Evaluation of the acute scarring response to the implant of different types of biomaterial in the abdominal wall

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Since the short-term, acute scarring process induced by a biomaterial may condition the evolution of the repair process, the present investigation evaluates the behavior of polytetrafluoroethylene (PTFE) and polypropylene (PL) biomaterials in the initial stages of repair. Three PTFE biomaterials (Mycro Mesh®, Dual Mesh® and Soft Tissue Patch®) and one PL biomaterial (Marlex®) were employed to repair defects created in the abdominal wall of New Zealand rabbits. Animals were sacrificed at 3 or 7 days. Specimens were obtained for light and scanning electron microscopy, and immunohistochemical analysis using the RAM-11 monoclonal antibody for rabbit macrophages. The PL implants showed substantial adhesion formation with viscera. Lower adhesion formation was detected in the PTFE implants. The evolution of the acute phase of the repair process was similar for each PTFE biomaterial. At 3 days post implant, an incipient neoperitoneum was detected which was fully established after 7 days. The behavior of the PL implant was similar, although a greater amount of reticular granulation was detected. The neoformed peritoneum was irregular. Few RAM-11-labeled macrophages were detected in all cases. The acute phase of the tissue repair process induced by the implant of PTFE and PL biomaterials generally proceeds along similar lines to a normal repair process. However, the use of microporous, laminar materials seems to favor the early establishment of a well-defined neoperitoneal layer.

1. Introduction

The tissue repair process of a wound, particularly the process of scarring, is initiated by humoral and cellular mechanisms which are ultimately responsible for its success. When such a repair process is interfered with by infection, a foreign body, etc. the final result is at times ostensibly modified [1]. Hence, when a biomaterial is used to repair a tissue defect or to provide strength to tissues, the environment in which the scarring process takes place is altered.

Most of the experimental studies performed to date to evaluate the behavior of prosthetic biomaterials in the repair of abdominal wall defects focus on the long-term repair process [2, 3]. Little attention has been paid to the short-term, acute response induced by the prosthetic biomaterials currently in use. Given that this acute phase is the determining factor for the long-term behavior and development of the implant, the present investigation aims to compare the acute scarring response to the implant of several biomaterials. The behavior of the implants within the first hours may reflect the possible long-term integration of the different prosthetic surfaces with recipient tissue.

2. Materials and methods

Eight male, white New Zealand rabbits weighing approximately 1800–2000 g were caged and maintained under constant light and temperature conditions (EEC 28871-22A9). Sterile, surgical technique was used to create two 2 × 2 cm abdominal wall defects involving all the layers of the wall with the exception of the skin in each of the experimental animals. The defects were repaired by use of a prosthetic patch of similar size to the defect composed of polytetrafluoroethylene (Mycro Mesh®, Dual Mesh® or Soft Tissue Patch®, Gore-Tex) or of polypropylene (Marlex®, Bard Card. Div.). Each animal was implanted with two different types of prosthesis such that each type of biomaterial was tested in four individual animals (Fig. 1). The polypropylene (PL) prosthesis Marlex (ML), consists of a PL monofilament woven to form a reticular structure of 1 mm pore size. The polytetrafluoroethylene (PTFE) prostheses differ in their structural characteristics. Thus, the Soft Tissue Patch (STP) has two laminar, microporous (30–60 mm) surfaces. Mycro Mesh (MM) is also laminar in structure but has 2 mm orifices evenly distributed over its surface conferring micro and

