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Short-term behavior of different polymer structure lightweight meshes used to repair abdominal wall defects

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Summary. Background. While lightweight (LW) polypropylene (PP) meshes are been used for hernia repair, new prosthetic meshes also of low-density and with large pores have recently been introduced composed of other polymer materials. This study compares the behavior in the short-term of two macroporous LW prosthetic materials, PP and non-expanded PTFE.

Methods. Partial defects were created in the lateral wall of the abdomen in New Zealand White rabbits and then repaired using a LW PP mesh or a new monofile, LW PTFE mesh. At 14 days postimplant, shrinkage and tissue incorporation, gene and protein expression of neo-collagens (qRT-PCR/immunofluorescence), macrophage response (immunohistochemistry) and biomechanical strength were determined.

Results. Both meshes induced good host tissue ingrowth, yet the macrophage response was significantly greater for the PTFE implants ($p<0.05$). Collagen 1/3 mRNA expression was greater for the PP mesh but differences lacked significance. Similar patterns of collagen I and III protein expression were observed in the neoformed tissue infiltrating the two meshes. After 14 days of implant, tensile strengths were also similar, while elastic modulus values were higher for the PTFE mesh ($p<0.05$).

Conclusions. In the short term, host collagen deposition and biomechanical performance seemed unaffected by the polymer structure of the implanted mesh. In contrast, the inflammatory response to mesh implant produced at this early time point was more intense for the PTFE.

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Introduction

The use of a biomaterial in the form of a mesh to repair a large abdominal wall defect is today virtually standard practice. The prosthetic material of choice for this purpose is sometimes large-pore polypropylene (PP) because of its good cost/benefit, biocompatibility and tolerance to infection (Alaedeen et al., 2007; Bellón et al., 1998).

Research and development in the field of biomaterials has nevertheless yielded new polymer materials of larger pore size conferring these designs the benefits of a lighter-weight implant able to better adapt to the biomechanics of the abdominal wall, thus improving compliance post-surgery and reducing the amount of foreign material implanted in the host. These new designs have led to the classification of macroporous meshes (Cobb et al., 2006) as heavy weight (HW) or light-weight (LW) respectively according to their density values.

The functional, morphological and histological properties of HW versus LW PP meshes have been addressed by several authors (Klinge et al., 2002; Bellón et al., 2009). The conclusions of these studies are that the lower amount of foreign material implanted when a LW rather than a HW material is used improves abdominal wall compliance to the extent that physiological compliance is achieved in the long term after a LW mesh is used for hernia repair. In a molecular and histological study (Pascual et al., 2008) examining the early host tissue incorporation of several meshes, it was observed that larger pore meshes induced the

genetic overexpression of collagen types I and III, the greater deposition of collagen type III, its faster conversion to collagen I, and that these features conferred the meshes greater tensile strengths 14 days after implant.

At least in theory, low-density meshes should show an improved foreign body reaction in that the amount of material implanted is minimized, thus reducing its contact surface with host tissue (Klosterhalfen et al., 2005). Effectively, studies have shown that a LW mesh will elicit a less intense acute inflammatory response compared to its HW counterpart (O'Dwyer et al., 2005; Weyhe at al., 2007). Hence, the development of reduced-material implants adapted to the physiological requirements of the anterior abdominal wall has served to improve both biocompatibility and patient comfort (Klinge 2007; Schumpelick et al., 1999).

Given these benefits of macroporous meshes, LW PP implants are currently widely used for hernia repair. However, several alternative materials based on the same concept of low density have recently appeared. These prosthetic materials have a similar large-pore structure but are composed of other polymers such as the new non-expanded polytetrafluoroethylene (PTFE) mesh, a loosely woven solid monofilament of PTFE (Bellón et al., 2002).

In this study, we compare the behavior of two LW meshes of similar pore size but of different polymer composition (PP versus PTFE) in the short term after implant. Having previously established the key role of mesh structure in host tissue regeneration (Pascual et al., 2012), this study was designed to determine whether the chemical composition of a mesh conditions the behavior of host tissue towards the implant regardless of its density or porosity.

The early host tissue incorporation process was examined in terms of mesh collagenization and the acute inflammatory reaction induced by the different polymer structures since both these processes are essential for good wound healing. Collagen deposition was also correlated with the biomechanical response of the implanted meshes. The time point of 14 days was selected since the initial acute phase reaction to mesh implantation is critical for the success or failure of the implant process.

Materials and methods

Experimental animals

The experimental animals were 16 male New Zealand White rabbits weighing approximately 2200 g caged under conditions of constant light and temperature according to European Union animal care guidelines (European Directive 609/86/EEC and European Convention of the Council of Europe ETS123). All procedures were approved by our institution's Review Board.

Prosthetic materials

The biomaterials used were (Fig. 1a,b):

- Optilene® mesh elastic (B/Braun, Germany): LW polypropylene (48 g/m^2); pore size 7.64±0.32 mm^2.
- Infinit® mesh (Gore and Associates, Arizona, USA): LW non-expanded polytetrafluoroethylene (PTFE) (70 g/m^2); pore size 4.05±0.22 mm^2.

