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Tissue reactions to lead samples in a late infection rat model

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Tissue reactions to rat lead samples, modelling for clinically used leads, were investigated in a late infection model, in which injection of bacteria was performed after a 3-week encapsulation process. At the site of injection, detachment of the original fibrous capsule, wound fluid infiltration, fibrin formation and granulocyte and macrophage infiltrations, occurred. Spreading of infection did not occur via the generally assumed direct bacterial adhesion to materials, but through blood vessels at the outside of capsules and through wound fluid passage at the interface and in the lumen of the lead sample. At day 5, infection had spread all over, but, apart from two small abscesses, seemed to be suppressed at day 10. However, probably due to luminal bacterial growth, at weeks 3 and 6 the reaction intensified showing larger abscesses with accumulations of lymphocytes. The results of this study represent a good basis for further studies aimed at developing infection-resistent lead material. Research efforts are first directed on modification of material surfaces to provide controlled release of antimicrobial agents.

1. Introduction

Synthetic materials are widely used in prosthetic implants and biomedical devices [1]. Whether the material is used in a permanent implant situation, as in pacemakers and heart valves, or for acute treatment of disease, as in oxygenators and blood pumps, it is necessary to be concerned about the possible adverse reactions resultant from the interaction with the surface of the foreign materials. Current clinical experience teaches us that one of the major clinical complications concerns the occurrence of implant- and device-associated infections [2–4]. Implant-associated infections are most often irreversible and necessitate implant removal in order to eliminate the infectious complications, in spite of the growing variety of available and potent antibiotics [5].

Implant- and device-associated infections are characterized by a high prevalence of *Staphylococcus* (S.) strains [2–4]. Implant-infections generally can be characterized as early (first post-operative months) or late. Early infections are probably secondary to intraoperative contamination or to early postoperative complications, such as wound infections. By contrast, late infections are thought to occur via a haematogenous route initiated by bacteraemia.

In a previous study [6], an early infection rat model was presented in which small polyurethane (PU) tubing segments were inoculated with a strain of *S. aureus* isolated from a patient with an infection of a PU-insulated lead. It was found that at least 10^6 bacteria were needed to provoke a clear a-specific inflammatory reaction and specific immunologic reaction.

In the present study we developed an *in vivo* rat model targeted at mimicking the case of a late infection. Late infection was represented by bacterial inoculation after a 3 week implant period, at which time the implanted lead samples were surrounded by a mature tissue capsule. Approximately 10^7 bacteria were used to introduce the infectious challenge to the implanted lead samples, after which the provoked tissue response was investigated at desired time points.

2. Materials and methods

2.1. Materials

Rat lead samples were constructed at Medtronic Bakken Research Center, Maastricht, The Netherlands. Basically, the rat lead samples are constructed from the same materials as actual clinically used leads, i.e. a polypropylene blue wire, a platinum/iridium (Pt/Ir)-coil-electrode and polyurethane (PU, Pellethane® 55D and 80A) tubing. In the case of the rat lead sample (see Fig. 1), the polypropylene blue wire (A) has a length of ±50 mm, around which the electrode (B) of about 40 mm is positioned. Both are covered by a 30 mm long PU insulation composed of

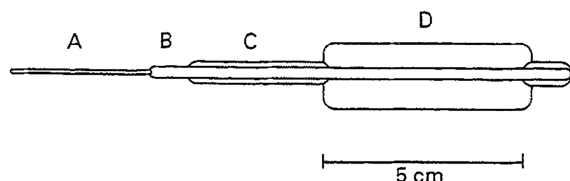


Figure 1 Rat lead sample consisting of a 50 mm long polypropylene blue wire (A), which in the scheme runs from left to right. Around the blue wire a Pt/Ir-coil-electrode of about 40 mm is positioned. This also runs to the right end. At the level (B) it is uncovered, but at the levels (C) and (D) both the blue wire and the electrode are covered by two sizes of polyurethane (PU) tubing. Small lumen 55D PU is present at C and at the right end. It was glued (not leakproof) to a ± 13 mm piece of large lumen 80A-PU (D).

three pieces of PU tubing glued together with PU-adhesive (not leakproof). The small lumen tubing (C) is made of 55D Pellethane®; the 13 mm long large lumen tubing (D) is made of 80A Pellethane®.

The rat lead samples were routinely sterilized by ethylene oxide.

2.2. Bacteria

A strain of *S. aureus* (code PW230693), obtained from a lead-associated infection was used for bacterial inoculation. *S. aureus* were routinely cultured on blood-agar plates (4% sheep erythrocytes; from Oxoid Ltd., Hampshire, UK).

S. aureus cultured for 18 h at 37 °C in brain heart infusion broth (from Oxoid) were washed three times with phosphate-buffered saline (PBS), pH 7.4 and resuspended in PBS. The bacterial suspension was spectrophotometrically adjusted to an optical density of 1.0 at 540 nm (PYE Unicam PU 8600 UV/VIS spectrophotometer from Philips, Eindhoven, The Netherlands), containing $\pm 10^9$ colony forming units (CFU) per ml.

After injection, the CFU-number was determined by spreading 0.1 ml portions from serial ten-fold dilutions of bacterial suspensions on blood-agar plates, which was followed by 18 h of incubation at 37 °C and CFU counting. From this it was calculated that the number of injected bacteria had been 2×10^9 per ml.

2.3. Implantation

NIH-guidelines for the care and use of laboratory animals (NIH Publication #85-23 Rev. 1985) have been observed.