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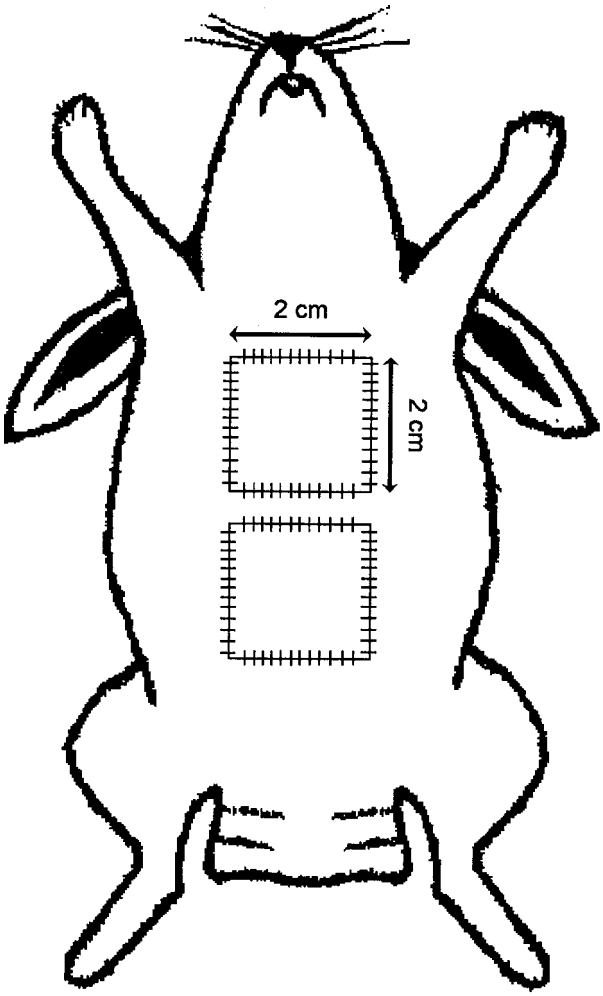

Figure 1 Diagram showing the defects created and repaired with biomaterials in the experimental animal.

macroporous properties upon the prosthesis. Dual Mesh (DM) is composed of a non-porous sheet of PTFE on one side and a layer of similar porosity to the STP on the other. The MM and DM implants are punched in a pyramidal pattern to provide one rough surface.

Each prosthetic patch was secured to the margins of the defect by continuous 4/0 polypropylene suture. The skin was closed over the implant also by continuous 4/0 polypropylene suture. Anaesthesia was induced by intramuscular injection of a mixture of ketamine hydrochloride ($70\,mg\,kg^{-1}$), diazepam ($1.5\,mg\,kg^{-1}$) and chlorpromazine ($1.5\,mg\,kg^{-1}$).

The animals were sacrificed at 3 or 7 days post-implant. At each of these follow-up periods, the prostheses were examined macroscopically to estimate the degree of adhesion formation with abdominal viscera and the presence of infection and/or rejection. Specimens of prosthesis and attached neoformed tissue were pro-cessed for light microscopy (using haematoxylin-eosin and Masson's tricome stains) and scanning electron microscopy. Specimens were also subjected to immunohistochemical labeling with the rabbit macrophage-specific monoclonal antibody RAM-11 (DAKO M-633) [4].

3. Results

There were no cases of infection and/or rejection of the prosthesis. Substantial adhesion formation with viscera was detected in the PL implant. Loose adhesions were observed in the macroporous areas of the MM. In contrast, no adhesions were induced by the STP or DM implants.

The evolution of the acute scarring process shown by microscopy was similar in the three PTFE implants. There was no cellular infiltration of the implants. A loose, reticular granulation tissue was observed running parallel to the main surfaces of the biomaterial. This type of tissue was most abundant on the subcutaneous or rough surface of the MM and DM implants. Few cells were detected with scarce accumulation of white blood cells on both prosthetic surfaces. The formation of granulation tissue containing a few white blood cells was also detected within the perforations of the MM.

The incipient formation of the neoperitoneal mesothelial layer could be observed by the third day post-implant. Mesothelial cells undergoing differentiation of spherical or flattened appearance were seen. At 7 days post-implant, this layer was fully formed (Figs 2–5).

