JOURNAL OF MATERIALS SCIENCE: MATERIALS IN MEDICINE 3 (1992) 126–136

A quantitative comparison of the cell response to commercially pure titanium and Ti–6Al–4V implants in the abdominal wall of rats

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Commercially pure (c.p.) titanium and Ti–6Al–4V implants were inserted in the abdominal wall of rats. The surrounding fluid space, inflammatory cells and fibrous capsule were evaluated after 1, 6 and 12 weeks. Light-microscopic morphometry demonstrated a fluid space around both implant materials which gradually decreased with time. Macrophages were preferentially distributed close to the implant surface in the innermost zone (0–25 μm from the surface). In contrast, fibroblasts and endothelial cells were located mainly in the outer three zones (25–100 μm from the surface). At all time periods studied and around both materials, lymphocytes were detected throughout the surrounding tissue. The outer border of the fibrous capsule, which consisted of macrophages, fibroblasts, endothelial cells and collagen, was difficult to define, in particular during the early phase of healing. At later time stages, 6 and 12 weeks, no difference in width (60–90 μm) was observed between the two materials. No major quantitative differences with respect to the number of different cells, fluid space width and fibrous capsule thickness were noted between the two materials studied. The observed mild inflammatory reaction and the absence of statistically significant differences between c.p. titanium and Ti–6Al–4V in soft tissue indicate that both materials could be suitable for use in soft tissues. In the context of previous comparative studies it may be concluded that the animal species as well as the different implantation locals play an important role in the determination of biocompatibility.

1. Introduction

Commercially pure (c.p.) titanium and Ti–6Al–4V represent some of the most frequently used implant materials in dental and orthopaedic surgery. The alloy has often been preferred to the pure metal because of its good mechanical properties combined with a high resistance to corrosion [1], material characteristics which are regarded as important for hard tissue implants. However, before the introduction of the titanium alloy there was no published evidence of its biocompatibility properties or biological behaviour, and still to this date there are no quantitative studies on hard tissue and very few investigations of soft tissue reactions to Ti–6Al–4V found in the literature. Recent hard-tissue studies [2–4] have presented experimental evidence of a qualitative as well as a quantitative nature which indicates that c.p. titanium is surrounded by more bone and is more rigidly fixated than is the titanium alloy. The mechanism responsible for this difference is not completely understood, although one potentially important contributing factor could be a leakage of element particles/aluminium from the alloy [4].

The purpose of the present study was to use similar implant materials to the ones used in our previous study in bone and to evaluate the cellular response around c.p. and alloyed titanium in soft tissue. It has been shown that solid implants of c.p. titanium have a low tendency to elicit inflammation in soft tissue in comparison with polymers [5, 6] as well as nitrogen ion-implanted c.p. titanium [7].

2. Experimental procedure

2.1. Animals and anaesthesia

Adult Sprague–Dawley rats (Alab, Södertälje, Sweden) weighing 250–300 g and fed on a standard pellet diet and water *ad libitum* were used. The rats were anaesthetized by i.p. injections of a mixture of nembutal (60 mg ml^{-1}), diazepam (5 mg ml^{-1}) and saline in 1:2:1 volume portions.

2.2. Implants and surgical technique

C.p. titanium and Ti–6Al–4V implant plugs consisting of a cylindrical rod (length 3 mm, diameter 2 mm)

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connected to the centre of a circular plate (thickness 0.5 mm, diameter 4 mm) were manufactured (Figs 1 and 2). The surface topography of the implants was examined with scanning electron microscopy (SEM) (Jeol JSM T 300, Japan) and with a Taylor Hobson Form Talysurf equipment (Rank Taylor Hobson Ltd, UK). With the latter equipment the surface structure of the implants was characterized. By performing numerical measurements of the average difference between the five highest peaks and the five lowest valleys within the traverse length (300 μm) on the unfiltered profile, the R_z value was calculated.

Prior to insertion the implants were cleaned in trichloroethylene and rinsed in absolute ethanol in an ultrasonic bath and finally sterilized in an autoclave. Each rat had two implants inserted in the abdominal wall, one of each metal, at a distance of 10 mm apart. The rod portion of the implant was inserted through a hole in the peritoneal membrane while the circular plate remained outside the peritoneum [5].

2.3. Preparation of the specimens

The follow-up time was 1, 6 and 12 weeks with seven animals in each group. At the day of sacrifice the rats were anaesthetized and fixed by perfusion via the left heart ventricle with 2.5% glutaraldehyde in 0.05 M sodium cacodylate, pH 7.4. The implants with surrounding tissue were left in glutaraldehyde overnight and then postfixed in 1% OsO_4 for 2 h. The samples were then dehydrated and finally embedded in epoxy resin (Agar 100, Agar Aids, Stansted, Essex, UK).

In order to prepare thin sections of the plastic-embedded tissue–metal interface, the bulk metal was removed using an electrolytical dissolution (electropolishing) technique [8]. In brief, the bottom plate portion of the implant was exposed by grinding and the rod portion connected to the sample holder (made of c.p. titanium) of an electrolytic cell. The assembly, except for the exposed surface of the bottom plate, was insulated by lacquer (Lacomit, Canning & Co., UK) and served as an anode when mounted in the electro-

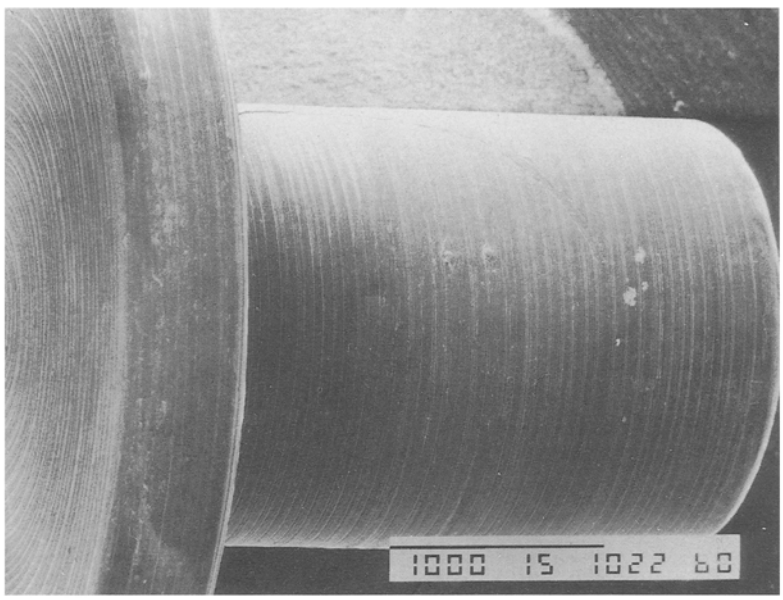


Figure 1 Scanning electron micrograph of a c.p. titanium implant. The cylindrical rod has a length of 3 mm and a diameter of 2 mm which is connected to the centre of a circular plate that has a thickness of 0.5 mm and a diameter of 4 mm.