Male AO-rats of approximately 3 months of age were ether anaesthetized. First, a rat lead sample was introduced in a sterile 1 ml syringe with the blue wire-end near the plunger. At the side of the shaved and disinfected back of the rat, an incision of $\pm$ 1 cm was made in the skin and a subcutaneous pocket was created parallel to the spine. The syringe with lead sample was then inserted in the subcutaneous pocket and the lead sample was pushed out of the syringe, while retracting the latter. Thus the lead sample had been introduced aseptically with the blue wire-end at the incision site. Thereafter, the skin was closed with one suture. Two lead samples per rat (total number of lead samples was eight) were subcutaneously implanted on each side of the back.

After 3 weeks, one lead sample was explanted to function as control ($t = 0$). Of the other leads, the skin at the former incision site was opened, and a small pocket was created by removing ± 2 mm of the tissue from the blue wire-end. Thereafter 10 µl of the *S. aureus* suspension containing $\pm 2 \times 10^7$ bacteria were injected into the pocket and the skin was closed again.

2.4. Sacrifice

Leads were explanted at day 1, 2, 5 or 10, and week 3 or 6 after introduction of the bacterial challenge.

First, the skin was carefully removed from the implant and surrounding capsule. To check for viable bacteria, a cotton swab was moved over the encapsulated implants, contacted with blood-agar plates and incubated for 18 h at 37 °C.

All implants were carefully dissectioned from the surrounding tissue. One complete 10-day lead sample was used to check for presence of live bacteria, by cross-cutting it into segments and contacting the different parts with blood-agar plates, followed by 18 h incubation at 37 °C. Furthermore, of the 3- and 6-week lead samples a small part of large lumen PU + surrounding capsule was taken for bacterial culture.

The other implants and the remaining parts of the 3- and 6-week lead samples were immersion-fixed in 2% (v/v) glutaraldehyde in PBS (pH 7.4) during at least 24 h at 4 °C. After removal of the Pt/Ir coil, usually three specimens (one taken from the blue wire-end, one from the small and one from the large lumen PU) were dehydrated in graded alcohols and embedded in glycolmethacrylate.

Semithin sections (2 µm) for light microscopical evaluations were routinely stained with toluidine blue.

3. Results

3.1. Macroscopy

Lead samples which had been implanted for 3 weeks were covered by a thin capsule (schematically represented in Fig. 2). This capsule is so thin that the implant with the different parts, such as the blue wire and large lumen PU, is easily recognized.

At day 1 after injection of *S. aureus*, the former thin capsule around the blue wire had turned into a clear red/yellow inflamed area with excessive wound fluid. At day 2 the inflamed area had spread to the electrode (B) and small lumen PU (C). At day 5 less wound fluid seemed present, but the inflammation had clearly spread all over the lead sample.

Five days later, i.e. at day 10, the inflammation seemed to be suppressed. A rather normal capsule was present with two distinct abscesses only at the blue wire-end.

However, at week 3, the reaction seemed to increase again, showing one large abscess at the blue wire-end, while at week 6 the lead sample seemed to be covered all over with abscess-like tissue.

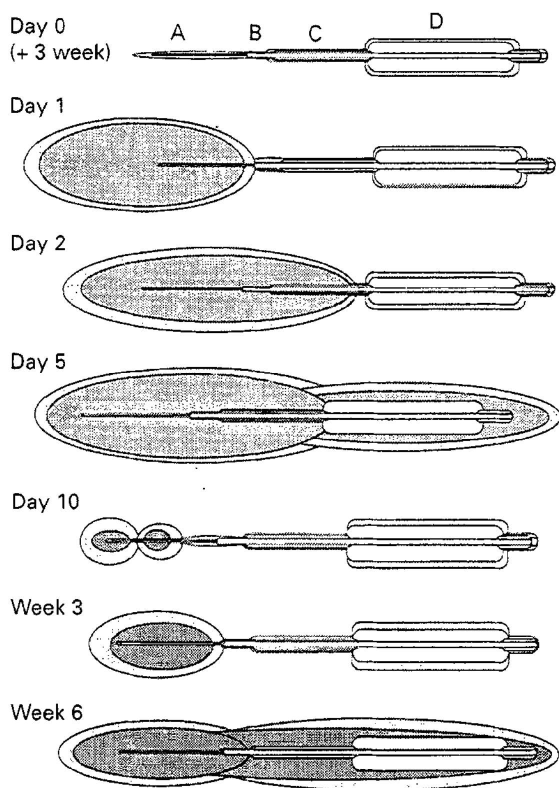


Figure 2 Scheme showing the macroscopic aspects of implanted lead samples. At day 0, i.e. after 3 weeks of implantation, lead samples were covered by a thin, 'light-grey-shaded' capsule. At day 1 after injection of *S. aureus*, the former thin capsule around the blue wire had turned into a clear red/yellow inflamed area with excessive wound fluid (medium grey shade). At day 2 the inflamed area had spread to the electrode and small lumen PU (B and C). At day 5 less wound fluid seemed present, but the inflammation had clearly spread all over the lead sample. At day 10 a rather normal capsule was present with only at the blue wire-end two distinct abscesses (dark grey shade). After 3 weeks, the reaction seemed to increase again, showing one large abscess at the blue wire-end, while at week 6 the lead sample seemed to be covered all over with abscess-like tissue.

3.2. Bacterial culture

After skin removal, a cotton swab was used to detect bacteria at the outside of the encapsulated implant. Only a few (1–5) colony forming units (CFU) could be cultured from the day 1, 2 and 5 implants. At later time points no live bacteria could be detected outside the capsules.

From one lead sample, which had been implanted for 10 days after infection, three samples consisting of both capsule and lead material were taken for bacterial culture. Numerous (uncountable) CFU were cultured from the blue wire-end with the abscesses, from the section at the margin of the small and large lumen PU (from C to D) and from the other end (of D).