The behavior of the PL prosthesis ML was similar to that of the three PTFE prostheses, although the reticular granulation tissue was more abundant and occupied all the internodal spaces of the ML mesh. There was also a

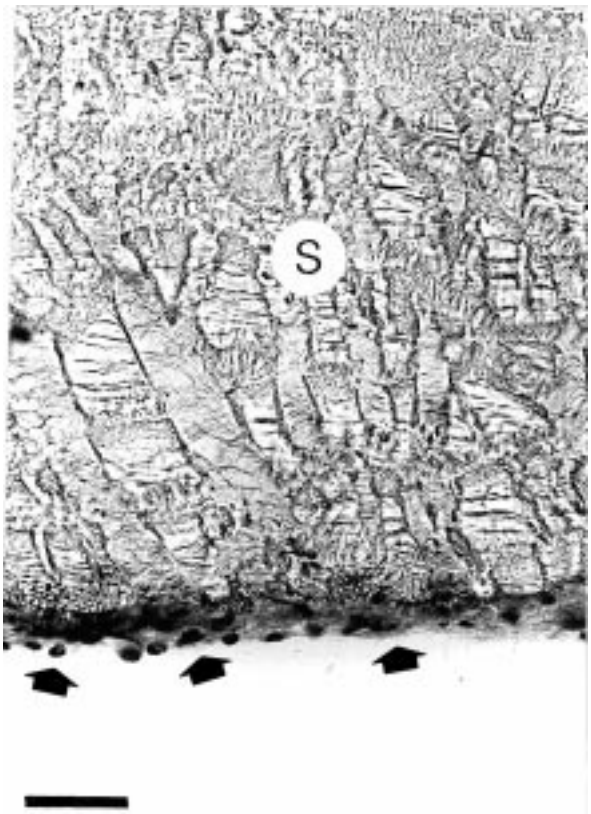

Figure 2 Neoperitoneum formed on a Soft Tissue Patch (s) implant three days post-implant. At this time, an incipient mesothelial cell layer (arrows) could be seen on the inner surface of the biomaterial (light microscopy × 20) (scale bar 100 μm).

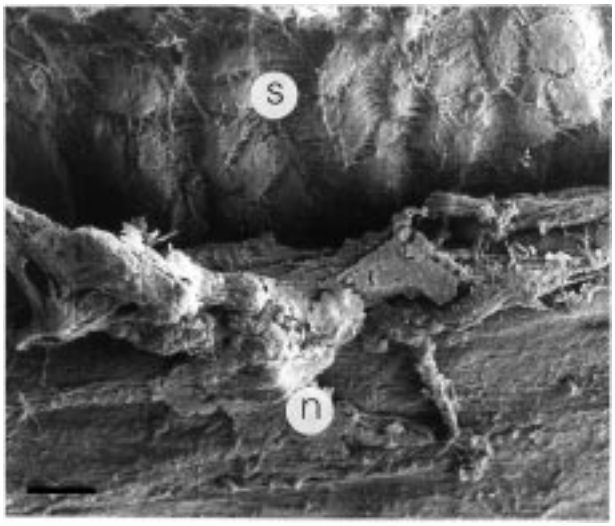

Figure 3 Scanning image of the interface formed between Soft Tissue Patch (s) and neoperitoneum (n) seven days after implant. Neoperitoneal fibers were distributed parallel to the surface of the implant (scanning electron microscopy × 125) (scale bar 10 μm).

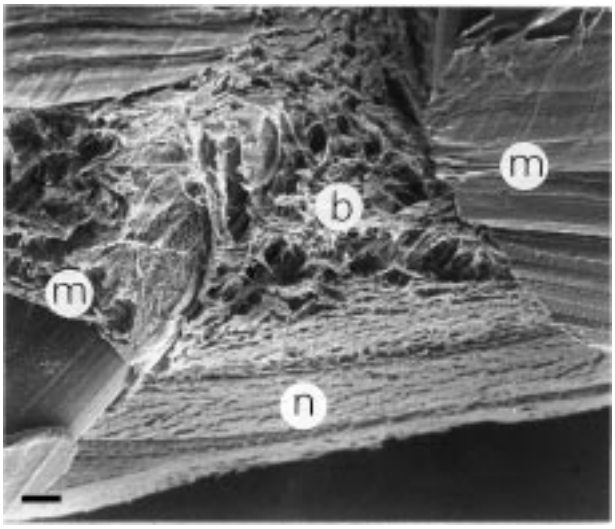

Figure 5 In the perforations of the Mycro Mesh implants (m), bridges of loose connective tissue (b) were formed seven days after implant. (n, neoperitoneum) (scanning electron microscopy × 250) (scale bar 100 μm).

