Long term comparative evaluation of two types of absorbable meshes in partial abdominal wall defects: an experimental study in rabbits

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Abstract

Purpose Synthetic prosthetic materials that are fully absorbable seek to reduce the host foreign body reaction and promote host tissue regeneration. This preclinical trial was designed to analyse, in the long term, the behaviour of two prosthetic meshes, one synthetic and one composed of porcine collagen, in abdominal wall reconstruction.

Methods Partial defects were created in the abdominal walls of New Zealand rabbits and repaired using a synthetic absorbable mesh (Phasix™) or a non-crosslinked collagen bioprosthesis (Protexa™). After 3, 6, 12 and 18 months, specimens were recovered for light microscopy and collagen expression analysis to examine new host tissue incorporation, macrophage response and biomechanical strength.

Results Both materials showed good host tissue incorporation in line with their spatial structure. At 18 months postimplant, Protexa™ was highly reabsorbed while the biodegradation of Phasix™ was still incomplete. Collagenization of both materials was good. Macrophage counts steadily decreased over time in response to Phasix™, yet persisted in the collagen meshes. At 18 months, zones of loose tissue were observed at the implant site in the absence of herniation in both implant types. The stress–stretch behaviour of Phasix™ implants decreased over time, being more pronounced during the period of 12–18 months. Nevertheless, the abdominal wall repaired with Protexa™ became stiffer over time.

Conclusion Eighteen months after the implant both materials showed good compatibility but the biodegradation of Phasix™ and Protexa™ was incomplete. No signs of hernia were observed at 18 months with the stress–stretch relations

being similar for both implants, regardless of the more compliant abdominal wall repaired with Protexa™ at short term.

Keywords Mesh repair · Bioprostheses · Abdominal wall repair · Hernia repair · Collagen mesh · Poly-4-hydroxybutyrate (P4HB)

Introduction

The repair of tissue defects in the abdominal wall, usually hernial defects, is a common general surgery procedure [1]. In current clinical practice, a prosthetic material is employed in over 90% of cases [2]. The biomaterials most frequently used are polymer materials such as polyester, polypropylene and expanded polytetrafluoroethylene. The most important characteristic is that they are permanent materials and thus persist over the patient's lifetime. Some induce intense foreign body reactions in the host tissue [3]. However, the repair and strength benefits they offer have led to significantly reduced hernia recurrence rates [4, 5].

When a hernial defect is repaired using one of these prosthetic materials, the ideal situation is that the least possible amount of foreign material persists in the patient. This has prompted the development of biomaterials that gradually become absorbed in the host and also ensure that patient's own tissue gradually replaces the implanted mesh as it is reabsorbed. An important requirement of these prosthetic materials, especially when used at the level of the abdominal wall, is that they should maintain the tissue's mechanical properties. In normal conditions, the abdominal wall rather than being static will be subjected to pressure changes [6]. Thus, an objective pursued by these totally absorbable materials is that the tissue regenerated after their biodegradation should fulfil these mechanical demands.

Recent research efforts directed at developing new hernia repair materials have tried to find the ideal balance between tissue repair and tissue regeneration at the implant site. With this purpose in mind, partially or totally biodegradable materials have been developed. Thus, two types of fully resorbable materials have been designed for hernia repair. One type are the so-called biomeshes derived from organic sources and there is a long list of such materials available on the market. Most are made of collagen derived from animal sources and pursue, as mentioned earlier, both the repair and regeneration of new tissue that is similar to the host tissue [7, 8]. Within these biomeshes, there are a further two types: those with crosslinks, which stabilize the collagen molecule preventing its rapid degradation, and those elaborated from non-crosslinked collagen, which undergo gradual degradation over a variable length of time [9–11]. These

biomeshes, especially non-crosslinked designs, still have properties that remain to be established such as their degradation time in the patient [12].

The second type of bioabsorbable material, or polymer materials, are biodegradable in the mid- or longer-term. Among these, we should mention Gore-BioA™ tissue reinforcement mesh (BioA™), consisting of a single synthetic resorbable fibre (polyglycolic acid: trimethylene carbonate PGA:TMC) [13]; TIGR™ matrix surgical mesh (TIGR™) composed of two synthetic fibre types (co-polymer glycolide-lactide trimethylene carbonate/lactide TMC) with a multifilament structure [14]; and Phasix™, a biosynthetic resorbable monofilament mesh (poly-4-hydroxybutyrate) [15]. The absorption times of these mesh materials are 6 months for BioA™ and slower times for the other two, from 18 months (Phasix™) to 3 years (TIGR™).

In a previous study [16], we observed the rapid degradation of BioA™ after 6 months of implant. This material was designed as tissue reinforcement but is not ideal for the definitive repair of tissue defects, as shown through clinical experience by high recurrence rates after hernia repair [17].

Experience with TIGR™ has been promising according to preclinical results [18], although unfortunately, these have not matched clinical outcomes [19].

In the present study, we selected, as a new contribution, the Phasix™ mesh based on scarce long term (> 1 year) preclinical data and a time of absorption that remains unclear. This material was faced to a non-crosslinked bioprosthesis with an apparently shorter absorption time in terms of the inflammatory reaction elicited by both materials, their integration with host tissue and their mechanical behavior evaluated by uniaxial tensile test, were also new contributions of our study, along with the comparison of postimplant degradation times.

Materials and methods

Experimental animals

Experimental procedures were conducted on 20 New Zealand white rabbits each weighing approximately 3200 kg. The study protocol adhered to ARRIVE (Animal Research: Reporting of in vivo Experiments) guidelines for the publication of animal studies [20].

The up-keep and handling of animals throughout the study was in accordance with the Guide for the Care and Use of Laboratory Animals of the National and European Institutes of Health (Spanish Law 6/2013, Spanish Royal Decree 53/2013, European Directive 2010/63/UE and European Convention of the Council of Europe ETS123). All procedures were performed at the Animal Research Centre of the Universidad de Alcalá (Madrid, Spain), which is registered with the Directorate General for Agriculture of the Ministry of Economy and

Technology Innovation of the Community of Madrid (ES280050001165) ensuring all facilities legally cover the needs and requirements of the research. The study protocol was approved by the Committee on the Ethics of Animal Experiments of the Universidad de Alcalá, Madrid, Spain.

Materials used in the study

Two absorbable meshes were used in this study (Fig. 1):

- Phasix™ (Bard, Davol Inc, USA). A reticular synthetic absorbable mesh composed of poly-4-hydroxybutyrate of porosity 0.258 mm^2, and thickness 0.508 mm. The Phasix mesh has a knitted mesh pattern similar to the conventional Bard polypropylene mesh (C.R. Bard, Inc). Its mechanical properties before implant also resemble those of the Bard mesh.
- Protexa™ (Tecnoss, Italy). A biological non-crosslinked collagen membrane of porcine origin, 1.4 mm thick. According to the manufacturer's specifications, a 10–15 min immersion period in sterile saline solution is necessary before implant.

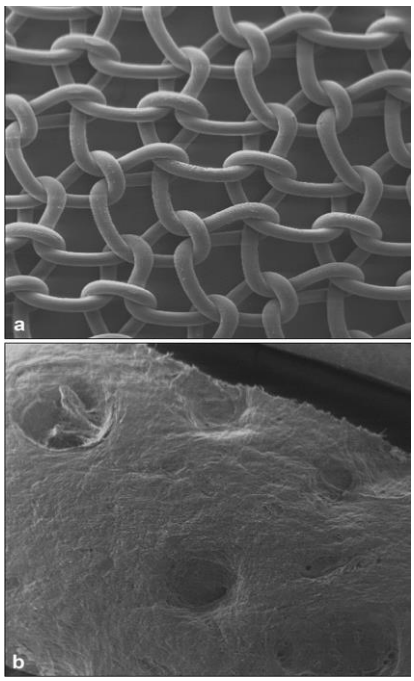

Fig. 1 Characteristics of the prosthetic materials. Scanning electron microscopy photographs revealing the reticular structure of: **a** Phasix™ (×20) and, **b** the laminar structure of Protexa™ (×20)

Surgical procedure

Preoperatively and once daily for the first 3 days postsurgery, animals were given analgesia (0.05 mg/kg buprenorphine, Buprecare, Divasa Farmavic, Spain). Rabbits were anaesthetized with a mixture of ketamine (20 mg/kg, Imalgene, Merial, Spain) and xylazine (3 mg/kg, Xilagesic 2%, Calier, Spain) administered intramuscularly.