Culture of capsule, PU, electrode and blue wire of a small part of the D-level, taken from the week 3 and week 6 lead samples, resulted in both cases in numerous CFU.

3.3. Microscopy (see also Table I)

The capsule around rat lead samples which had been implanted for 3 weeks is thin and strong. Around the blue wire and PU, it generally consists of one or two layers of parallel-to-the-interface oriented flat macrophages or giant cells, followed by some 10 layers of parallel-to-the-interface oriented fibroblasts alternating with newly formed collagen (Fig. 3a and b). Further outward a layer of non-oriented connective tissue can be identified, in which usually some small blood vessels are present. The first cell layer around the Pt/Ir coil (B) clearly shows a less pronounced cellular orientation, probably as result of the coil-surface topography.

At *day 1* after injection of *S. aureus* a clear tissue reaction was observed. The capsule around the blue wire-end had turned into a detached inflamed tissue with extended areas with wound fluid, fibrin, increased number and size of blood vessels, swelling of endothelium, and haemorrhages, marked by the presence of loose erythrocytes. Granulocytes and macrophages are actively infiltrating (Fig. 4a). *S. aureus* are observed, single or in clusters, both in granulocytes and macrophages (Fig. 4a). Although macroscopically not observed, microscopy clearly shows that these effects reach the level of the uncovered coil (B). Normal cell layers are present at the interface at the level of the small lumen PU (C). However, relatively far from this interface, increase in number and size of blood vessels, swelling of endothelium and mast cell degranulation, the latter needing precise identification since the granules look very similar to *S. aureus*, are observed (Fig. 4b). At the level of the large lumen PU (D) a normal capsule is present.

At *day 2* after injection of *S. aureus* the inflamed area has spread to the small lumen PU (C). Increase in number and size of blood vessels, swelling of endothelium as well as haemorrhages are observed further from the interface. An increase in infiltration as well as in degeneration of granulocytes and macrophages is observed. The first signs of degeneration of cells is often observed as the presence of vacuoles, which result from digestion of *S. aureus* and other cellular remnants. Degeneration is most obvious around the blue wire and uncovered coil (B), with, as a final result, large accumulations of nuclear material. Clear identification of *S. aureus* is difficult; some loose bacteria as well as phagocytosed bacteria are present in the wound fluid at the level of the electrode. Macrophages with included particles are also observed near blood vessels relatively far from the interface, thus suggesting removal of phagocytosed particles from the interface. The original capsule, still recognized from some fibroblast/collagen layers, has detached (Fig. 5). The fibroblasts show spherical instead of spread morphologies.

The capsule around the small lumen PU (C) is, in part, normal (Fig. 6). However, nearby the blood vessels have increased in number and size, while the endothelium has swollen. Macrophages with phagocytosed particles are present near these blood vessels while a small cluster of *S. aureus* is observed in between collagen and fibroblasts (Fig. 6b). In contrast, the capsule around the large lumen PU (D) looks more activated since it, in part, contains infiltrations with granulocytes and macrophages at the interface. This is explained from a possible intraluminal bacterial

TABLE I

t = day 1	inflammation: around A/B C: D:	wound fluid/fibrin, grans, MØ, SA outside: large blood vessels quiet capsule; outside: mast cell degranulation quiet capsule
t = day 2	inflammation around A/B/C B C D	as above + more compact (less fibrin/wound fluid) + increase grans/MØ- degeneration outside: transport of SA by MØ via vessels loose SA in wound fluid, degenerating fibros transport SA by MØ via blood vessels in part: quiet capsule outside: blood vessels with MØ + SA wound fluid/fibrin in capsule
t = day 5	inflammation: around A/B/C/D	wound fluid/pus (degenerated cellular/proteinaceous material) fibroblast degeneration/fibroblast activation relatively less grans/MØ intraluminal: SA-accumulations
t = day10	abscess A–D	pus; more compact/fibros quiet capsule intraluminal: SA-accumulations
t = week 3	abscess A/B/C/D	SA-accumulations, pus/fibrin, grans/MØ around: fibros/compact quiet capsule
t = week 6	'abscess' A–D	large lymphocyte-accumulation first layer: degenerated cells, grans outside: lymphocytes

A = polypropylene blue wire; B = Pt/Ir-coil-electrode; C = small lumen PU; D = large lumen PU; grans = granulocytes; MØ = macrophages; SA = Staph.aureus; fibros = fibroblasts

