Wound healing phenomena in titanium fibre mesh: the influence of the length of implantation

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In previous experiments a new type of percutaneous device for implantation in soft tissue was designed, containing a sintered titanium fibre mesh. The devices are inserted by a so-called ‘two-phase’ surgical technique with an intervening healing period of 3 months between insertion of the subcutaneous and percutaneous parts. From a clinical point of view, this time interval is too long. The aim of this study was to investigate whether it was possible to reduce the intervening healing period. The implants were inserted in the backs of nine goats. In each goat, six implants were placed with intervals of 1 week. Consequently, at the end of the experiment, in each goat six implants were present with implantation periods ranging from 1 to 6 weeks. After 6 weeks, the animals were killed and the implants with surrounding tissue were processed histologically. Analysis demonstrated that during the first 2 weeks an inflammatory response was present. Thereafter, no difference in tissue response was found between the various implantation periods. In conclusion, the experiment suggests that for titanium mesh percutaneous devices a 3-week healing period is sufficient between the installation of the subcutaneous and percutaneous parts. © 1996 Elsevier Science Limited

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It is well known that considerable difficulties exist with the maintenance of percutaneous devices1-9. The problems encountered are mainly concerned with exit-site infections and related complications.

Therefore, in our laboratory research has been directed toward the development of a more successful percutaneous device10-14. These efforts have resulted in a percutaneous device provided with a subcutaneous flange made of a porous titanium fibre mesh structure15. In addition to the design of the implant, a so-called two-stage surgical procedure was used for placement of the devices. During the first surgical session the subcutaneous component was inserted. After a healing period of 3 months the percutaneous part of the implant was adapted to the subcutaneous component. Because this healing period of 3 months was considered to be too long for application in peritoneal dialysis, an experiment has been performed to determine whether it is possible to reduce this period16. For this purpose, titanium fibre meshes were inserted subcutaneously in rabbits with implantation periods ranging from 5 to 12 weeks. Histological and histomorphometrical analysis of the tissue response to the implants showed that there was no difference between the various implantation periods. Considering these results, further reduction of the healing period has to be evaluated. The purpose of this experiment was to analyse the tissue response to the titanium fibre structures with implantation periods varying from 1 to 6 weeks.

MATERIAL AND METHODS

Implant materials

Figure 1 shows the porous fibre mesh used in this experiment. The mesh was fabricated by interengaging and intertwining a multiplicity of commercially pure titanium fibres (Bekaert, Belgium). After compression the fibre structures were sintered to bond the fibres at their points of contact. The fibre diameter was 50 μm. The volumetric porosity was 86% and the weight of the mesh sheet was 600 g/m². Scanning electron microscopical examination revealed on the surface of the titanium fibres the presence of irregular, rather profound longitudinal grooves. As demonstrated by energy dispersive spectroscopic analysis, the surface of the fibres was composed of titanium17.

Implantation procedure

Nine healthy, adult (2-3 years of age), female Saane goats weighing about 60 kg were used in the experiments. In each goat, six implants were placed,
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three on the left and three on the right side of the spinal column. The implants measured 2.0 × 1.5 cm². The implants were inserted with intervals of 1 week. Consequently, at the end of the experiment, in each goat six implants were present, with implantation periods ranging from 1 to 6 weeks.

Before insertion the implants were sterilized in an autoclave. Surgery was performed under local anaesthesia. The back of the goats was shaved, washed and disinfected with iodine. Paravertebrally, only on one side of the spinal column, a longitudinal incision of about 3.0 cm was made through the full thickness of the skin. Subsequently, lateral to the incision a subcutaneous pocket was created by blunt dissection with scissors. The fibre mesh was inserted in this pocket. Finally, the wound was carefully closed with vicryl 3-0.

To assure complete randomization, the position of the various implants into the back was based on a split plot design¹⁴. Balancing was done by Latin square to exclude experimental influences. The experimental protocol used for these studies was approved by the Institutional Animal Care and Use Committee and adhered to the National Institutes of Health guidelines for the use of experimental animals.

**Histological evaluation techniques**

One week after the last surgical procedure the animals were killed using an overdose of Nembutal¹⁶. After the animals were killed, the implants with their surrounding tissues were excised immediately and fixed in 10% buffered formalin. The tissue specimens were then trimmed to remove excess tissue and embedded in methyl methacrylate. After polymerization, thin (10 μm) histological sections were prepared using a modified diamond-blade sawing microtome technique¹⁸,¹⁹. The sections, containing the implants and the surrounding tissues attached to them, were stained with Methylene Blue and basic fuchsin and investigated by light microscopy.

To assess the soft tissue response to the implants, both histological and histomorphometric evaluations were performed. The histological evaluation consisted of a thorough description of the observed tissue reaction. For the histomorphometric evaluation the following parameters were assessed:

1. The characteristics of the capsule surrounding the implant and the characteristics of the tissue inside the interstices of the implant as rated according to a method that has already been described extensively in a previous paper (Table 1)¹⁷. In summary, the evaluation of the interstitial tissue was only qualitative. The semiquantitative capsule classification consisted of a capsule thickness measurement based on the observed number of fibroblasts. The qualitative rating of the capsule and interstitium consisted of numerically rating the tissue morphology (fibrous tissue, maturity, etc.).