The animals were positioned backside-down. After shaving and disinfection, two incisions were made on both sides of the linea alba. Two 3-cm-long × 3-cm-wide pieces including the external and internal oblique abdominal muscles were then excised bilaterally to create two partial defects comprising the anatomical planes of the internal and external oblique muscles, while sparing the transverse muscle and parietal peritoneum. These defects were repaired by fixing a mesh of the same size as the defect to its edges using a running 4/0 polypropylene suture which was interrupted at the four corners. In each animal, the mesh used was Phasix™ on the right side of the linea alba and Protexa™ on the left. After mesh placement, the skin was closed with a 3/0 polypropylene running suture. The animals were kept in individual cages and checked daily.

At the time points 3, 6, 12 and 18 months postsurgery, five animals per implant group were anaesthetized and sacrificed humanely using sodium pentobarbital 20% (Dolethal, Vetoquinol SA, France). Subsequently, with the animal placed on its back, the skin was dissected away and the implant area was carefully inspected to check for prosthetic shrinkage. Next, the full thickness of the abdominal wall was harvested to obtain both implants and surrounding tissue. Each mesh sample was divided into two 1.5-cm-width × 7-cm-long pieces. A piece was used for the biomechanical study, and the other was reserved for histological analysis, scanning electron microscopy and immunohistochemical tests.

Experimental design

Twenty New Zealand white rabbits were used in the study:

- Phasix™ (n = 20); right side of the linea alba.
- Protexa™ (n = 20); left side of the linea alba.

Experimental animals were further subdivided by sacrifice time: 3, 6, 12 or 18 months postimplant (n = 5 each).

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 (3 × 3 cm). At the end of the implant period, the outlines of

the meshes were traced on the templates before their removal. The surface area of the templates could them be determined by computerized image analysis using Image J software (https: //imagej.nih.gov/ij /). Results are expressed as the area (cm^2) occupied by each implant at the time of sacrifice.

Morphological analysis

Standard fixation procedures were used for both microscopy techniques. For light microscopy, samples were fixed in F13 solution. After 7 days, the fixatives were removed and the samples were dehydrated and embedded in paraffin. Tissue blocks were cut into 5 µm sections and placed onto slides coated with poly-lysine (SIGMA, Merk, USA). Finally, the samples were hydrated, stained with haematoxylin eosin, Masson's trichrome (Goldner-Gabe), and with Sirius red. Samples were examined under a light microscope Zeiss Axiophot (Zeiss, Germany). Sirius red staining was also observed under polarized light, which allowed observation of collagen type I and III. Type I collagen, appears as a reddish-orange stain, while type III collagen takes on a yellowish-green stain when observed under the polarized light microscope.

For scanning electron microscopy (SEM), samples were fixed with 3% glutaraldehyde, dehydrated and mounted on stubs using double-sided tape. Critical point was reached in a critical point dryer (E-3000; Polaron, United Kingdom) with carbon dioxide. Samples were then metalized with gold palladium and examined in a Zeiss scanning electron microscope DSM-950.

Postimplantation mesh reabsorption

To determine the degree of absorption of the implanted prosthetic materials, we measured the diameter of the Phasix™ filaments and the thickness of the Protexa™ sheet in a morphometric study. Measurements were obtained on digitized images of 20 histological sections per sample. Images were captured using a digital camera coupled to the microscope (Axiocam HR, Zeiss) and analyzed by Axiovision AC 4.1 (Zeiss) image analysis software.

Results are provided as the percentage of prosthetic material remaining referred to the initial diameter of the Phasix™ filaments or thickness of the Protexa™ lamina.

Macrophage response

For their identification, macrophages were immunohistochemically stained with the monoclonal antibody to rabbit macrophages RAM11 (DAKO M-633, USA). Paraffin sections were hydrated and after blocking inspecific binding with bovine serum albumin (SIGMA), incubated with primary antibody RAM11 overnight.

Sections were then covered with the secondary antibody and biotin for 60 min, and subsequently incubated with avidin for 60 min. A chromogenic substrate containing naphthol phosphate and fast red was used to develop the positive reaction. Cell nuclei were counterstained for 5 min in acid haematoxylin.

Labelling was quantified in images of ten microscopy fields per sample captured by a digital camera fitted to the light microscope (Axiocam HR, Zeiss) and analyzed with Image J.

Biomechanical assay

Immediately after animal sacrifice, implant samples were collected in minimal essential medium (MEM) to preserve them correctly until the biomechanical tensiometric tests. Strip length, width and thickness were determined with a digital caliper, making three measurements of each variable on each strip. The strips (1.5 ± 0.13 × 7 ± 0.21 cm) were clamped vertically between the grips and tests were performed under displacement control in an Instron 3340 Microtester (Illinois Tool Works, Glenview, IL, USA) with a 50 N full-scale load cell. To avoid sample slippage and premature failure, we used 250 N capacity pneumatic grips with serrated surfaces. The displacement rate was 5 mm/ min until sample rupture. Strip elongation was expressed as stretch (−), computed as stretch = ($L0$ + ΔL)/$L0$, where $L0$ is the initial length between grips and ΔL is the upper grip displacement. The de stress (Mpa) was estimated as $P = N/A0$ where N is the load applied and A0 the initial cross section of the specimen.

At 3 months, the thickness of abdominal wall repaired with Protexa™ was 4.83 ± 0.7 cm and 4.53 ± 0.89 cm at 18 months. The thickness varied from 4.11 ± 0.66 cm at 3 months – 4.74 ± 0.83 cm at 18 months for the wall repaired with Phasix™.

Sample failure stress and stretch were computed as the maximum stress and stretch sustained by the mesh and recorded as mean ± standard deviation.

Statistical analysis

Mean data of the shrinkage, tensiometric analysis, postimplantation mesh thicknesses and immunohistochemical studies within the same prosthetic material for the different study times were compared using the Mann–Whitney U test implemented in the GraphPad Prism five package (GraphPad Software, Inc., USA). Significance was set at $P < 0.05$.

Results

There were no complications related to surgery, anaesthesia or prosthetic material infection.

Macroscopic inspection of the Phasix™ implants revealed good tissue ingrowth. In one Phasix™ implant, a lateral desinsertion was observed at 3

months. Filaments were observed at all study times without signs of evident resorption.

Desinsertion was also observed in two of the Protexa™ implants one at 3 months and the other at 6 months. At 12 months, one of these meshes had not become integrated within the host tissue and another appeared folded over itself. At 18 months, behaviour was more consistent and in three of the five animals, absorption of the material was evident and only small mesh fragments could be observed. In the other two animals, the implants were similar in appearance to the earlier time points.

Shrinkage

Implant shrinkage was assessed at the moment of animal sacrifice.

In the Phasix™ implants, no modifications in the area occupied by the prosthetic material were noted at 3 and 6 months postimplant. At 12 months, a slight distension of the recipient implant zone was detected. At 18 months, there was a significant increase in the recipient zone in relation to its size at 6 months which appeared distended (Fig. 2).

For Protexa™, implant sizes were similar at the time points 3–6 and 12 months. At 18 months, the implant zone was significantly distended in comparison with earlier time points (Fig. 3).