Experimental design

The two different biomaterials were implanted in 16 animals to give two experimental groups of 8 animals each (PP and PTFE). Four animals of each group were used for the morphological and gene and protein expression studies and the other four for the shrinkage

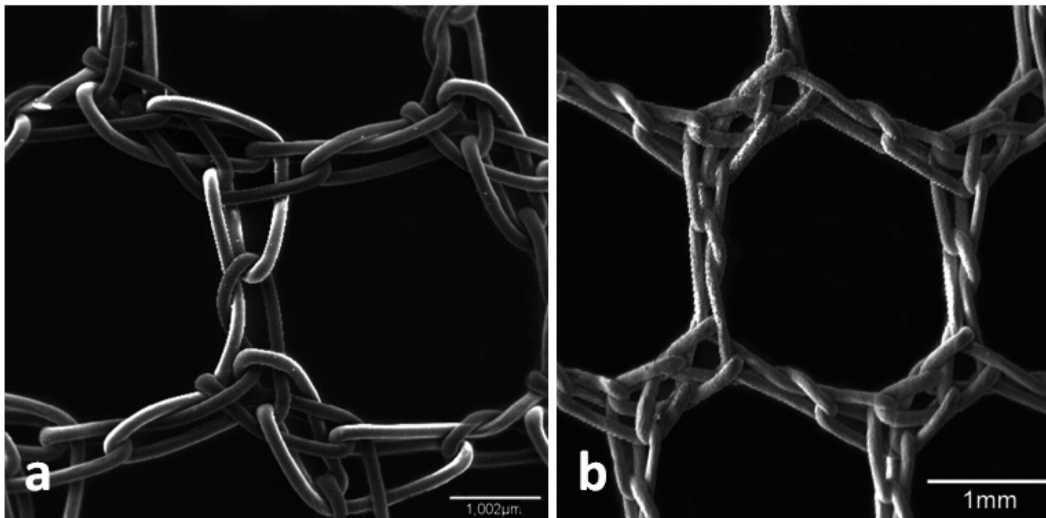

Fig. 1. Scanning electron microscopy images of the different biomaterials used in this experimental study. **a.** Optilene® elastic (PP) and **b.** Infinit® mesh (PTFE). x 15

and biomechanical studies. Animals were euthanized in a CO_2 chamber after 14 days of implant.

Surgical technique

To minimize pain, all animals were given 0.05 mg/kg buprenorphine (Buprecare®, Divasa Farmavic, Barcelona, Spain) 1 hour before the surgical procedure. Anesthesia was induced with a mixture of ketamine hydrochloride (Ketolar, Parke-Davis, Spain) (70 mg/kg), diazepam (Valium, Roche, Spain) (1.5 mg/kg), and chlorpromazine (Largactil, Rhone-Poulenc, Spain), (1.5 mg/kg) administered intramuscularly.

Using a sterile surgical technique, 4x4 cm defects were created in the lateral wall of the abdomen comprising the planes of the external and internal oblique muscles and sparing the transversalis muscle, parietal peritoneum and skin. The defects were then repaired by fixing a mesh of the same size to the edges of the defect using a running 4/0 polypropylene suture interrupted at the four corners. The skin was closed by 3/0 polypropylene running suture. Over the following three postoperative days, meloxidyl® (Esteve, Spain) (0.1 mg/kg) was administered orally by mixing with water for pain relief.

Shrinkage

Shrinkage of the implanted meshes was determined by image analysis. For this purpose, we designed a set of transparent templates of the same dimensions as the original meshes (4x4 cm). At the end of the implant period, the outlines of the meshes were traced on the templates before removing them from the animal. The surface areas of the templates could then be determined by computerized image analysis using the Image J software. Results were expressed as the percentage size reduction experienced by each implant. To examine the initial stage reaction, mesh shrinkage was determined 14 days after implant.

Morphological analysis

Light microscopy

For light microscopy, specimens were collected from the mesh/host tissue interface. The samples were fixed in F13 solution, embedded in paraffin and cut into 5-μm sections. Once cut, the sections were stained with Masson's trichrome (Goldner–Gabe) and examined under a light microscope (Zeiss Axiophot, Carl Zeiss, Oberkochen, Germany).

Gene and protein expression of collagens

Real time RT-PCR

Tissue fragments 1 cm^2 in size were obtained from the central mesh zone and stored at -80°C until use. RNA was extracted using guanidine-phenol-chloroform isothiocyanate procedures with trizol (Invitrogen, Carlsbad, CA, USA). The RNA was recovered from the aqueous phase and precipitated by adding isopropanol and incubating overnight at -20°C. Complementary DNA was synthesized using 200 ng of total RNA by reverse transcription (RT) with oligo dT primers (Amersham, Fairfield, USA) and the M-MLV reverse transcriptase enzyme (Invitrogen). RT reactions were run in the absence of M-MLV to confirm the RNA lacked genomic DNA.

cDNA was amplified using the following primers: collagen 1 (sense 5'-GAT GCG TTC CAG TTC GAG TA-3' and antisense 5'-GGT CTT CCG GTG GTC TTG TA-3'; collagen 3 (sense 5'-TTA TAA ACC AAC CTC TTC CT-3' and antisense 5'-TAT TAT AGC ACC ATT GAG AC-3'; and GAPDH (sense 5'-TCA CCA TCT TCC AGG AGC GA-3' and antisense 5'-CAC AAT GCC GAA GTG GTC GT-3').

The RT-PCR mixture contained: 5 μl of the inverse transcription product (cDNA) diluted 1:20, 10 μl of iQ SYBR Green Supermix (Bio-rad, Laboratories, Hercules, CA, USA) and 1 μl (6 μM) of each primer in a final reaction volume of 20 μl. RT-PCR was performed in a StepOnePlus Real-Time PCR system (Applied Biosystem, Foster City, California, USA). Samples were subjected to an initial stage of 10 min at 95°C. The conditions for cDNA amplification were: 40 cycles of 95°C for 15 s, 60°C (collagens I and III) or 55°C (GAPDH) for 30 s and 72°C for 1 min. Negative controls containing ultraPureTM DNase, RNase free distilled water (Invitrogen) were run in each reaction. Products were electrophoresed on a 2% agarose gel, stained with SYBR Green II RNA gel stain (Invitrogen) and visualized with UV light.

