Histol Histopathol (2014) 29: 139-149
DOI: 10.14670/HH-29.139

http://www.hh.um.es

Histology and Histopathology
Cellular and Molecular Biology

Long term behavior of biological prostheses used as abdominal wall substitutes

G. Pascual[2], S. Sotomayor[2], P. Pérez-López[1], J. Buján[2] and J.M. Bellón[1]

[1]Department of Surgery and [2]Department of Medical Specialities, Networking Research Center on Bioengineering, Biomaterials and Nanomedicine (CIBER-BBN), Faculty of Medicine, Alcalá University, Alcalá de Henares, Madrid, Spain

Summary. Background. Despite their degradation in the host organism, the benefits of collagen bioprostheses remain unclear. This study addresses the absorption and long-term host tissue incorporation of several collagen biomeshes. Material and methods. Partial ventral hernial defects created in the abdominal wall of rabbits were repaired using the crosslinked meshes Permacol® or CollaMend®, or the non-crosslinked Surgisis®, Tutomesh® or Strattice®. After 90 and 180 days of implant, morphological studies and morpho-metric analysis of the thickness of the meshes were performed. Immunofluorescence confocal microscopy combined with differential interference contrast (DIC) imaging was used to distinguish newly formed collagen from that comprising the mesh. The macrophage response was examined by immunohisto-chemistry. Results. At 90 days, the thinner non-crosslinked biomeshes Tutomesh and Surgisis were more fully degraded with much of their collagen replaced with loose connective tissue. By 180 days, both implants had been practically fully absorbed. In contrast, in Strattice only the outermost third was infiltrated by neoformed tissue. On both surfaces of the crosslinked meshes, a fibrous capsule with host cells lining its perimeter was observed at both time points, though at 180 days these cells had penetrated the mesh interior. At both implant times, Strattice showed the higher expression of collagen type I while collagen III expression was similar for all the meshes. The non-crosslinked materials elicited lower macrophage counts at both time points, significantly so for Strattice. The macrophage response decreased over time for all the meshes but Surgisis. Conclusions. Strattice, the thicker, more compacted non-crosslinked mesh showed the best balance between tissue incorporation and absorption while eliciting a minimal foreign-body reaction in the long-term.

Key words: Abdominal wall repair, Bioprosthesis, Collagen mesh, Hernia repair

Offprint requests to: Mª Gemma Pascual González, Department of Medical Specialities, Faculty of Medicine, Universidad de Alcalá,, Ctra. Madrid-Barcelona, Km 33,600, 28871, Alcalá de Henares, Madrid, Spain. e-mail: gemma.pascual@uah.es

Introduction

Biomaterials used in surgery to repair or substitute different tissues can either be synthetic or biological in nature. The recent surge in the use of biomaterials and their expanded indications include their use to strengthen or substitute tissue defects that affect the abdominal wall.

The biomaterial industry has developed prosthetic materials derived from live animal (xenografts) or human (allografts) tissues, which supposedly achieve repairs similar to those offered by the use of autologous tissues. These materials are the so-called bioprostheses, or biological prostheses, elaborated out of denatured, acellular collagens. Bioprostheses represent a significant advance since, as a basic characteristic, they become degraded until their complete elimination in the recipient organism (Badylack et al., 1998; Abraham et al., 2000).

The gradual degradation of a bioprosthesis in the host will determine the formation in its place of a neotissue, which in the long term will completely replace the biomaterial. In ideal conditions, the goal is to achieve not only the repair of the damaged zone but also the regeneration of an adequately organized tissue that

will promote angiogenesis and even recruit growth factors acquiring characteristics similar to those of healthy tissue (Menon et al., 2003).

To pursue this goal, the degradation/regeneration process needs to be controlled by pretreating the bioprostheses with different substances (glutaraldehyde, hexamethylene diisocianate, etc.) that confer these materials initial stability in an environment where they are susceptible to degradation by collagenases. In 1975, Oliver et al. showed that creating covalent bonds within the collagen molecule stabilizes its structure making it more resistant to the actions of these collagenases. These crosslinks make the triple helix structure of collagen solid and thus impair its rapid degradation or reabsorption in the host (Liang et al., 2004). It has also been shown that the process of crosslinking modifies the structure of the extracellular matrix and this could delay cell infiltration and potential remodeling of the matrix (Butler, 2006).