Morphological analysis and mesh reabsorption

Three months after implant, a disordered host tissue infiltrate was observed around the Phasix™ filaments. This infiltrate consisted of repaired connective tissue with macrophages, giant foreign body cells and fibroblasts, along with an extracellular matrix rich in collagen I as shown by Sirius red staining. In interfilament zones, the major tissue present was adipose tissue, among which disperse connective tissue appeared. The appearance of these implants at 6 months was similar to that observed during the previous time point except that the connective tissue surrounding the filaments contained both collagen type I and III fibres. At 1 year, in a small number of prosthetic filaments, macrophage-like cells appeared to infiltrate the filaments. The tissue enveloping the filaments was similar to that observed at the earlier time point. At 18 months, prosthetic filaments were still visible though appeared more disorganized and with greater signs of resorption. The tissue surrounding the implants was practically fully replaced with adipose tissue (Fig. 4), although the mechanical behavior of the repair area was not compromised by this great development of adipose tissue in the long term.

Morphometric measurements indicated the diameters of the poly-4-hydroxybutyrate filaments of Phasix™ remained intact at 3 months, with no signs of degradation. At 6 months, there was a slight reduction in filament diameter of

close to 10%, and this advanced significantly until 18 months when resorption had reached 30%. All differences between time points were significant (**P < 0.01) (Fig. 4 m).

At 3 months, the Protexa™ implant was encapsulated by a thin layer of inflammatory cells and connective tissue. In the new tissue growing on the Protexa™ implants, collagen fibres (mostly type III) ran parallel to the mesh surface. Only in those zones in which the fibres of this lamina were not as compact, leaving small spaces, did host cells penetrate into the implant interior, and small blood vessels could even be seen. At 6 months, it was possible to observe some zones in which cells had practically colonized the full thickness of the material interspersed with others in which this only occurred in the outer layers. Zones of calcification were also evident. Around these zones, the inflammatory reaction was especially intense. This also occurred in zones closest to the host tissue, where giant foreign body reaction cells flanking the implant were abundant and even infiltrated it is outermost layers. At 12 months, in the attachment zones to the host tissue, this new tissue grew between the layers comprising the material separating it into thin sublayers. The inflammatory reaction was especially intense at this stage as were zones showing calcification. Areas furthest from fixation points showed a similar appearance to earlier time points with scarce cell infiltration into the matrix. In the connective tissue encapsulating the material, larger amounts of collagen type III were observed. At 18 months, a different behaviour was observed. In three of the Protexa™ implants, evident signs of absorption appeared including significant thinning while the remaining two implants showed the same appearance as at 12 months. In the connective tissue around the implants, again there was a predominance of mature type collagen (Fig. 5).

Our morphometric study revealed no modifications in the thickness of the Protexa™ biomaterial at 3 months postimplant. The absorption process started after this time period and there was evident and steady thinning in subsequent time points. By 18 months, more than half the initial prosthetic thickness had been reabsorbed. Thickness differences were significant when the 3-month group was compared to the other implant times (Fig. 5m).

Macrophage response

The macrophage response to the implants was assessed by examining RAM11-positive cells in the implant area. Macrophage labelling varied between mesh types: while in the Phasix™ implants it was detected in the periphery of the filaments and sometimes isolated in interfilament zones, in the Protexa™ implants, RAM11 positive cells could be seen along all the prosthetic perimetry and infiltrating the biomaterial interior (Figs. 6, 7).

In the Phasix™ implants, moderate amounts of macrophages and giant foreign body cells persisted without significant modifications until 12 months. At 18

months, a significantly reduced number of inflammatory cells was observed compared to 3 and 12 months (Fig. 6).
RAM11 positive cell percentages for the Protexa™ implants at 3 and 6 months postimplant were also moderate. However, Protexa™ showed an active inflammatory reaction with many macrophages and foreign body giant cells appearing over time. This increase was significant at 12 and 18 months such that significant differences emerged versus 3 and 6 months (Fig. 7).

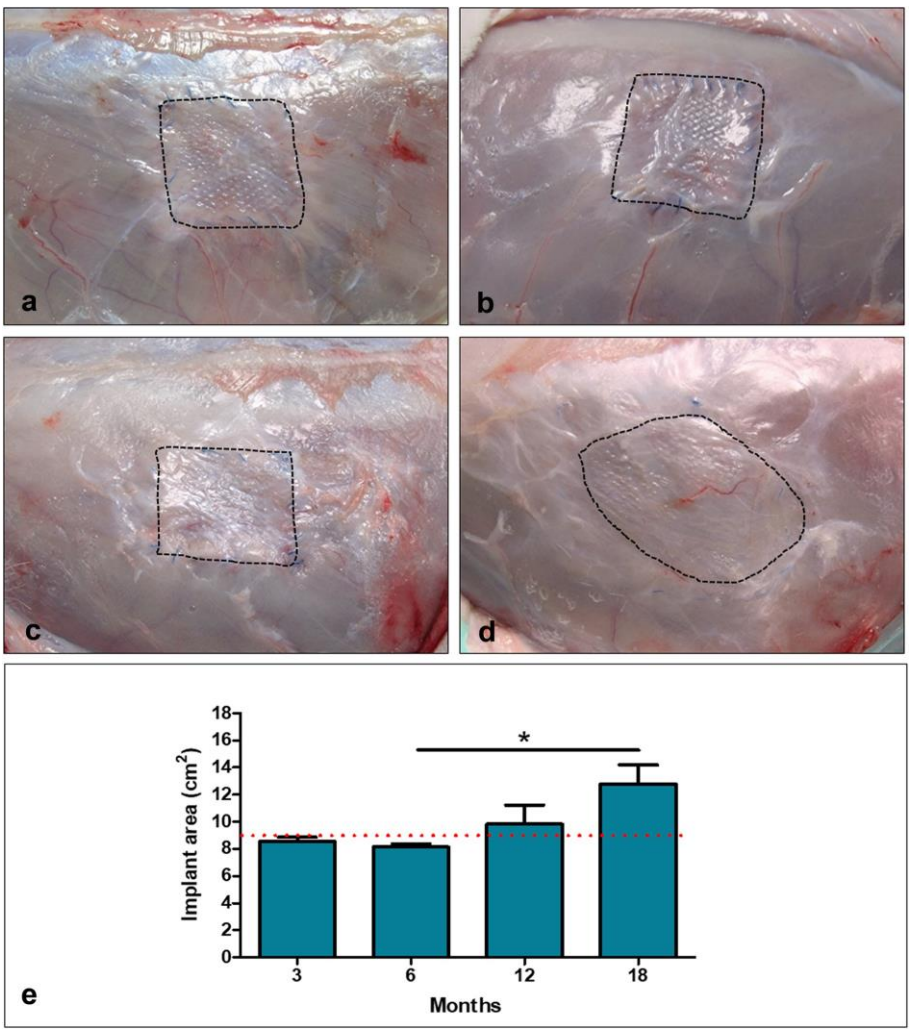

Fig. 2 Macroscopic images of the Phasix™ implant site. Macroscopic view of the implants at 3 (**a**), 6 (**b**), 12 (**c**) and 18 (**d**) months postsurgery. The implant contour is indicated with a black dotted line. **e** Implant area measurements recorded in each study group. Red dotted line corresponds to the implant area at the beginning of the study (9 cm^2). Significant distension of the implant area was observed at 18 months in relation to 6 months (*$P < 0.05$)

Biomechanical assay

Biomechanical behaviour for the Phasix™ implants is shown in Fig. 8. Stress–stretch curves for each animal (n = 5), representative of the final study time and reparative process (18 months), can be seen as and example in