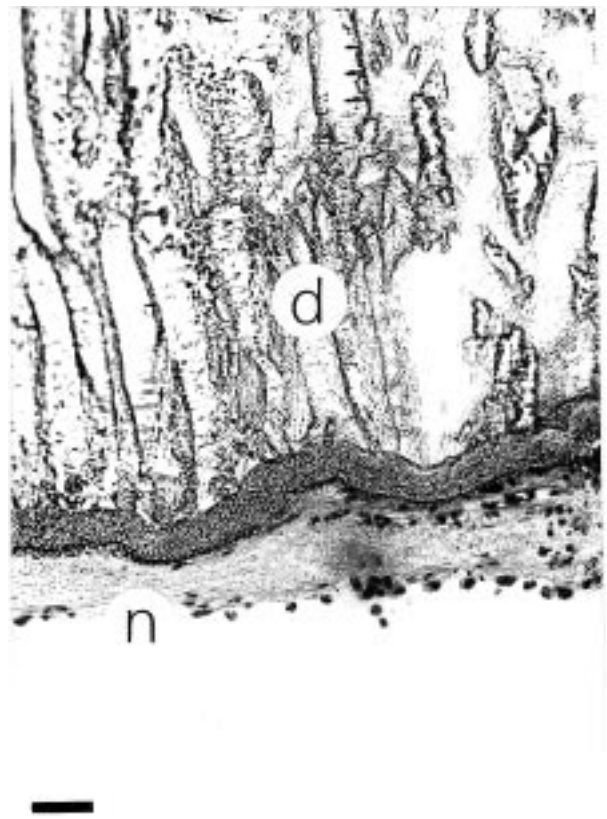

Figure 4 Neoperitoneum (n) established seven days after implant on a Dual Mesh (d) prosthesis. The non-porous PTFE layer impedes cellular colonization of the biomaterial (light microscopy × 20) (scale bar 100 μm).

scarce presence of cells in the tissue and only a few white cells were seen around the PL monofilaments. However, the newly formed peritoneum was irregular and at times discontinuous at both follow-up periods (Figs 6 and 7).

Few macrophages labeled with RAM-11 were detected at each follow-up time in each of the implants confirming the scarce accumulation of white blood cells observed. No differences in the number of labeled macrophages were detected between the different biomaterials. Only a few labeled cells were restricted to the surfaces of the PTFE implants and to the areas around the PL monofilaments of ML.

4. Discussion

The initiation of the repair process in the presence of a prosthetic material used for the repair of an abdominal wall defect is yet to be established. Given that the start of the scarring process may condition its future development, any type of interference at the onset may lead to anomalies. The presence of a foreign body such as a biomaterial could induce modifications to the normal repair process. The aim of the present investigation was to evaluate the onset of this process after the implant of two types of biomaterial often used in clinical practice, PTFE and PL. Experimental findings to date only include the long-term evolution of the repair process in the presence of these materials [5–7]. The present results show that there are, in fact, differences with respect to the neoformed tissues which depend upon the structure of the prosthetic material employed and mainly upon its porosity. Thus, the greater porosity of ML permits the establishment of granulation tissue throughout the prosthesis shortly after implant, presumably due to the easy passage of cells. In contrast, in the PTFE prostheses, the amount of granulation-type, neoformed tissue is much reduced due to low porosity. These observations are consistent with previous findings of long-term evaluations by the present author and others [8–11]. From the first moments after implant, this type of tissue was seen running parallel to both surfaces of the PTFE while the structure and disposition of neoformed tissue in the presence of ML was very disorganized. This is in accordance with observations made in previous studies with regards to the long-term structure of neoformed

