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Distribution of cells in soft tissue and fluid space around hollow and solid implants in the rat

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The development of the tissue surrounding an implanted material is anticipated to be regulated by the biological factors in the interface as well as the physicochemical properties of the implant material. In the present study light microscopic morphometry and transmission electron microscopy were used to evaluate the distribution of cells adjacent to the implant surface of different implant designs (hollow and solid implants) and materials (titanium and polytetrafluoroethylene). An increased number of leukocytes, predominantly PMN, was retrieved from the exudate inside hollow implants 1 and 9 days after surgery. In contrast, the increased cellularity in the soft tissue around the hollow implants was mainly due to an increased number of monocytes/macrophages and fibroblasts. The presence of a fluid space around both hollow and solid implants was revealed by the use of an electropolishing technique and ground sections. In the fluid space around solid titanium the concentration of leukocytes and the proportion of PMN decreased between 1 and 7 days. After 1 day the majority of leukocytes were freely suspended in the fluid and were rarely directly apposed to the implant surface. A majority of the monocytes/macrophages present in the fluid space after 7 days were attached to the fibrin matrix at the border between the fluid space and the reorganized tissue. Our studies demonstrate that hollow implants promote the influx and a persistence of PMN in the interior of the implant in comparison with the tissue surrounding the hollow and solid implants. Furthermore, during the first week after implantation inflammatory cells are not preferentially distributed directly on the titanium implant surface.

1. Introduction

Different non-biological materials (i.e. metals, ceramics and polymers) are used to replace damaged tissue and restore organ functions. The cellular and humoral reactions to non-biological implants are critical for the long-term outcome of an implanted device. A crucial part of this response is most likely played by the cells close to or in contact with the material surface. Previous studies [1–5] have shown that different materials elicit different inflammatory responses including leukocyte accumulation and release of inflammatory mediators. However, the mechanisms by which implants influence the tissue response are to a large extent unknown.

In studies of implant-soft tissue interactions *in vivo* it is of importance to use an experimental system which allows monitoring of activity of cells and composition of the tissue fluid in the interface zone. The fluid space adjacent to the implant surface cannot, for quantitative and sampling reasons, be analysed by conventional biochemical methods. Therefore, a hollow implant allowing retrieval of the tissue exudate for analysis *in vitro* was introduced [1]. Previous studies have shown that a fluid space containing cells is present during the early phase (1–2 weeks) after introduction of an implant in soft tissue [4–6]. However, the distribution of different types of inflammatory cells in this fluid space has not been evaluated quantitatively.

The aim of the present study was to analyse the number, types and distribution of inflammatory cells in the fluid space and tissue around polytetrafluoroethylene (PTFE) and titanium implants in soft tissue. The experiments were performed in the abdominal wall of rats using two types of implant configurations: hollow and solid implants.

2. Materials and methods

2.1. Animals

Male Sprague-Dawley rats (B & K Universal AB, Sollentuna Sweden), weighing about 250 g were used

(number of animals, titanium 1 d: 6; 3 d: 9; 9d: 7 and polytetrafluoroethylene (PTFE) 1 d: 11; 3 d: 9 and 9 d 7 rats). The rats were received at least 1 week before surgery and kept at the laboratory animal facility under good laboratory practice conditions. The experimental protocol was approved by the Animal Use Committee.

2.2 Anaesthesia

At least six animals were used in each group (see 2.1.). The rats were anaesthetized by an intraperitoneal (i.p.) injection (0.15 ml/100 ml body weight) of a mixture of nembutal (Mebumal vet®., ACO, Solna, Sweden, 50 mg/ml), diazepam, (Stesolid®, Dumex, Copenhagen, Denmark, 5 mg/ml) and saline in 1 2:1 volume proportions.

2.3. Implantation

2.3.1. Hollow implants

The present hollow implant model has been used in a series of experiments in order to analyse the cellular content retrieved from the interior of the hollow implants [1, 7–9]. In the present experiment morphology of the surrounding soft tissue was evaluated. A right paramedian incision was made through the shaved and cleaned (Jodopax, Ferrosan, Sweden) abdominal skin and further through the ventral part of the rectus sheath. The rectus muscle was then blunt divided longitudinally and a pouch between the muscle and the dorsal portion of the rectus sheath was created.

The hollow implant (with an upper part and screw made of c.p. titanium, and an exchangeable bottom portion consisting of either c.p. titanium or PTFE (Fluorseals SPA, Grumello del Monte, Italy) was placed with its neck portion penetrating through the muscle and with the bottom portion on the dorsal rectus sheath (Fig. 1). Prior to the sealing with a screw, the interior of the implant was filled with 30 µl of Hank's balanced salt solution (HBSS). Finally, the skin was closed with two silk sutures (Ethicon ® Inc, Sommerville, NJ, USA).

2.3.2. Solid implants

Machined, solid implants of c.p. titanium were inserted in the rat abdominal wall according to a previously described procedure [10] in order to analyse

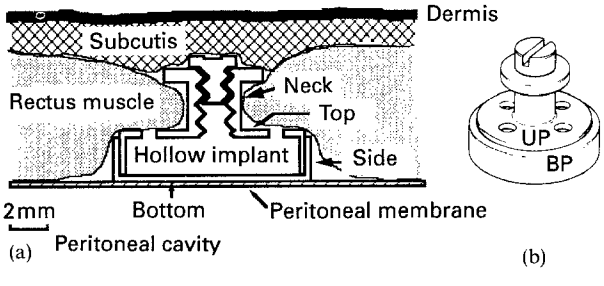


Figure 1 (a) A schematic cross-section of a hollow implant *in situ*. Different regions analysed are indicated in italics; (b) details of the implant (UP: upper portion; BP: bottom portion).