### Table 1 Histological grading scale for soft-tissue implants

<table>
<thead>
<tr>
<th>Reaction zone</th>
<th>Response</th>
<th>Score</th>
</tr>
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<tbody>
<tr>
<td>Capsule quantitatively</td>
<td>thickness rating: 4</td>
<td></td>
</tr>
<tr>
<td></td>
<td>1–4 fibroblasts 3</td>
<td></td>
</tr>
<tr>
<td></td>
<td>5–9 fibroblasts 2</td>
<td></td>
</tr>
<tr>
<td></td>
<td>10–30 fibroblasts 1</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Not applicable 0</td>
<td></td>
</tr>
<tr>
<td>Capsule qualitatively</td>
<td>capsule tissue is fibrous, mature, not dense, resembling connective or fat tissue in the non-injured regions 4</td>
<td></td>
</tr>
<tr>
<td></td>
<td>capsule tissue is fibrous but immature, showing fibroblasts and little collagen 3</td>
<td></td>
</tr>
<tr>
<td></td>
<td>capsule tissue is granulous and dense, containing both fibroblasts and many inflammatory cells 2</td>
<td></td>
</tr>
<tr>
<td></td>
<td>capsule consists of masses of inflammatory cells with little or no signs of connective tissue organization 1</td>
<td></td>
</tr>
<tr>
<td></td>
<td>cannot be evaluated because of infection or other factors not necessarily related to the material 0</td>
<td></td>
</tr>
<tr>
<td>Interstitium qualitatively</td>
<td>tissue in interstitium is fibrous, mature, not dense, resembling connective or fat tissue in the non-injured regions 4</td>
<td></td>
</tr>
<tr>
<td></td>
<td>tissue in interstitium shows blood vessels and young fibroblasts 3</td>
<td></td>
</tr>
<tr>
<td></td>
<td>invading the spaces; few macrophages may be present 2</td>
<td></td>
</tr>
<tr>
<td></td>
<td>tissue in interstitium shows giant cells and other inflammatory cells in abundance but connective tissue components in between 1</td>
<td></td>
</tr>
<tr>
<td></td>
<td>implant cannot be evaluated because of problems that may not be related only to the material to be tested 0</td>
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Figure 2 Schematic drawing of the subcutaneous implant showing the fields of cell counting used in the histomorphometric analysis.

Figure 3 At 1 week implantation, the capsule surrounding the titanium fibre mesh consists mainly of inflammatory cells and blood vessels. Original magnification ×25, bar = 122 μm.

Figure 4 Inside the porosity of the mesh, inflammatory cells and blood vessels are present after 1 week implantation. Original magnification ×63, bar = 49 μm.

presence of connective tissue or fat tissue) and cellularity (presence of fibroblasts, macrophages and giant cells).

2. The tissue response as quantified by counting the number of blood vessels and inflammatory/foreign body cells in the surrounding fibrous capsule and interstitial tissue. For this purpose, the microscopic images were projected with a total magnification of ×400 on a colour monitor using a colour camera attached to the light microscope. Subsequently, the occurrence of blood vessels and foreign body giant cells was calculated in 14 predetermined fields (Figure 2). Depending on the total length of the section, the fields were positioned at regular 2-mm intervals: six fields (with one side bordering the implant surface) in the capsule surrounding the fibre mesh, six in the middle of the interstitium and two at both ends of the interstitium. The occurrence of inflammatory cells in each field was estimated by superimposing a net ruling, consisting of 176 smaller squares (1.5 x 1.5 cm²) on the colour monitor. Then, the number of inflammatory cells in five marked squares per field was counted.

All histomorphometric procedures were done blindly by two different operators. Differences in scores were discussed. The data presented are based on 'consensus between observers' about the differing judgements.

RESULTS

Tissue response

Descriptive light microscopic evaluation
Evaluation of the prepared sections showed that the tissue reaction to the titanium meshes with an implantation period of 1 week was mainly characterized by an inflammatory response. The implants were surrounded by a thick layer of inflammatory cells and blood vessels (Figure 3). Inside the porosity of the mesh mainly inflammatory cells were observed, although some fibroblasts were also present (Figure 4). Occasionally, accumulations of erythrocytes were found inside the mesh. Already at 2 weeks of implantation the inflammatory response around the mesh, as well as in the interstitium, decreased. The inflammatory layer surrounding the implants was almost totally replaced by a fibrous tissue capsule consisting of fibroblasts and collagen. Also inside the mesh a cellular fibrous tissue was now present. Furthermore, the thickness of the reactive tissue capsule was less compared with the inflammatory cell layer surrounding the 1-week specimens.