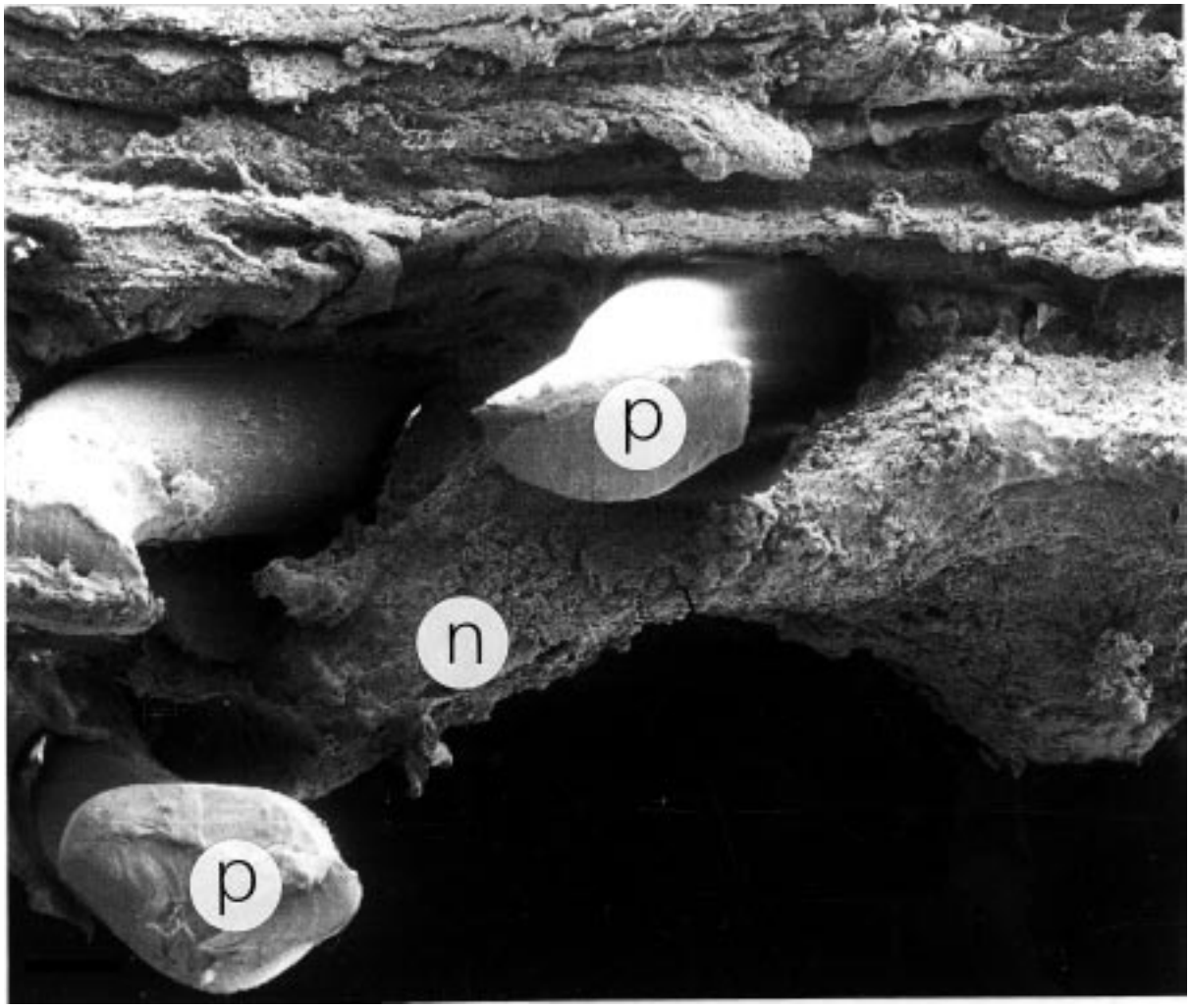

Figure 6 Three days after implants Marlex meshes (p) were surrounded by a reticular granulation tissue. Neoperitoneum (n) had a rough texture (scanning electron microscopy × 50) (scale bar 100 μm).

tissue in the presence of both types of implant, and indicates the great influence of the initiation of the repair process on its future development.