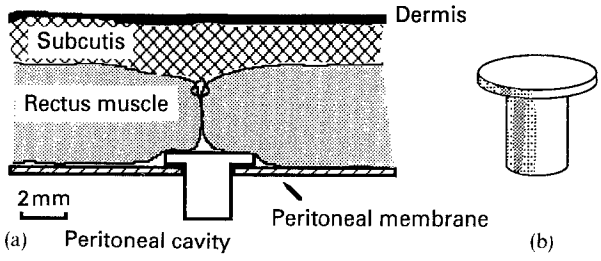


Figure 2 (a) A schematic cross-section of a solid implant *in situ*; (b) details of the implant.

the number and distribution of inflammatory cells in the fluid space and soft tissue around solid implants. The implants consisted of a cylindrical rod connected to the centre of a plate (Fig. 2). In brief, the same dissection technique as described above was used. The dorsal portion of the rectus sheath and the peritoneal membrane was penetrated by the rod portion protruding into the peritoneal cavity (Fig. 2). The muscle fascial layer and skin was sutured as described above.

2.4. Tissue preparation

2.4.1. Hollow implants

The rats with hollow implants were killed by an i.p. injection of a mixture of nembutal and diazepam in 1:1 proportions (0.35 ml/100 g body weight) after 24 h, 3 and 9 days. Immediately after sacrifice the skin was opened and the exudate collected by washing five times with HBSS using a syringe attached to a pipette giving a total volume of about 150 µl. Thereafter, the hollow implant and the surrounding tissue were excised *en bloc*. The tissue surrounding the hollow implant was trimmed to a thickness of about 2 mm and the specimen was fixed in 2.5% glutaraldehyde in 0.1 M sodium cocodylate, pH 7.2 and then in 1% osmium tetroxide for 2 h followed by dehydration in ethanol and embedding in epoxy resin (Agar-Aids, Stanstead, Essex, UK). After separation of the embedded tissue from the hollow implant, one µm thick sections were cut and stained with Richardsons's solution (1% Azur II, 1% methylene blue in 1% disodium tetraborate) for light microscopy (LM).

2.4.2. Solid implants

The rats with solid implants were fixed by perfusion via the left heart ventricle with 2.5% glutaraldehyde in 0.05 M sodium cacodylate, pH 7.4 after 24 h and 7 days. The implants were excised *en bloc* with the surrounding tissue and postfixed in 1% osmium tetroxide for 2 h. Specimens, embedded in epoxy resin as described above, were treated with an electrochemical procedure [11] in order to remove the bulk metal and make sectioning of the intact interface zone possible. In brief, the circular plate of the embedded solid implant was exposed by grinding and the rod was connected to an electrode. Similarly, the circular bottom portion of six hollow titanium implants was exposed by grinding and the upper part of the plate connected to an electrode. The surface of the specimen except for the ground surface of the implant was

insulated by lacquer and the specimen put in an electrolyte (5% perchloric acid, 35% n-buthanol and 60% mehtanol) and cooled to -30 °C. Electropolishing was performed at 22 V for up to 3 h with the specimen as anode. When the plate portion was completely removed the specimen was rinsed in water and re-embedded in epoxy resin before sectioning: 1 μm thick sections were cut with glass knives and stained as above for LM examination. For transmission electron microscopy (TEM), selected areas were cut with diamond knives and sections were contrasted with uranyl acetate and lead citrate.

About 10 μm thick ground sections [12] of 12 hollow implants (with the implant and surrounding tissue embedded in LR White) were also prepared.

2.5. Hollow implant exudate

After aspiration of the content, retrieved from the interior of the hollow implant, the total number of exudate leukocytes was counted in a Bürker chamber [13]. The viability was determined by trypan blue dye exclusion.

2.6. Morphology

2.6.1. Hollow implants

The tissue surrounding the hollow implants was analysed histologically by light microscopy. The cells were divided into four groups: (A) polymorphonuclear granulocytes (PMN), cells with a multilobulated nuclei often more intensive stained than nuclei of other cells; (B) monocytes/macrophages, slightly elongated with less intensely stained centrally located non-lobulated nucleus; (C) fibroblast-like cells (in text fibroblasts), with a smooth contour (length > three times the width) often with a prominent, oval, weakly stained nucleus, often with a prominent nucleolus; (D) multinuclear giant cells (MNC). LM morphometry was performed in a Leitz Dialux 20 EB light microscope (using 100 × oil-immersion objective and 10 × oculars (1000 × magnification)). In each specimen the cell content in five different circular areas of each region (neck, top, side and bottom) around the hollow implant (Fig. 1) was evaluated.

The number of cells were counted in a circular area (diameter 137.5 μm, area 14.850 μm^2) tangential to the border between the tissue and the fluid space. This approach was chosen as, it was not possible to exactly define the position of the implant surface in these specimens. The values given are calculated mean values of each area (neck, top, side and bottom).

2.6.2. Solid implants

Light microscopic morphometry of the tissue surrounding the solid titanium implants was performed in 1 μm thick sections using a Nikon FXA microscope with × 40 objective and a × 10 eye-piece fitted with a square-grid. In each section four different areas along the interface of the plate portion were analysed. The first area was located 200 μm from the edge of the implant and the remaining three areas located consecutively, separated by 120 μm, along the interface. Each area was subdivided into the fluid space and pheripheral to this four zones (each 20 μm deep and 80 μm length) (Fig. 4). Zone 1 encompassed the tissue immediately adjacent to the fluid space (0–20 μm from the fluid space) and zone 4 was located furthest away (60–80 μm) from the fluid space. In each zone the number of PMN, monocytes/macrophages, fibroblasts, mast cells, endothelial cells, multinuclear giant cells, lymphocytes and unidentified cells was determined. A separate analysis of the concentration and distribution of different cells in the fluid space was performed directly in the microscope (× 40 objective, × 10 oculars) (Leitz Metalux 3 equipped with a Microvoid image analysis system). The area of the fluid space, the length of the implant surface and the tissue, cell types and numbers and their distribution (free, adherent to implant surface of tissue) were determined

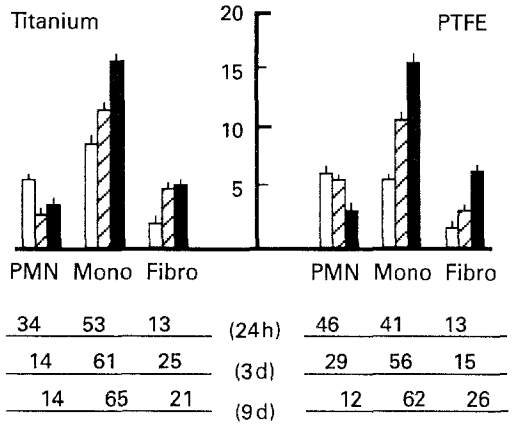


Figure 3 Morphometric data obtained from the tissue around the titanium and PTFE hollow implants (mean values obtained from the entire implant). The bars show the number (mean ± SEM) of PMN, monocytes/macrophages (Mono) and fibroblasts (fibro) per unit area 24 h (□), 3 days (▨) and 9 days (■) after surgery. The proportion of each cell type (%) after 24 h, 3 days and 9 days is given below the bars.