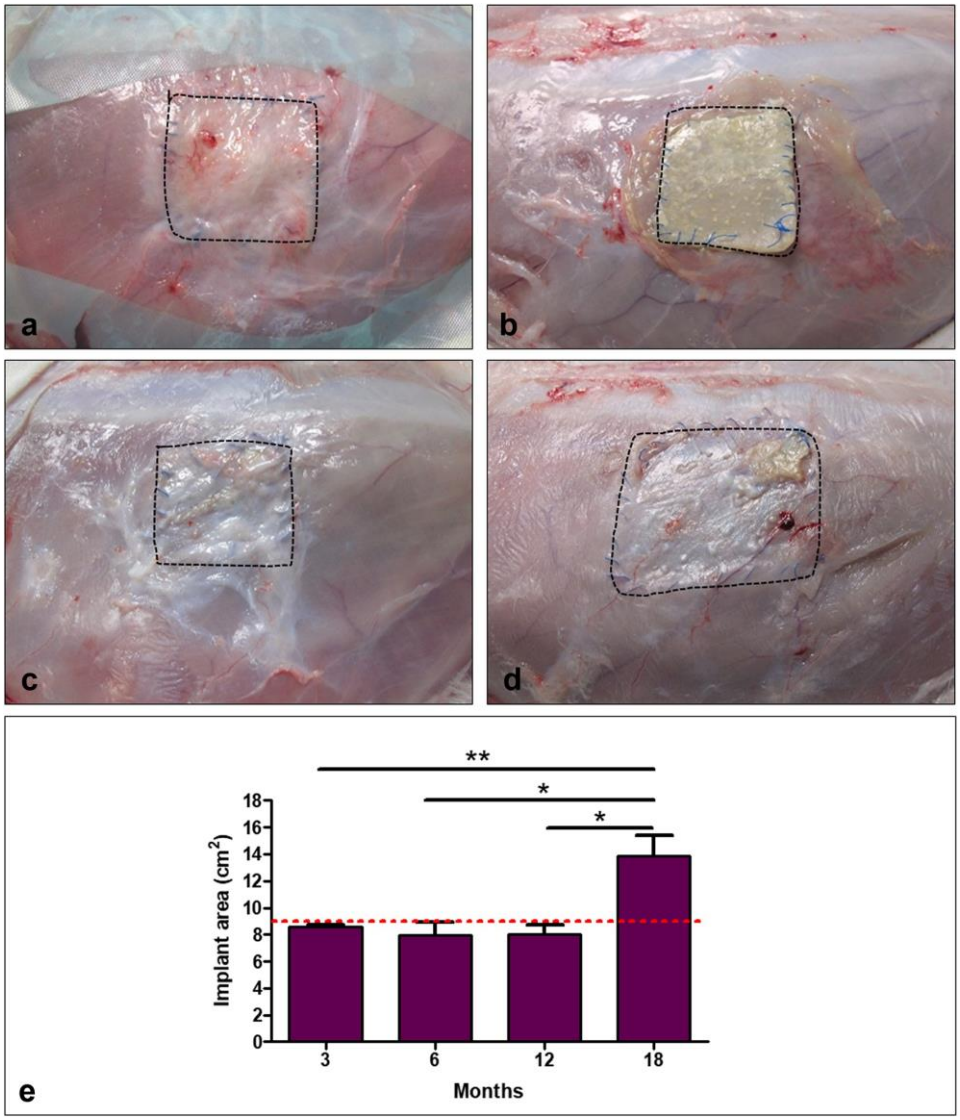

Fig. 3 Macroscopic images of the Protexa™ implant site. Macroscopic view of implants at 3 (**a**), 6 (**b**), 12 (**c**) and 18 (**d**) months postsurgery. The implant contour is indicated with a black dotted line. **e** Implant area measurements recorded in each study group. Red dotted line corresponds to the implant area at the beginning of the study (9 cm^2). Significant distension of the implant area was observed at 18 months versus all remaining time points (*P < 0.05, **P < 0.01)

Fig. 8a. Analyzing the mean stress–stretch curves (truncated at 1.5 strech) for the different study times, the behavior at 3 and 6 months were similar and stiffer than 12 and 18 months after implantation (Fig. 8b). Similar behavior is observed in the rupture, the failure stress decreases from 0.9 ± 0.19 MPa at 3 months to 0.69 ± 0.09 MPa (Fig. 8c) and the failure strech increases from 1.57 ± 0.04 at 3 months to 1.71 ± 0.11 as the implant is being reabsorbed (Fig. 8d).

The biomechanical behaviour of Protexa™ implants is shown in the Fig. 9. Stress–stretch curves for each animal (n = 5), representative of the final time point of the study and reparative process (18 months), is illustrated in Fig. 9a. From the analysis of the mean stress–stretch curves (truncated 1.5 stretch) for the different study times, the stiffness of the abdominal wall increases gradually and significantly with time. At 3 months postimplant the stiffness was very low, increasing significantly along the study time. As the implant time progressed, the implant area gradually stiffens and this was accompanied by an increase in the failure stress (Fig. 9b). Stress differences were significant at the three longest study times when compared to 3 months postimplant (Fig. 9c).

The failure stress increased from 0.32 ± 0.06 MPa at 3 months to 0.63 ± 0.25 MPa (Fig. 9c). The failure stretch did not change significantly for the analysed time points. The stretch value varied from 1.81 ± 0.1 at 3 months to 1.61 ± 0.2 at 18 months, as the implant was gradually reabsorbed (Fig. 9d).

Figure 10 shows a comparison between the two prosthetic materials at the initial and final study times. At short term (3 months), the abdominal wall repaired with Protexa™ mesh was more compliant (less stiff) compared to the wall repaired with the Phasix™ mesh.

However, at long term (18 months), once the meshes have been partially reabsorbed, to a greater extent in Protexa™, and the repair tissue has been formed, the behaviour of the abdominal wall was homogenized, with stress–stretch relations being similar for both implants (Fig. 10b).