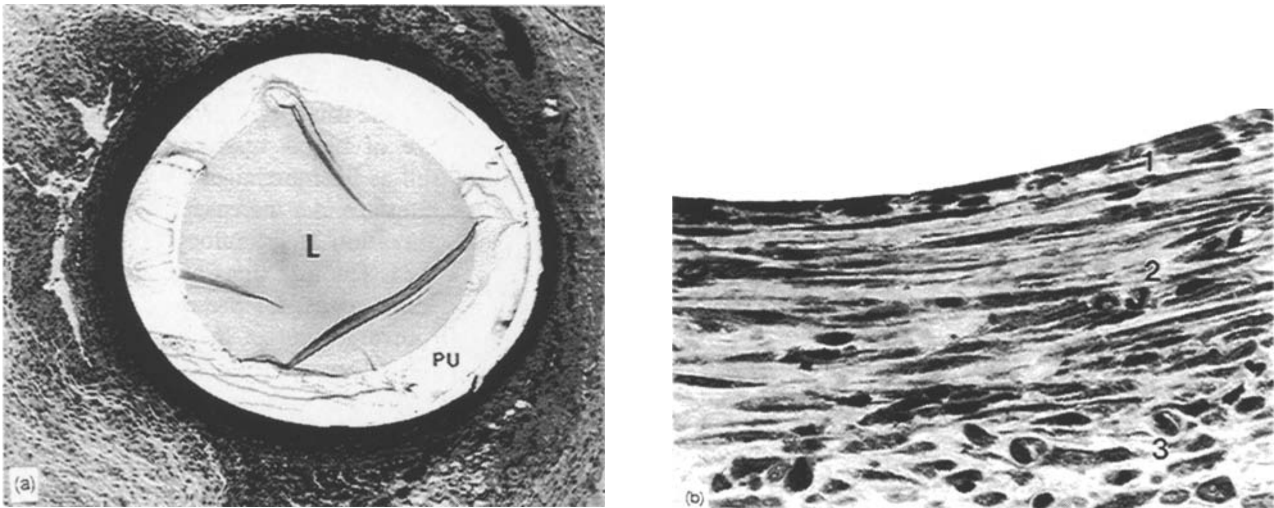
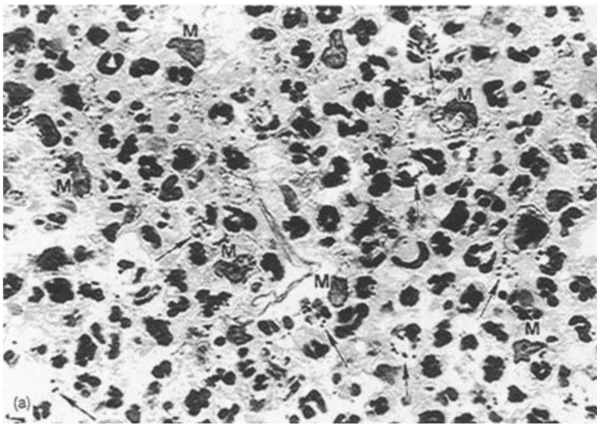


Figure 3 The capsule around the small lumen PU of a control rat lead sample, which had been implanted for 3 weeks. (a) Light-micrograph (made at 40 ×) showing PU in cross-section. The lumen (L) is empty, which may be for real, or which may have occurred due to removal of the electrode. (b) Magnification (made at 400 ×). The capsule usually consists of one or two layers with macrophages or giant cells (1), followed by some 10 layers of parallel-to-the-interface oriented fibroblasts and newly formed collagen (2). The third layer represents a 'loose connective tissue', in which usually some small blood vessels are present (3).

transport, which at the non leakproof fixation point from (C) to (D) may result in the observed discrepancy between the two levels.

At *day 5* after injection of *S. aureus* a first layer of pus, i.e. an amorphous structure of wound fluid, fibrin, and (remnants of) granulocytes, macrophages, fibroblasts and *S. aureus*, was present at the interface from the blue wire to the other end (Fig. 7). This is consistent with the macroscopic observation of inflammation all over the lead sample. The pus layer was thicker at the blue wire-end than at the other end and enclosed by an oriented tissue capsule consisting of fibroblasts and collagen. In between collagen and fibroblasts, new granulocytes and macrophages as well as receding macrophages with phagocytosed particles are present. Further from the interface, the collagen bundles in this layer become larger and an increase of capillaries is observed. Larger blood vessels are present in a third layer, which contains more collagen, but has less orientation. Phagocytosing macrophages are also observed here, while lymphocytes are occasionally present.



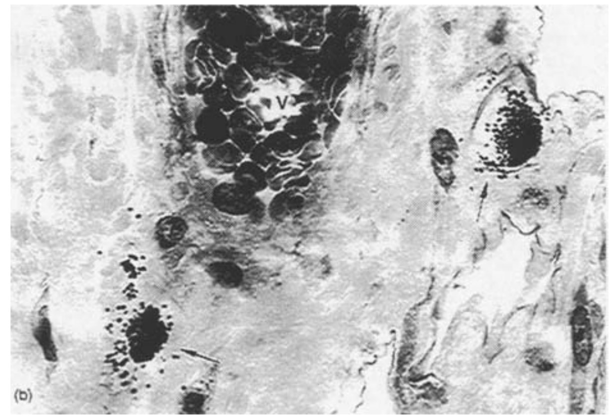
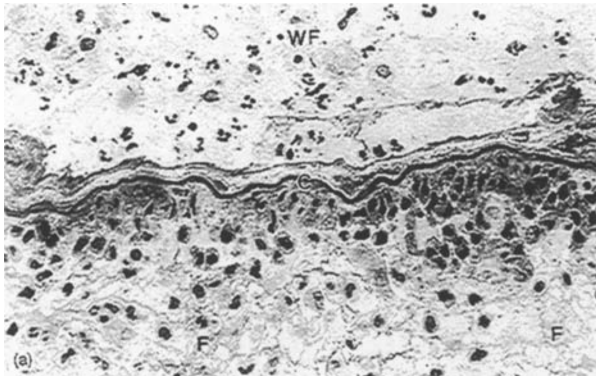


Figure 4 Rat lead sample at day 1 after injection of *S. aureus*. (a) Light micrograph (made at 1000 ×) of the capsule around the blue wire-end. Many granulocytes (with polymorph nuclei) and less (usually 1:9) macrophages (M; large light-stained nuclei) have infiltrated. *S. aureus* are observed as singles or in clusters. All seem intracellularly localized, both in macrophages and granulocytes (arrows). (b) Light micrograph (made at 1000 ×) of the capsule around the small lumen PU. Relatively far from the blue wire blood vessel-activation (V) and mast cell degranulation (arrows) occurred.