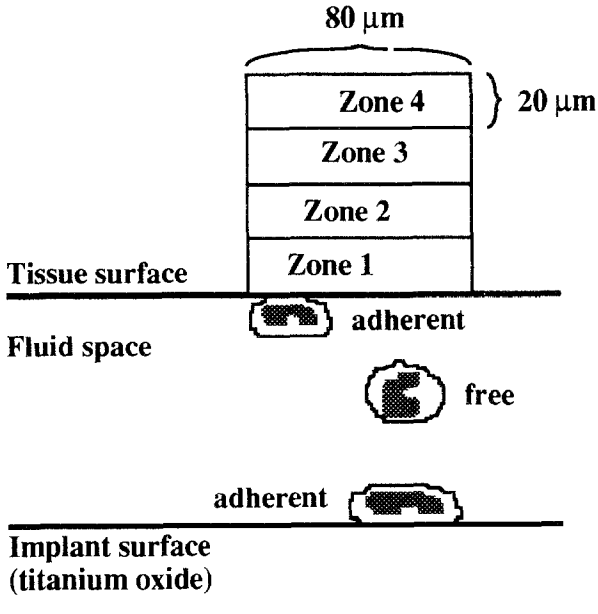


Figure 4 A schematic of the fluid space and tissue adjacent to solid implants showing the different areas used for morphometry.

by analysing consecutive areas of the fluid space along the entire implant surface (Fig. 2).

3. Results

3.1. Hollow implant exudate

In the exudate retrieved from hollow titanium implants, the highest number of inflammatory cells was found after 3 days (Table I). In the PTFE hollow implant a progressive increase in cell numbers with time was found (Table I). Previous analysis of the hollow implant exudates of both titanium and PTFE [1, 7, 8] have shown a predominance of PMN (> 70%) and a high viability (> 75%) at least up to 9 days after insertion.

3.2. Morphology of the fluid space and tissue surrounding hollow implants

In ground as well as electropolished sections of hollow implants (Figs 5–7) the presence of a fluid space containing inflammatory cells and proteinaceous material was evident. This fluid space was in direct communication with the interior of the implants via the holes in the top region (Fig. 1). After 24 h inflammatory cells, predominantly PMN, were located in the fluid space. PMN were also frequently observed at later time intervals (about 50% of cells in fluid space at 6 and 9 days).

Although macrophages and fibroblasts predominated in the tissue a considerable number of PMN were found also in the tissue around both types of implants at 6 and 9 d (in contrast to that found in around solid implants) (Figs 6 and 7).

After 6 and 9 days the tissue peripheral to the fluid space was organized as a fibrous capsule consisting of flattened macrophages and fibroblasts separated by collagen bundles. Newly formed blood vessels were located in the loose connective tissue outside the capsule. Observations on a few (two) specimens indicate that this capsule was more prominent after 2 months, at this time interval the fluid space was absent.

TABLE I The number of leukocytes ($\times 10^6$/ml) (mean ± SEM) harvested from the hollow implant exudates at each time interval.

	1 day	3 days	9 days
Titanium	1.9 ± 0.2	4.1 ± 0.8	2.6 ± 0.7
PTFE	2.2 ± 0.3	6.1 ± 0.8	11.6 ± 2.1

3.3. LM morphometry of tissue surrounding hollow implants

The absolute and relative numbers of different cell types outside the hollow implants were evaluated. It should be pointed out that this morphometric evaluation did not include the fluid space located at the implant surface and assumed to communicate with the implant interior of the hollow implant (see 3.2). The values given (Fig. 3) are the means of values obtained from 6–11 animals at every time interval (see 2.1)

