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# Interface formed between visceral peritoneum and experimental polypropylene or polytetrafluoroethylene abdominal wall implants

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The objective of this work was to study the healing process at the interface between biomaterial and visceral peritoneum. Implants of polytetrafluoroethylene (ePTFE) and polypropylene prostheses were introduced into the abdominal wall of New Zealand rabbits. The behaviour of the biomaterials was analysed using light and scanning electron microscopy and immunohistochemistry in which a specific anti-rabbit macrophage monoclonal antibody (RAM 11) was employed. According to macroscopic observation, there was significantly fewer adhesions prosthesis-viscera to ePTFE than to polypropylene implants. After ePTFE implantation, restoration of the peritoneum took place in an orderly fashion. When polypropylene was used, the peritoneum formed was a disorderly tissue in which small areas of haemorrhage and necrosis could be seen to coincide with the appearance of adhesions. The number of labelled macrophages peaked 14 days after ePTFE or polypropylene implantation, after which it decreased gradually. It is concluded that, given the low rate of adhesion provoked by PTFE, this material is ideal for implants contiguous to the peritoneal cavity viscera. The macrophage response does not determine the use of one material or the other. The structure of the newly formed peritoneum and development of adhesions depends on the porosity of the biomaterial.

## 1. Introduction

The use of biomaterials is one of the technical alternatives available for the surgical treatment of abdominal wall defects such as hernia or eventration. The two most frequently employed prosthetic materials are knitted macroporous polypropylene, and microporous expanded polytetrafluoroethylene (ePTFE).

On certain occasions, especially since the introduction of laparoscopic surgical techniques, these biomaterials are placed in such a way that they are contiguous to the organs of the peritoneal cavity. The prosthesis/visceral peritoneum interface is still a subject of controversy. The rate of adhesion at this interface differs from one material to another, a fact which must be taken into account.

Likewise, the publication of clinical, and even experimental, reports of visceral erosion produced by polypropylene prostheses [1], which can lead to the formation of fistulas [2–4], makes it imperative that this interface be studied in depth.

The purpose of this work was to carefully assess the healing process involving biomaterials and the visceral peritoneum, using conventional histological methods as well as scanning electron microscopy (SEM) and immunohistochemical techniques.

## 2. Materials and methods

A total of 24 animals (male New Zealand white rabbits), weighing between 1800 and 2000 g, were used.

The prosthetic materials employed were knitted macroporous polypropylene mesh (Marlex$^{R}$; Bard Card. Div.), implanted in a group of 12 animals, and 1 mm thick microporous expanded polytetrafluoroethylene (ePTFE) (Soft TISSUE PATCH®; Gore-Tex), implanted into the remaining 12 rabbits. Anesthesia was achieved with a mixture of ketamine hydrochloride (70 mg/kg), diazepam (1.5 mg/kg) and chlorpromazine (1.5 mg/kg) i.m. In some cases, additional anesthesia was administered intraperitoneally.

Using a sterile technique, a 7 × 5 cm defect, involving all the layers of the anterior abdominal wall excluding the skin, was created in each animal. Fragments of the prosthetic materials cut to the same dimensions were implanted into the defect in such a way that they were contiguous to the intestinal loops (internal aspect) and with the subcutaneous cellular tissue or skin (external aspect). The prostheses were secured to the margins of the defect by a continuous 4/0 polypropylene suture that was interrupted only at the corners of the implant.

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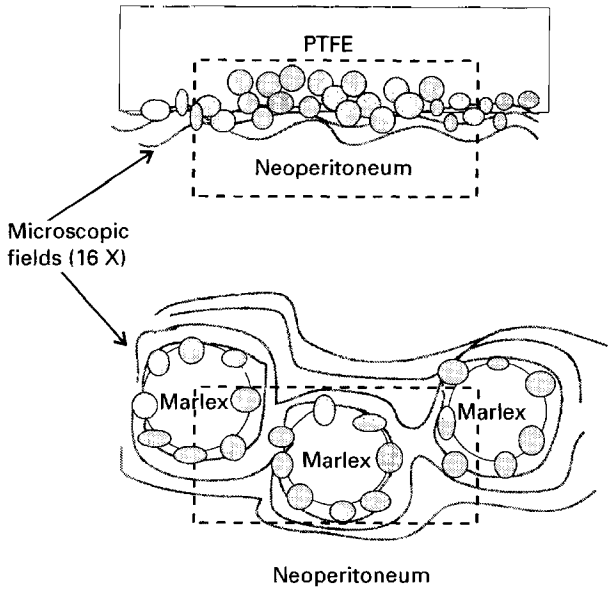


*Figure 1* Diagram showing the method for quantifying labelled macrophages (◎) in both biomaterials. PTFE has a layered, microporous structure. Marlex mesh is a single polypropylene monofilament woven into a loose net with macroporous structure. Owing to this, the labelled cells were quantified by studying 30 light microscopic fields (16x) for each study time and for each biomaterial.