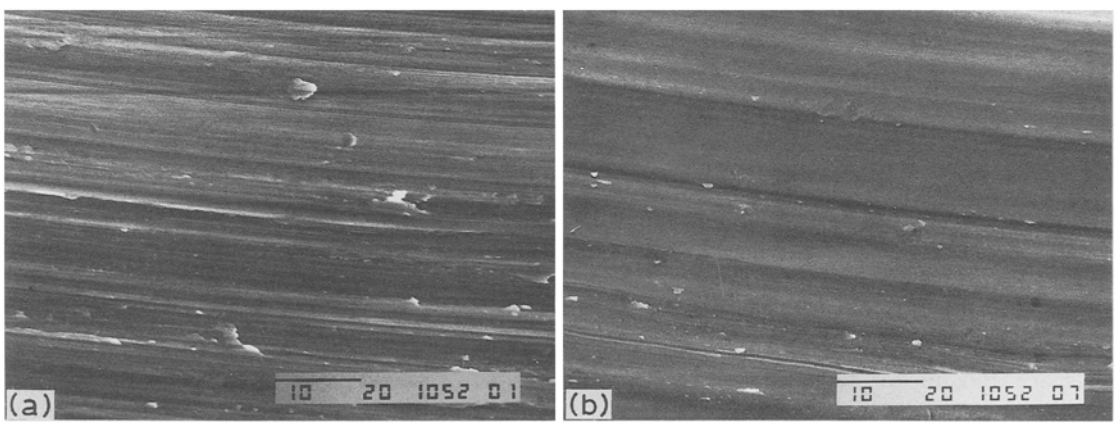


Figure 2 Scanning electron micrographs of (a) c.p. titanium and (b) Ti–6Al–4V surfaces. The surfaces examined were on the circular plate facing the rod portion of the implant (corresponding to the area used for morphologic analysis). Grooves and ridges, typical for machined metal surfaces, are present on both implant surfaces. Bar = 10 μm.

polishing equipment. The sample was surrounded by a cylindrical platinum cathode and electropolishing performed in an electrolyte (5% perchloric acid, 35% n-butanol and 60% methanol) under cooling and vigorous stirring. The current was intensiostatically controlled until the intended value of 24 V was reached, whereafter the potentiostatically controlled process was continued until the plate portion was completely removed. The specimen was disconnected from the cell, rinsed in water and re-embedded in epoxy resin. Sections were then cut on an ultramicrotome using glass knives for 1 μm semi-thin sections (light microscopy, LM). Sections for LM were stained with Richardson's solution, i.e. 0.5% Azur II and 0.5% methylene blue in 1% disodium tetraborate. Selected areas were cut and stained with uranyl acetate and lead citrate for transmission electron microscopy (TEM) (Philips EM 400 or Zeiss CEM 902).

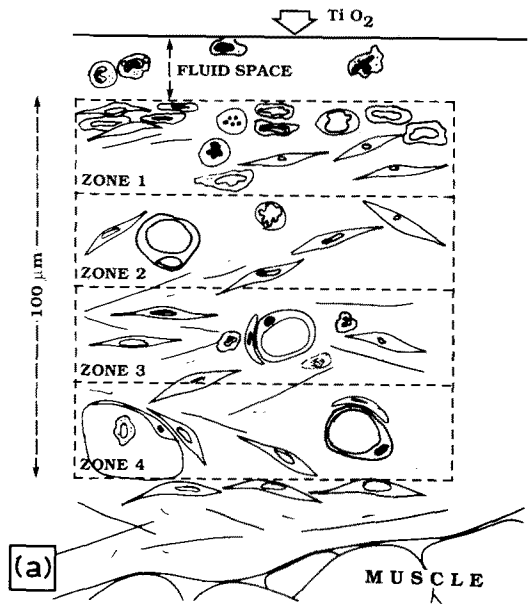
2.4. Morphometry

Semi-thin sections were analysed essentially according to Röstlund *et al.* [7] using a Leitz microscope with a 40 × objective and a 10 × eyepiece fitted with a square grid. The tissue used for LM morphometry was located adjacent to the surface of the plate portion of the implant facing the peritoneal layer (Fig. 3a). A dense line, i.e. the metal oxide that remained after the electrochemical removal of the bulk metal, could be observed in the sections. The width of the space (fluid space) between this line and the organized tissue was determined. The tissue peripheral to the fluid space was divided into four zones (each 25 μm depth × 100 μm length; 2500 μm^2) with zone 1 encompassing the part of the tissue immediately adjacent to the fluid space (0–25 μm from the fluid space) and zone 4 furthest away from the fluid space (75–100 μm from the fluid space). In each zone, the number of polymorphonuclear granulocytes (PMNGs), monocytes/macrophages and fibroblasts, as well as other cells i.e. mast cells, mononuclear cells, endothelial cells, extravascular erythrocytes and unidentified cells, were determined. In each section, fluid space and cell measurements were made in five different areas along the interface. The first area measured was located 250 μm from the rim of the implant and the remaining four areas were located consecutively, separated by 150 μm, along the interface. The thickness of the newly formed tissue around the implants (mean at five points along the interface and with equal distances apart) was determined on each section by measuring the width of the organized connective tissue (Fig. 3b).

2.5. Statistics

One section per animal was quantitatively analysed and mean values (cell numbers per zone, the width of the fluid space and fibrous capsule) of five different measurement areas calculated. The statistical evaluation was performed on the mean values (plotted data in tables) in each group (1 week: c.p. titanium $n = 5$, Ti–6Al–4V $n = 7$; 6 weeks c.p. titanium $n = 7$, Ti–6Al–4V $n = 7$; 12 weeks c.p. titanium $n = 6$, Ti–6Al–4V $n = 5$).

Multivariate analysis of variance, modified Hotelling T^2 test and analysis of variance were used for determination of significant differences at the 5% level.



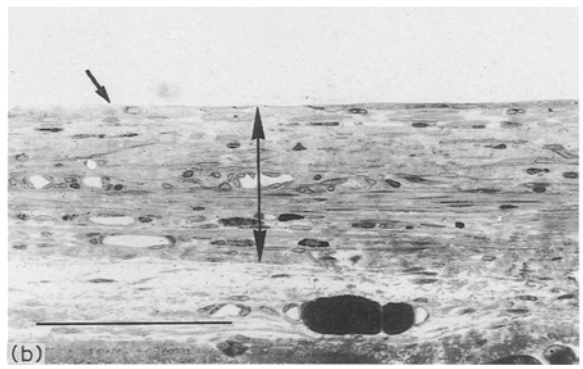


Figure 3 (a) Schematic diagram of the implant-close fluid space and tissue used for morphometry. (b) Survey light micrograph showing the connective tissue close to the implant surface. The small arrow points to the implant surface whereas the double-headed arrow shows the width of the organized connective tissue, regarded as the fibrous capsule (Ti–6Al–4V implant at 12 weeks of follow-up. Bar = 100 μm).

3. Results

3.1. Surface topography

Implant surface topography was evaluated with SEM and profilometer measurements. SEM did not reveal any major differences in the topography (Fig. 2a and b). With the Form Talysurf equipment the microsurface irregularities were calculated, giving R_z values of 3.0 for c.p. titanium and of 1.7 for the alloy.

All 21 animals survived the follow-up periods. One week after insertion two of the seven c.p. titanium implants were detected in the peritoneal cavity, whereas all alloy implants were present at the surgical site. After 6 weeks of follow-up, all 14 implants could be investigated. After 12 weeks, one c.p. titanium and two alloy implants were detected in the abdominal cavity.