Gene expression was normalized against the expression recorded for the constitutive gene glyceraldehyde 3-phosphate-dehydrogenase.

Immunofluorescence

To detect the protein expression of collagens I and III, tissue fragments were fixed in F13 fluid, embedded in paraffin and cut into 5 μm-thick sections. Once cut, the sections were deparaffinated, hydrated, equilibrated in PBS buffer and incubated with the monoclonal antibodies anti-collagen I (Sigma, St. Louis, MO, USA) and anti-collagen III (Medicorp, Montreal, Canada). The secondary antibody used was conjugated with rhodamine. An immunofluorescence technique was used to detect the antigen-antibody reaction. Cell nuclei were counterstained with DAPI. Samples were examined under a confocal microscope Leica SP5 (Leica Microsystems, Wetzlar, Germany) to detect fluorescence.

Macrophage response

For immunohistochemistry, a specific monoclonal

antibody to rabbit macrophages, RAM 11 (DAKO M-633, USA) was applied to paraffin-embedded sections. The alkaline phosphatase-labeled avidin-biotin method was performed as the following steps: incubation with the primary antibody (1:50 in tris-buffered saline or TBS) for 30 minutes, incubation with immunoglobulin G (IgG) and biotin (1:1000 in TBS) for 45 minutes, and labeling with avidin (1:200 in TBS) for 30 minutes. These steps were conducted at room temperature. Images were developed using a chromogenic substrate containing naphthol phosphate and fast red. Nuclei were counterstained for 5 min in acid hematoxylin. RAM-11-labeled macrophages were quantified according to a method described elsewhere (Bellón et al., 1994).

Biomechanical strength

To determine the biomechanical strength and modulus of elasticity of the meshes after implant, strips of the different biomaterials 1 cm wide and 5 cm long were tested in an INSTRON 3340 tensiometer (static load 500N) (Instron Corp., UK). The cross-head speed was 5 cm per min and recording speed 2 cm per min.