Today, there are several biological prostheses available on the market. These bioprostheses are classified according to: the species of origin (animal (xenogenic) or human (allogenic)), the tissue source (dermis, pericardium, or intestinal submucosa) and whether or not they are crosslinked. However, clinical experience in the use of bioprostheses is still limited for several reasons. First, existing inert materials (polypropylene, polyester, expanded polytetrafluoroethylene) offer good outcomes in patients, although the lack of degradation of these materials does not allow for their true incorporation in host tissue (Liang et al., 2004; Bellón et al., 1996). Second, these biomeshes have so far been ascribed precise indications and they have been mainly used in zones compromised by infection (Bellón et al., 1998; Catena et al., 2007). Finally, their high cost has also limited their use (Franklin et al., 2008).

To date, the literature lacks sufficient long-term follow up data for valid conclusions to be drawn regarding the benefits of bioprostheses. In the present study, we assessed the behavior of five biological prosthetic materials of clinical use. These materials were all xenogenic yet varied in terms of their tissue source (dermis, intestinal submucosa or pericardium), thickness and whether they were crosslinked or not. Our main objective was to gain insight into the long-term host tissue incorporation of these different bioprostheses, mainly in terms of their collagenization in a partial abdominal wall defect model. This process of collagenization is central to adequate wound repair, and will determine which of the different meshes is likely to induce the formation of a neotissue of adequate characteristics.

Material and methods

Experimental animals

The experimental animals were 30 male New Zealand White rabbits weighing approximately 2500 g caged under conditions of constant light and temperature according to European Union animal care guidelines (European Directive 2012/63/UE and European Convention of the Council of Europe ETS123). All procedures were approved by our institution's review board.

Prosthetic materials

The non-crosslinked collagen meshes tested were:

- Surgisis® (Cook Inc., West Lafayette, IN, USA): porcine small submucosa, thickness 0.1 mm.
- Tutomesh® (Tutogen Medical GmbH, Nümberg, Germany): collagen I membrane derived from bovine pericardium, thickness 0.5 mm.
- Strattice® (LifeCell Corporation, Branchburg, NJ, USA): tissue matrix derived from porcine dermis, thickness 1.2 mm.

The crosslinked collagen meshes tested were:

- CollaMend® (Bard Inc., Cranston, RI, USA): porcine dermal collagen treated with 1-ethyl-3-(3-dimethylaminopropyl) carbodiimide (ECD), thickness 1 mm.
- Permacol® (Covidien, Dublin, Ireland): porcine dermal collagen treated with hexamethylene diisocianate (HMDI), thickness 1 mm.

Surgical technique

To minimize pain, all animals were given 0.05 mg/kg buprenorphine (Buprecare®, Divasa Farmavic, Barcelona, Spain) 1 hour before surgery and once a day during the three days after 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. No gaseous anesthetic agent was needed during the course of surgery.

Using a sterile surgical technique, and after making an incision in the skin some 6 cm in length, 3 x 3 cm defects were created in the right and left sides of the abdomen, comprising the planes of the external and internal oblique muscles yet sparing the transversalis muscle and parietal peritoneum. The defects were then repaired by fixing a mesh of the same size to the edges of the defect by a running 4/0 polypropylene suture interrupted at the four corners. The skin was closed by running suture using 3/0 polypropylene.

Before sacrifice, the animals were daily inspected to check for signs of dehiscence of the skin wound, seroma formation, wound infection and/or mesh incompatibility.

Experimental design

A total of 30 animals were implanted with 2 different biomaterials (60 implants of the 5 meshes)

alternating the side of implant (3 right; 3 left of each one), to establish 5 groups of 12 implants each.

At each of the established time points, 90 and 180 days postimplant, 15 animals were sacrificed in a CO_2 chamber and specimens of the bioprostheses obtained for analysis. Since each animal was implanted with two different meshes, at each time point we obtained 30 implants comprising 6 specimens of each of the different biomeshes.

Morphological analysis

Specimens were analyzed by morphological analysis by light microscopy (LM) and scanning electron microscopy (SEM).

For LM, a sample of tissue from each of the animals included in the study were fixed in F13 solution, embedded in paraffin, sliced into 5 μm sections, and stained with hematoxylin and eosin and Masson's trichrome (Goldner-Gabe). These specimens were examined under a Zeiss light microscope (Carl Zeiss, Oberkochen, Germany).