3.2. Light microscopy

In all sections examined at 1 week after surgery a fluid space was present between the surface oxide, which was observed as a thin dark line, and the surrounding tissue (Fig. 4a and b). The width of the fluid space varied within the same section. This space contained scattered inflammatory cells and in some sections also erythrocytes. Although not quantified, the majority of cells in the fluid space were monocytes and macrophages. One week after surgery, the mean width of the fluid space was 28 μm for c.p. titanium and 67 μm for the alloy (the latter value was due to one extremely large width observed in one animal) (Fig. 7). Irrespective of material, the fluid space diminished with time. After 6 weeks the space was on average smaller around the c.p. titanium implants (mean 5 μm) in comparison with the titanium alloy (mean 26 μm). At 12 weeks the tissue was very close to the surface oxide. No statistically significant differences in fluid space width were observed between the materials at any time period (Fig. 7).

Around both materials a connective tissue capsule had formed (Figs 3b, 5, 6 and 8). This capsule consisted mainly of elongated fibroblasts, macrophages and blood vessels. After 1 week the tissue was not well organized and the outer limit of a supposed fibrous capsule was less distinct (Fig. 4a and b). Therefore, we judged measurements of fibrous capsule thickness at 1 week as uncertain. After 6 weeks a slightly thinner capsule was observed around the c.p. titanium im-

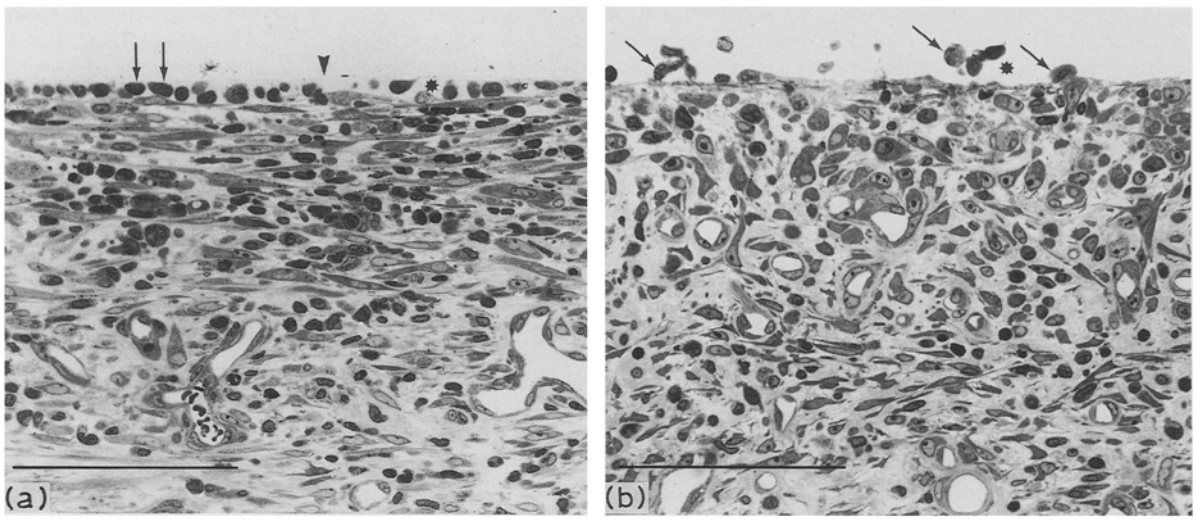


Figure 4 Light micrographs of tissue adjacent to implants. The bulk metal was removed electrochemically. (a) C.p. titanium 1 week after insertion. A fluid space (asterisk) separates the implant surface (thin line marked by arrow-head) from the tissue. Macrophages (some of which are indicated by arrows) are present in the fluid space and tissue. Bar = 100 μm. (b) Ti–6Al–4V 1 week after insertion. The fluid space contains scattered macrophages (arrows). These cells are also present in the richly vascularized connective tissue. No sharp outer boundary of a supposed fibrous capsule may be determined. Fluid space (asterisk). Bar = 100 μm.

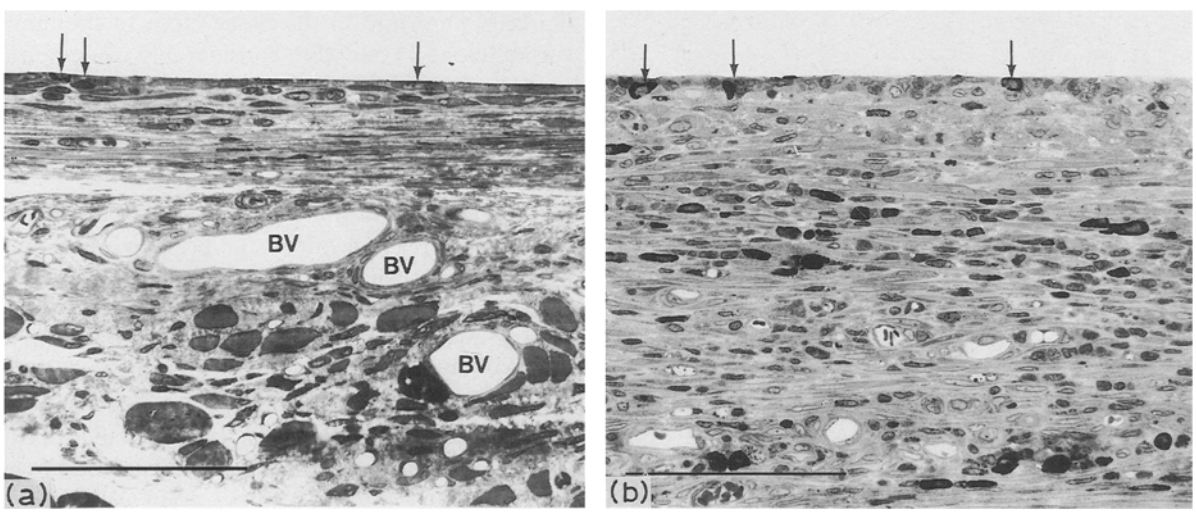


Figure 5 Light micrographs of tissue adjacent to implants. The bulk metal was removed electrochemically. (a) C.p. titanium 6 weeks after insertion. Macrophages (some of which are indicated by arrows) are located close to the implant surface. A dense connective tissue is present (consisting of elongated fibroblasts and macrophages). Large blood vessels (BV) are detected 1–200 μm from the implant surface. Bar = 100 μm. (b) Ti–6Al–4V 6 weeks after insertion. A rather dense connective tissue has been formed. Macrophages occupy the tissue close to the implant surface. Mast cells are present in the connective tissue capsule and are in some places located immediately adjacent to the implant surface (arrows). Bar = 100 μm.