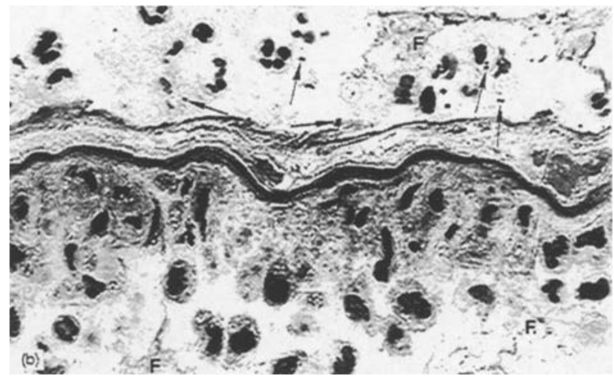
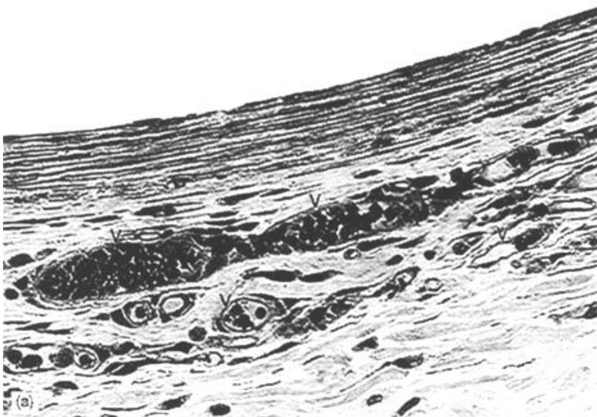


Figure 5 Rat lead sample at day 2 after injection of *S. aureus*. (a) Light micrograph (made at 400 ×) of the capsule around the electrode. The original capsule, still recognized from some cellular/collagen (C) layers, has detached. The original cells, mostly fibroblasts have rounded off. Infiltration of wound fluid resulted in fibrin formation (F) in the capsule. Wound fluid (WF) and infiltration of many granulocytes and macrophages also occurred near the interface. (b) Magnification (made at 1000 ×) further indicating fibrin (F), wound fluid and many cellular remnants, originating from degenerating cells and *S. aureus* (arrows).



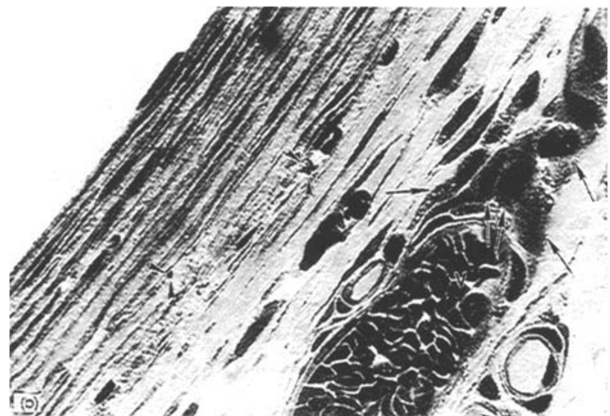


Figure 6 Rat lead sample at day 2 after injection of *S. aureus*. (a) Light micrograph (made at 400 ×) of the intact capsule around the small lumen PU. At the interface the original structure with oriented cellular/collagen layers is recognized. V: blood vessels. (b) Magnification (made at 1000 ×) showing several macrophages (arrows) containing phagocytosed particles near one of the, clearly activated, blood vessels (V) and two small clusters of *S. aureus* (arrow head).

Some proteinaceous material was preserved in the lumen of the large lumen PU (D). It contained wound fluid, fibrin, cellular remnants and small colonies of *S. aureus* (similar to the day 10 specimen, except for smaller colonies, see Fig. 8b). Evidence for direct adhesion of bacteria to PU was not found.

At *day 10* after injection of *S. aureus*, apart from two abscesses located further down the blue wire (similar

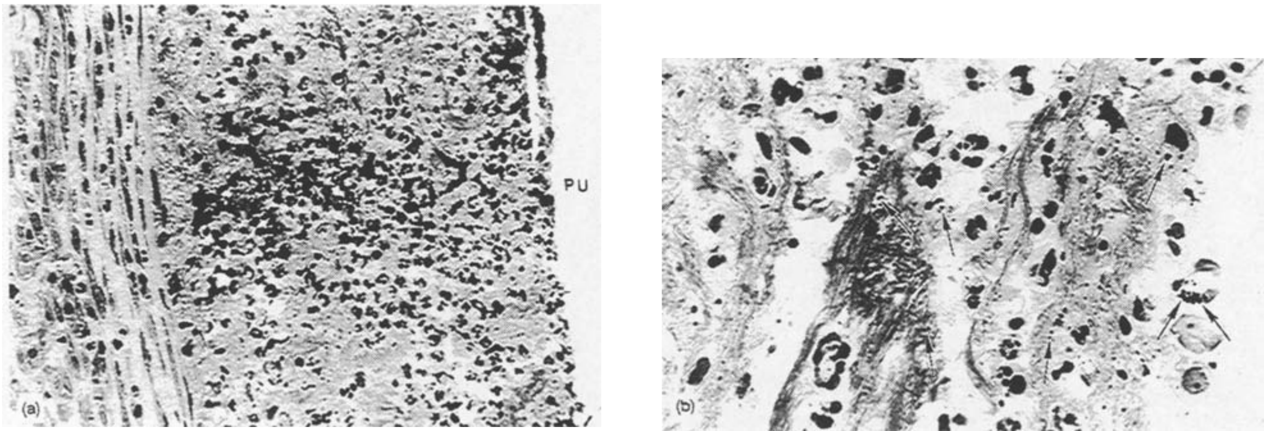


Figure 7 Rat lead sample at day 5 after injection of *S. aureus*. (a) Light micrograph (made at 400 ×) of the tissue around small lumen PU. At the interface first a thick layer of pus is present. The following layer consists of oriented cellular/collagen layers. (b) Light micrograph (made at 1000 ×) of the interface around the large lumen PU(D). The pus layer consists of wound fluid, fibrin and (remnants of) granulocytes, macrophages, fibroblasts and *S. aureus* (arrow).