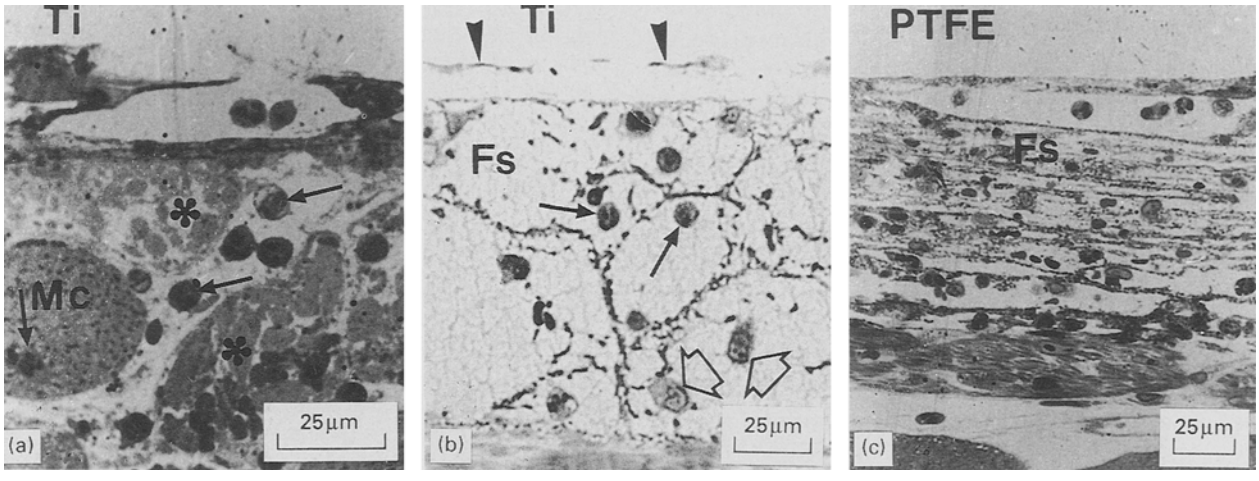


Figure 5 Light micrographs of the tissue close to titanium (a, b) and PTFE (c) hollow implants 24 h after surgery. Examples of two different preparation techniques were shown: conventional separation before embedding in epoxy (a, c) and (for titanium) electrolytical dissolution (b). The sections prepared with electrolytical dissolution technique allow an analysis of the preserved fluid space (Fs) between the implant surface and the tissue. (a) Inflammatory cells, predominantly PMN (some of which are indicated; arrows), are observed in the connective tissue between muscle cells (Mc) and in the superficial part of the tissue. Part of the tissue display signs of damage, including fragmentation of the muscle cells (*). Several leukocytes are detected within the muscle cells. The surface of the tissue towards the (removed) titanium (Ti) implant is interrupted by a space which contains inflammatory cells. Conventional separation technique. (b) Light micrograph of the fluid space (Fs) between the surface (arrow-heads) of an electrolytically removed hollow titanium (Ti) implant. The space contains inflammatory cells (PMN: arrows; monocytes/macrophages: open arrows) and proteinaceous material, partly arranged in a network. A few PMN are detected close to the surface of the tissue whereas no cells are adherent to the implant surface. (c) Light micrograph of the Fs between a (removed) hollow PTFE implant and the tissue. Inflammatory cells are distributed in a network of proteinaceous material.

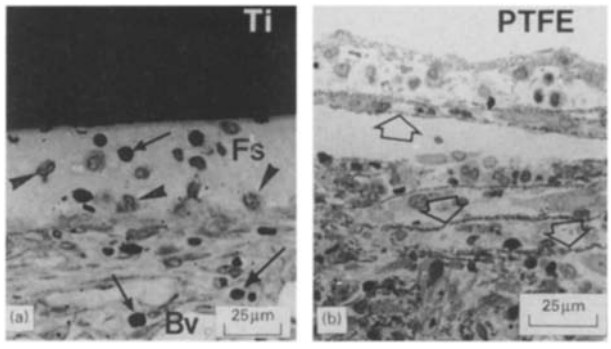


Figure 6 Light micrographs of the tissue adjacent to hollow titanium (Ti) and PTFE implants 6 days after surgery. (a) Ground section. PMN (some of which are indicated by arrows) and monocytes (arrowheads) are located in the tissue as well as in the fluid space (Fs) between the titanium (Ti) surface and the organized tissue. Bv = blood vessel. (b) Inflammatory cells are distributed in the tissue adjacent to the (removed) PTFE implant. Strands of proteinaceous material are indicated (open arrows). The superficial part of the tissue (towards the implant surface) is separated from the remaining tissue (possibly due to the separation technique used).

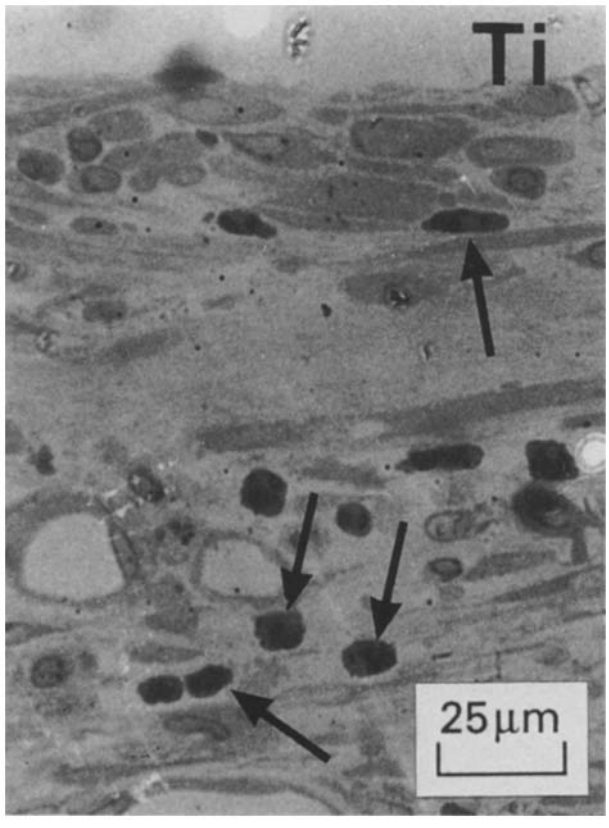


Figure 7 Light micrograph of the tissue adjacent to a conventionally separated hollow titanium (Ti) implant 9 days after surgery. The organized tissue contains inflammatory cells, including PMN (some of which are indicated by arrows) mainly located close to blood vessels deep in the tissue.

The changes in concentration and types of inflammatory cells in the tissue outside hollow implants did not follow the same pattern as in the exudate. Around both materials an increase (50–100%) in total cell numbers was observed between 24 h and 9 days (Fig. 3). This was due to an increased number of monocytes/macrophages and fibroblasts. The increase was about the same for both types of implants (Fig. 3). For both types of implants the highest concentration of PMN was found after 24 h. For titanium implants the concentration of PMN decreased already after 3 days, whereas the major reduction of PMN around PTFE hollow implants occurred between 3 and 9 days (Fig. 3). Around titanium implants monocytes/macrophages constituted the major cell type at all time periods. Around PTFE hollow implants the concentrations of PMN and monocytes/macrophages cells were about the same after 24 h; the monocytes/macrophages then increased gradually to be the most abundant cell type at 3 and 9 days (Fig. 3). The increase of fibroblasts around titanium was most pronounced between 24 h and 3 days, whereas a similar increase was noted between 3 and 9 days around PTFE hollow implants (Fig. 3). After 9 days fibroblasts constituted 21% of the cells in all regions around the titanium implant and 26% around PTFE implants (Fig. 3). Irrespective of material, few MNC were observed (data not shown).