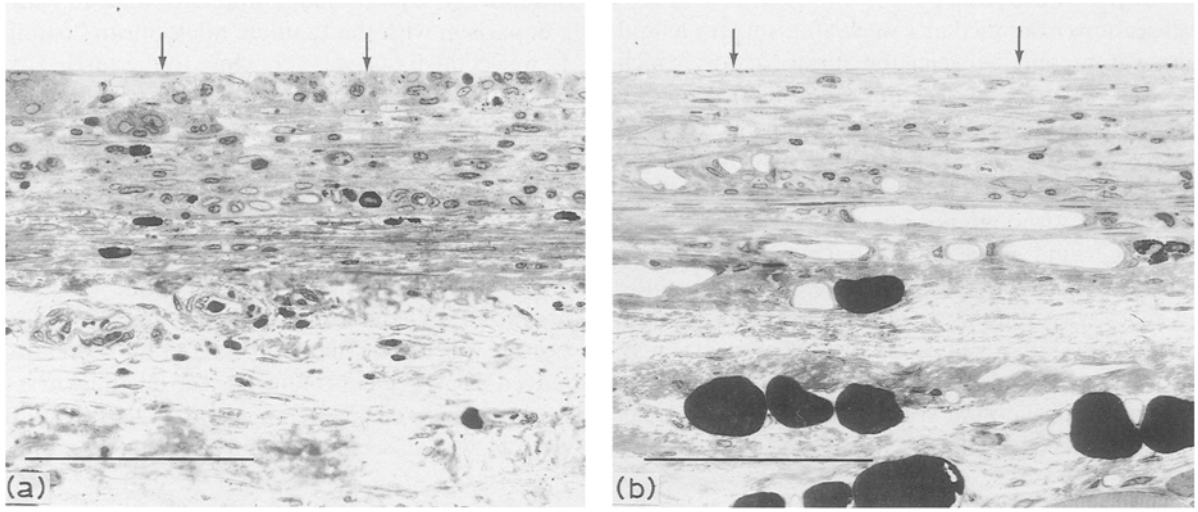


Figure 6 Light micrographs of tissue adjacent to implants. The bulk metal was removed electrochemically. (a) C.p. titanium 12 weeks after insertion. The tissue close to the implant consists of an about 100 μm wide dense connective tissue. The implant surface is marked by arrows. Bar = 100 μm. (b) Ti–6Al–4V 12 weeks after insertion. A dense connective tissue has been formed around the implant. A fluid space is not detected, instead cells are closely attached to the implant surface (marked by arrows). Blood vessels are frequently detected, in particular in the outer part of the dense connective tissue. Bar = 100 μm.

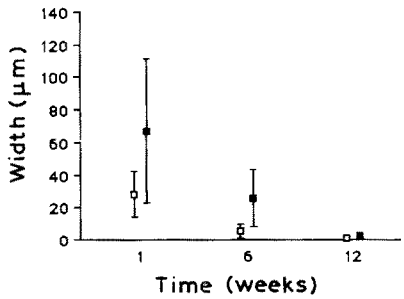


Figure 7 Width (mean ± SEM) of fluid space around (□) c.p. titanium and (■) Ti–6Al–4V implants 1, 6 and 12 weeks after surgery.

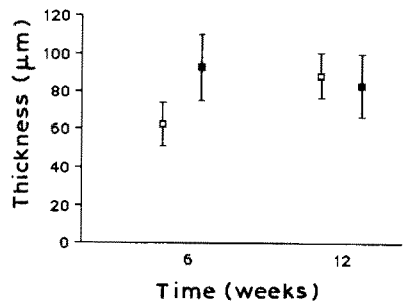


Figure 8 Thickness (mean ± SEM) of fibrous capsule around (□) c.p. titanium and (■) Ti–6Al–4V implants 6 and 12 weeks after surgery.

plants (63 μm) than around the alloy implants (93 μm). The values did not differ significantly from those obtained from 12 weeks (Fig. 8).

LM morphometry revealed the presence of different cell types in the tissue around the implants. The majority of cells, irrespective of the material, was macrophages and fibroblasts. On average, a slightly larger number of macrophages was observed around c.p. titanium implants (Fig. 9). Irrespective of material and observation time, the number of macrophages decreased with increasing distance from the implant surface. Thus, macrophages were preferentially distributed close to the implant surface in zone 1.

Fibroblasts were detected throughout the observed area and were more frequent in zones 2 to 4 than were macrophages (Fig. 10). Independent of the follow-up period, a peak in the fibroblast numbers around the c.p. titanium and alloy implants was observed in zones 2 and 3, that is 25–75 μm from the surface. No significant differences in the number of fibroblasts were observed between the two materials.

With the exception of macrophages, the majority of mononuclear cells in the tissue appeared to be small lymphocytes (Fig. 11). These cells appeared at all time periods and around both materials (data not shown). However, in comparison with fibroblasts and macrophages the number of mononuclear cells was low (in general less than one per zone).

The morphometric analysis revealed that endothelial cells were rare in the tissue close to the implant surface (zone 1) but were considerably more frequent in the outer zones (significant difference) around both materials (data not shown). No major differences were noted between the materials and at different time periods after surgery.

Mast cells could not be detected in the tissue at 1 week after insertion but a significant increase in cell numbers was detected after 6 and 12 weeks (data not shown). No statistically significant difference between the two materials was observed. Around both materials PMNGs were infrequently detected. The total number of PMNGs in the tissue decreased with time.

Multinucleated giant cells were extremely rare in the observation area around the implants after 1 week. However, after 6 and 12 weeks a few cells were observed on the surface of both materials (data not shown).

In all sections, cells were present which could not be identified. The number of unidentified cells was about 4% of the total number of cells counted (c.p. titanium: 3.86% and titanium alloy: 3.90%).

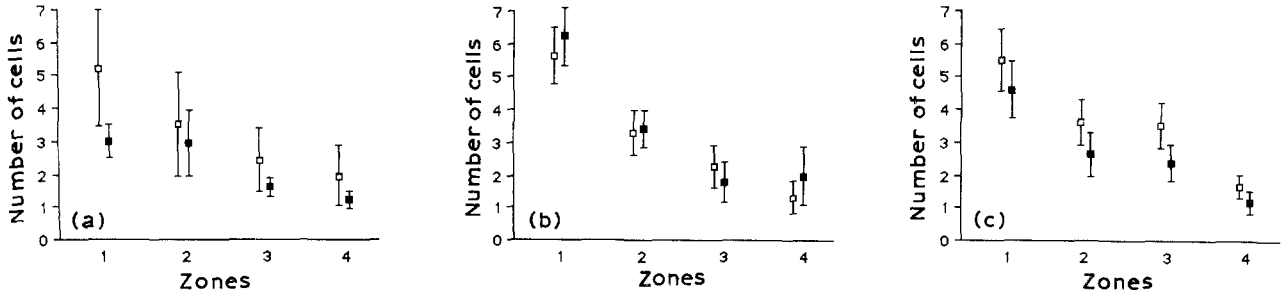


Figure 9 Distribution of macrophages (No. of cells/2500 μm²) in tissue adjacent to (□) c.p. titanium and (■) Ti–6Al–4V implants (a) 1, (b) 6 and (c) 12 weeks after surgery. Zone 1: 0–25 μm, zone 2: 25–50 μm, zone 3: 50–75 μm and zone 4: 75–100 μm from the fluid space. Data are mean ± SEM.

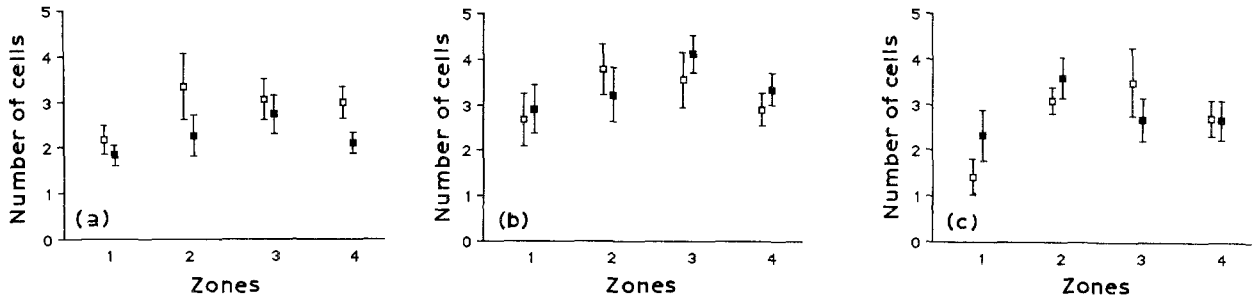


Figure 10 Distribution of fibroblasts (No. of cells/2500 μm²) in tissue adjacent to (□) c.p. titanium and (■) Ti–6Al–4V implants (a) 1, (b) 6 and (c) 12 weeks after surgery. For definition of the zones, see Fig. 9. Data are mean ± SEM.