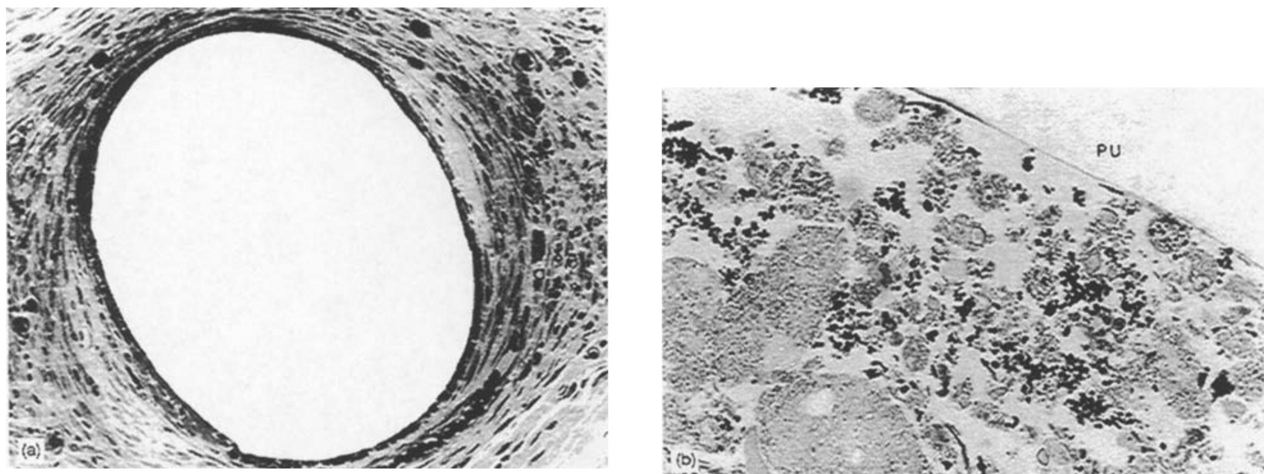


Figure 8 (a) Light micrograph (made at 200 ×) of the normal capsule around the blue wire at day 10 after *S. aureus* injection. (b) Light-micrograph (made at 1000 ×) of a rat lead sample at week 3 after *S. aureus* injection. The lumen of PU (at the level of C) contains proteinaceous material with cell remnants and large colonies of *S. aureus* (arrows). Direct adhesion of *S. aureus* to PU was not observed.

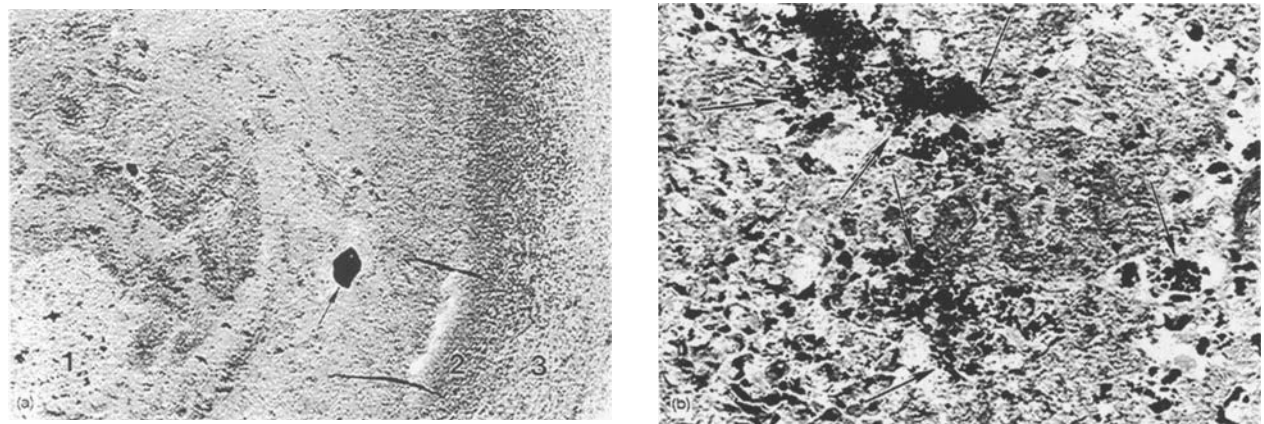


Figure 9 Rat lead sample at week 3 after injection of *S. aureus*. (a). Light micrograph (made at 50 ×) of part of the abscess. The centre (1) is amorphous with a.o. bacterial colonies and accumulations of nuclear material. Further layers (2, 3) are characterized by an increase in recognition of single cells. The third layer consists of numerous phagocytosing macrophages. Arrow: a large nuclear accumulation. (b) Magnification (made at 1000 ×) showing the centre of the abscess with bacterial colonies (arrows) in between fibrin and cellular remnants.

morphology to that depicted for the week 3 specimen, see Fig. 9), normal encapsulation of the complete lead sample seems to have occurred. However, at high magnification clear remainders of the former infectious complication can be observed by way of phagocytosing macrophages in between the collagen and near the blood vessels.

In the PU-lumen (C) again some proteinaceous material remained (Fig. 8b). It contains wound fluid, fibrin, cell remnants and large colonies of *S. aureus*.