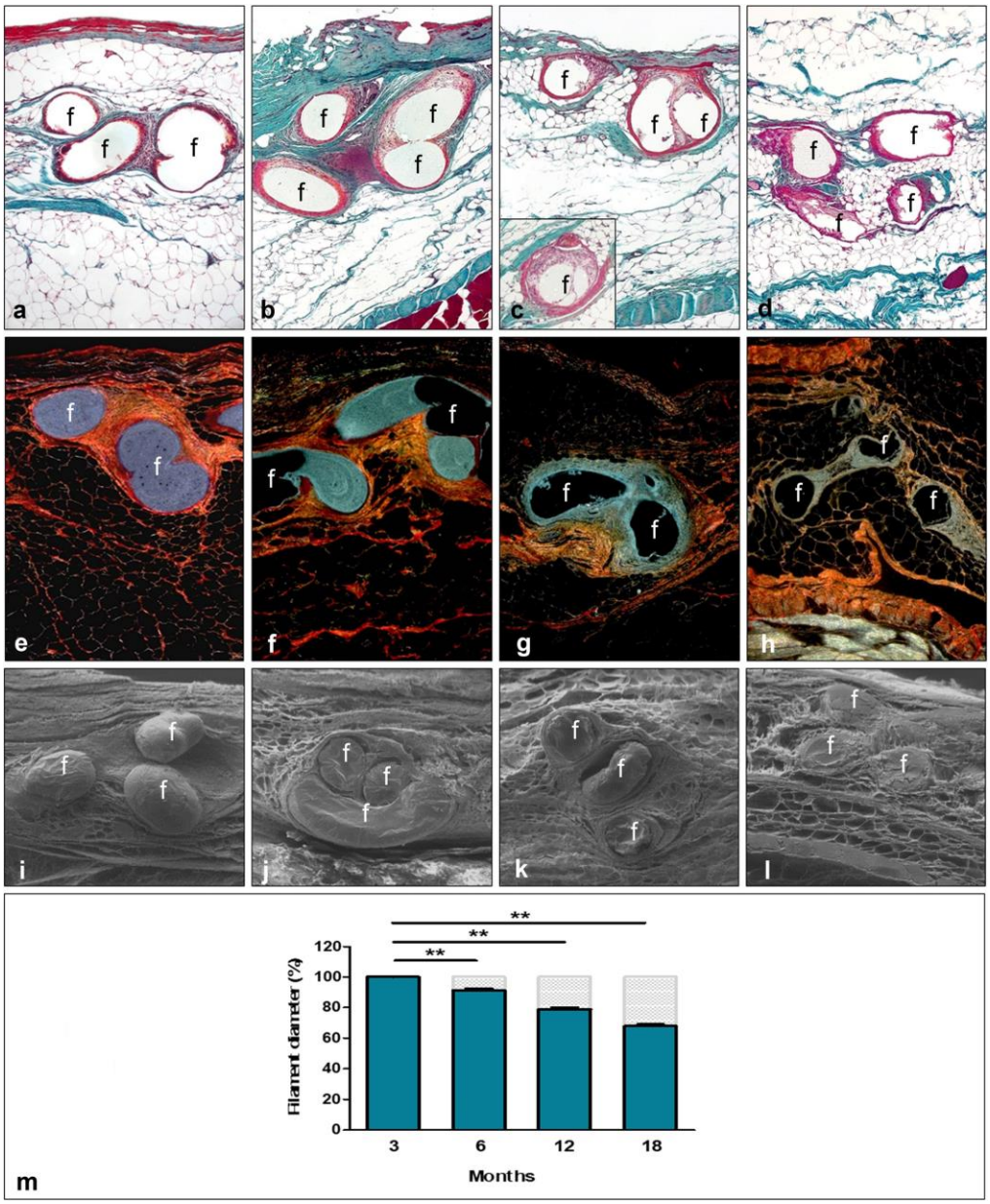

Fig. 4 Morphological analysis and resorption of Phasix™. Cross section showing host tissue ingrowth around the Phasix™ filaments at postimplant months 3 (**a**), 6 (**b**), 12 (**c**) and 18 (**d**). Prosthetic filaments were surrounded by inflammatory cells and embedded in adipose tissue (inset: detail of host cell infiltration in one filament, ×200). Masson's trichrome (Goldner-Gabe) staining (×100). Sirius red staining showing collagen deposition in the neoformed repair tissue at 3 (**e**), 6 (**f**), 12 (**g**) and 18 (**h**) months (×100). Type I collagen appears red, and type III collagen yellow-green. (**i-1**) Scanning electron micrographs showing Phasix™ filaments at all the study times (×100). (*f*: Phasix™ filament). *m* percent of not resorpted prosthetic materials for Phasix™. Shaded areas indicate absorption of the material in the different study times (**$P < 0.01$ versus the rest of the at groups)

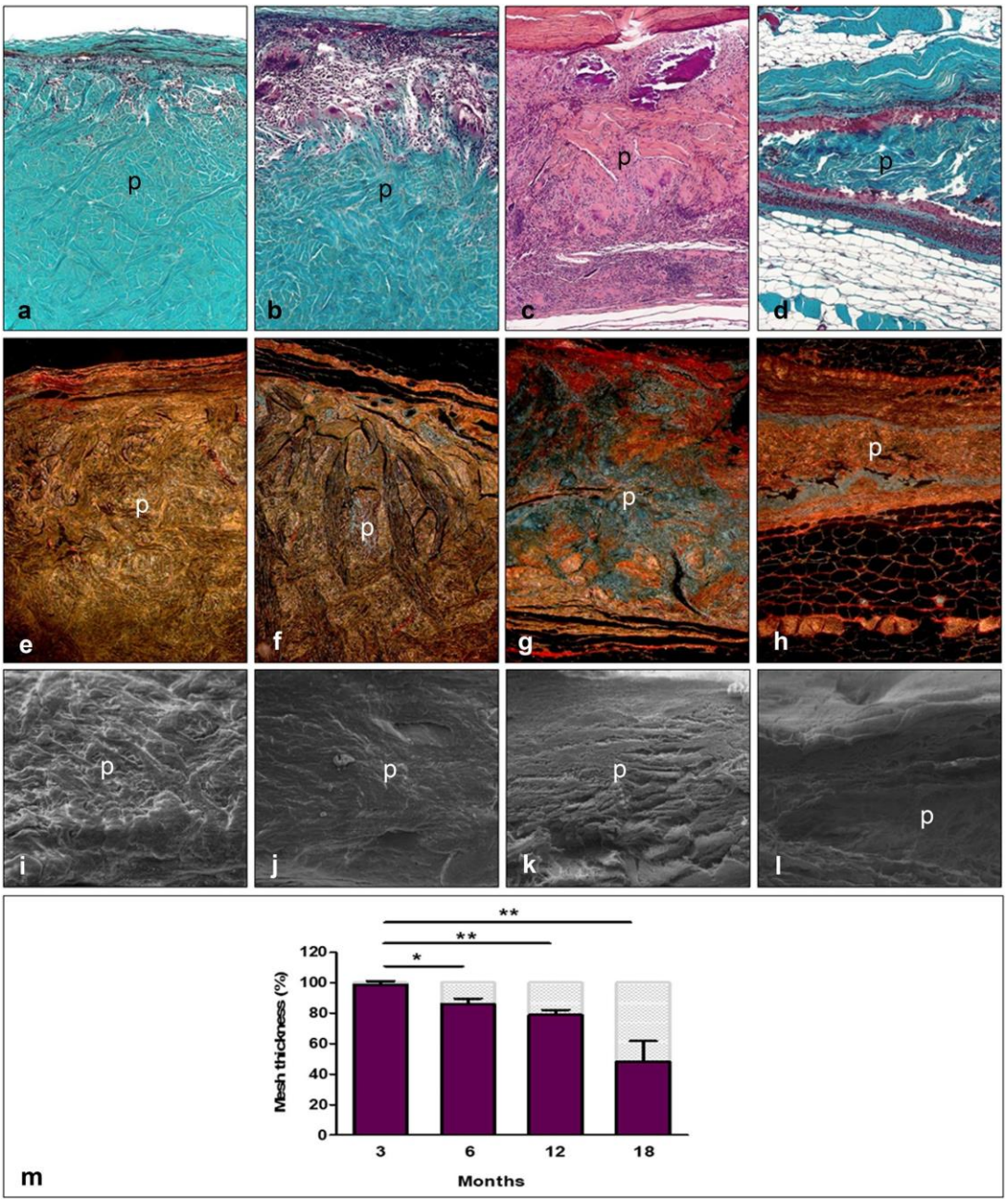

Fig. 5 Morphological analysis and resorption of Protexa™. Representative light microscopy cross-sectional images of the Protexa™ implant repair site at 3 (**a**), 6 (**b**), 12 (**c**) and 18 (**d**) months. The implant was surrounded by a layer of connective tissue and inflammatory cells, with no significant cell infiltration noted before 12 months of follow-up. Masson's trichrome (Goldner-Gabe) staining (×100). Sirius red stained images showing collagen deposition in the neoformed repair tissue at 3 (**e**), 6 (**f**), 12 (**g**) and 18 (**h**) months (×100). Type I collagen appears red, and type III collagen yellow-green. Scanning electron micrographs showing Protexa™ implants at 3 (**i**), 6 (**j**), 12 (**k**) and 18 (**l**) months (×100). (*P* Protexa™; * calcification). **m** Absorption extent of Protexa™. Shaded areas represent the percentage of resorpted prosthetic material (*$P < 0.05$, **$P < 0.01$)

