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Wound-healing phenomena around percutaneous devices implanted in rabbits

J. A. JANSEN, J. P. C. M. VAN DER WAERDEN, K. DE GROOT
Biomaterials Research Group, University of Leiden, Rijnsburgerweg 10, 2333 AA Leiden, The Netherlands

The major factor determining percutaneous implant success is the formation of a stable skin–implant junction. The objective of this study was to gain more insight into the mechanisms underlying implant–skin reactions. For this purpose, plasma-sprayed, dense hydroxyapatite and titanium implants were inserted into the tibia and on the cranium of 15 rabbits. The implants were left *in situ* for 9 months. Clinical and histological investigations were performed. It was found that especially direct attachment to bony skeletal tissues favours the longevity of percutaneous implants. No differences in soft tissue reaction between the various implant materials was observed.

1. Introduction

There are many different applications for percutaneous implants in medicine. Presently, some of the uses include: catheter perfusion or dialysis, skeletally attached artificial limbs, power supply to cardiac-assist devices, and artificial hearing aids. However, a limiting factor for the extended use of percutaneous devices is that most of the implant systems fail over prolonged periods of implantation [1]. The most common failure modes are marsipulization, permigration and infection [2]. As all of these failure modes are related to the skin reaction to the implant, the formation of a stable skin–implant junction in the area where the implant protrudes through the skin appears to be the major factor in determining the success of percutaneous implants.

In previous reports [3, 4] we demonstrated that stabilization of the percutaneous device is a method for achieving a long-term failure-free percutaneous passage. The required stabilization was obtained by: (1) skeletal attachment of the percutaneous implant or (2) subcutaneous insertion of the implant on a bony surface. The direct or indirect attachment of the devices to the bony skeletal tissues reduced the movement of the percutaneous implant relative to the surrounding tissues and benefited the longevity of the percutaneous implant.

The purpose of this paper is to describe the results of further investigations with these percutaneous implants in rabbits.

2. Materials and methods

2.1. Implants

Two types of implants were used for the experiments: (1) flange-shaped dense ceramic implants for insertion on the cranium of rabbits and (2) hydroxylapatite-(HA-)coated titanium implants for insertion into the tibia of rabbits.

2.1.1. Flange-shaped ceramic implants (Fig. 1)

The implants feature an extracutaneous and subcutaneous flange of diameter 6 and 10 mm, respectively. The diameter of the percutaneous component was 3 mm and the length of the implants was 9 mm.

The implants were prepared from commercially available HA powders [5]. By compression and subsequent sintering at 1300° C, 97 to 99% dense HA was obtained. The HA consisted of 90% apatite and 10% Whitlockite.

2.1.2. HA-coated titanium implants (Fig. 2)

Three versions of implants were constructed. The basic shape of all implants was that of a cylinder with a length of 15 mm and a diameter ranging from 2 to 3 mm. The enossal part of all the implants was made of Ti_6Al_4V and was coated with a layer of HA. The HA coating was applied to a thickness of approximately 50 μm using a plasma-spray technique [6]. For the skin-penetrating component, two different

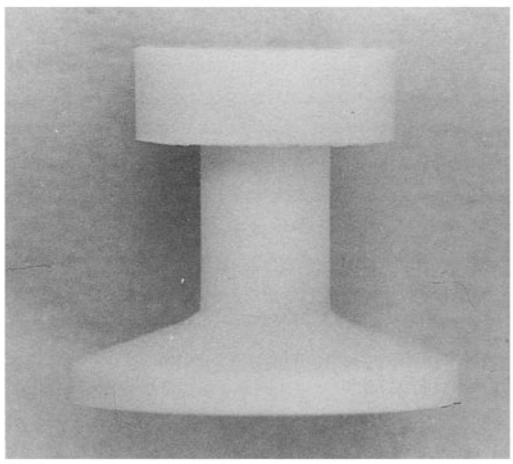

Figure 1 Flange-shaped cranium implant.

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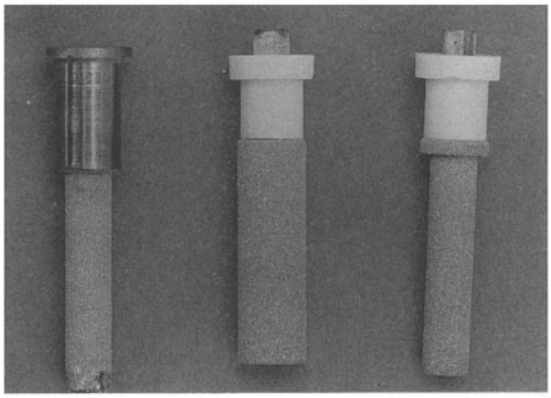

Figure 2 Various types of tibia implants.