For SEM, three samples of each of the groups were fixed in 3% glutaraldehyde, placed in Millonig buffer (pH 7.3) and dehydrated in a graded ethanol series. Critical point was reached in an E-3000 Polaron instrument (Polaron Ltd., England). Finally, the specimens were metalized with gold palladium and examined under a Zeiss scanning electron microscope (DSM-950).

Morphometric analysis of the thickness of the materials

The reduction of the initial thickness of the collagen layers of the different biomaterials at the different study times was estimated by morphometric assessment in 10 histological sections (in microscopy fields of magnification 10x) per group. In each tissue section, two random measurements were made of the thickness of the biomaterial. Images for analysis were captured using a digital camera fitted to a light microscope (Axiocam HR, Zeiss). The software used for these determinations was the Axiocam image analyzer (Axiocam HR, Zeiss).

Collagen expression. Immunofluorescence microscopy

Collagen was detected in the implants by immunofluorescent labeling. Samples of tissue from each of the animals included in the study were fixed in F13 solution, embedded in paraffin and cut into 5 μm-thick sections. Once cut, the sections were deparaffinated, hydrated and equilibrated in PBS buffer. Non-specific protein interactions were blocked with BSA 3% and the samples were then incubated with the monoclonal antibodies anti-collagen I (Sigma, St. Louis, MO, USA) and anti-collagen III (Medicorp, Montreal, Canada). The antigen-antibody reaction was detected using a secondary antibody conjugated to rhodamine. Negative controls were run using BSA 3% instead of the primary antibody. Cell nuclei were counterstained with DAPI. Samples were examined under a confocal microscope Leica SP5 (Leica Microsystems, Wetzlar, Germany) to detect fluorescence. This work was performed by the confocal Microscopy Service of the UAM and CIBER-BBN located at the facilities of the Cell Culture Unit: www.uah.es/enlaces/investigacion.sthm. Differential contrast (DIC) images were also obtained using this microscope. These images were merged with the fluorescence images to differentiate the newly formed collagen in the repair tissue from the collagen comprising the biological prosthesis. In this way, the newly synthesized collagen, will be located with the anti-collagen antibody. However, the completely denatured collagen, which forms the implanted bioprostheses, it is logically not revealed with the antibody, but can be seen translucent with the help of DIC images, allowing the differentiation of both.

Macrophage response. Immunohistochemistry

To assess the macrophage response produced in the implants, macrophages were immunohistochemically detected in the paraffin-embedded sections of all the samples of tissue from each of the animals included in the study using a monoclonal antibody to rabbit macrophages, RAM 11 (DAKO M-633, USA) in the alkaline phosphatase-labeled avidin-biotin method. Histological sections were deparaffinated, hydrated, equilibrated in tris buffered saline (TBS), blocked with BSA 3% and incubated with the primary antibody (1:50 in tris-buffered saline or TBS) overnight. A secondary antibody conjugated to biotin (Sigma B0529, St. Louis, MO, Usa) (1:300 in TBS) was incubated for 1 hour and labeled with avidin (Sigma E2636, St. Louis, MO, Usa) (1:200 in TBS) for 1 hour. 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 minutes in acid hematoxylin. RAM-11 labeled macrophages were quantified by performing counts in 20 microscopic fields (x20) for each biomesh, in each of the groups. Quantification was performed by 2 independent observers in a blinded fashion.

Statistical analysis

Results are expressed as the mean ± SEM. Data for the different biomeshes were compared using the Mann-Whitney test implemented in the Graph Pad Prism 5 package (GraphPad Soft-ware, Inc., La Jolla, CA, USA). Significance was set at $p<0.05$.

Results

There was no mortality among the animals undergoing surgery. The implants were well-tolerated in

that no signs of infection and/or rejection were observed.

Seroma at the level of subcutaneous tissue was the most common complication noted. The CollaMend group showed 2 implants with seroma and another 2 cases were observed in Surgisis. No seromas were observed in Permacol, Tutomesh and Strattice at 90 and 180 days postimplant. By 180 days, the Tutomesh prosthesis had almost completely disappeared macroscopically, and the implant area appeared to be distended.

