either the choice of implant material or to the method of implantation.

The aim of this study is to address the issue of biomaterials: Is the predictable development of osseointegration dependent on the use of pure titanium? If not, is there still a differentiated response to materials of different composition?

Materials and methods

Implants

Cylindric implants of standard size were used (Figure 1). Four groups of metallic materials were studied:

1) Commercially pure titanium (ATi24, Avesta Jernverk AB, Avesta, Sweden) was machined to size on a lathe and then washed in a mild soap solution.

Figure 1. The standard shape and size (mm) of the implants

University of Lund Department of Orthopedics, Malmö General Hospital, Malmö, Sweden

Correspondence: Dr. Lars Linder, Department of Orthopedics, Gävle Hospital, S-801 87 Gävle, Sweden
2) Ti-6Al-4V alloy (Tivanium®) implants were custom-made by Zimmer, Inc. After being machined to size, they were dry-blasted, cleaned ultrasonically, passivated in 40 percent nitric acid at room temperature, and finally rinsed in water.

3) Cast Vitallium® alloy (ASTM F75-82) implants were custom-made by Howmedica, Inc. Following the casting process, the implants were sandblasted, ultrasonically cleaned, and passivated in a 20 percent nitric acid solution at 55 °C for 30 min.

4) AISI 316 stainless-steel implants had two surface finishes. Half of the implants were machined to size on a lathe and left without further treatment. The other half were polished with various polishing pastes until they attained the polish of a bone screw or bone plate. Regardless of treatment, all the steel implants were washed in a mild soap solution.

Before implantation, each implant was alternately ultrasonically cleaned in 70 percent ethanol and trichloroethylene. The implants were then packed individually, and finally steam autoclaved.

Animals

Thirty-eight adult 2.4-4.5-kg albino rabbits of both sexes were used. The observation times were 4 and 11 months, with 19 animals in each group. Each animal in turn received one implant of pure titanium and one implant of one of the other materials in each tibia (Table 1).

Surgical procedure

The animals were operated on under general anesthesia, but 1 percent mepivacain (Carbocain®, Astra, Sweden) was infiltrated around the operative field as well. In addition to standard clinical aseptic conditions, 75 mg of cefuroxim (Zinacef® Glaxo, England) was given intravenously before surgery.

The operative technique has been described in detail by Linder and Lundskog (1975). Through a medial skin incision at the knee and after excision of the deep fascia, the periosteum over the medial aspect of the upper tibial metaphysis was exposed. A 4.0-mm diameter periosteal defect was made with a circular punch. In the center of this defect, a 3.6-mm hole was drilled through the cortex with a flat drill, guided by a central pilot hole. During drilling, the bone was cooled by saline irrigation.

The implants, which were on an average 3.57 mm in diameter and thus slightly undersized in relation to the holes, were macroscopically stable when pressed down against the cortex. As an additional stabilizer, a suture running from the medial collateral ligament insertion to the patellar tendon insertion was tied through the slot in the implant.

Analysis

The plugs were removed by using a trephine drill with an inner diameter of 6.0 mm. The entire implant/tissue specimen thus obtained was fixed for about 7 days in 3