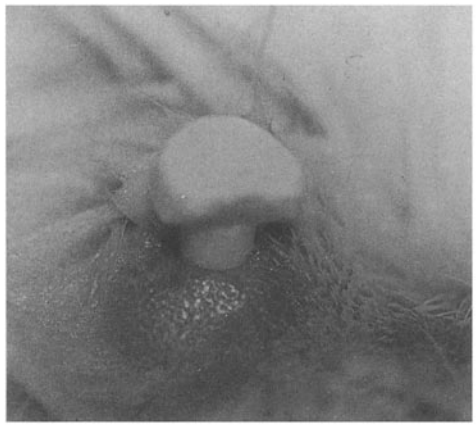

Figure 3 Clinical appearance of a cranium implant, showing no adverse tissue reaction, 9 months after insertion.

materials were used. The tibia implants had partly a percutaneous surface made of dense HA and partly a surface made of Ti_6Al_4V.

2.2. Animals

Fifteen adult New Zealand White rabbits were used in this experiment. During surgery the rabbits were sedated by intramulscular injection of Hypnorm (Duphar, Amsterdam). In all rabbits the area of implantation was anaesthetized by subcutaneous administration of Lidocain.

The implants were inserted on the cranium and into the tibia of the rabbits. Before insertion the implants were sterilized in an autoclave. A total of 15 cranium and 30 tibial implants were placed. The implants were inserted under aseptic conditions. The implanation procedures were described in [3, 4]. In brief, for the cranial implants an incision was made above the frontal area of the skull, a subcutaneous pocket was created and the flange-shaped implant was inserted. For the tibia implants a longitudinal incision was made on the medial surface of both legs. Then a hole was drilled and the implants were installed.

The implants were left *in situ* for 9 months. All rabbits were inspected weekly and during these inspections the percutaneous implantation sites were carefully cleaned. Monthly radiographs were taken of each tibia implant. At the end of the implantation time the rabbits were killed by injecting Nembutal peritoneally.

2.3. Histological procedures

After killing the rabbits, the implants with their surrounding tissues were excised immediately and fixed in 10% buffered formalin solution for histological processing. The tissue specimens were embedded in methylmethacrylate. After polymerization non-decalcified thin (10 μm) sections were made using a standard diamond-blade sawing microtome technique [7]. This simple method provides sections that can be used directly for light microscopy without the need of extended grinding or polishing procedures. The sections give clear cellular detail of the original tissue–implant interface.

3. Results

3.1. Clinical observations

The results of the percutaneous implants in the rabbits are outlined in Table I.

Of the cranium implants, one implant was lost because the implant had fractured. The rest of the cranium implants functioned without any clinical sign of inflammation (Fig. 3). Although there was macroscopically no inflammatory reaction, in three cases the skin was retracted from the implant neck and the subcutaneous flange was exposed. The skill implants were not immobile, but could still be moved.

Of the tibia implants, four showed an inflammatory reaction, which occurred directly following placement

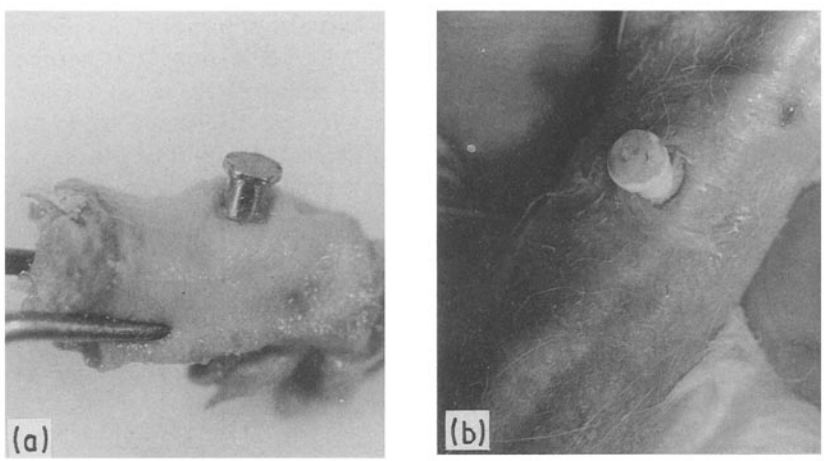

Figure 4 Clinical picture of two successful tibia implants. (a) Tibia implant with a percutaneous component made of Ti_6Al_4V and (b) tibia implant with an HA percutaneous component.