The tissue response to the fibre meshes with an implantation period of 3–6 weeks was relatively uniform (Figure 5). These implants were surrounded by a medium thin fibrous tissue capsule containing fibroblasts, collagen, blood vessels and some inflammatory cells. Occasionally, nerve bundles were observed close to the mesh material. Inside the implants, the mesh porosity was filled with components of early-stage connective tissue, including blood vessels (Figure 6).

Histomorphometric evaluation
Figure 7 shows the data of the histomorphometric rating of capsule and interstitium characteristics for all implantation periods. Statistical analysis of these data, using a one-way analysis of variance (ANOVA) and a multiple comparison procedure (Newman–Keuls), showed that a significant difference existed in capsule
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Figure 5 After 2 weeks implantation, the tissue capsule consists mainly of fibroblasts and collagen. The inflammatory response, together with the number of blood vessels, decreased. Original magnification x25, bar = 122 µm.

Figure 6 Inside the mesh material early stage connective tissue is present at 3 weeks implantation. Original magnification x63, bar = 49 µm.

Figure 7 Comparative rating of capsule quantity, capsule quality and interstitium quality using the histomorphometric grading scale.

Figure 8 Comparative rating of: a, inflammatory cells/6375 µm²; b, foreign body giant cells/224310 µm²; c, blood vessels/224310 µm².

The quantity between 1-week and 3-6-week implants (P < 0.01). In addition, a significant difference was demonstrated in capsule and interstitium quality between 1-week and 2-6-week specimens (P < 0.01 in both cases).

Figure 8 provides data on the occurrence of inflammatory and foreign body giant cells, as well as blood vessels, in the fibrous capsule and interstitial tissue. One-way ANOVA and a multiple comparison procedure (Newman–Keuls) indicated that a significant difference existed in the number of inflammatory cells occurring in the middle of the interstitial tissue between an implantation period of 1 week compared to 3–6 weeks (P < 0.01).

For all implantation periods, no foreign body giant cells were found in the fibrous capsule. Although it appeared that at 1 week of implantation less giant cells were present in the interstitium, no significant difference in foreign body giant cell occurrence existed for the various implantation periods.

Statistical analysis of the blood vessel data revealed that there was no significant influence of the implantation period.
tion period on the number of blood vessels in both capsule and interstitium. The diameter of the blood vessels varied from 5 to 100 μm.

Finally, the number of inflammatory cells, foreign body giant cells and blood vessels present in the middle part of the mesh material compared to the endings of the interstitium was not significantly different.

DISCUSSION AND CONCLUSIONS

The success of implanted biomaterials is dependent on many factors, which all have to do with the final tissue response to the materials. In the cascade of events that follow after the first injury to insert a material, the duration and level of the initial inflammatory response play an important role. With regard to the implant material used, the possibility of evoking an adverse healing response depends on material variables like bulk chemistry, surface texture and implant design. For example, in another study we compared in goats the subcutaneous tissue reaction to implants provided with either the currently used Dacron velour versus titanium fibre mesh. Four months after implantation, histological and histomorphometrical evaluation showed that the soft tissue response inside the Dacron velour was mainly inflammatory, while inside the porosity of the titanium mesh a more mature collagenous tissue was found. In this context, it has to be emphasized that titanium is considered to be a very biocompatible material. Therefore, it is not surprising that the overall healing process to the titanium fibre mesh, as used in this study, shows a striking similarity to the regular sequence in all connective tissue wound healing processes. Although the exact time depends on the original tissue location, the initial inflammatory and proliferative phase of wound healing takes about 2 weeks, followed by a remodelling phase. The histomorphometric evaluation of the soft tissue response to the titanium fibre mesh material, as used in the present study, showed that during the first 2 weeks after implantation a tissue response occurred resembling an early tissue reaction characteristic for a surgical trauma without an implant. When this response faded away, which was generally after no longer than 2–3 weeks, fibroblasts started to proliferate. This was followed by the deposition of collagen inside the titanium mesh. Unfortunately, the histological preparation technique used makes it impossible to obtain additional information about the quality of the collagen matrix produced.

Considering our previous study in rabbits dealing with the healing response between 5 and 10 weeks, we now observed no difference between the number of foreign body giant cells as found in the end and the middle parts of the interstitium. Two explanations can be given for this discrepancy in observations. First, it is possible that the implantation period is too short to result in a different reaction, certainly when it is recognized that mechanical trauma, as a product of the flexibility properties of the implant, is responsible for the adverse reaction at the ends of the mesh implants. Second, the implantation site as used in this study in goat is more flattened than in the previous study in rabbit, consequently resulting in less mechanical trauma.

Finally, comparison of our studies in goat and rabbit shows that with the applied histomorphometrical techniques no gross differences can be demonstrated between the 3–6 and 5–10 week healing periods.

Therefore, on the basis of the results we assume that the intervening healing period between the first and second stage surgical procedure for placement of our experimental titanium mesh percutaneous device can be reduced to 3 weeks. This observation can lead to a better clinical acceptance of this device for human applications like peritoneal dialysis, although it has to be emphasized that extrapolation of experimental animal results to the human situation is not always straightforward.

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