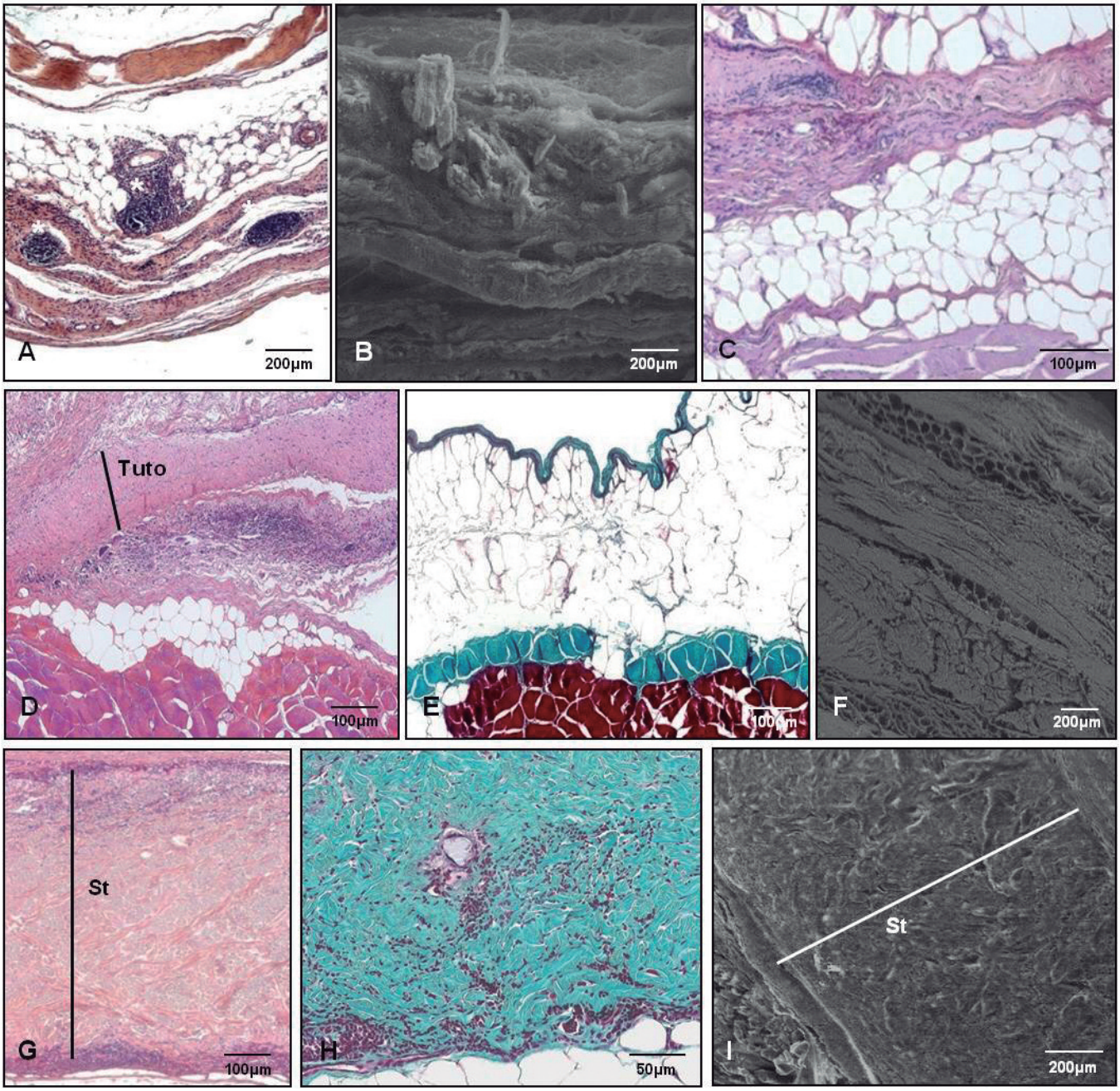

Fig. 1. Morphological analysis. Non-crosslinked biomeshes. **A.** Surgisis remains were surrounded by granulomas (*) at 90 days. **B.** SEM image of Surgisis transversal section. **C.** Surgisis, 180 days. The biomaterial had been replaced by host tissue. **D.** Tutomesh implant, 90 days. **E.** At 180 days, the implant area was occupied by adipose tissue. **F.** Panoramic view of Tutomesh sample, 180 days. **G.** Strattice appeared encapsulated by connective tissue and inflammatory cells at 90 days postimplant. **H.** Strattice 180 days postimplant. **I.** SEM image. There were no signs of Strattice degradation before 180 days postimplantion (50x). SIS, Surgisis; Tuto, Tutomesh; St, Strattice.