Preoperative antibiotic prophylaxis with cefazolin (0.125 mg/kg body weight) was administered in every case.

Studies were performed in animals sacrificed at 14, 30, 60 and 90 days, with macroscopic examination of tolerance to the biomaterial, presence of infection, existence of points of relaxation in the abdominal wall and possible presence of adhesions to the visceral peritoneum.

After sacrifice, samples of the prosthesis/visceral peritoneum interface were taken for histologic study of the integration and behaviour of the prosthetic material in this zone.

Light microscopy and SEM were performed, as was immunohistochemical labelling. The latter involved the use of RAM-11 (DAKO), a specific monoclonal antibody to rabbit macrophages.

The fragments studied under light microscope were fixed in 10% formaldehyde, embedded in paraffin and cut into 5–7 μm thick sections for staining with hematoxylin-eosin and Masson's trichrome (Goldner–Gabe variant).

The samples for SEM were sectioned in such a way as to allow the interface to be studied. They were fixed in 3% glutaraldehyde and stored in Milloning's buffer (pH 7.3), to be dehydrated in graded concentrations of acetone. The critical point was determined in a Polaron E-3000 with CO2. The samples were coated with gold-palladium and studied under a Zeiss DSM-950 scanning electron microscope.

For immunohistochemical processing, an alkaline phosphatase-labelled avidin-biotin method was employed. The preparations were incubated with specific monoclonal antibody to rabbit macrophages in paraffin-embedded samples. The images were developed using either a chromogenic substrate, containing fast red and α-naphthol in some cases, and a silver substrate in others.

The labelled macrophages were quantified at each time point by studying 30 light microscopic fields (16 ×) randomly selected for each study time from the prosthesis/peritoneum interface formed with each biomaterial (Fig. 1). The results obtained were analysed by a descriptive statistical procedure to determine the arithmetic mean and standard deviation. The Student–Newman–Keuls test for the comparison of means was performed for each biomaterial. Owing to the different structures of the PTFE – layered and microporous – and the polypropylene mesh – which is a single monofilament woven into a loose net with large gaps – it was not possible to compare them by statistical methods.

## 3. Results

### 3.1. Macroscopic findings

Of the 12 animals that received ePTFE implants, only two presented adhesions between the prosthesis and the intestinal loops. In both cases, the adhesions were located in the peripheral areas of the implant, coinciding with the suture threads.

In the group that underwent polypropylene mesh implantation, adhesions were observed in 10 cases; in four they were extensive and of solid consistency.

In no case was infection or rejection detected.

### 3.2. Microscopic findings

Fourteen days after implantation of the ePTFE prostheses, the formation of a loose, poorly vascularized neoperitoneum of orderly structure was observed. There was evidence of foreign body reaction characterized by the appearance of a cell layer composed of monocytes/macrophages at the prosthesis/neoperitoneum interface.

Between 30 and 60 days, there was a clear progression of the development of the neoperitoneum, which became more extensive, compact and vascularized and showed greater cellularity. The foreign-body reaction induced by the ePTFE, characterized by the presence of macrophages (Fig. 2) (Table I) and giant foreign body cells, diminished progressively.

At 90 days of implantation, we found a compact, well vascularized neoperitoneum running parallel to the prosthesis, coating the surface of the biomaterial (Fig. 3). The tissue had a normal aspect and was rich in nuclei of elongated morphology which are typical of fibroblasts. At this stage, the foreign-body reaction detected was minimal.

With respect to the cellular and fibrillar colonization of the interior of the biomaterial, we have observed that, from day 14 to 90, the number of cells that penetrated the mesh rose progressively, although their depth never surpassed the innermost third of the prosthesis, even at day 90.