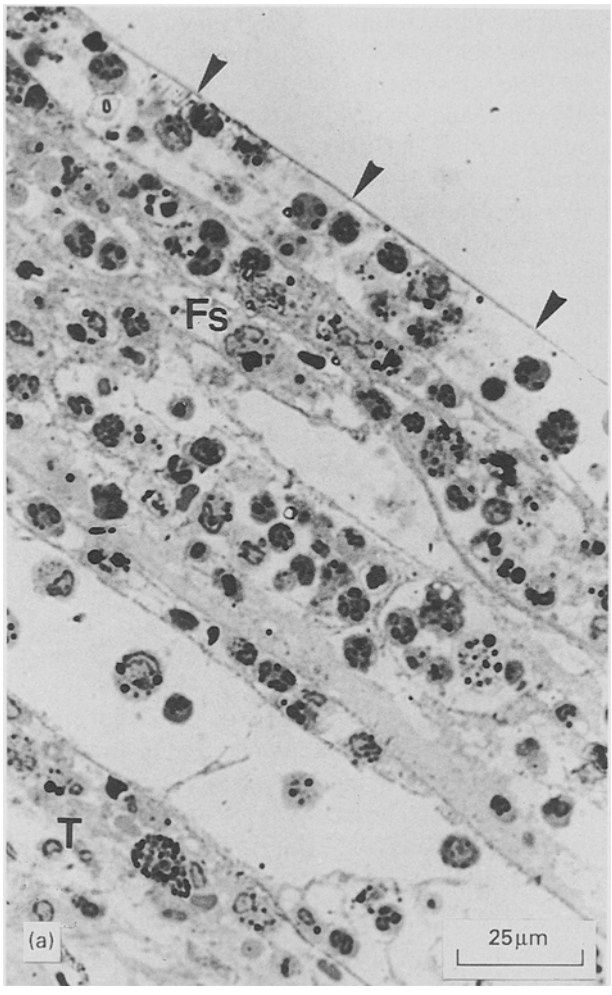
In general, the morphometric analyses of the individual regions (data not shown) around different portions of the hollow implants (Fig. 1) showed a similar pattern to that described above (data not shown). Around both materials PMN were more frequently observed in the side and bottom regions.

3.4. Morphology of the fluid space and tissue surrounding solid implants

The study on solid titanium implants was performed on specimens processed by electropolishing and was focused on the morphology of the implant-close fluid space. The thin oxide (which remained after electropolishing) was visible in the light microscope and allowed an analysis of the cell distribution (Fig. 8).

After 1 day a large number of inflammatory cells, mainly PMN but also monocytes/macrophages, were detected both in the fluid space and scattered throughout the tissue around the implants (Fig. 8a). Major components of the fluid space were inflammatory cells and proteinaceous material including strands of fibrin (often arranged in a network). Inflammatory cells were commonly observed in this network or attached to the surface of the strands (Fig. 8a). The inflammatory cells were only occasionally adherent to the implant surface. Adherent cells were flattened while the majority of the inflammatory cells present in the fluid space had an oval or circular shape.

After 7 days predominantly monocytes/macrophages were detected in the fluid space. At this time period, several layers of elongated monocytes/macrophages and fibroblasts formed a distinct border between the fluid space and the tissue (Fig. 8b). Fibrin strands were present in the fluid space-tissue border and also to some extent deeper in the tissue. Monocytes/macrophages, with varying shape, were often attached to the tissue surface. A general impression was that the presence of proteinaceous material was markedly reduced after 7 days.



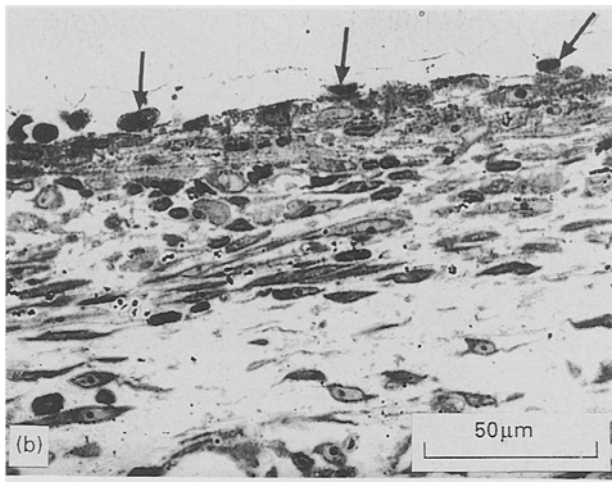


Figure 8 Light micrographs of the fluid space and tissue adjacent to solid titanium implants 1 and 7 days after surgery. Electropolishing technique. (a) The implant surface (arrow-heads) is separated from the tissue (T) by a fluid space (Fs) which contains numerous inflammatory cells, mainly PMN. A majority of the cells are trapped in a proteinaceous network. 1 day after surgery. (b) Macrophages (some of which are indicated by arrows) are located in the narrow fluid space. The cell-rich innermost portion of the organized tissue contains mainly macrophages and fibroblasts. 7 days after surgery.

3.5. LM morphometry of fluid space and tissue surrounding solid implants

The data on cell numbers, cell types and their distribution in the fluid space is given in Table II. In general, it was difficult to delineate the exact border between the cut (injured) tissue and the fluid space after 1 day. Therefore, evaluation of the distribution of inflammatory cells in the tissue was not performed at this time interval.

The width of the fluid space was markedly wider after 1 day (112 ± 29.6 μm) than after 7 days (17.6 ± 3.3 μm). However, the dimensions of the fluid space varied between different animals, in particular after 1 day, and also at different locations along the implant surface.

After 7 days the concentration of cells in the fluid space had decreased (Table II). A major difference between the two time intervals was the proportion of inflammatory cells. After 1 day PMN predominated whereas after 7 days monocytes/macrophages were the exclusive cell types detected. Another major observation was the different pattern of inflammatory cell distribution in the fluid space after 1 and 7 days. After 1 day the majority of cells (about 85%) were free in the fluid space and only a minor fraction were adherent either to the implant (6%) or tissue surface (9%). At 7 days only about 16% of the cells were free; the remainder were adherent to the tissue (55%) and a smaller fraction to the implant (29%).