Morphological analysis

90 Days postimplant

Non-crosslinked biomeshes. At this time point, the two thinner non-crosslinked implants, Tutomesh and Surgisis, were the most degraded, both showing extensive absorption of the collagen mesh and its replacement with loose connective tissue (Fig. 1a,b,d). The reduced thickness and layered structure of these biomeshes conditioned the remodeling process. Thus, extensive host tissue ingrowth was observed in these implants along with neoformed connective tissue and a reduction in the initial thickness of the collagen layers. These processes constitute the degeneration of the material and its gradual replacement by host tissue.

The also non-crosslinked yet thicker Strattice biomesh showed scarce evidence of the absorption of its comprising collagen sheet (Fig. 1g). However, traces of neoformed tissue were seen to penetrate the outer third of the implant. At this time point, the inflammatory reaction was restricted to the biomesh area.

Crosslinked biomeshes. At 90 days, the histological behavior of the crosslinked biomeshes was similar to that observed for Strattice. No signs of degradation were detected, though the structure of CollaMend was less compacted than that of Permacol (Fig. 2a,b,d). In both cases, a fibrous capsule enveloped the implant material, separating the neoformed connective tissue from the mesh; some capsule cells were observed inside the biomesh, although these were fewer in number in the case of CollaMend (Fig. 2a,d).

180 Days post-implant

Non-crosslinked biomeshes. In the longer term, Tutomesh and Surgisis were completely absorbed (Fig.1 c,e,f), although in some areas some remains of Tutomesh were observed, and were almost fully replaced by a dense connective tissue, tissue that has collagen fibers as its main extracellular matrix constituent mainly of collagen type I. This area was rich in white adipocytes in the case of Tutomesh (Fig. 1e) and rich in blood vessels