In the process of neoperitoneum formation following implantation of polypropylene mesh, by day 14, a loose scar tissue had developed which we found to be unorderly (with the fibres arranged concentrically to the polypropylene filaments) and rich in cells and blood vessels. Moreover, the formation of one or two

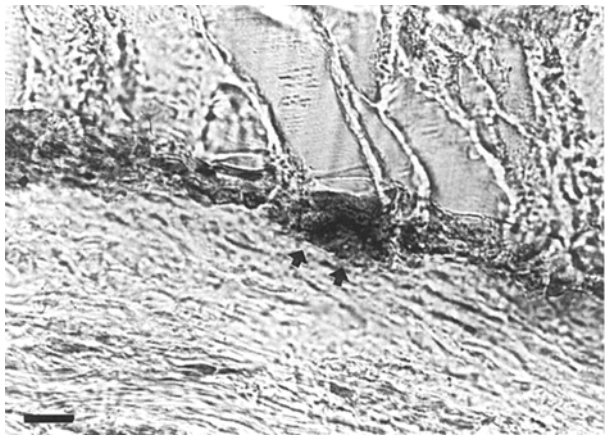

*Figure 2* Immunohistochemical labelling (➡) with the anti-rabbit macrophage monoclonal antibody RAM-11 of the prosthesis/neoperitoneum (N) interface after PTFE implantation (16x). Scale bar = 10 μm.

TABLE I RAM-11 labelled macrophage counts in ePTFE implants (cells per microscopic field × 16)

| 14 days | 30 days | 60 days | 90 days |
|---|---|---|---|
| 19 | 23 | 15 | 10 |
| 28 | 18 | 18 | 7 |
| 34 | 25 | 16 | 5 |
| 40 | 22 | 14 | 0 |
| 28 | 29 | 17 | 4 |
| 36 | 17 | 13 | 2 |
| 26 | 19 | 15 | 5 |
| 41 | 21 | 12 | 9 |
| 44 | 20 | 11 | 12 |
| 28 | 16 | 13 | 7 |
| 30 | 18 | 14 | 6 |
| 41 | 18 | 12 | 6 |
| 17 | 20 | 10 | 4 |
| 35 | 25 | 17 | 5 |
| 44 | 23 | 9 | 3 |
| 37 | 21 | 13 | 6 |
| 29 | 26 | 12 | 5 |
| 28 | 20 | 14 | 4 |
| 35 | 17 | 17 | 5 |
| 37 | 25 | 11 | 7 |
| 45 | 23 | 12 | 7 |
| 27 | 20 | 10 | 6 |
| 23 | 26 | 13 | 6 |
| 29 | 24 | 10 | 2 |
| 40 | 21 | 10 | 4 |
| 39 | 23 | 11 | 9 |
| 38 | 26 | 15 | 7 |
| 36 | 24 | 9 | 10 |
| 35 | 20 | 16 | 7 |
| 30 | 27 | 13 | 7 |
| Mean ± S.D | Mean ± S.D | Mean ± S.D | Mean ± S.D |
| 33 ± 7.26 | 21.9 ± 3.3 | 13 ± 2.54 | 5.9 ± 2.5 |

Student–Newman–Keuls test $p = 0.01$ in every case

cell layers of RAM-11 positive macrophages was observed (Fig. 4), with nuclei typical of the cells associated with foreign body reaction, surrounding each filament of the mesh.

By day 30, the loose connective tissue that we observed at 14 days was confined to the internodal spaces in the polypropylene, having been replaced by a tissue exhibiting a more orderly structure and abundant fibroblasts. Neovascularization continued to develop notably. Between 60 and 90 days postimplantation, we observed an increase in the extension and compactness of the neoperitoneum, covering the prosthetic mesh (Fig. 5).

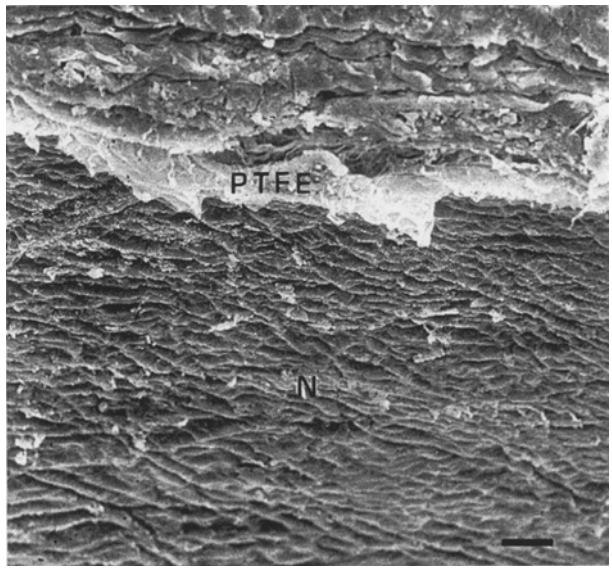


*Figure 3* View from the peritoneal aspect of the newly formed tissue (N) 90 days after implantation (scanning electron microscopy) (PTFE = prosthesis) (200x). Scale bar = 100 μm.