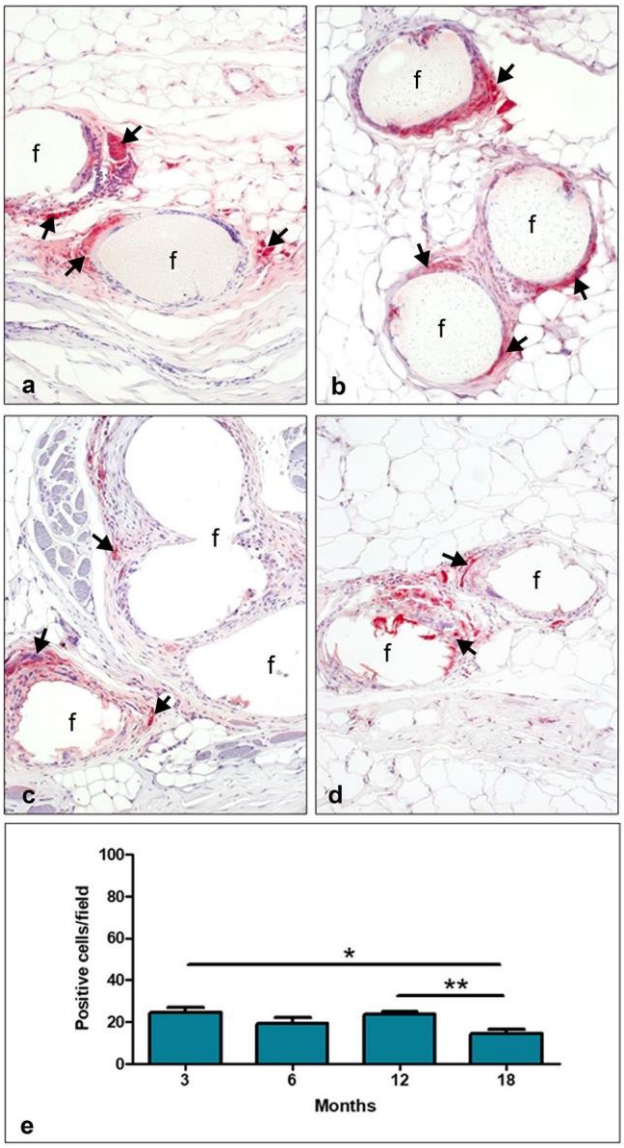

Fig. 6 Macrophage response to Phasix™ implants. Immunohistochemical labelling at the different study times for rabbit macrophages (arrows), 3 (**a**), 6 (**b**), 12 (**c**) and 18 (**d**) months, using the RAM-11 monoclonal antibody (×200). (*f*: Phasix™ filament). (**e**) Macrophage positive cells per field were significantly reduced at 18 months versus 3 (*$P < 0.05$) and 12 months (**$P < 0.01$) postimplant

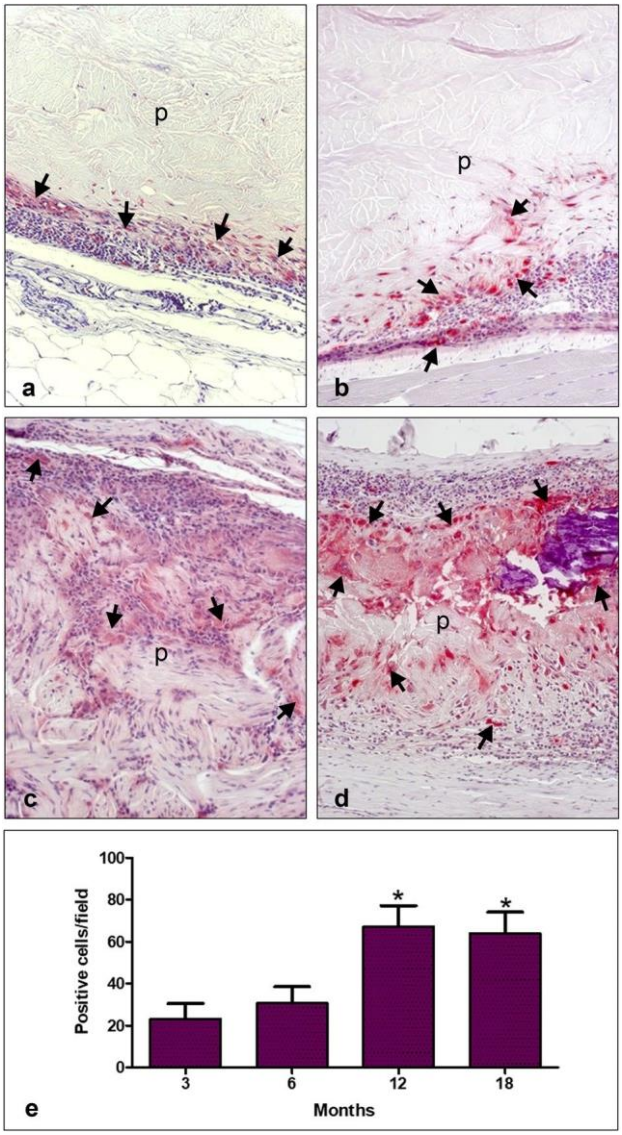

Fig. 7 Macrophage response to Protexa™ implants. Immunohistochemical labelling at the different study times, 3 (**a**), 6 (**b**), 12 (**c**) and 18 (**d**) months, for rabbit macrophages (arrows) using the RAM-11 monoclonal antibody (200x). (*P*: Protexa™). (**e**) Macrophage positive cells per field were significantly increased at 12 and 18 months versus 3 and 6 months postimplant (*$P < 0.05$ vs. 3 and 6 months)

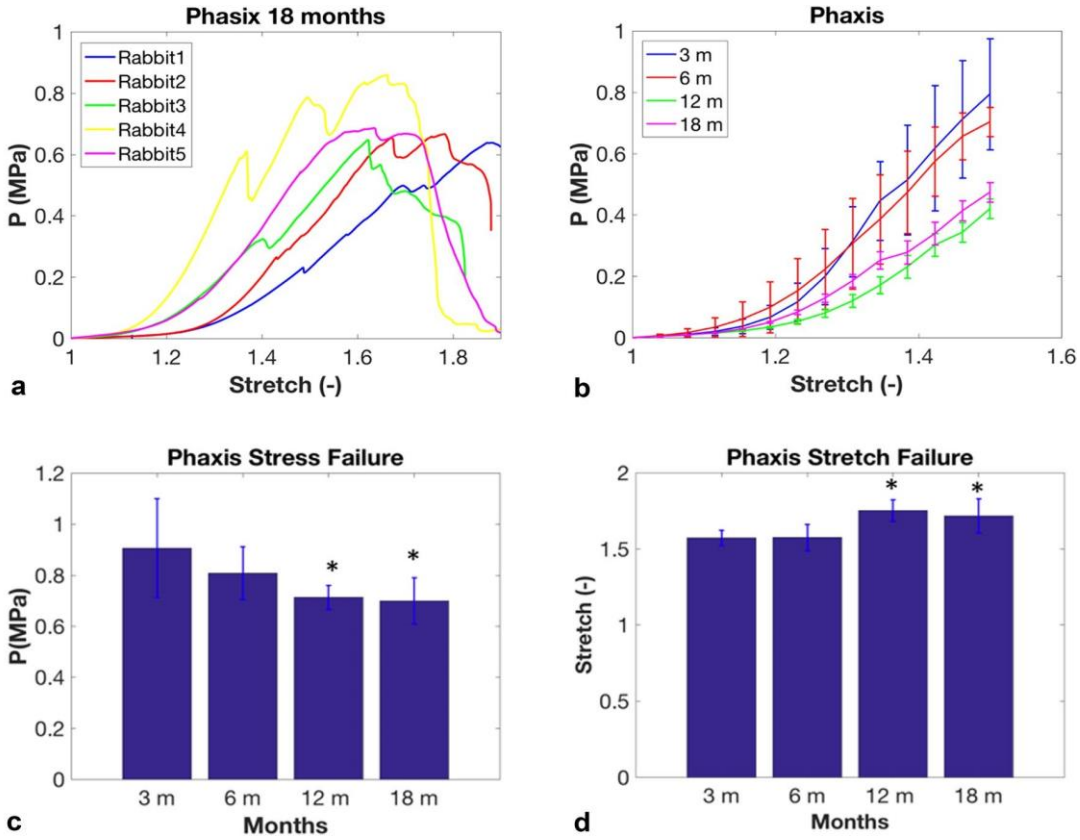

Fig. 8 Mechanical behaviour of Phasix™ implants. **a** Stress–stretch curves for each animal after 18 months, at the final study time. **b** Meand and standard desviation stress–stretch curves after 3, 6, 12 and 18 months (curves were truncated at 1.5 before the maximum stress value). **c** Mean failure stress values obtained in each group. **d** Mean failure stretch values obtained in each group. (*P < 0.01) significant differences were recorded for Phasix™ at 12 and 18 months vs. 3 months