The strips obtained at 14 days post-implant included

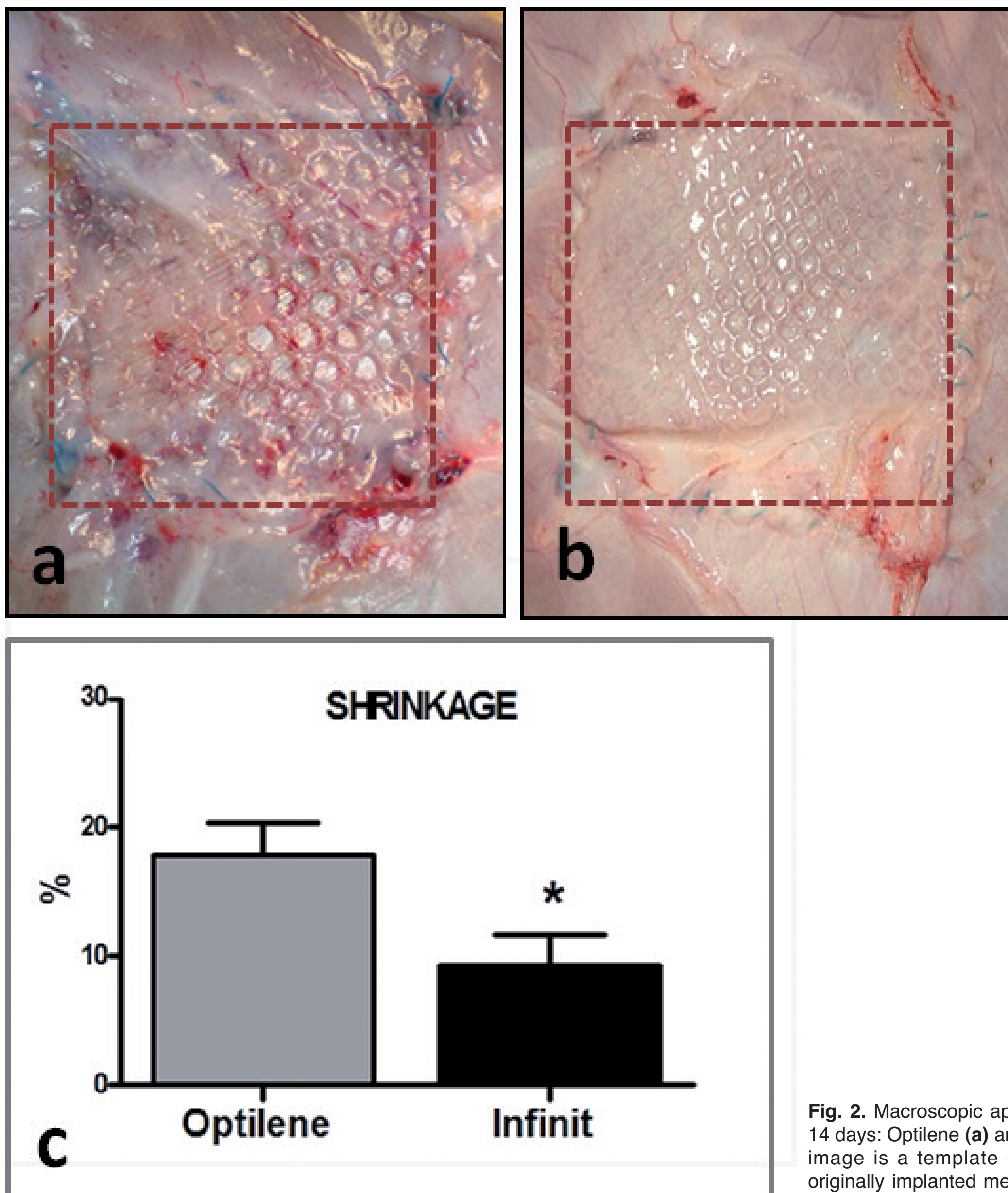

Fig. 2. Macroscopic appearance of the implant site after 14 days: Optilene **(a)** and Infinit **(b)**. Superimposed on the image is a template of the same dimensions as the originally implanted meshes (4x4 cm). **c.** % shrinkage of the implanted mesh.

the mesh and infiltrated host tissue. All tests were conducted immediately after animal sacrifice.

Statistical analysis

Statistical analysis was performed using the Graph Pad Prism 5 package. Shrinkage percentages, collagen 1 and 3 mRNA expression, RAM-11 positive cells, biomechanical strength and modulus of elasticity values were compared between the two study groups using the Mann-Whitney U test. The level of statistical significance was set at $p<0.05$.

Results

There were no cases of mortality or signs of infection and/or rejection of the implants in the animals. Seroma was detected in two of the animals with PTFE implants at 14 days post-surgery.

Shrinkage

Shrinkage values determined at 14 days postimplant indicated a significantly greater ($p<0.05$) percentage size reduction for Optilene® (17.82±2.60%) compared to Infinit® (9.33±2.35%). (Fig. 2).

Morphological analysis

Light microscopy

A similar wound healing process was observed in the

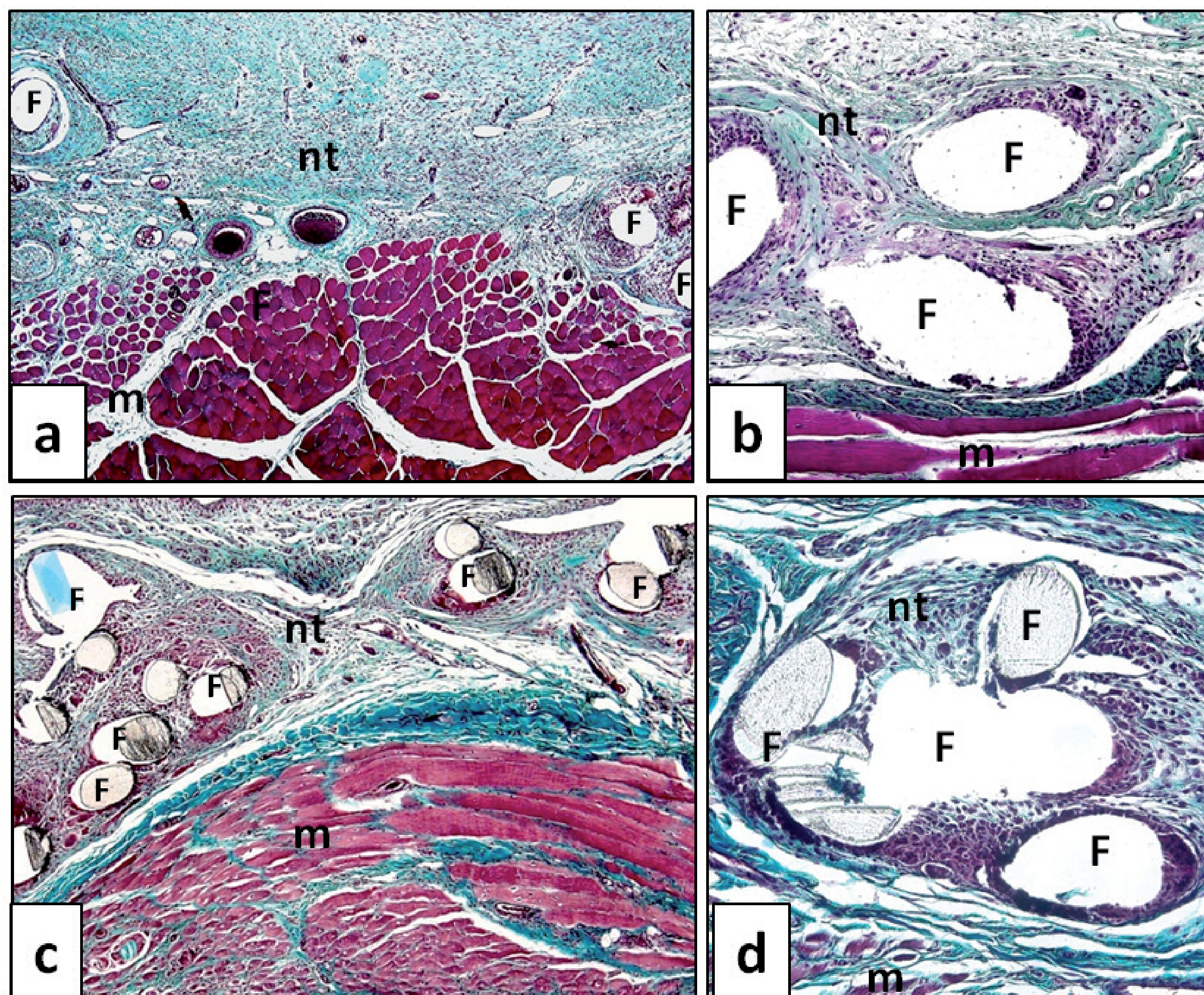

Fig. 3. Histological findings on Masson's trichrome stained sections of the implanted meshes. Optilene **(a/b)** and Infinit **(c/d)** after 14 days of implant in the experimental animals. F: prosthetic filaments, m: muscle tissue, nt: neoformed tissue. a, c, x 50; b, d, x 160