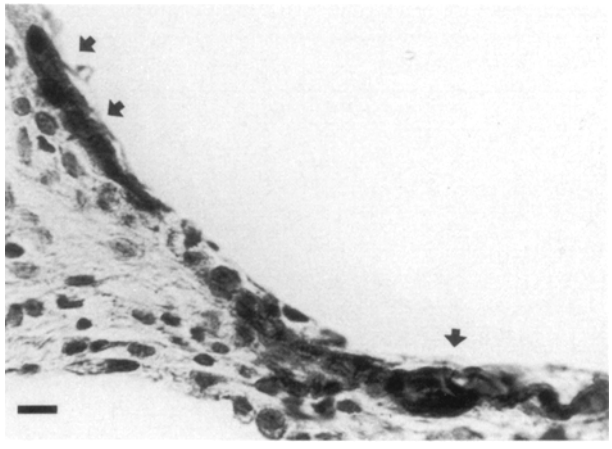

*Figure 4* RAM-11 labelled macrophages (➡) surrounding a polypropylene monofilament (PL) (40x). Scale bar = 10 μm.

One relevant histological finding in the polypropylene implants was the presence, at the prosthesis/neoperitoneum interface, of small areas of haemorrhage and necrosis, coinciding with the appearance of adhesions between the prosthesis and the organs of the abdominal cavity. This was observed at all the study times.

In these implants, the immunohistochemical study showed that the number of labelled macrophages peaked at 14 days, (PTFE = 33 ± 7.26; Marlex = 10.9 ± 1.66) (mean ± SD) after which it gradually decreased until day 90 (PTFE = 5.9 ± 2.5; Marlex = 1.8 ± 1.5) (mean ± SD) (Table II) (Figs 6 and 7).

When the two biomaterials are compared, it should be pointed out that, in the case of ePTFE, the reduction in the number of macrophages was accompanied

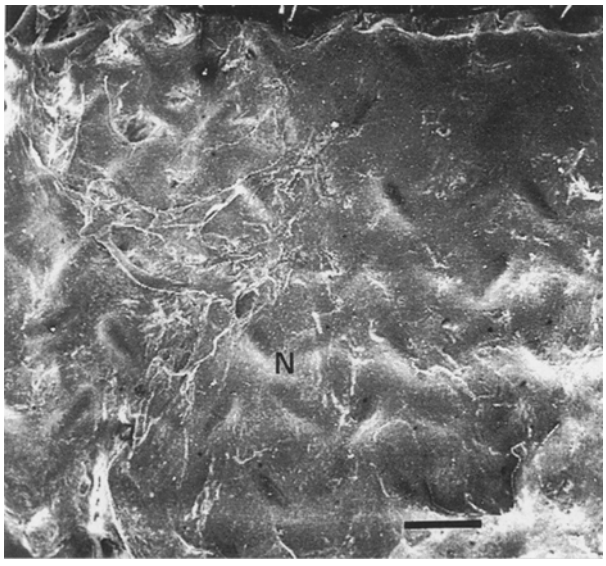


*Figure 5* General view of the newly peritoneum (N) 30 days after polpropylene implantation (scanning electron microscopy) (14x). Scale bar = 1 mm.

TABLE II RAM-11 labelled macrophage counts in Marlex implants (cells per microscopic field × 16).