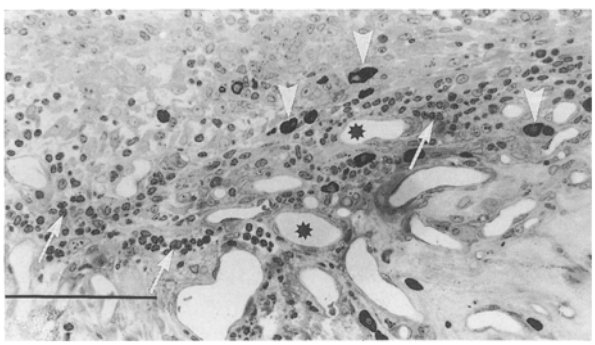


Figure 11 Light micrograph of tissue close to a c.p. titanium plug 12 weeks after insertion. Several lymphocytes (arrows), mast cells (arrow heads) and blood vessels (asterisk) are present. Bar = 100 μm.

3.3. Transmission electron microscopy

In the electron microscope the attention was mainly focused on the organization of cells located close to the implant surface, that is within zone 1. As shown in Fig. 12, the implant surface appeared in sections as a dark line, about 10 nm wide, which represents the oxide layer constituting the surface of the implant [8]. No apparent differences in the structure or dimensions of the oxide layer between the two types of implant could be distinguished. Neither was there any apparent feature in the structure or relative amount of different cell types located close to the implants which could be related to any type of implant material. Therefore the observations described below are valid for both types of implant.

As described, the implants were after 1 week surrounded by a fluid space (Fig. 12) containing proteinaceous material and scattered inflammatory cells, mainly monocytes/macrophages. Few cells were

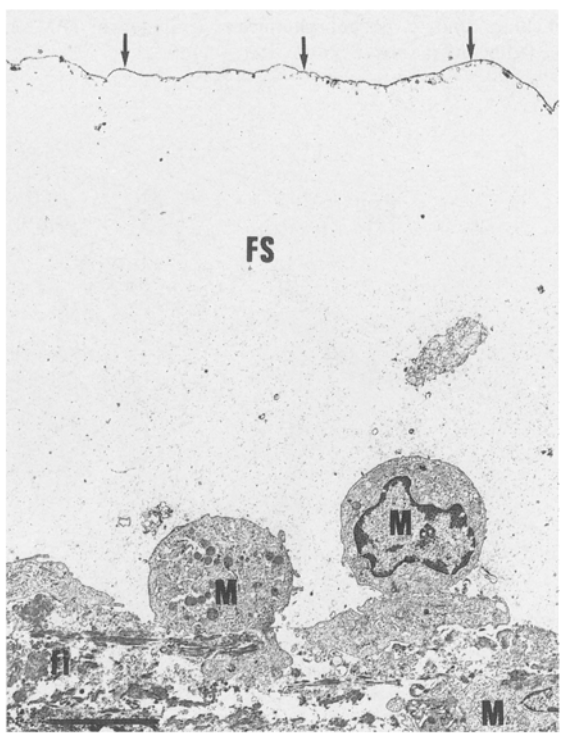


Figure 12 Low-power TEM micrograph of a Ti–6Al–4V implant 1 week after insertion. The implant surface, marked by the oxide layer (arrows) remaining after electropolishing, is separated from the reorganizing tissue by a fluid space (FS). Macrophages (M) and fibrin (fi) deposits are bordering the fluid space. No cells are attached to the implant surface. Bar = 5 μm.

attached to the implant surface. The border of the tissue towards the fluid space was formed by distinct strands of fibrin to which macrophages, most often elongated with cytoplasmic extensions, were adhering (Figs 12–14). Adjacent to this border zone, macrophages

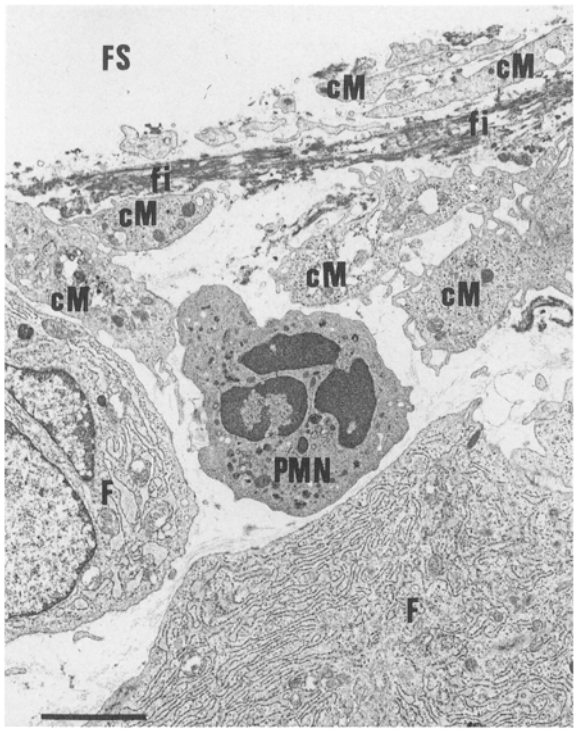


Figure 13 C.p. titanium implant after 1 week. The border between fluid space (FS) and tissue is formed by numerous cytoplasmic extensions (cM), belonging to macrophages and a layer of fibrin (fi). Fibroblasts (F) containing large amounts of rough endoplasmic reticulum and a polymorphonuclear granulocyte (PMN) are located below the tissue border. Bar = 3 μm.

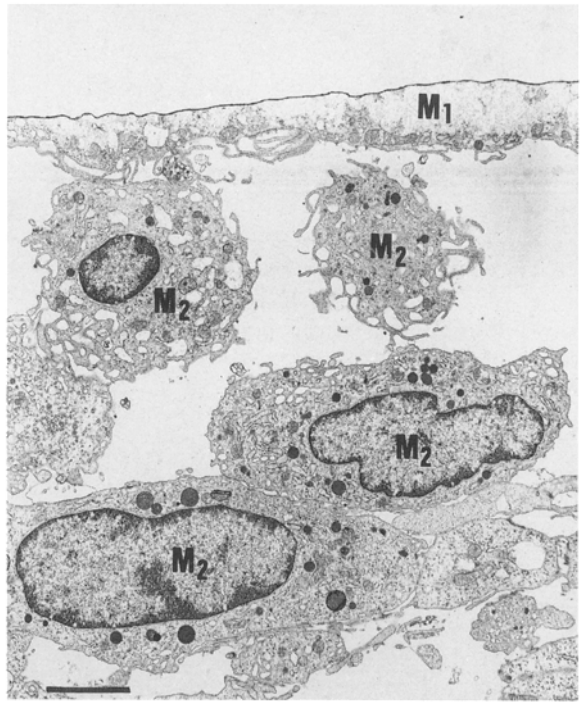


Figure 15 C.p. titanium implant after 12 weeks. An attenuated macrophage (M_1) is adhering to the implant surface. The adjacent tissue consists of loosely packed macrophages (M_2) ("phagocytic type") characterized by an irregular cell surface and numerous vesicles and lysosomes in their cytoplasm. Bar = 3 μm.