<table>
<thead>
<tr>
<th>Implant material (Number of implants)</th>
<th>Periosteal proliferation</th>
<th>Cartilage-like areas</th>
<th>Dark-staining line</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>0</td>
<td>+</td>
<td>++</td>
</tr>
<tr>
<td>Pure titanium (19)</td>
<td>8</td>
<td>5</td>
<td>6</td>
</tr>
<tr>
<td>Tivanium® (5)</td>
<td>--</td>
<td>2</td>
<td>3</td>
</tr>
<tr>
<td>Vitallium® (5)</td>
<td>--</td>
<td>1</td>
<td>4</td>
</tr>
<tr>
<td>Steel, rough (4)</td>
<td>1</td>
<td>1</td>
<td>2</td>
</tr>
<tr>
<td>Steel, polished (5)</td>
<td>2</td>
<td>--</td>
<td>3</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Implant material (Number of implants)</th>
<th>Periosteal proliferation</th>
<th>Cartilage-like areas</th>
<th>Dark-staining line</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>0</td>
<td>+</td>
<td>++</td>
</tr>
<tr>
<td>Pure titanium (19)</td>
<td>--</td>
<td>4</td>
<td>15</td>
</tr>
<tr>
<td>Tivanium® (5)</td>
<td>--</td>
<td>1</td>
<td>4</td>
</tr>
<tr>
<td>Vitallium® (5)</td>
<td>--</td>
<td>1</td>
<td>4</td>
</tr>
<tr>
<td>Steel, rough (4)</td>
<td>--</td>
<td>1</td>
<td>4</td>
</tr>
<tr>
<td>Steel, polished (4)</td>
<td>--</td>
<td>1</td>
<td>3</td>
</tr>
</tbody>
</table>
percent glutaraldehyde in 0.15 molar cacodylate buffer at 4 °C. Decalcification was carried out in 10 percent EDTA for up to 2 weeks. Post fixation was done in 2 percent osmium tetroxide for 6 h. The specimen was then washed in sodium cacodylate buffer, taken through a graded series of ethanol, and finally transferred to propylene oxide. The specimen was then embedded in epoxy resin (Agar Resin 100, Agar Aids, Stansted, Essex, England). After polymerization of the epoxy, sectors of the now plastic-embedded tissue were separated from the implant (Linder 1985). In most cases, three or four sectors were processed for histology, comprising more than half of the circumference of the implant. A completely clean separation of embedment and implant was required for the interface studies (Figure 2). A clean separation indicates that the separation has occurred in a plane less than 10 nm from the implant surface (Lausmaa and Linder 1988).

The broken-off sectors of the embedment were glued to plastic blocks with epoxy and were then mounted on an LKB ultrotome making 1–2 μm sections parallel to the long axis of the implants. The sections were mounted on glass slides and were stained with hematoxylin-eosin, van Gieson, toluidine blue-basic fuchsin, and methylene blue.

The sections were scrutinized for evidence of a direct contact between implant and surrounding bone. They were also analyzed morphometrically using a Merz grid to estimate the amount of periosteal and endosteal new bone formation. The total length of bone-implant contact in each section was also measured.

Results

There was no postoperative wound infection. On gross examination the tissue surrounding the implants was free of discoloration, and the implants were all macroscopically stable in the bone.

Microscopic findings

In 73 of the 76 cases, the typical tissue reaction was a periosteal and endosteal proliferation of new bone bordering one half to three fourths of the implant circumference without an intervening soft-tissue membrane (Figure 3). Whereas the extent of endosteal proliferation was quite constant regardless of implant material and observation time, the periosteal new-bone formation varied greatly at 4 months to become uniformly distributed at 11 months (Table I). There was no infiltration of inflammatory cells at the interface.

In no case was the entire implant surrounded by a soft-tissue membrane. However, three cases (two pure titanium, one polished stainless steel—all 4 months' observation time) had an atypical appearance with only small areas of direct bone/implant contact and with the major part of the interface consisting of a soft-tissue membrane, apparently formed by downward growth from the periosteum. In these cases, there was no bone proliferation periosteally.

In sections stained with methylene blue or toluidine blue-basic fuchsin, particularly at 4 months, small areas of cartilage-like appearance were observed (Figure 4), with no relation to a particular material (Table 1). Another finding of uncertain significance was the dark-staining line seen on the edge of the bone at the interface (Figures 4 and 5). This line was never continuous; it was seen without relation to a particular material; and it was not related to the presence of the cartilage-like areas mentioned above. It was also more common at 4 months (Table 1).

A thin, clear zone peripheral to the stained edge of the tissue (Figure 5) was seen around all of the materi-
Figure 3. Low-power view of the typical tissue reaction to the implants, in this case pure titanium. The contour of the removed implant (I) is clearly seen. Soft tissue covers the upper end of the implant. The interface between the horizontal markers has a direct bone-implant contact without an intervening, continuous soft-tissue membrane. The periosteal new bone formation in this case is rated ++ (van Giesoo, x25).