| 14 days | 30 days | 60 days | 90 days |
|---|---|---|---|
| 6 | 10 | 7 | 2 |
| 11 | 7 | 4 | 4 |
| 12 | 7 | 4 | 1 |
| 11 | 9 | 3 | 0 |
| 9 | 5 | 3 | 0 |
| 10 | 5 | 4 | 5 |
| 11 | 12 | 7 | 3 |
| 11 | 7 | 2 | 2 |
| 10 | 8 | 2 | 1 |
| 13 | 8 | 4 | 1 |
| 8 | 5 | 5 | 0 |
| 10 | 6 | 7 | 1 |
| 12 | 4 | 6 | 0 |
| 11 | 8 | 7 | 3 |
| 13 | 10 | 4 | 1 |
| 10 | 6 | 4 | 3 |
| 10 | 5 | 5 | 1 |
| 9 | 7 | 7 | 1 |
| 11 | 7 | 3 | 3 |
| 13 | 6 | 4 | 1 |
| 14 | 7 | 3 | 3 |
| 10 | 5 | 3 | 1 |
| 12 | 3 | 6 | 1 |
| 13 | 7 | 5 | 2 |
| 11 | 7 | 6 | 0 |
| 9 | 7 | 4 | 3 |
| 8 | 6 | 3 | 5 |
| 9 | 2 | 3 | 0 |
| 10 | 8 | 7 | 4 |
| 12 | 7 | 4 | 2 |
| Mean ± S.D | Mean ± S.D | Mean ± S.D | Mean ± S.D |
| 10.9 ± 1.66 | 6.7 ± 2.01 | 4.5 ± 1.58 | 1.8 ± 1.5 |

Student–Newman–Keuls test $p = 0.01$ in every case

by a decrease in other cell types associated with a foreign-body reaction. This did not occur in the case of polypropylene mesh, where other cells, mainly giant foreign-body cells and white cells, accumulated up to day 90 of the study. The application of the Student–Newman–Keuls test to the labelled cell counts in each

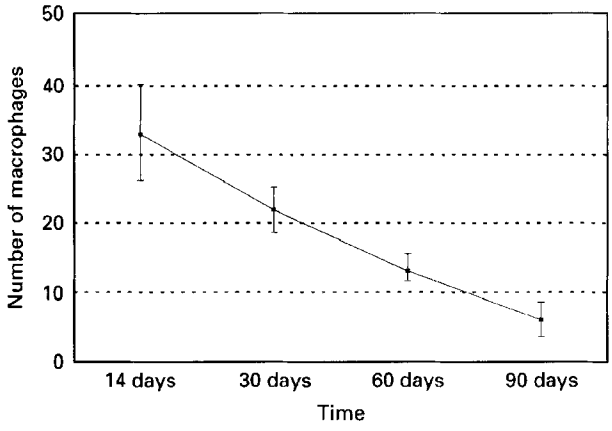


*Figure 6* Reduction of the number of macrophages on ePTFE implants between days 14 and 90.

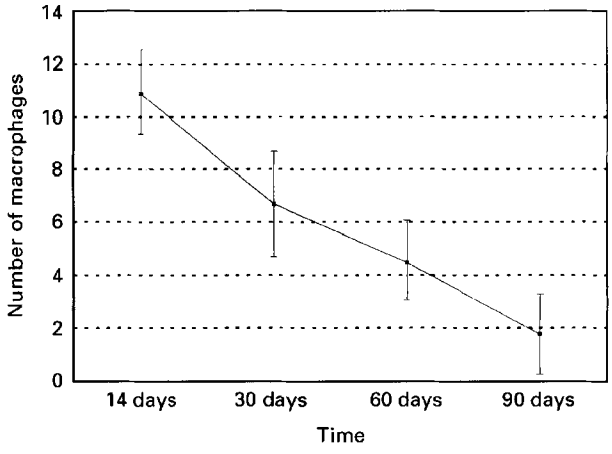


*Figure 7* Development of the macrophage response to polypropylene implants, which is similar to that observed with PTFE implants.

biomaterial revealed statistical significance ($p = 0.01$) in every case, confirming the reduction of the number of macrophages between days 14 and 90 in both models. Although the development of the reactions to the two biomaterials over the course of time could not be statistically compared, owing to the different porosity of the two biomaterials, the pattern appeared to be similar.

## 4. Discussion

One of the most controversial aspects of the use of biomaterials to repair abdominal wall defects is the behaviour of these materials at the prosthesis/visceral peritoneum interface. The appearance of adhesions between prosthetic materials and the intestinal loops is a well-known event. This can lead to the onset of obstructive phenomena as well as other complications. Thus, we considered that a study to compare the interface formed with two of the biomaterials most widely employed for this purpose, the ePTFE patch and polypropylene (Marlex) mesh, could determine which of the two biomaterials would be more suitable for use in the clinical situation, when contiguous to the intestinal loops.

Our results showed a moderate foreign-body reaction to the ePTFE implants, as well as the formation of a neoperitoneum exhibiting an orderly structure, a finding that coincides with those of Law and Ellis [5]. In any case, the process of tissue formation was not completed in 8 weeks, as stated by Elliot and Juler [6], but continued even after postimplantation day 90.