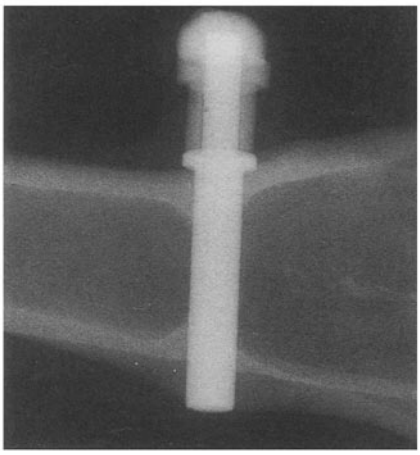

Figure 5 Radiograph of a tibia implant 9 months postoperatively.

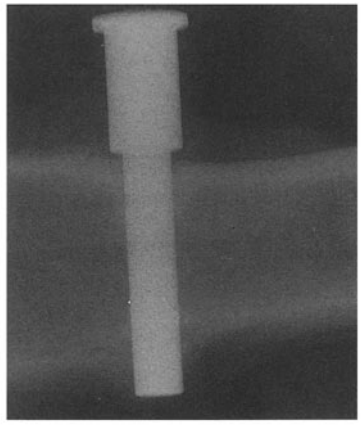

Figure 6 Radiograph demonstrating the dislocation of a tibia implant.

of the implants. All of the other implants showed an excellent healing and tissue adaptation without clinical signs of failure (Fig. 4). The implants were immobile during the whole experimental period. The radiographs demonstrated that there was no bone resorption, rather new bone was being formed around the neck of the implant (Fig. 5). Although the implants had a good fit in the predrilled hole, in some instances the implants were extruded from the implant bed. This dislocation caused a small space between the tibial bone and the percutaneous part of the implant (Fig. 6).

3.2. Histological observations

Light-microscopic analysis of the cranium implants did not confirm the clinical findings. Most sections demonstrated downgrowth of the epidermis along the subcutaneous flange of the implants, and a sinus tract was formed between the migrating epidermis and the implant surface (Fig. 7). The sinus was filled with keratin. In most cases, when epidermal downgrowth occurred, the superficial epidermis remained in close contact with the neck of the implant. Despite the process of sinus tract formation, no inflammatory reaction was observed in the dermal connective tissue (Fig. 8). In all specimens a fibrous capsule was formed around the entire subcutaneous flange of the cranium implants. The connective tissue fibres were orderly arranged parallel to the implant surface (Fig. 9).

Histological evaluation of the tibia implants showed a major difference in epidermal tissue response compared with the cranium implants. Although, similarly to the cranium implants, a sinus was formed by epidermal migration along the implant surface, the epidermal downgrowth did not progress any further than a maximum depth of 2.5 mm (Fig. 10). The sinus was also filled with keratin. At the bottom of the sinus the epidermis ended in apposition to the implant surface. The epidermis at the bottom was thin. In some cases the morphological arrangement of the implant–dermal junctional epithelium showed the same characteristics as a normal junctional epithelium surrounding natural teeth (Fig. 11). The dermal connective tissue was free of inflammatory cells. There were no differences in wound healing around the titanium and HA implants.

Examination of the bone–HA coating interface revealed that organized bone tissue was in intimate contact with the HA-coated implant surface without any intervening fibrous tissue layer (Fig. 12). There were no signs of coating loss by biodegradation or resorption. At the cortical level of the bone no angular resorption was observed.

Occasionally, as also demonstrated radiographically, the implants were dislocated after insertion. In these cases epidermal downgrowth reached to the HA-coated part of the implants (Fig. 13). At the base the skin tissue was in contact with the HA-coated surface. The space between the base and the percutaneous part of the implants was filled with keratin. The tissue immediately surrounding the implants also showed a lack of inflammation and there was a good bone adaptation.