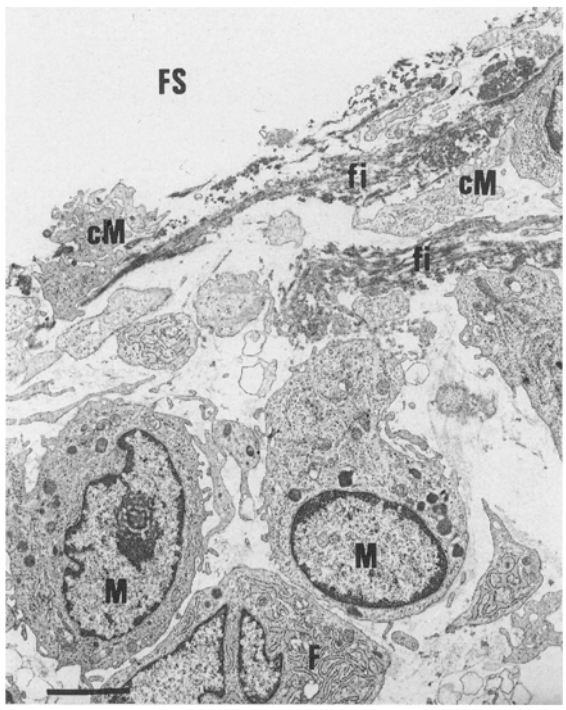


Figure 14 C.p. titanium implant after 1 week. Another illustration of the tissue–fluid space (FS) border formed by cytoplasmic extensions (cM) of macrophages. In deeper parts macrophages (M) and a fibroblast (F) are located. Fibrin (fi). Bar = 3 μm.

(Fig. 14) and fibroblasts containing large amounts of rough endoplasmic reticulum, indicating a high protein-synthetic activity, were present.

After 6 and 12 weeks the fluid space was generally absent and cells had established contact with the implant surface. In some areas the cells close to the implant were rather loosely arranged (Fig. 15) but in general they were densely packed (Fig. 16). The by far predominant cell type in contact with the implant surface was macrophages. Most macrophages at the implant surface were closely applied to the surface, and a common feature was the subplasmalemmal accumulation of actin filaments (Fig. 16); this is a common feature of cells attached to a surface, as for instance a culture dish.

Fibroblasts in contact with the implants were in general separated from the surface by 1 to 3 layers of macrophages (Figs 16 and 17). The ultrastructure of the macrophages (within zone 1) after 6 and 12 weeks was variable. For descriptional purposes three main phenotypes could be distinguished. The first one, the "phagocytic macrophage" (Fig. 15), was characterized by an irregular plasma membrane forming numerous folds and by a cytoplasm containing vesicles, vacuoles and lysosomes but only small amounts of rough endoplasmic reticulum. The second type, the "secretory macrophage" (Fig. 16), was characterized by a bulky cytoplasm containing large amounts of rough endoplasmic reticulum and well-developed Golgi areas. The third type, the "quiescent macrophage" (Fig. 17), was characterized by an elongated cell shape with a nucleus occupying a relatively large fraction of the cell volume and with a cytoplasm containing low concentrations of any particular organelle. This type of macrophage was more common close to the implant surface after 12 weeks and was also found in the organized fibrous capsule surrounding the implants.

In deeper parts of the tissue, roughly corresponding

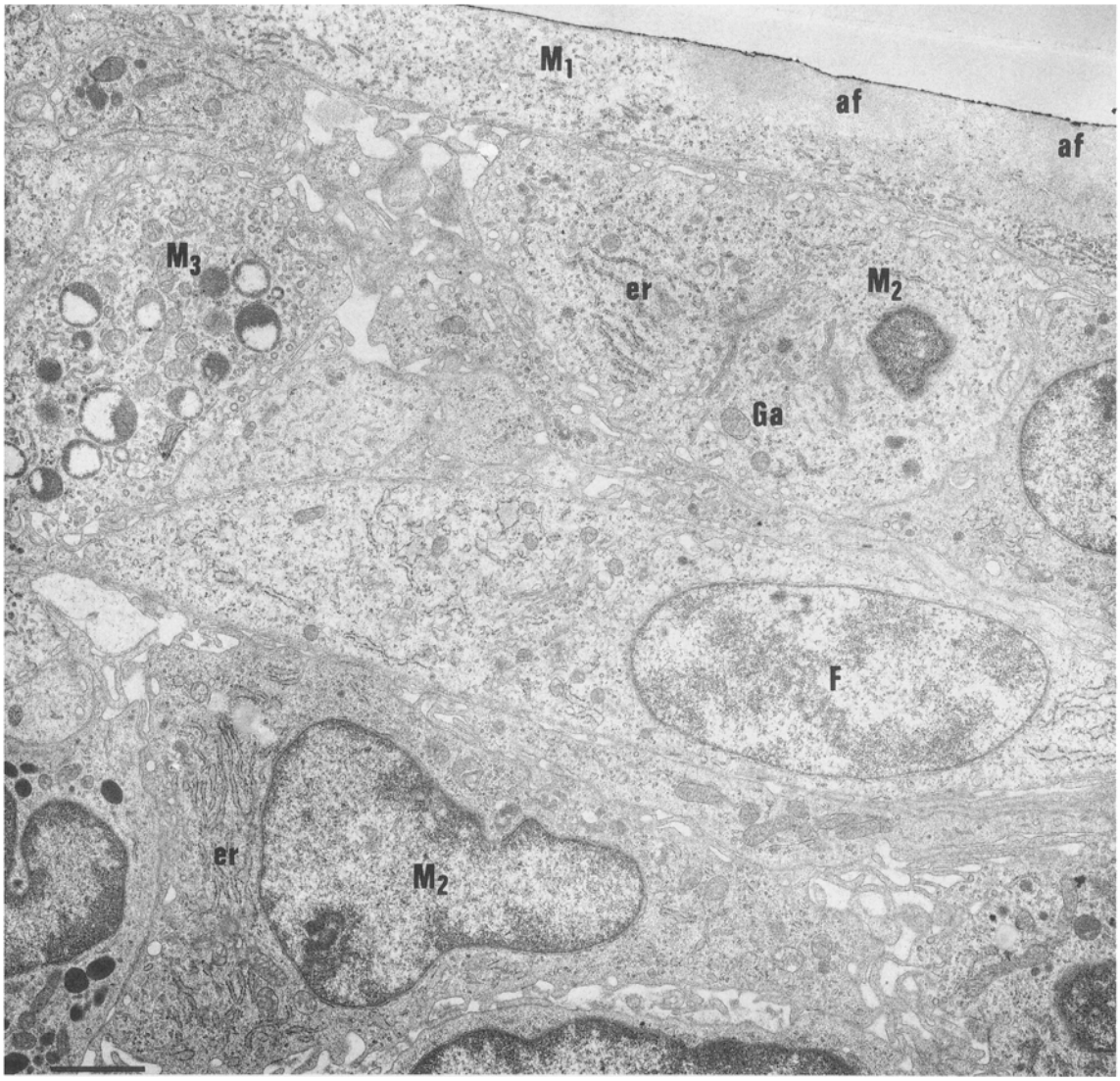


Figure 16 Ti–6Al–4V implant after 6 weeks. A flattened macrophage (M_1) is adherent to the implant surface. The cytoplasm adjacent to the implant surface contains accumulations of actin filaments (af). Two macrophages (M_2 "secretory type") containing large amounts of rough endoplasmic reticulum (er) and a well-developed Golgi area (Ga) and another macrophage (M_3 "phagocytic type") containing numerous vesicles and lysosomes are located at some distance from the implant together with a fibroblast (F) undergoing mitosis. Bar = 2 μm.