According to some authors [5, 7–9], in the case of polypropylene, the formation of the neoperitoneum results in the development of a compact, resistant scar tissue, the structure of which is less orderly than that observed with ePTFE.

On the other hand, we found less adhesions to the intestinal loops when ePTFE was implanted than when the biomaterial used was polypropylene mesh. The high rate of adhesion formation with polypropylene in rabbit and in rat was also observed by other authors [5, 6, 10, 11]. Only Jenkins *et al.* [12], working in rats reported less adhesions with polypropylene than with ePTFE. This finding could support the assertion of Pans and Pierard [13] that the animal species employed in the experimental models should be taken into account.

In our study, we observed a correlation between areas of haemorrhage with fibrin deposition and adhesion formation, a finding which agrees with the reports of other authors [14–16].

We consider that the progressive and orderly peritoneal neoformation that followed the implantation of ePTFE, which was influenced by the structure of the biomaterial and consisted of a compact lamina of very low porosity (10–20 μm) and smooth texture, could at least partially explain why the formation of adhesions was intrinsically limited. This is corroborated by the fact that adhesions appeared only in the areas of the sutures, which, in our experimental design, were made of polypropylene. Given the speed with which the neoperitoneal healing process takes place, and because its very structure is more aggressive consisting of a mesh made up of a braide i monofilament with high porosity and a rough surface, polypropylene would provoke a high rate of adhesions in visceral repair.

However, despite this drawback, polypropylene had the advantage of becoming totally integrated into the newly formed tissue. This integration occurred very rapidly, in agreement with the observations of Murphy *et al.* [11], who reported a greater deposition of collagen in the polypropylene prosthesis. These authors also concur with us in that they found the initial arrangement of the collagen fibres to be concentric to the polypropylene monofilaments.

On the other hand, the ePTFE did not integrate with the tissue, but became encapsulated by the peritoneum [8].

With respect to the foreign-body reaction and the development of the macrophage response, we observed some similarities between the two biomaterials, which behaved identically, resulting in a maximum number of macrophages between 14 and 30 days postimplantation, counts which diminished progressively until day 90 of the trial.

The reports of Leibovich and Ross [17] and Mustoe *et al.* [18] demonstrate the major role of the macrophages in tissue repair. Taking into account that it is in the initial stages of the healing process that these cells are relevant [19], this result is to be expected since the number of these cells should diminish as the repair process progresses.

An increase in the number of labelled macrophages would also indicate chronic foreign-body reaction. The number of labelled cells differ in the two biomaterials because they depend on the biomaterial surface area exposed in each case, which is lesser when polypropylene is employed. At any rate, the presence of macrophages in both biomaterials at day 90 of the study may indicate the continuation of the neoperitoneum healing process beyond that time. For this reason, we do not agree with the observations of the Elliot and Juler [6], who state that the healing process in ePTFE implants is completed 8 weeks after implantation. Moreover, there are reports in the literature that refer to continuity of the healing process after day 90 [9].

However, these findings disagree with the behaviour observed under light and electron microscopy. In the case of ePTFE, the reduction of the number of macrophages is accompanied by a decrease in the numbers of other cells typical of foreign-body reaction. With polypropylene implants, as the macrophage counts diminished there was an accumulation of other cell types such as fibroblasts, plasma cells and lymphocytes. By day 90, granulomas had formed around the polypropylene monofilaments. The giant foreign-body cells were very abundant at 90 and 120 days, a fact reported by Dabrowiecki *et al.* also [9].

In both biomaterials, the presence of macrophages leads us to suppose that the healing process continues beyond the end of our study period. The difference between the two biomaterials at day 90 was noteworthy since the polypropylene mesh was invaded by newly formed interstitial material of a markedly fibrous texture and high cellularity, while the neoperitoneum that formed on the ePTFE consisted of a lamina of looser connective tissue made up of fewer cells.

Thus, from the analysis of our results, we can conclude that:

- Given the low rate of adhesions provoked by ePTFE, this biomaterial can be implanted contiguous to the organs of the abdominal cavity.
- The capability of polypropylene to become integrated with the tissue to be healed makes it more suitable for placement in areas in which there is no intimate contact with the abdominal organs.
- The foreign-body reaction and macrophage response do not determine the usefulness of either of the two biomaterials.
- Finally, we consider that the structure and, particularly, the porosity of the biomaterial are major factors that influence both the structure of the newly formed peritoneum and the formation of adhesions between the prosthesis and the viscera of the abdominal cavity.

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