The small differences observed in the three PTFE implants may also be explained by the structure of the prosthesis. The rough PTFE surface may provoke a greater formation of granulation tissue without involving loss of structural organization. In a similar manner, the formation of granulation tissue bridges in the perforated areas of the MM implant does not alter the organized structure of the neoformed tissue.

The absence of a large number of white blood cells confirms the great physicochemical stability of PTFE and PL; no foreign body reaction towards the biomaterials was observed. This fact was also supported by the scarce number of macrophages labeled with the RAM-11 monoclonal antibody. The number of macrophages observed in the presence of PTFE and PL implants in previous studies is greater after 14 days than that observed here [2, 4, 12, 13]. This indicates that the repair process occurring in the presence of these biomaterials follows a similar course to the normal repair process. The sequence of appearance of the different cell types or number of such cells seems to be unaffected by their presence. Moreover, the fact that there were no differences between the two types of biomaterial in terms of the macrophage response, confirms the ideal nature of these materials for use as a tissue substitute in the abdominal wall.

Finally, it may be of interest to mention that the higher degree of adhesion formation induced by PL in comparison to PTFE is also consistent with the findings of previous long-term investigations [2, 14, 15]. This, once again, highlights the effect of the structure of the prosthesis on the evolution of the scarring process.

It may be concluded that, in general, the acute phase of the tissue repair process in the presence of the biomaterials PTFE and PL follows a similar course to that of a normal repair process. The structural characteristics of the biomaterial, particularly its porosity, condition the evolution of the repair process and the structure of neoformed tissue shortly after implant. Thus, the use of a laminar, microporous material such as PTFE favors the early formation of a well-defined neoperitoneal layer. Both types of biomaterial are well-tolerated by the recipient organism without the induction, at least

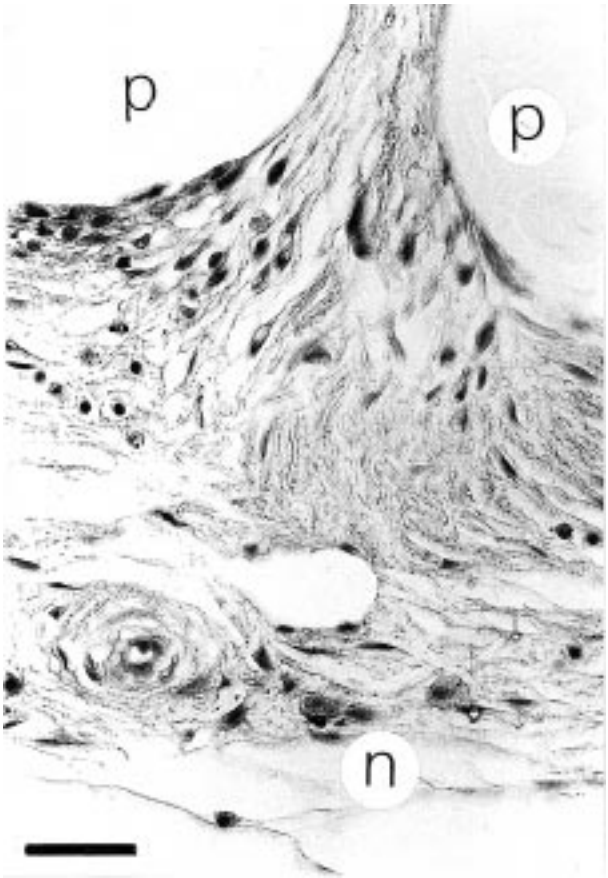

Figure 7 The reticular tissue was substituted on the Marlex implants (p) by a well-vascularized fibrous tissue seven days post-intervention. Its structured was very disorganized (n, neoperitoneum) (light microscopy × 20) (scale bar 100 μm).

in the short-term, of a chronic foreign body reaction. The macrophage response to the biomaterials does not differ from that occurring in a normal repair process.

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