4. Discussion and conclusions

The outcomes of this study confirm partly the findings of our earlier experiments. The results demonstrated that a good and durable percutaneous passage can be obtained when provisions are met to minimize the mechanical stresses at the interface between the implant and the skin. It is also shown that, in contrast with the tibia implants, epidermal downgrowth around the cranium implants is retarded but not completely inhibited. Therefore, it can be concluded that only stabilization of percutaneous implants is not enough to prevent epidermal migration. Stabilization,

TABLE I Survey of the clinical findings

Implants	Skin retraction from implant neck	Signs of inflammation	Undisturbed wound healing	Implants failed due to loss
Cranium HA	3		11	1
Tibia HA		2	17	
Tibia Ti_6Al_4V		2	9	

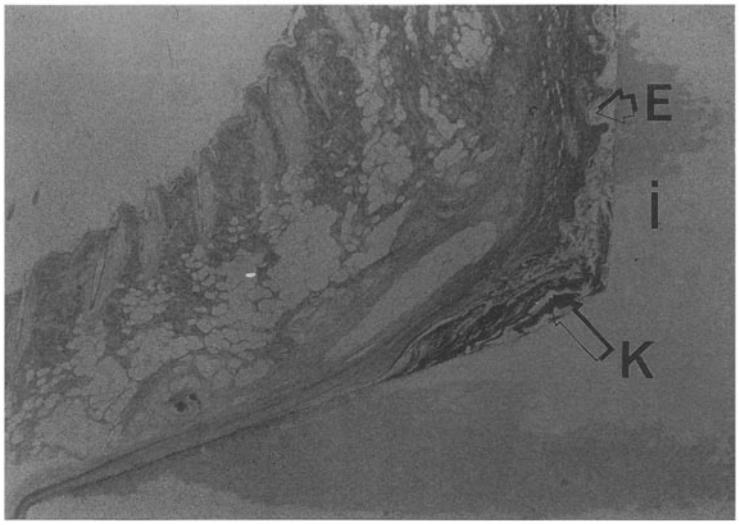

Figure 7 Histological section of a skull implant 9 months after implantation. The epidermis (E) surrounding the implant (i) shows a tendency to grow downwards. The sinus tract is filled with keratin (K). The superficial epidermis remained in close local contact with the implant surface. Original magnification $\times 72$, bar = 300 μm.

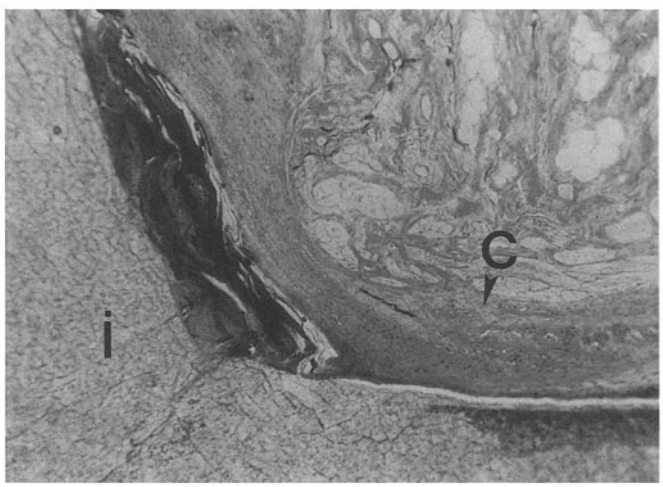

Figure 8 Despite the downgrowth of the epidermis along the implant surface (i), the connective tissue (C) is always free of inflammation. Original magnification $\times 200$, bar = 100 μm.

without proper fixation (e.g. into bone), will lessen but not eliminate the mechanical interface stresses.

On the other hand, it should be noted that there are no signs of acute or chronic inflammation in the soft tissues surrounding the cranium implants. Apparently, the integrity of the percutaneous area is maintained due to the lack of mechanical trauma. Therefore, it may be hypothesized that the presence of a fibrous capsule around an implant does not impede the development of an adequate epithelium–implant attachment, which forms a barrier against the ingress of bacteria or injurious agents.