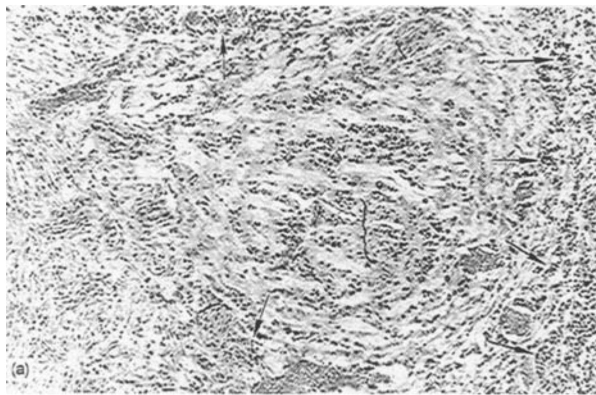
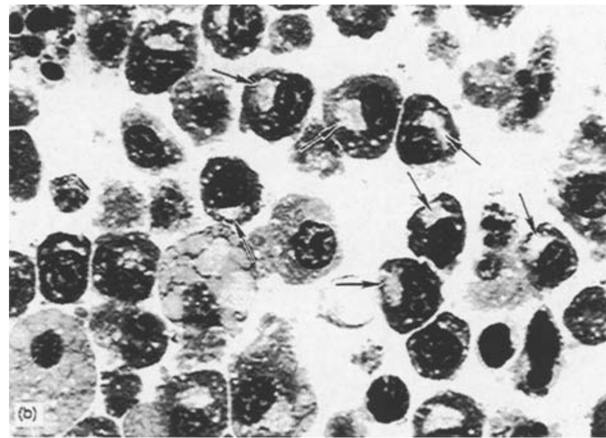



Figure 10 Rat lead sample at week 6 after injection of *S. aureus*. (a) Light micrograph (made at 100 ×) of a lymphocyte accumulation near the blue wire. The central part of this accumulation contains more plasma cells and macrophages, while smaller cells such as T-lymphocytes are found at the margin (arrows). (b) Light micrograph (made at 1000 ×) of, mainly, plasma cells, recognized from the excentric nucleus and the light pale centrosome (arrows), present in the outer layer of the capsule around the blue wire.

Direct adhesion of *S. aureus* to PU was not observed.

At *week 3* after *S. aureus* injection normal encapsulation is observed with the exception of one large abscess (Fig. 9a). The centre of the abscess is amorphous and contains fibrin, cellular remnants among which accumulations of nuclear material and bacteria in sometimes very large colonies (Fig. 9b) are present. The interior of the large encapsulating layer clearly consists of numerous remnants of phagocytosing macrophages, indicated by the vacuoles. Further outward many fresher macrophages with less vacuoles, as well as granulocytes and lymphocytes can be identified. Loose erythrocytes indicating small haemorrhages and capillaries are also present. Increasing presence of collagen and increasing flattening of fibroblasts results in orientation. Most larger blood vessels as well as haemorrhages are found at the outside of this layer. At one point an accumulation of lymphocytes with mainly plasma cells, recognized from the excentric nucleus and light pale centrosome, is observed (see also week 6 specimen, Fig. 10b). Also at this outer level macrophages with phagocytosed material are found, especially near the blood vessels, thus indicating removal. Finally, the encapsulating layer is followed by a layer characterized by poor cellular and high non-oriented collagen presence.

At *week 6* after injection of *S. aureus* the lead sample is completely surrounded by abscess-like tissue, as observed macroscopically. At all levels, flat fibroblasts as well as newly arriving granulocytes and macrophages can be observed at the implant interface, which denotes, concomitant with blood vessels and haemorrhages at the outside of the capsule, the activated state. Lymphocyte accumulations are observed in the outer capsule at each level. At the level of the blue wire, these accumulations are largest (Fig. 10a). The central part of one of the accumulations contains more plasma cells and phagocytosing macrophages, while smaller T-cell-like lymphocytes (with darker stained nuclei) are found at the margin. This accumulation is located just at the outside of the capsule surrounding the blue wire. In the outer layer of this capsule again specific plasma cell accumulations are present near some large blood vessels (Fig. 10b), while in the loose connective tissue around the capsule a combination of plasma cells and T-lymphocytes is infiltrating.

4. Discussion

An *in vivo* rat model has been developed to describe the events that take place in a late biomaterial-associated infection. Late infection was represented by injection of bacteria at the skin incision site after a 3-week implant period.

The results show that injection of *S. aureus* at first induced local, i.e. at the blue wire (Fig. 1), detachment of the initial fibrous capsule, wound fluid infiltration, fibrin formation and granulocyte and macrophage reactions. These reactions then spread along the lead sample. Bacteria were removed via phagocytosis, while fibroblast proliferation induced thick encapsulation of the implant. Spreading of infection did not occur via direct bacterial adhesion to PU, but through blood vessels at the outside of the capsules and through wound fluid passage at the interface and in the lumen of the lead sample. After day 5 infection apparently was in a controlled state. Apart from two small abscesses, this resulted in relatively controlled circumstances at day 10. However, probably due to the possibility of growing in the lumen, where granulocytes and macrophages were unable to cope with the bacteria, at weeks 3 and 6 the reaction had intensified. By now, the tissue response had turned into a specific inflammatory reaction as denoted by accumulations of lymphocytes.

Before the bacterial challenge was introduced, lead samples were implanted for 3 weeks at which time they were surrounded by a matured thin capsule without any further exceptional observations.