Figure 4. Tissue section in a case of pure titanium at 4 months. A small area with the appearance of chondroid tissue is indicated by arrows. This area is also bordered by a dark-staining line. However, the rest of the interface shows a direct bone-implant contact (methylene blue, x150).

als, although with different frequency (20/38 pure titanium implants, 1/10 Tivanium* implants, 4/10 Vitallium* implants, and 14/18 of the steel implants). Peripheral to this clear zone was the epoxy, which means that the clear zone must have formed before the embedding was separated from the implant.

Minute (< 1 µm) metallic particles were seen on the edge of the tissue sections in 9/10 Tivanium* and 3/10 Vitallium* cases. There was no cellular reaction to these particles.

Discussion

Comments on method

Implant biocompatibility is not defined by a single parameter, but on recordings of biological responses in standardized test systems; our views on biocompatibility are modified with improved sensitivity of our test methods.

As pointed out by Ling (1986), the same implant material can provoke a spectrum of reactions in the tissue depending on its mechanical environment during the healing phase. For this reason, great care has been taken in this study to provide identical healing conditions for all of the materials, and to study only materials and surface finishes that are in clinical use today. Moreover, the histologic method has been developed solely for metallic bulk implants (Linder 1985).

In situ embedding of tissue and implant preserves the interface in its entirety, and a cellular reaction should therefore not escape detection. Consequently, with the present method the apparent lack of a tissue reaction is not false, as may be the case if cells are lost during embedding or torn out with the implant in a mechanical test prior to embedding. Instead, the lack of inflammatory change and a direct bone-implant contact should be regarded phenomenologically as absolute and significant observations.
Comments on findings

The aim of this investigation was not primarily to grade the biocompatibility of the materials. The fact that 73/76 cases fulfilled the definition of osseointegration (Brånemark et al. 1977; Albrektsson 1985) does not necessarily imply that the materials have identical biocompatibility; it shows that a certain latitude is acceptable to the tissue and that osseointegration as such may not be an absolute discriminator of biocompatibility. However, this theoretical uncertainty should not obscure the important message of the findings: Osseointegration is a response of bone to a tolerable implant material inserted under tolerable conditions.

In 3/76 cases the implants were incompletely invested in bone. A toxic influence is unlikely, because the bone present at the interface was newly formed and appeared viable. Mechanical instability or insufficient primary bone-implant contact is a more probable explanation (Carlsson et al. 1988). With the exception of these 3 cases, the bony reaction was remarkably consistent; and of the recorded histologic parameters, only three showed variation—periosteal proliferation, dark-staining lines, and cartilage-like areas at the interface. The significance of the two last-mentioned is unknown; they were either histologic artifacts or in vivo events. However, the variation was related to the time of observation rather than the nature of the implanted material.

Clinical relevance

Implants of pure titanium can regularly become osseo-integrated in the human tibia in arthrosis and rheumatoid arthritis (Linder et al. 1988). It is extremely unlikely that this applies exclusively to implants of pure...
titanium. Indeed, Hicks suggested as early as 1958 that "zero reaction" (an interface without a fibrous membrane) was the normal response of bone to an inert metal. This prophetic statement was based on histologic observations of steel implants and made at a time when pure titanium was hardly used.

When seen in the clinical perspective, the results of the present study strongly indicate that failure to obtain osseointegration of a joint prosthesis is not due to the use of, for instance, titanium alloy or chrome-cobalt alloy, but instead to an inability to create a biological environment suitable for bone formation. This includes both the surgical handling of the bone as a tissue and the postoperative mechanical conditions at the interface.

The clinical debate today is focused on whether or not to use bone cement. If formulated in that way, the discussion may well miss the essence of the problem, because both cemented and uncemented implants have been shown to be anchored in bone, provided proper healing conditions prevail (Ling 1986). However, the clinical reality today is that bony anchorage is unpredictable and that fixation in fibrous tissue is the most common finding (Thomas et al. 1987). This shows that the biological healing potential of bone tissue is not utilized to its full extent. If the problem were instead stated as osseointegration or fibrous fixation, its solution would call for a tissue-oriented approach rather than a material-oriented one. This should result in new surgical techniques and implant designs that together would give the surgeon better control of the healing conditions and thereby a possibility to create a predictable interface tissue.

References


Acknowledgements

Ms Lisbeth Lindberg prepared all the histologic sections with great skill and patience.

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