In addition to percutaneous implant failure, the above hypothesis also has consequences for the interpretation of reasons underlying failure or success of permucosal dental implants. Permucosal implants are anchored into the alveolar bone and penetrate the oral mucosa. Two types of bone responses to these implants can be distinguished. The first type involves the formation of a connective tissue capsule around the implants. The second type is characterized by direct bone–implant contact without an intervening connective tissue layer. It is supposed [8–10] that neither bone response guarantees success if no healthy and firmly gingival attachment apparatus is present. It is also known from histological studies [11–13] that, besides a periodontal type of breakdown of the soft tissues, a connective tissue capsule is present around the enossal part of failing permucosal implants. The results of this study now demonstrate that the

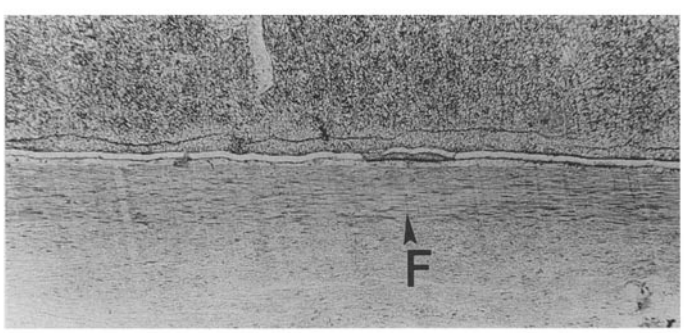

Figure 9 A thin fibrous tissue capsule (F) surrounds the subcutaneous flange of the cranium implants. Original magnification $\times 200$, bar = 100 μm.

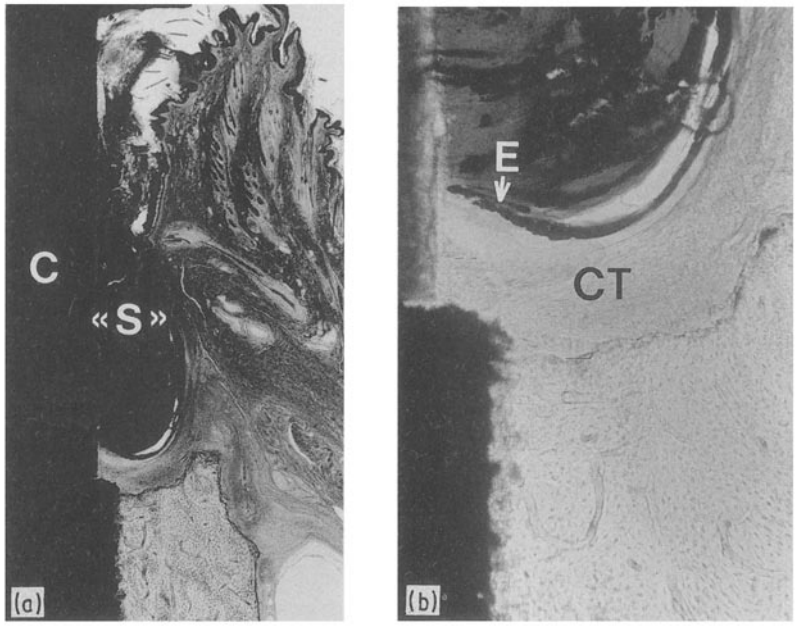

Figure 10 Histological reaction of a tibia implant with a ceramic percutaneous component (C). A limited downgrowth of the epidermis is observed. The epithelium (E) at the bottom of the sinus is thin. The sinus tract (S) is filled with keratin. The connective tissue (CT) below the epithelium is free of inflammation. (a) Original magnification ×50, bar = 400 μm and (b) original magnification ×200, bar = 100 μm.

presence of a capsule does not prevent the formation of a functional epithelial attachment. Therefore, failure of dental implants must be attributed to a pathological mobility of the implant in the implant socket causing destruction of the gingiva–implant attachment. It thus appears, consistent with the statement of Brånemark *et al.* [14], that: (1) direct bone bonding has to be preferred for the long-term functioning of dental implants and (2) capsule formation has to be considered as an unfavourable situation. However, at this point it may be appropriate to mention that, even in the case of a proper bony fixation, enossal implants will still fail if the permucosal–cutaneous seal is disturbed. This seal can be maintained only when the underlying connective tissue is attached or closely apposited to the implant surface via perpendicular or circular collagen fibres, respectively [15, 16].

In summary, our experiments have demonstrated that direct attachment to bony skeletal tissues favours the long-term results with percutaneous implants. These findings may have several clinical applications. However, in many situations where percutaneous conduits are indicated, solely soft-tissue anchored systems have to be used. Therefore, all experimental efforts have to be directed to the development of new devices that prevent epidermal migration alongside the implant surface and can be implanted in soft tissues without any bony support.