animals implanted with both mesh types. Hence, after 14 days, the two biomaterials had become infiltrated by a disorganized, well-vascularized, loose connective scar tissue. This neoformed tissue surrounded the prosthetic filaments, filling all existing gaps (Fig. 3a,c). Collagen fibers ran parallel to the mesh surface in zones far from the filaments or were arranged concentrically to these filaments in areas closer to the implant edges. Around the prosthetic filaments, there was an evident yet moderate inflammatory reaction (Fig. 3b,d).

Gene and protein expression of collagens

Real time RT-PCR

After implant, the PP biomaterial (Optilene®) showed a more intense pattern of mRNA expression for collagen 3 (immature) and 1 (mature), than the PTFE mesh, whose expression of these mRNAs was low. Despite this difference in relative amounts of mRNAs, we detected no significant differences in collagen 1 and 3 mRNA expression patterns between the PP and PTFE meshes ($p>0.05$) or within each mesh group (Fig. 4).

Immunofluorescence

In the short term, collagen III protein was mostly expressed in the neoformed tissue surrounding the prosthetic filaments, regardless of their composition. Both groups showed a homogeneous distribution of the immature form of collagen (collagen III protein) throughout the newly formed tissue (Fig. 5b,d). However, a slightly more intense pattern of immunostaining for this collagen was observed in the Infinit mesh® (Fig. 5d).

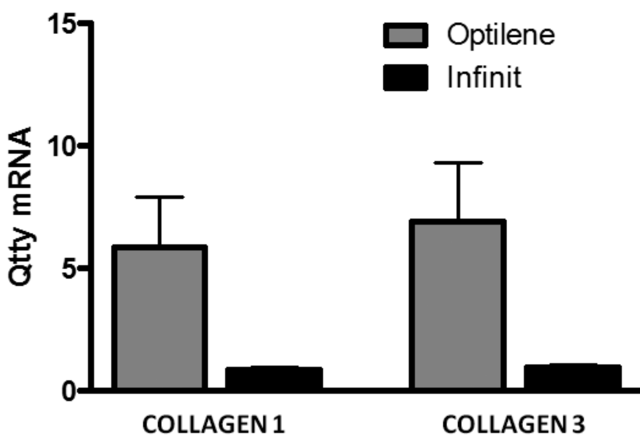

Fig. 4. Relative amounts of collagen 1 and 3 mRNA in the Optilene® and Infinit® meshes determined by qRT-PCR. No significant differences were observed between the groups ($p>0.05$). Results are the mean ± SEM of three experiments performed in duplicate. Gene expression was normalized to values recorded for the GAPDH gene.

Compared with collagen III, immunostaining for the mature form of collagen (collagen I) was very weak (Fig. 5a,c). In the PP meshes labeling was virtually undetectable and in the PTFE implants it lacked a fibrillar appearance and was restricted to small areas of the neoformed tissue close to the prosthetic filaments (Fig. 5a,c).

Macrophage response

In both study groups, macrophage cells were detected in the neoformed tissue between the mesh filaments (Fig. 6a,d). Most inflammatory cells concentrated around the filaments where, besides macrophages, multinucleated foreign-body giant cells, typical of a wound repair response, could be seen. These cells appeared mostly around the filaments of PTFE (Infinit®) (Fig. 6c,d).

Macrophage numbers were significantly higher for the PTFE meshes compared to the PP implants ($p<0.05$) (Fig. 6e).

Biomechanics

The tensile strengths, or breaking points (Fig. 7a,b), recorded for the two groups of meshes implanted for 14 days did not vary significantly (Optilene 3.026±0.336 N, Infinit 2.812±0.223 N) ($p>0.05$) (Fig. 7c). However, a significantly higher postimplant elastic modulus was recorded for the PTFE ($p<0.05$) compared to the PP meshes (Fig. 7d).

Discussion

The most recent modifications made to PP meshes have pursued the idea of minimizing the material implanted in the host without compromising their mechanical resistance and this has led to the development of today's light-weight meshes (Klosterhalfen et al., 2005). Similarly, attempts to improve the tissue incorporation and tensile strength of conventional laminar PTFE prostheses have given rise to large pore meshes made by interweaving a solid monofilament of non-expanded PTFE (Bellón et al., 2009).

Studies conducted on these new macroporous meshes have revealed that rather than the chemical composition of the biomaterial, it is their loosely woven structure that determines their tissue behavior. Some years ago, our group created a macroporous mesh (Bellón et al., 2002) using CV-4, an expanded-PTFE suture thread. In contrast, the mesh examined here is composed of a non-expanded PTFE monofilament knitted to create a large pore size making it a LW mesh. Although this type of mesh in the meantime has been withdrawn from the European market, we believe it is very important to understand the behavior of this new

abdominal prosthesis.