After injection of *S. aureus*, the results show that spreading of infection in this model seemed to occur via blood vessels outside the capsules and wound fluid passage at the interface and in the lumen of the lead

samples. This is based on five observations:

(1) the presence of live bacteria outside the capsules during the first days, as detected by culture,

(2) blood vessels activation and mast cell degranulation observed at the outside of the capsules,

(3) the small colony of bacteria found near the intact interface at level C at day 2,

(4) detachment of the original capsule and wound fluid infiltration at the interface, and

(5) the presence of bacterial colonies in the PU lumen (D) at day 5.

Thus, spreading of infection did *not* occur via direct adhesion to lead materials. This could also be concluded from the intraluminal observations (Fig. 8b). This is in contrast with the generally reported mechanism for implant-related infections that assumes that bacterial adherence is its prelude, followed by formation of biofilm-enclosed microcolonies [7–10].

In the case of the rat lead samples, wound fluid passage and the presence of relatively large lumina probably are the decisive factors. Capillary action will probably, immediately after bacterial challenge, provoke entry of wound fluid into the sample's lumina. Then only few bacteria are needed to establish large colonies later, as was also confirmed by bacterial cultures at day 10, and week 3 and 6. The natural defence mechanism will not be able to cope with bacterial growth in the lumen, and although it is unknown if the rat would have been able to cope with bacterial growth present only in abscesses around the lead sample (if the lead sample had been made without lumina), intraluminal bacterial growth represents a continuous danger for revival of infectious complications. This is supported by other reports [9, 11]. Supposedly, the occurrence of late infections and reappearing infections also may be the result of lumina (or pores). It has been hypothesized [9] that bacteria, introduced during surgery at the implant site, may find this sheltered environment that protects them from the host defence mechanism. As long as a hostile environment is faced, the bacteria will remain in a low metabolic state. However, as soon as the surroundings are in a quiet state, i.e. the wound healing response has finished, so called 'swarmer cells' may release and cause new or recurred infectious complications [9].

From this study the fate of the infected lead samples is unknown. In this respect we refer to an observation once made with PU tubing segments (unpublished results), where we saw a breakthrough of the infection towards the skin, inducing a fistula, which the rat eventually removed, including the tubing segment, by gnawing.

We hypothesize, also in the case of solid biomaterials, that wound fluid passage, may play a more crucial role than direct bacterial adhesion. In fact, it may well be that the presence of the capsule at the interface is crucial in spreading the infection, since a (detaching) capsule presents an excellent passageway for wound fluid and migrating bacteria. This seems in agreement with the fact that infections also occur in non-biomaterial related circumstances where passage via fluid and/or capsules is evident.

Blood vessel activation, wound fluid, fibrin, cellular extravasation and bacterial accumulation are the visible results of the infectious challenge, originating from a complex variety of reactions [12]. For example, the coagulase-positive *S. aureus* produces the enzyme coagulase, which may be one of the factors involved in the formation of excessive fibrin [13]. Furthermore, the presence of bacteria creates a whole cascade of reactions, which involve many biochemical factors from the coagulation cascade, complement system and further inflammatory response, such as platelet factors, complement factors and cytokines, the latter released from granulocytes, macrophages, mast cells and lymphocytes [14–16]. Transport of these factors, as well as of bacteria, via blood vessels to places remote from the interface induces the observed microvascular thrombohaemorrhagic changes because of their effects on the endothelium [16] and on mast cell degranulation at those locations. The latter itself results in additional release of factors, thus also from that point further spreading the inflammation reaction, etc.

As observed, in first instance the tissue response is an a-specific inflammation reaction marked by infiltration of granulocytes and macrophages followed by ingrowth of fibroblasts. Subsequently also a specific immuno-reaction is provoked, which agrees with our previous observations [6], in which we stated that at least 10^5 or 10^6 bacteria are needed to induce these reactions. The specific immuno-reaction, which in this model is not a memory-reaction since the rats had been bred germ-free, is probably initiated by macrophages, which have been in contact with *S. aureus* or related chemotactic factors, and thereafter migrate towards nearby lymph nodes [14–16]. Here, interactions between macrophages and T-lymphocytes (To-cells) will result in helper functions of other T-lymphocytes (Th2-cells) inducing proliferation and maturation of B-cells. This results in immunoglobulins (Ig)-producing plasma cells. Ig forms at the implantation site complexes with (remnants of) *S. aureus*, thus attracting, for example, new macrophages to phagocytose [14–16]. This sequence of events takes approximately one week, which explains the absence of lymphocyte accumulation in the first week (small numbers of lymphocytes were always observed but not mentioned). If, furthermore, the a-specific reactions are unable to cope with the bacterial challenge, the specific immuno-reaction will be the result. At weeks 3 and 6 the lymphocytes present in the surrounding loose connective tissue have probably migrated from nearby lymph nodes; however, it is not impossible that also local proliferation has occurred.

In conclusion, the results of this study using a late infection model show that spreading of infection occurs in a way that is different from the generally proposed mechanism.

Although injection of such large numbers of bacteria seems exaggerating, the results of this study form a good basis to evaluate various anti-infection technologies for their efficacy *in vivo*. Currently, efforts are being made to develop materials with enhanced resistance to infection, for which several options are under consideration, all directed to modification of the implant surface.

The first option is to develop materials that provide controlled release of antimicrobial agents at the implant site [17–19]. Furthermore, surface modification techniques to improve tissue adherence are under investigation [20]. The initial tissue response, generally perceived as biocampatible and unable to cope with this secondary bacterial challenge, confirms that encouragement of proper tissue adhesion and integration at the implant interface is of major importance to prevent (secondary) infectious complications [20]. Thus, detachment of tissue capsule and subsequent bacterial passage along the implant interface may be prevented. The presented model is thought to be very suitable for demonstrating potential efficacy.

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