The concentration of cells in the tissue was more than two-fold higher than that in the fluid space at 7 days. The majority of cells in the tissue around the solid titanium implant was monocytes and fibroblasts (Table III). Monocytes were preferentially distributed close to the tissue–fluid space border in zone 1, whereas fibroblasts were more frequent in the outer zones (zone 2–4). Other cell types were rare in the observation area around the solid implants after 7 days. When

TABLE II Solid titanium implants. Cell distribution in fluid space (Fs). Mean values from consecutive areas (1 day: range 3–7; 7 days: range 5–12). The mean total area (1 day: mean 179 800 μm², range 77 500–326 200 μm², 7 days: mean 52 600 μm², range 8400–99 400 μm²). The total number of cells counted in Fs was 1 day: 798 (range 112–399); 7 day: (range 12–92)

Days	Total number of leukocytes (/1600 μm²)	PMN (%)	Mono (%)	Free (%)	Tissue adherent (%)	Implant adherent (%)
1	2.8 ± 1	63	37	85	9	6
7	2.1 ± 0.8		100	16	55	29

TABLE III Solid titanium implants. Cell distribution in soft tissue 7 days after implantation. Lymphocytes, multinuclear giant cells, endothelial cells, mast cells and unidentified cells are listed together in the Table (other cells).

Zone	Total number of leukocytes (1600 μm²)	Mono (%)	Fibroblast (%)	Other (%)
1	6.3 ± 2	81	10	9
2	4.5 ± 1.4	76	22	2
3	4.9 ± 1.9	59	27	14
4	3.8 ± 1.4	68	19	13

taking the concentrations of cells in both the fluid space and the tissue into consideration, the morphometric data revealed that the proportion of inflammatory cells which were free in the fluid space, adherent to the organized tissue and the implant were only 1.5%, 5% and 2.8%, respectively (Table II).

3.6. Electron microscopy

After 24 h the fluid space had a proteinaceous content of low, but generally of homogeneous electron density. Strands of fibrin were concentrated to, and associated with, the implant surface (Fig. 9) but were also present in central areas of the fluid space where they were associated with cells.

The predominant cell type in the fluid space was PMN but monocytes, and to some extent also red blood cells were present. The majority of inflammatory cells had a spherical shape and appeared to be freely suspended in the fluid (Fig. 9a). However, inflammatory cells within the fluid space also formed aggregates with closely apposed plasma membranes (Fig. 9b). PMN and monocytes associated with the surface often had a more flattened shape. Cells associated with the surface were almost never seen directly

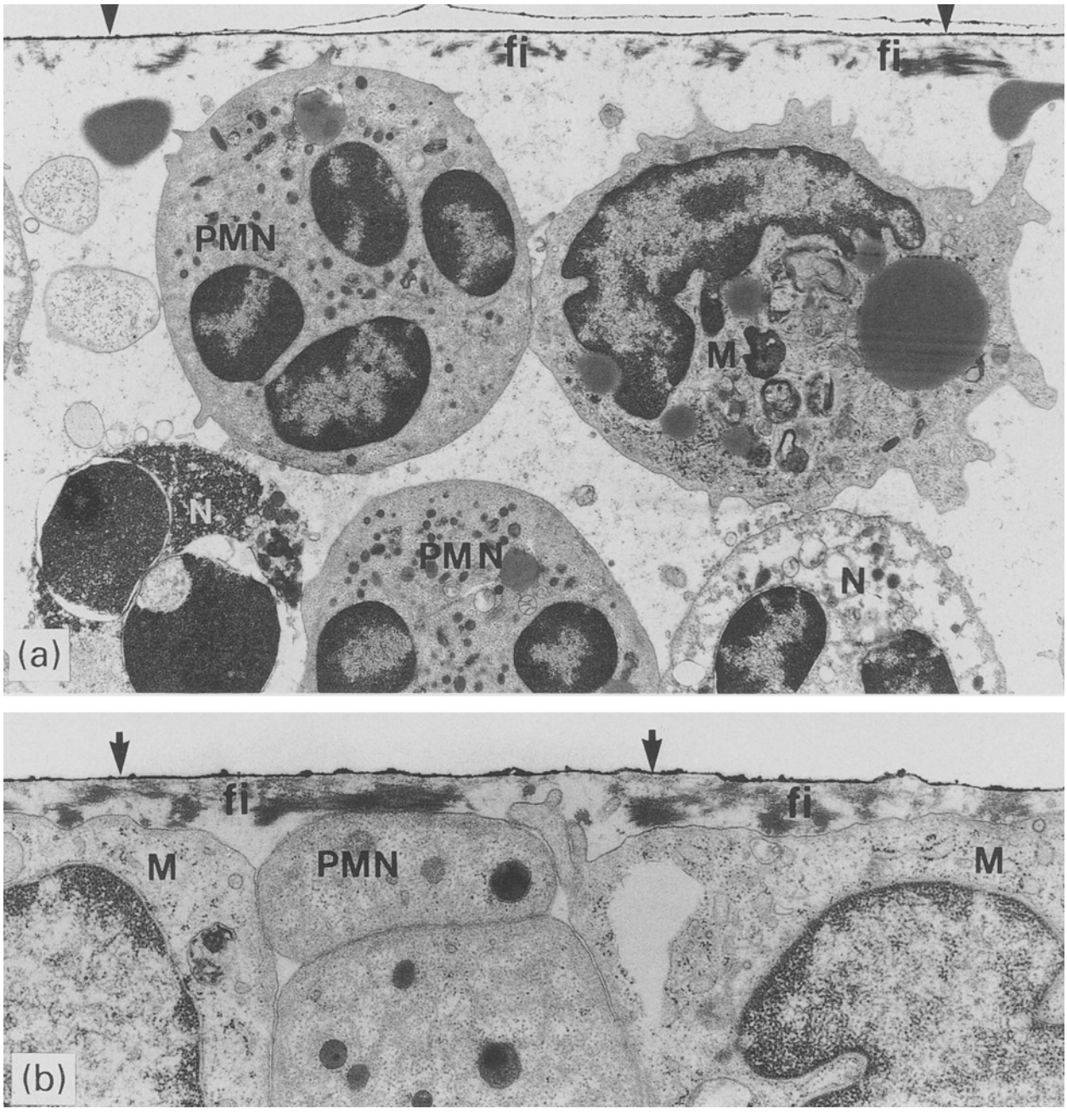


Figure 9 Electron micrographs of cells located in the fluid space close to the surface of solid titanium implant after 1 day. Specimens were prepared by the electrochemical method and the implant surface is represented by the remaining surface oxide appearing as a dense line (arrows). (a) PMN and a monocyte/macrophage (M) are present close to the surface but none of the cells are attached to surface (× 6000). (b) Monocytes/macrophages (M) and PMN which in the LM are categorized as "adherent to the implant". The cells are not in direct contact with the surface but separated from it by a layer of fibrin (fi). This is the typical cell-surface relation 1 day after implant insertion (× 16 000).