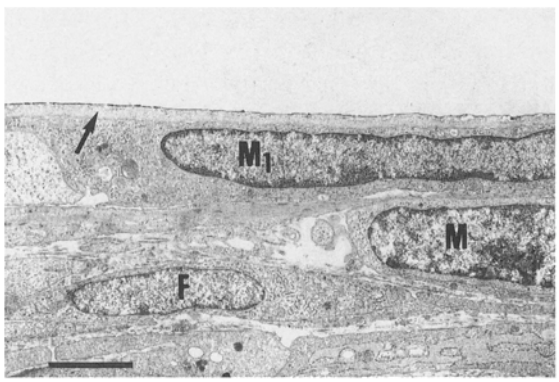


Figure 17 Ti–6Al–4V after 12 weeks. A flattened macrophage (M_1 "quiescent type") is separated from the implant surface by a thin rim of cytoplasm belonging to another macrophage. A macrophage (M) and a fibroblast (F) can also be identified. Bar = 3 μm.

to zone 2 as described above, fibroblasts and macrophages were predominant and these cells were arranged in a complex pattern together with capillaries, lymphocyte-like cells (Fig. 18), eosinophilic leukocytes and mast cells (Fig. 19). The extracellular matrix contained collagen fibrils which however were rarely encountered in zone 1 close to the implant surface (Fig. 19).

4. Discussion

The presence of inflammatory cells near-by implant surfaces has been reported around most types of biomaterials in hard and soft tissue and around both solid and particulate materials [9]. However, comparative and quantitative data on the cell types and their distribution close to implants are yet incomplete. Several factors may account for this. One reason is that few techniques which enable the study of cells, their distribution and degree of activation may actually be applied to the study of implant-close tissue. Conventional biochemical assays may in general not be applied due to the inaccessability and small amount of peri-implant fluid. Another reason, in particular for metal implants, is the difficulty of adequately preserving an intact metal–tissue interface for the study of implant-close cells. Since the disruption of an intact

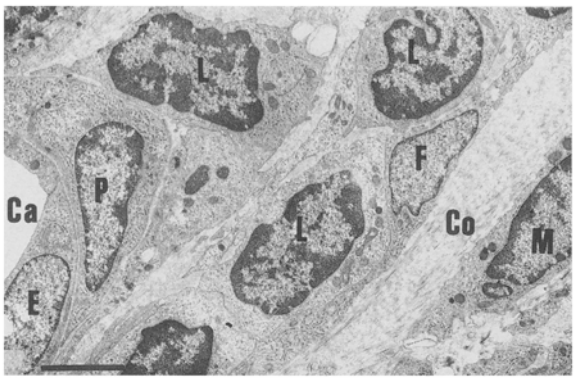


Figure 18 Ti–6Al–4V implant after 12 weeks. Lymphocyte-like cells (L) are located about 50 μm from the implant surface close to a capillary (Ca); E = endothelial cell, P = pericyte. A fibroblast (F) and a macrophage (M) as well as collagen fibrils (Co) are also present. Bar = 3 μm.

material surface–tissue interface and a loss of implant-close cells is an invariable result of preparation techniques, which separate implant and tissue before fixation, and resin-embedding, previous attempts to relate cells to the implant surface have been impeded.

In the present study the inflammatory reaction and the early healing of titanium and titanium alloy were quantitatively evaluated using sections with an intact metal oxide–tissue interface [8]. Although few quantitative differences existed between the two materials, macrophages, fibroblasts and endothelial cells were distributed in the surrounding tissue in a characteristic way. A prominent finding around both materials and at all time periods was the preferential distribution of a large number of macrophages close to the implant surface. This finding indicates that the surface properties of the material and the activation of cells and mediator systems by the surface may affect the recruitment as well as the persistence of cells close to the surface. The factors which govern the recruitment of monocytes and macrophages to the implant–tissue interface and their persistence are not fully understood.

Several chemotactic factors may be implicated: complement factor C5a [10] and interleukin 1 [11], have been detected in the exudate around polymers [12] and both factors have a chemotactic effect on several different cell types [13–15]. In the exudate around c.p. titanium, leukotriene B_4, which is chemotactic both for PMNGs and monocytes/macrophages [16–18], has been detected [6]. Another possibility is that metal ions and metal particles derived from the c.p. titanium and the Ti–6Al–4V surface had influenced the recruitment of macrophages. In fact, an *in vitro* study has shown cobalt, chromium and nickel ions to affect human granulocyte polarization and migration stimulated by chemotactic agents [19].