In our study, partial defects were created in the abdominal wall of the rabbit to avoid involving the peritoneum in the repair process. After sacrificing the animals, seroma was detected in two of the animals with PTFE implants. This means there was a more intense inflammatory reaction in these animals, in agreement with our immunohistochemistry results. Thus, using the anti-RAM-11 macrophage monoclonal antibody, the PTFE implants showed a significantly augmented macrophage reaction over that shown by the PP implants. This observation is in line with the findings of a recent study (Jacob et al., 2012) in which the Infinit® mesh was compared with a mesh (Optilene LP) similar

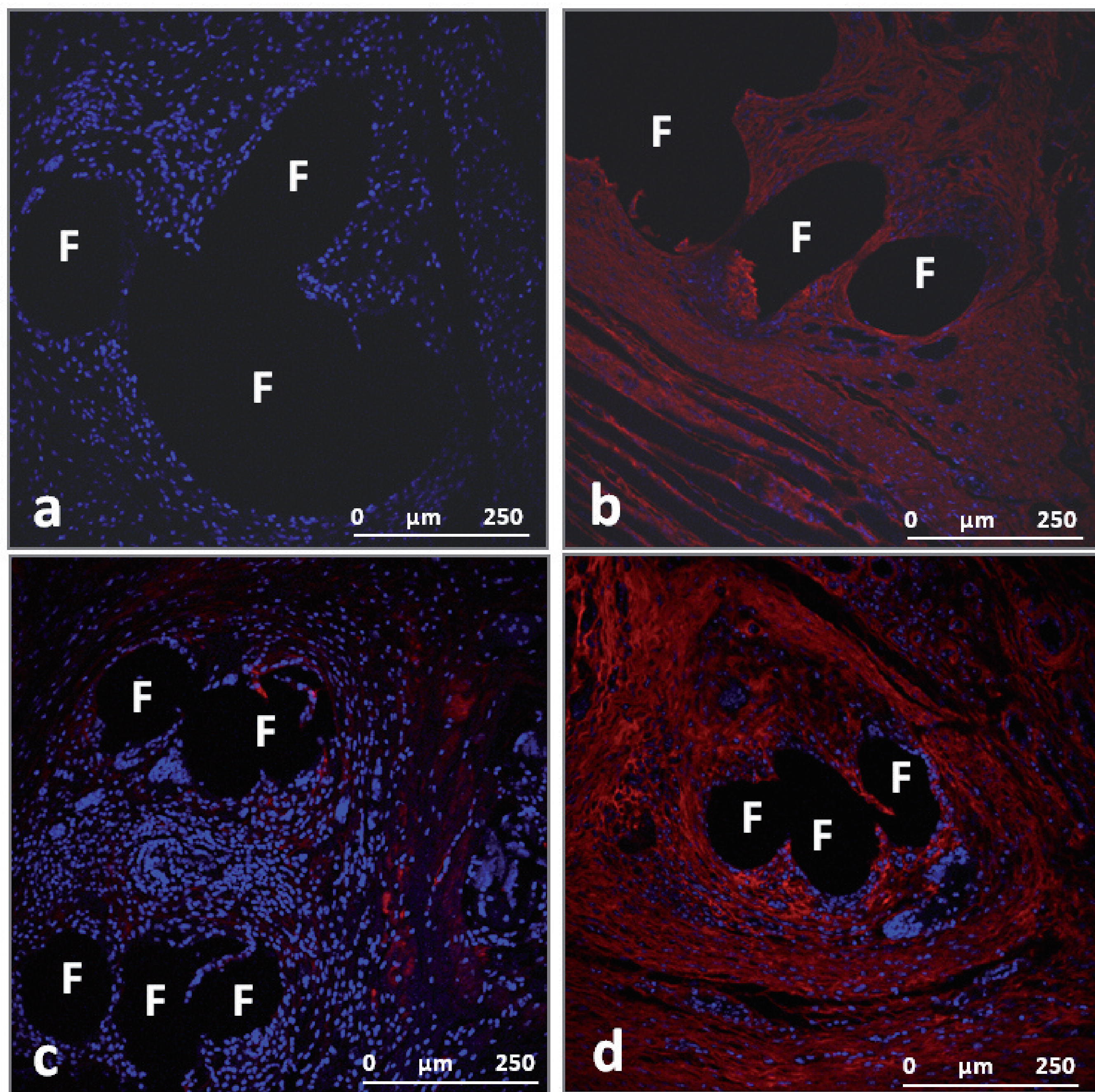

Fig. 5. Collagen I and III protein expression around the mesh filaments of the biomaterials at 14 days post-implant. Collagen appears as red fluorescence upon laser scanning confocal microscopy. Cell nuclei appear blue (DAPI). Collagen I **(a, c)** and Collagen III **(b, d)** . Optilene® **(a, b)** and Infinit® **(c, d)**, 14 days. F: Prosthetic filaments. x 200