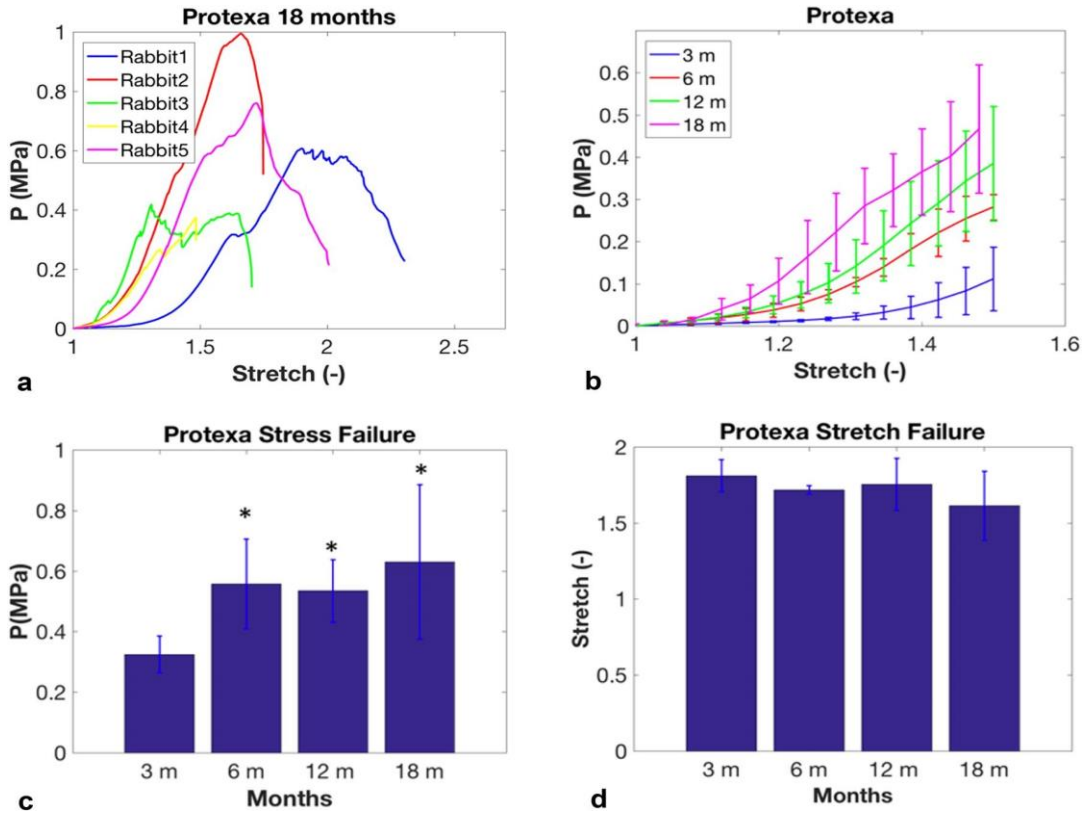

Fig. 9 Mechanical behaviour of Protexa™ implants. **a** Stress–stretch curves for each animal after 18 months, at the final study time. **b** Mean and standard desviation stress–stretch curves after 3, 6, 12 and 18 months (curves were truncated at 1.5 before the maximum stress value). **c** Mean failure stress values obtained in each group. **d** Mean failure stretch values obtained in each group. (*P < 0.01) Significant differences were recorded for Protexa™ versus 3 months

Discussion

New prosthetic materials for hernia repair are constantly being developed. Requirements of a limited inflammatory reaction leaving a minimum of foreign material in the recipient have prompted the appearance first of natural biomeshes and subsequently of synthetic materials, both fully biodegradable in the long term.

To date, synthetic biodegradable materials (Vycril®, Dexon®) have had a short resorption period of around 80–90 days. The most recent material (BioA™) shows a degradation time of under 6 months [16]. This latter implant is consequently ineffective for abdominal wall hernia repair and is normally used only for tissue reinforcement.

Biomeshes were initially accepted for the repair of a hernial defect, especially if there was risk of infection or an existing infection in the recipient tissue. While in clean surgeries, outcomes have been acceptable, especially when the biomesh is placed in visceral contact [21], this has not been the case in contaminated surgical fields [22]. Clinical experience in this last type of setting has led to highly variable results as there have been scarce prospective long term studies. Thus, some clinical trials have suggested the improved behaviour of conventional polypropylene prostheses, especially large pore designs, over that shown by biomeshes [23]. Another drawback of the clinical use of these materials has been their elevated costs.

In the present experimental study, we have analyzed the postimplant behaviour of a biodegradable acellular dermal porcine non-crosslinked biomesh (Protexa™) and a synthetic material (Phasix™) of natural origin. The latter is composed of PHT4 (poly-4-hydroxybutyrate), a fully reabsorbable polymer produced by the microorganism *Escherichia coli* K12 via transgenic fermentation techniques. This material has already been clinically tested for other uses, namely as a suture material [24] and in breast implants [25]. PHT4 is a high-strength polyester that degrades in a steady manner into a natural metabolite [4-hydroxybutyrate (4HB)] that is normally present in human tissues and is eliminated via the Krebs cycle as carbon dioxide and water [24]. Hence, it is biocompatibility has been well established [26].

The use of these fully reabsorbable meshes pursue the idea of providing the necessary support at the repair site during the initial wound healing period while allowing tissue ingrowth, remodelling and progressive transfer of mechanical load from the mesh to the host tissue over time. A recent review [27] about resorbable synthetic meshes for abdominal wall repair has revealed the need for long term studies with this type of materials that show the potential advantages for their clinical practice.

We selected follow-up times for our study extending into the long term period (up to 18 months), according to the biodegradation characteristics of both materials. The experimental model, the rabbit, is considered optimal for this type of preclinical trial [28].

Few experimental studies examining the behaviour of Phasix™ have included a postimplant follow up period as long as 18 months. This is effectively the degradation time provided by the manufacturer. Martin et al., in a 18-month follow-up study [29] described Phasix™ as an attractive option with the handling advantages of a polypropylene mesh, and host tissue-based repair free of permanent material retention.

The same literature situation has been found for Protexa™ although some preclinical trials have examined another biological material, also non-crosslinked, in which tests were conducted at one year postimplant [30].

Our macroscopic findings were the absence of rejection or infection following the implant of both the synthetic and natural material. Interestingly, at 18 months, zones of loose, relaxed tissue at the repair site were detected in both implants without any hernia sac as such with intraabdominal contents. These detached tissue zones have been described in human clinical practice [31] and we have also observed them in experimental BioA™ implants [16].