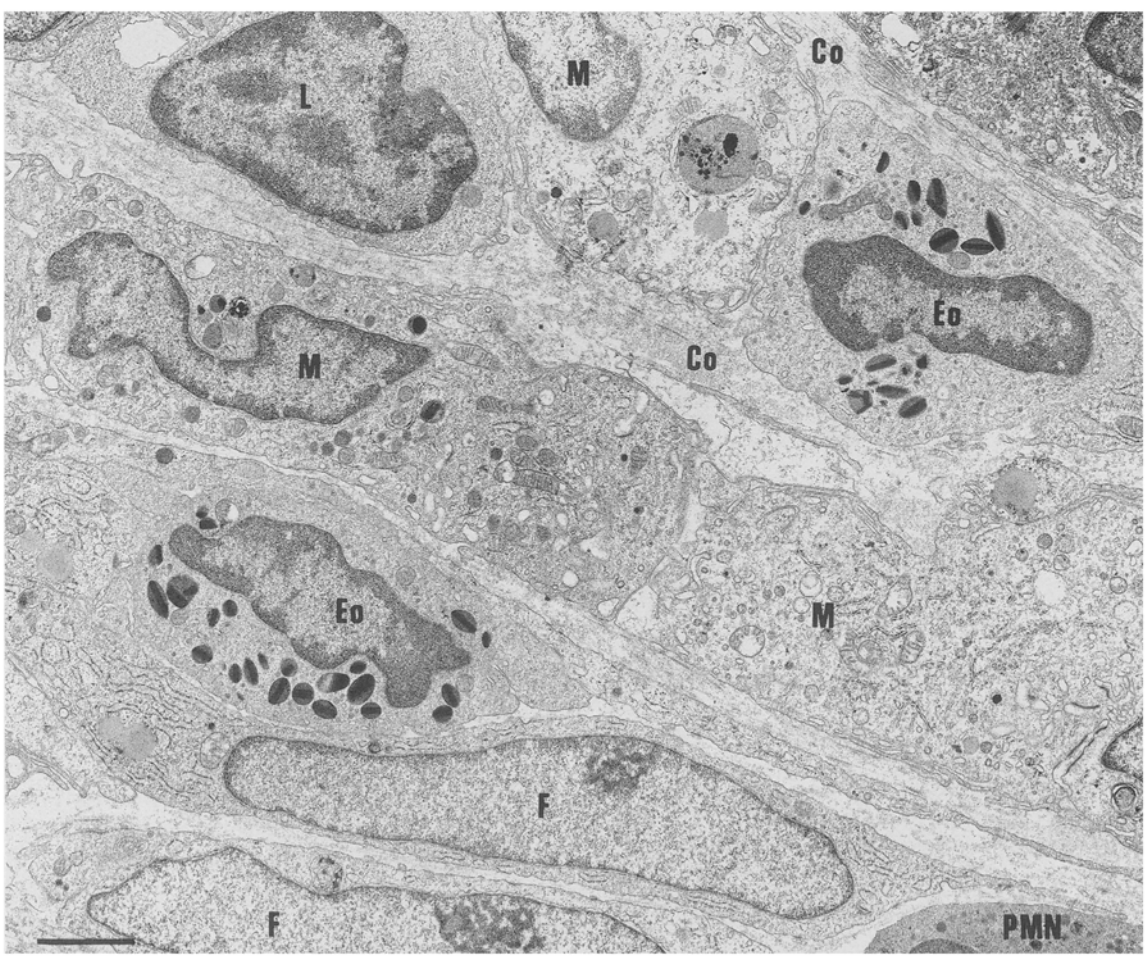


Figure 19 C.p. titanium implant after 12 weeks. Tissue located about 30 μm from the implant surface. The electron micrograph illustrates the complexity of the tissue organization. Macrophages (M), fibroblasts (F), eosinophil leukocytes (Eo), a polymorphonuclear granulocyte (PMN) and a lymphocyte-like cell (L) can be identified. Collagen fibrils (Co) are present in the extracellular matrix. Bar = 2 μm.

In the present study small mononuclear cells, light-microscopically different from macrophages, were located in the tissue adjacent to the implant. The ultrastructural observation of lymphocytes in the fibrous capsule suggests that the majority of these mononuclear cells were in fact lymphocytes. Thus, these cells were detected already after 1 week and persisted throughout the observation period up to 12 weeks. These findings corroborate previous experiments using immunocytochemistry [20]. These authors found that c.p. titanium implants inserted in rat muscle were surrounded by Ia-expressing cells and T-suppressor/cytotoxic cells [20]. Since these immunocompetent cells may be anticipated in immune responses, e.g. type IV hypersensitivity reactions, it is interesting that such cells have also been observed around clinically well-functioning bone-anchored hearing aids of pure titanium [21]. The activity and role of the lymphocytes around the titanium materials is at present unknown.

Following the acute inflammatory reaction after insertion, the repair phase in the tissue resulted in the formation of a fibrous capsule, external to the inner layer of macrophages. In the capsule, apart from fibroblasts and collagen, several different cell types were present, including macrophages, lymphocytes and endothelial cells. After 1 week the outer limit of a fibrous capsule was hard to detect and, thus, not subjected to quantitation.

Previous studies focused on the biocompatibility of metal as well as polymer implants have used the width of the capsule as a measure of the degree of biocompatibility. Laing *et al.* [22] divided different implant materials into four different groups, depending on the capsule thickness around the materials 6 months after implantation in rabbit muscle, and concluded that titanium and titanium alloy usually fell within groups 1 and 2, that is with capsule thickness 2–30 μm. In the present study the capsule width varied between 60 and 90 μm and no significant differences were observed between the materials or between 6 and 12 weeks. Several factors may account for the differences observed in the study of Laing *et al.* [22] and the present one. Firstly, differences in the definition of fibrous capsule width may be one possible reason. Laing *et al.* used "pseudomembrane thickness" and did not apparently include the width of the external "replacement of muscle with fibrous tissue and fat". Most likely, the pseudomembrane described by Laing *et al.* is equivalent to the innermost zone (zone 1), with an abundance of macrophages, in the present experimental study. Secondly, the different species and implantation time might be important. In a recent study in rat muscle, Therin *et al.* [23] concluded that the membrane thickness was not a time-dependent parameter nor material-dependent. In the latter study the authors found, in agreement with our data, no differences between c.p. titanium and Ti–Al–V with regard to capsule thickness or number of inflammatory cells.

The type of cells and tissue in contact with the implant may be crucial for the long-term fixation and function of the implant. In a previous study in rabbit tibia, c.p. titanium had a significantly higher degree of bone-to-implant contact and removal torque than had Ti–6Al–4V implants [3]. The reasons for these differences in bone, but not in soft tissues as revealed by the present study, are not understood at present. Apart from species differences (rabbit versus rat) several factors could account for the observed findings, such as differences in implant designs and shape (threaded fixtures versus plugs) and in micromovements and the fact that different tissues were studied (bone cells and mineralized matrix versus inflammatory cells and connective tissue). In both studies the c.p. titanium and Ti–Al–V surface topographies were rather similar. In none of the models were wear particles a conspicuous finding. Most likely, the micromotion of the abdominal plugs was more extensive than for the corresponding threaded implants in bone. Another possible explanation for the differences may be that corrosive processes and a leakage of metal ions into the surrounding tissue could influence the tissue response. The implant materials (c.p. titanium and Ti–Al–V) consist of oxides with different physicochemical characteristics [24–26]. Although both materials may be considered corrosion-resistant, it is known that all metals, even the most passive ones such as Ti and Ti–Al–V, release metal ions after implantation [27]. Partly due to technical difficulties in measuring element content and distribution, in particular in the bone immediately adjacent to implants, the majority of studies have focused on the distant organ accumulation of ions [28–30]. However, metal also accumulates around prostheses in humans, most likely as a result of both wear and corrosion [31, 32]. Therefore it is possible that metal ions, if accumulated locally, could affect interfacial cell function differently depending on the host tissue. In soft tissues titanium is seldom detected close to the implant surface of c.p. titanium plugs [33]. However, in bone titanium and aluminium are detected around Ti–Al–V implants [4]. Since Al ions inhibit mineralization *in vivo* [34] and aluminium is detected in the bone within 100 μm of the Ti–Al–V surface [4] it is possible that our observations of different biocompatibility between c.p. titanium and Ti–Al–V in bone but not in soft tissues could be due to a local effect of the metal ions on bone mineralization.

In conclusion, the present study revealed no major differences in the number of inflammatory cells, fluid space and fibrous capsule width between c.p. titanium and Ti–6Al–4V implant after insertion in the abdominal wall of rats up to 12 weeks. Since these findings in soft tissue are not in agreement with our earlier reported findings in rabbit bone, we conclude that for examination of the tissue response around implants both factors related to the species and the host tissue are of a great importance when determining the biocompatibility of a metal.

Acknowledgements

Mrs Lena Emanuelsson, Miss Petra Hammarström, Miss Maria Johannesson and Mrs Gunnel Bokhede are acknowledged for their skilful assistance. This study was supported by the Gothenburg Medical Society, King Gustaf V 80-year Fund, the Medical

Faculty, University of Gothenburg, the Swedish National Association against Rheumatism, the Swedish National Board for Technical Development (90-00536, 90-1480) and the Swedish Medical Research Council (grant Nos 06533, 09289 and 09495).

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Received 13 February
and accepted 5 March 1991