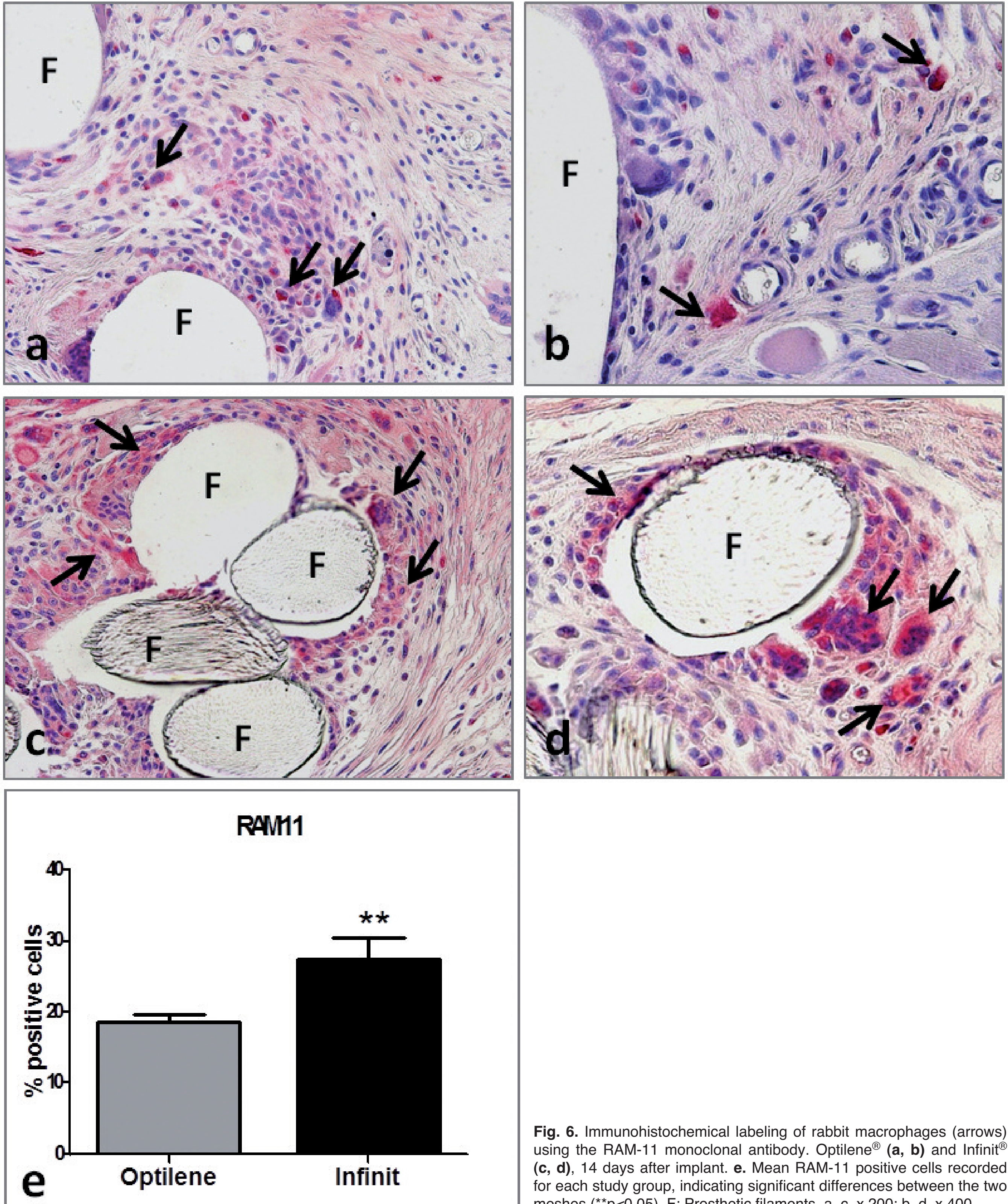

Fig. 6. Immunohistochemical labeling of rabbit macrophages (arrows) using the RAM-11 monoclonal antibody. Optilene® **(a, b)** and Infinit® **(c, d)**, 14 days after implant. **e.** Mean RAM-11 positive cells recorded for each study group, indicating significant differences between the two meshes (**$p<0.05$). F: Prosthetic filaments. a, c, x 200; b, d, x 400

to the PP mesh used here but with a smaller pore size. The authors of this study also detected a significantly greater macrophage response for the PTFE meshes despite the fact that it was a long-term study (94 days post-implant) conducted a different experimental animal and model to ours.

This behavior is also similar to that exhibited by some absorbable materials in the early postimplant course (Klinge et al., 2001; Rosch et al., 2003) possibly with clinical implications like seroma. Several factors (Post et al., 2004; Kayaoglu et al., 2005; Di Vita et al., 2008) influence the inflammatory response to an implanted prosthesis such as density, pore size, type of material, fiber structure, texture and mesh construction. However, a review (Weyhe et al., 2007) of the European medical literature on the outcome of hernia mesh repair concluded that mesh pore size and filament structure were the most important determinants of the foreign body reaction produced after mesh implantation.

In the present experimental study, we compared the short-term post-implant behavior of a conventional PP-LW mesh to that of a new LW mesh composed of non-expanded PTFE. In terms of host tissue incorporation, both LW mesh types showed good behavior. The behavior of the PTFE meshes showed little resemblance to that of the classic microporous ePTFE prosthetic materials, which gradually become encapsulated by host tissue.