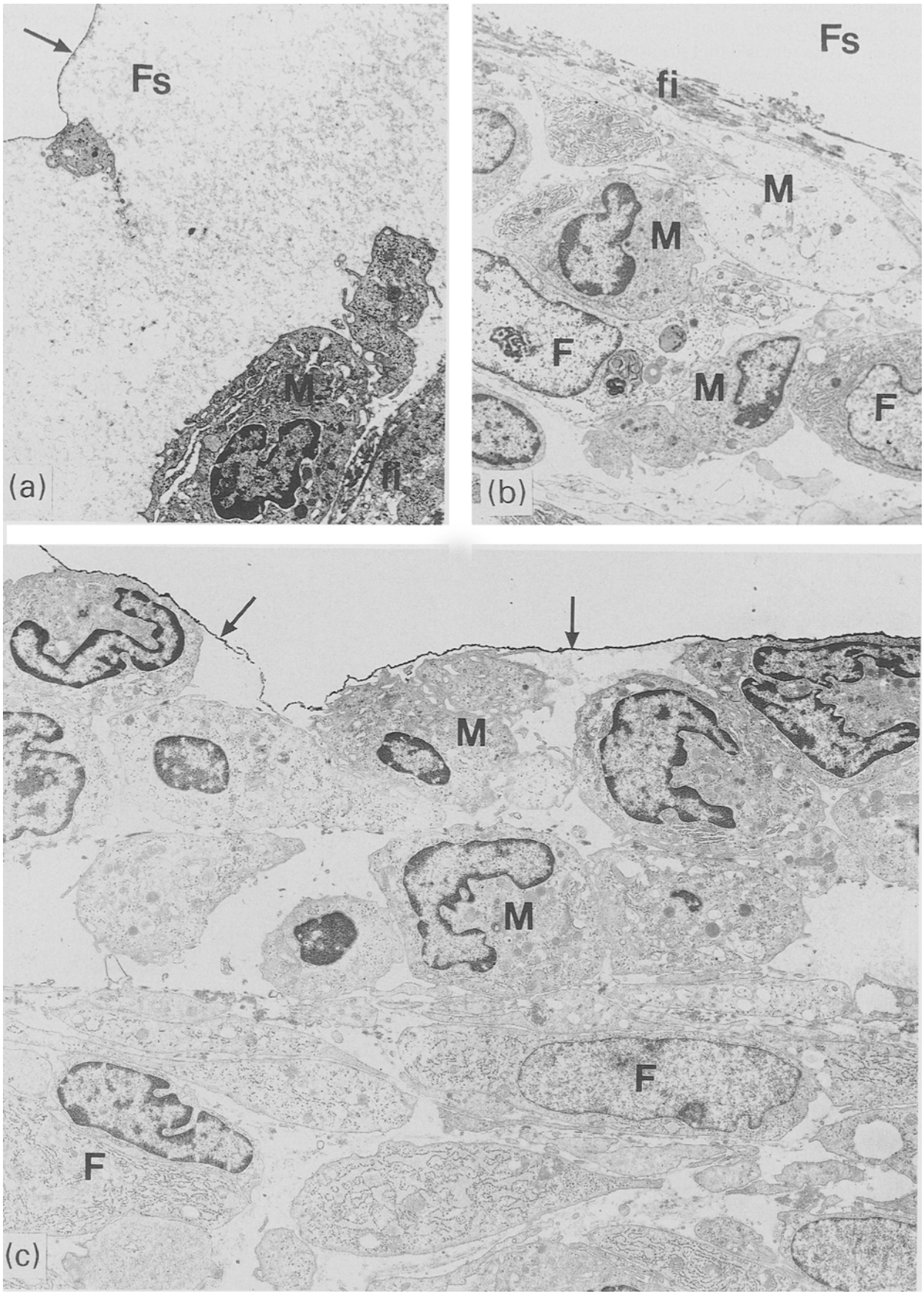


Figure 10 Electron micrographs of cells located close to solid titanium implants 7 days after insertion. (a) Except for a cell process (arrowhead) belonging to a macrophage and in contact with the implant surface, no cells are present in the fluid space (Fs). A macrophage (M) is attached to a layer of fibrin (fi) which constitutes the border between the fluid space and the tissue (× 2800). (b) The border between the fluid space and the tissue is formed by a layer of fibrin. Fibroblasts (F) and macrophages (M) are the main cell types in the tissue (× 3300). (c) In general, the distinct fluid space containing rather few cells is present after 1 week. However already after 1 week, but more typically after 2–3 weeks, the fluid space disappear and cells reach contact with the implant surface (arrows). Macrophages (M) almost invariably form the first and second innermost cell layers closest to the implant, while fibroblasts (F) are located further away. The macrophages adhere closely to the implant surface without any intervening layer of fibrin as seen at 1 day after implantation (cf. Fig. 8b) (× 3700).

apposed to the implant surface but were attached to the surface via fibrin strands (Fig. 9a and b).

After 7 days the fluid space was demarcated from the reorganizing tissue by a distinct border made up of fibrin strands oriented parallel to the implant surface (Fig. 10b), which served as a matrix to which flattened macrophages and, in the deeper portions, also fibroblasts adhered. On the fluid space side the macrophages attached to the fibrin matrix had a spherical or oval shape with profiles bulging into the fluid space; these cells were categorized as "adherent to the tissue" in the light microscopic quantification reported above. In most locations the fluid space contained rather few free cells (almost invariably macrophages) and cells adherent to the implant surface. The degree of apposition of the plasma membrane to the implant surface varied. A close contact, with an intervening space less than 0.05 μm was frequently (Fig. 10a) observed. In some locations, typically at the edge of the plate portion or in the angle between the rod and plate portions, the fluid space had largely disappeared and was replaced by two or three layers of often loosely packed macrophages (Fig. 10c). During the following 2–3 weeks the fluid space disappeared also along other parts of the implant. Along the entire implant macrophages formed the innermost two or three layers of cells which separated the implant from fibroblasts.