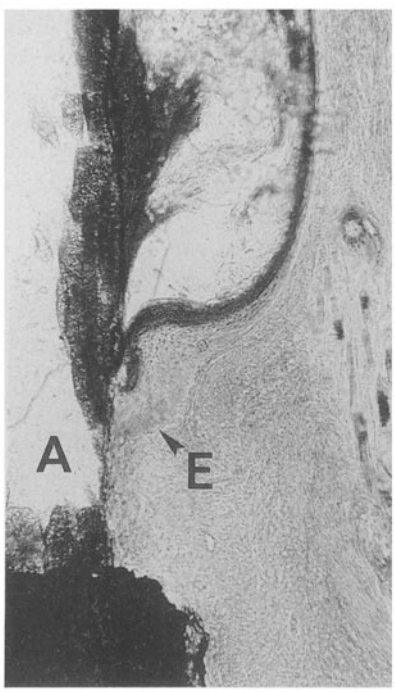

Figure 11 Percutaneous passage of a tibia implant with a percutaneous part made of HA (A). The epithelium (E) terminates in direct apposition to the implant and shows the same characteristics as the gingiva–tooth attachment apparatus. Original magnification ×200, bar = 100 μm.

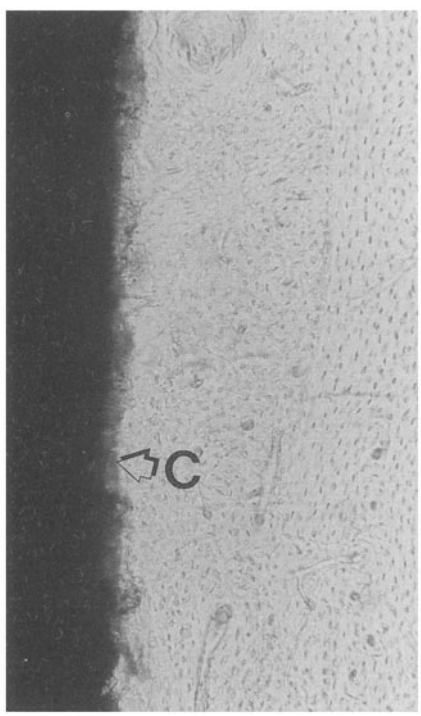

Figure 12 Non-decalcified section, showing the intimate contact between bone and HA coating (C). No intervening fibrous tissue layer is observed. Original magnification ×200, bar = 100 μm.

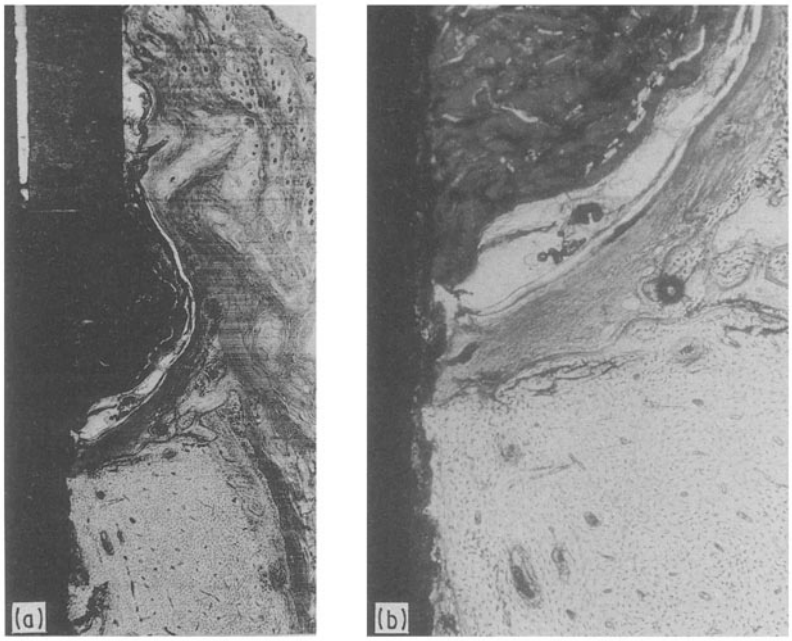

Figure 13 Light micrograph showing the skin tissue adjacent to a dislocated tibia implant. (a) Original magnification ×72, bar = 300 μm and (b) original magnification ×200, bar = 100 μm.

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