Two weeks after implant, the immature form of collagen, collagen III, was the predominant collagen type present. Similar expression of the protein was observed for the two different polymer structures. At this time point, collagen I expression was low. This is as expected, as the mature form of collagen is synthesized

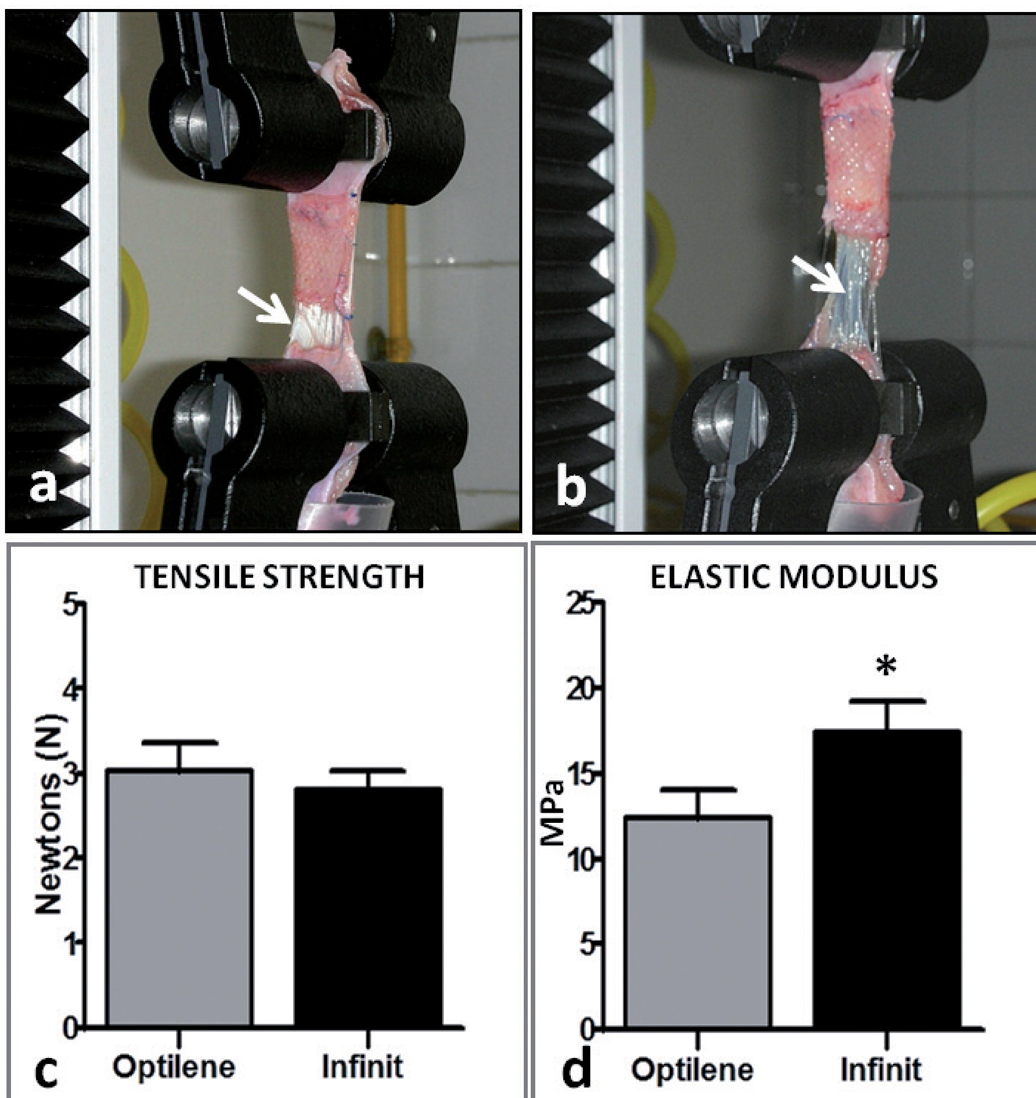

Fig. 7. a, b. Tissue rupture points (arrows) during the development of the biomechanical study. **c.** Biomechanical resistance values (Newtons) obtained for Infinit® and Optilene® at 14 days post-implant indicating no significant differences. **d.** Modulus of elasticity recorded for the two mesh types. Significant differences were observed between Infinit® versus Optilene® (*$p<0.05$).

and secreted in the extracellular matrix later than the immature form. In prior work (Pascual et al., 2012), we observed that in the mid to long term (3 and 6 months post-implant) these LW meshes (Optilene® and Infinit®) induced the greater protein expression of mature collagen. Other authors (Greca et al., 2001, 2008) have also reported greater type I collagen deposition following the implant of large-pore prosthetic materials.

In an earlier study (Pascual et al., 2008), we also compared the influence of porosity on the behavior of different meshes, this time in a full-thickness abdominal wall defect model, and noted significantly higher collagen 1 and 3 gene expression levels for the larger pore meshes at 14 days post-implant. In the present study, we observed similar behavior at this time point; both LW implants showed higher gene expression levels for both collagen types than the levels recorded in another study in the long term (Pascual et al., 2012). This suggests that in the short term messenger RNA expression peaks, and consequently so does its transcription. The higher, though not significant, relative amount of mRNA observed in our PP meshes than PTFE meshes could point to less effective protein translation since similar protein levels were detected by immunofluorescence.

Our biomechanical results indicated similar tensile strengths at 14 days for the PP and PTFE meshes. Our previous studies have shown that this behavior persists in the long term, and that tensile strength increases over time (Pascual et al., 2012). In another study, we effectively observed that by 90 days post-implant, similar biomechanical strengths were attained by several different implants (Bellón et al., 2007).

The elastic modulus recorded after implant was significantly higher ($p<0.05$) for the PTFE than PP meshes, although as for their mechanical strength, similar values seem to be attained in the longer term for both implants as indicated in a previous study (Pascual et al., 2012) in which both the resistance to breakage and elasticity of several implants were increasingly conditioned by the in-growing host tissue.

In conclusion, our findings suggest no effects of the chemical composition of LW meshes on the short-term collagenization of neoformed tissue and its consequent resistance to traction. Both these factors are crucial for the early failure or success of an implant. In contrast, the macrophage response does seem to depend on polymer structure and was appreciably more intense in response to PTFE than PP.

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