4. Discussion

Insertion of an implant in most instances causes a tissue injury which leads to an inflammation. In order to examine the role of the inflammatory cells for implant healing the use of a combination of different morphological and biochemical techniques is required. The number as well as the activity of the inflammatory cells may be analysed in the tissue fluid retrieved from the interior of the hollow implants [7]. However, the number and distribution of cells adjacent to an implant surface is technically difficult to study. By using a combination of ground sections (10 μm thick) and specimens prepared by an electrolytical dissolution technique (semithin and ultrathin sections) [11] it was possible to evaluate the distribution of cells around hollow and solid implants in the present study.

A characteristic feature of the arrangement of the tissue around hollow as well as solid implants was the presence of a fluid space containing inflammatory cells. Our morphometric studies revealed a higher concentration of inflammatory cells in the tissue than in the fluid space. Further, PMN and macrophages were not preferentially located on the implant surface. The inflammatory cells were either detected free in the fluid space (PMN) after 1 day or adherent to the border between the fluid space and the tissue (monocytes/macrophages after 7 days). Previous studies [14] have shown that fibrinogen and fibronectin immunoreactivity is preferentially located in the fluid space–tissue border. Therefore, it is possible that these proteins may influence the function of cells in this location.

A difference in number of leukocytes inside the hollow implant was found between the two materials at time points beyond 3 days. After 9 days the number of leukocytes in PTFE hollow implants was more than four times higher than with the titanium implants. This finding corroborates previous observations using this hollow implant model [1, 7, 8]. In these previous studies it has been shown that PMNs predominate in the hollow implant exudate (of both titanium and PTFE) at least up to 9 days after implantation (more than 70% PMN) [7, 8]. Morphometry of the soft tissues around the hollow implants revealed an increased cellularity over time, which was mainly due to an increased number of monocytes/macrophages and fibroblasts, whereas the number of PMN decreased over time. This is in agreement with the observations of cell kinetics in wound healing and our present observations on the cell numbers and types in the tissue around the solid implants [1, 7, 8]. However, the observations in the present study clearly show that there was a difference in tissue with respect to the presence of PMN around hollow and solid implants. Around solid implants, PMN were almost completely absent from the fluid space and the tissue after 7 days whereas PMN still constituted a considerable fraction of the inflammatory cells in the fluid space and the tissue around hollow implants after 9 days. Thus, there are several findings which need further consideration, including the discrepancy between the relative number of PMN and monocytes/macrophages in hollow implants and surrounding tissue, the different inflammatory reaction around hollow and solid implants and the material-related difference in number of leukocytes retrieved from the two types of hollow implants.

We have no evident explanation for any of these findings. Most likely, there are multifactorial reasons for the discrepancy between the results obtained with the two types of hollow implants (titanium and PTFE) and implant designs (hollow versus solid implants). Factors of possible importance include differences in shape and dimension of solid and hollow implants, differences in chemical and physical surface-properties leading to differences in adsorption of proteins [15, 16] and subsequent adhesion of leukocytes [17]. Also material degradation, and leakage of material constituents and activation of the complement system may play a role. An explanation for the relatively greater increase of inflammatory cells in the chamber as compared to the tissue could be that a generation of chemotactic substances (i.e. LTB_4, C5a and IL-1) is promoted in the interior of the hollow implant [7] leading to a selective and prolonged influx of PMN to the interior of the implant. Generation of such leukotactic factors inside the hollow implant may also secondarily influence the accumulation of inflammatory cells in the surrounding tissue. This might explain the presence of high numbers of PMN around hollow implants at time intervals when they have completely disappeared from the tissue surrounding solid implants. However, we cannot exclude that other factors may be of importance for the selective accumulation of PMN in the hollow implants. It appears quite possible

that the oxygen tension [18] and also pH are low inside the hollow implants, a condition which could favour the selective survival of PMN. A retention, perhaps selective for PMN, may cause an increased aggregation and adhesion of cells in the hollow implant impairing their ability to return to the tissue and thus, leading to a gradual accumulation in the hollow implants. Another mechanism leading to a predominance of PMN over monocytes/macrophages in the hollow implant could be a selective retention of these latter cells in the tissue caused by their immobilization due to adherence to extracellular matrix components. This possibility gains support from our morphometric findings (around solid implants) showing that only a minor fraction of monocytes/macrophages are free in the fluid space 7 days after implantation, and our immunohistological observations [14] showing that fibronectin and fibrin, providing attachment sites for macrophages, are concentrated at this site rather than at the implant surface after 7 days.

The analysis of the cell types and numbers in the soft tissue around hollow implants revealed differences in cell composition between some of the regions. For titanium implants, titanium was exposed in all regions. Thus, differences may be due to factors not directly related to the material used, for instance, the implant design and vicinity to different types of tissues (i.e. dense and loose connective tissue, muscle and fat tissue). Also such physical circumstances as tension, compression and micromovements of the tissues could contribute to regional variations in cell numers. For PTFE hollow implants the soft tissues were in contact with PTFE only in the side and bottom regions. The absolute number and percentage of PMN was higher 24 h and 3 days after implantation in the neck and top regions around PTFE chambers. This difference cannot be explained by PTFE itself, since titanium was facing the tissue for both types of implants. A possible reason for the relatively high concentration of PMN in these regions is that these regions are located closest to the holes connecting the chamber with the fluid space and therefore are exposed to the highest concentration of leukotactic factors originating from the hollow implants interior.

Hollow implant models are of interest in the studies of early inflammatory response. A prerequisite for a valid analysis is that a free communication exist between the inside and outside of the hollow implant. Our own experience with titanium and PTFE hollow implants indicate that both the pore size and the degree of inflammation may influence the rapidity whereby the pores become occluded by soft tissue (titanium 15 days after implantation and PTFE 9 days post-surgery). As a result of these findings, we believe that the hollow implant model should not be utilized beyond these time-points. With this limitation in mind the hollow implant model is a useful tool for the analysis of early events in association with implants.

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