The tissue integration behaviour of Phasix™ was good in terms of adequate collagenization and angiogenesis around prosthetic filaments, similar to that observed for non-absorbable polypropylene mesh [32]. Instead, Protexa™ became encapsulated as usually occurs with the more laminar materials [16]. Degradation of the Phasix™ filaments steadily declined over time from 3 months after surgery, and the same occurred with the Protexa™ sheet. Both prosthetic materials remained in the recipient zone at 18 months. In relation to Phasix™, these data are inconsistent with that described by Deeken et al. [33], who 1 year after implant were unable to find remains of this material. However, our findings are in line with those of Martin et al. [29], who reported visible filaments at 18 months postimplant. In a recent clinical study [34], the authors observed prosthetic material remains in a Phasix™ implant biopsy 22 months after implant. Therefore, it seems that the biodegradation of this polymer mesh extends beyond that indicated by previous preclinical trials including those conducted by its manufacturing company.

There are no 18-month postimplant biodegradation data available for Protexa™. Our 1-year results are in agreement with those of Mulier et al. [30], who employing a noncrosslinked bioprosthesis (Strattice™) observed its progressive reabsorption until 12 months postimplant. Other authors [35] also observed remains at this time point of another biomesh (Surgisis™).

Our study of the foreign body reaction induced by both materials assessed via macrophages detected at the different time points postimplant revealed a progress decline in these cells between 3 and 18 months elicited by the Phasix™ implants. This finding is consistent in the shorter term (12 months) with the findings of others [33], and at 18 months, with the report by Martin et al. [29] of a “moderate inflammatory reaction” noted at all the study’s time points. This behaviour coincides with that observed with non-absorbable prosthetic materials

such as polypropylene in response to which the macrophage response decreases progressively even at shorter study times [36].

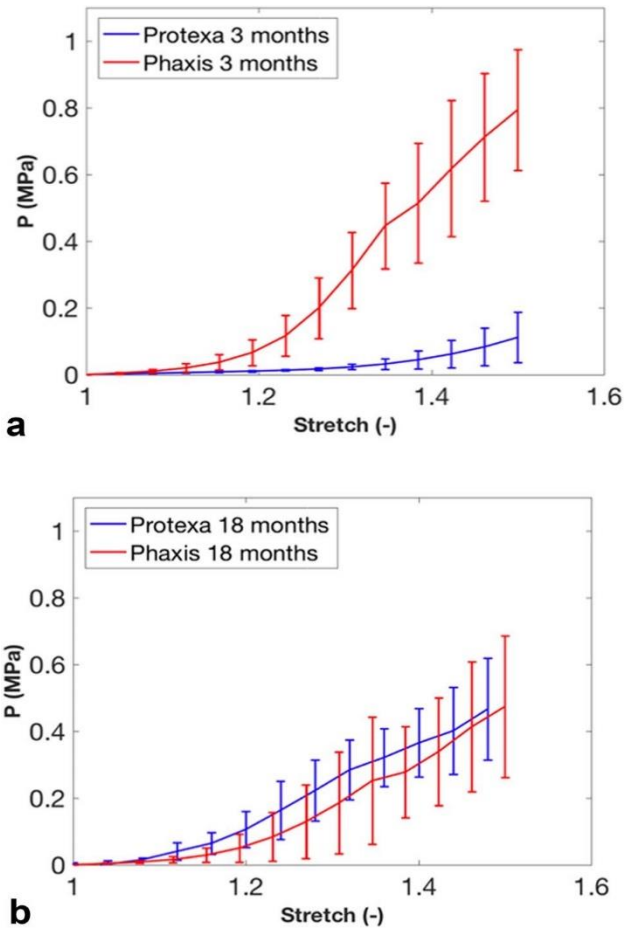

Fig. 10 Comparison between the mechanical behaviour of Phasix versus Protexa™. **a** 3 months postimplant and **b** 18 months postimplant

In response to the Protexa™ implants, labelling for macrophages was increased at 12 and 18 months. This has also been reported for the use of non-crosslinked meshes at 12 months [35]. This augmented response is likely related to the high degree of resorption that occurs at these time points.

As a result of the current study, the stress–stretch behaviour of the abdominal wall repaired with Phasix™ decreased over time, being more pronounced in the period 12–18 months of implant due to the gradual resorption of the implant. This fact is interesting as, despite the presence of relaxed tissue in the implant zone, we found no zones with hernial defects. These findings are somewhat in

contradiction with those reported by other authors [29] that only analysed the tensile strength, i.e., they considered forces and displacements. Therefore, a direct comparison is not possible. On the other hand, the mechanical response of the repaired abdominal wall with Protexa™ became stiffer over time due to the synthesis and deposition of collagen in the neoformed tissue over the hernial defect. Over the postimplant period of 3–18 months, the failure stress of the defects repaired with Protexa™ increased significantly. The tissue remodelling that takes place in this collagen implant may explain this observation, as despite the resorption shown by this material, the neoformed tissue replacing the mesh shows intense collagenization. Accordingly, Sirius red staining revealed large deposits of collagen type I forming structures that are able to withstand the tensile stress responsible for these mechanical characteristics of the repaired tissue. This fact is also observable in the stress–strain curves of
Protexa™ where at 3 months, the repaired zone was more compliant with a reduced failure stress. As time progresses the repaired zone gains the ability to withstand increasing stresses.

While preclinical or experimental studies have been scarce, so have clinical trials. We can also say that, overall, patient follow up in clinical studies has been short. In a study by Plymale et al. [37], in which Phasix™ was used to repair ventral hernias, no recurrence was observed during 2 years of follow up. In contrast, a prospective study at 18 months, in inguinal hernia surgery, [38] detected recurrence in 9% of cases.

We should mention other polymer materials such as TIGR™ of long term resorption (up to 3 years), which have shown good preclinical results [18]. Nevertheless, the clinical utility of this material is unclear because of hernia recurrence [19]. For Protexa™, a 1-year clinical trial in patients with abdominal hernia has revealed its good behaviour even in an emergency setting [39].

Hence, it seems that the use of reabsorbable materials such as those examined here whether of biological or synthetic origin requires caution. We are aware of the limitations of our study, including the animal model and the insufficient study time required until the complete degradation of the materials. It should also be noted that the absorption rates of the material may also be related to the animal species, body temperature, and also the metabolic activity of the species. In our experience, although the rabbit model has provided optimal results in terms of tissue repair, immune responses and biomechanical behaviors, it is necessary to transfer the model to human clinical practice. Very longterm prospective clinical trials are still needed to assess their real efficacy.

In conclusion, the findings of our study indicate that: (a) both materials showed good compatibility with host tissue, the biodegradation of Phasix™ being incomplete and that of Protexa™ being in a more advanced phase at 18 months; (b) the macrophage reaction diminished progressively in response to Phasix™, while it remained elevated for Protexa™; (c) at long term the mechanical

behaviour of the abdominal wall was homogenized, with stress–stretch relations being similar for both implants, regardless of the more compliant abdominal wall repaired with Protexa™ at sort term.

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Compliance with ethical standards

Conflict of interest We have no conflicts of interest to disclose.

Ethical approval The study protocol was approved by the Committee on the Ethics of Animal Experiments of the Universidad de Alcalá,
Madrid, Spain.The study protocol was approved by the Committee on the Ethics of Animal Experiments of the Universidad de Alcalá, Madrid, Spain.

Human and animal rights The up-keep and handling of animals throughout the study was in accordance with the Guide for the Care and Use of Laboratory Animals of the National and European Institutes of Health (Spanish Law 6/2013, Spanish Royal Decree 53/2013, European Directive 2010/63/UE and European Convention of the Council of Europe ETS123). All procedures were performed at the Animal Research Centre of the Universidad de Alcalá (Madrid, Spain), which is registered with the Directorate General for Agriculture of the Ministry of Economy and Technology Innovation of the Community of Madrid (ES280050001165) ensuring all facilities legally cover the needs and requirements of the research.

Informed consent For this type of study, formal consent is not required.

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