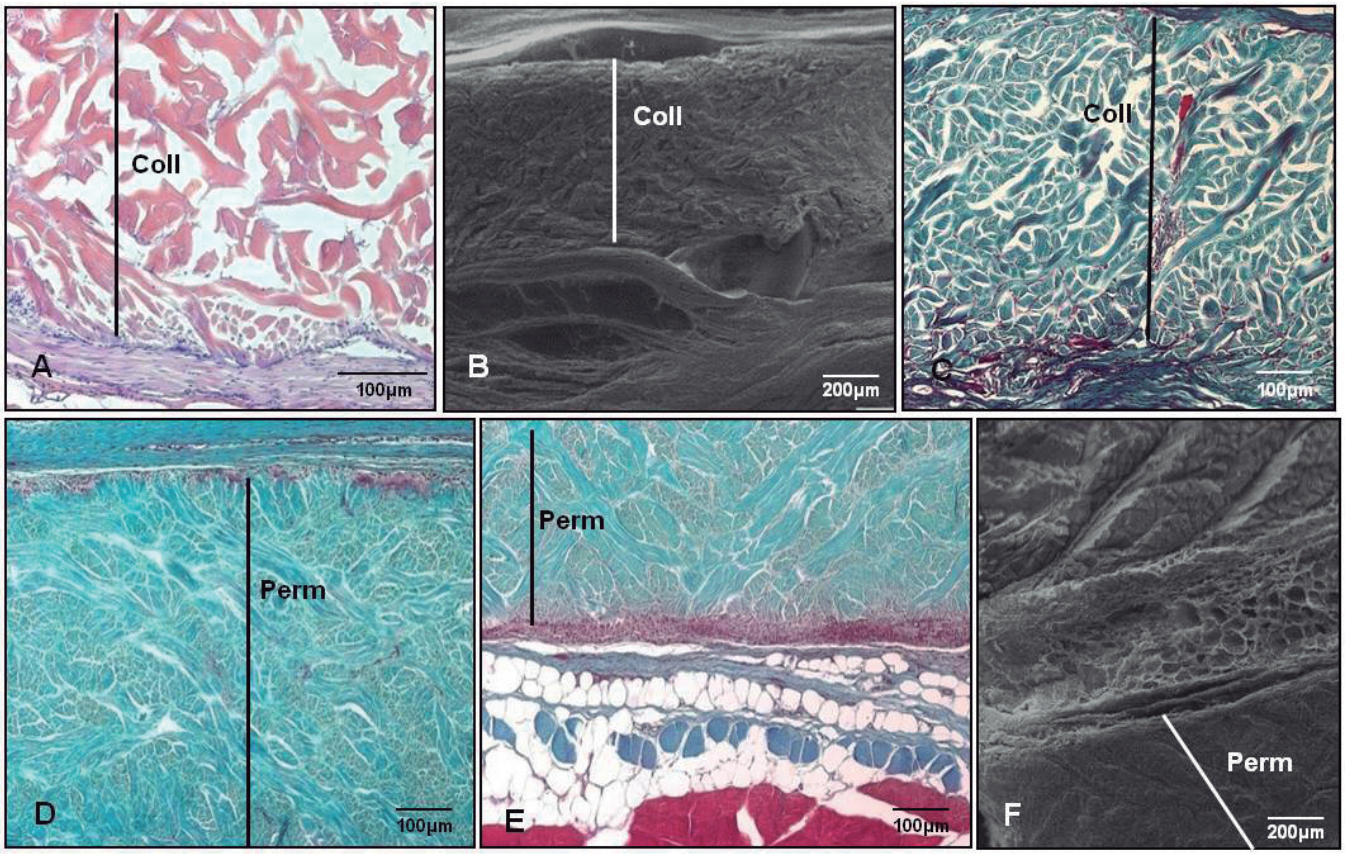

Fig. 2. Morphological analysis. Crosslinked biomeshes. **A.** A fibrous capsule could be observed around Collamend 90 days. **B.** SEM, CollaMend, 90 days. **C.** Collamend 180 days postimplant. **D.** No signs of biomaterial degradation were observed in Permacol implant at 90 days. **E.** There are no significant changes in the histological behavior of the Permacol at 180 days compared with previous study time. **F.** SEM detail of the previous image. Coll, CollaMend; Perm, Permacol.

in that of Surgisis (Fig. 1c). Compared to 90 days, at 180 days the Strattice mesh was more extensively infiltrated by neoformed tissue and the inflammatory reaction around this biomaterial was significantly reduced (Fig. 1h,i).

Crosslinked biomeshes. The earlier histological behavior of CollaMend and Permacol persisted in the long term and we could still observe the fibrous capsule on both prosthetic surfaces and cells lining its perimetry (Fig. 2c,e,f). At this time point, however, a large number of these cells could also be seen in the implant interior. Areas of neoformed connective tissue expanded over the study period from the outer prosthetic zone towards the inside, their presence being more intense in the greater irregularities of the material (Fig. 2c). In these implants, neoformed connective tissue density increased and small blood vessels appeared. Cell colonization was limited to the outermost third of the meshes. At this time point, signs of degeneration of the implanted collagen and its gradual replacement by host tissue started to emerge.

Morphometric analysis of the thickness of the materials

The thickness of the biomaterial at the different study times was expressed as the percentage of thickness with respect to the normal thickness for each of the different meshes (Fig. 3).

The two lower thickness non cross-linked prostheses (SIS/Tuto), showed a very important resorption, that of the SIS being almost complete 90 days of implant (Fig. 3).

The rest of the bioprosthesis showed a percentage reduction of thickness much less important. The prosthesis that showed higher resorption at 180 days was colamend (Fig. 3). Statistically significant differences ($p<0.05$) were observed between sis/tuto, compared with the rest of the meshes at 90 and 180 days postimplant.

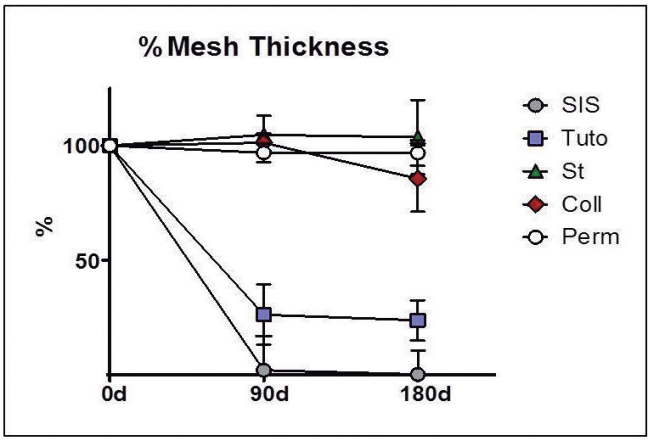

Fig. 3. Percentage of mesh thickness at the different study times. SIS and Tuto showed a very important degradation, that of the SIS being almost complete 90 days of implant. The rest of the bioprosthesis showed a percentage reduction of thickness much less important. Statistically significant differences (*$p<0.05$) were observed between sis/tuto, compared with the rest of the meshes at the different study times.

Collagen expression. Immunofluorescence microscopy

The immunofluorescence confocal microscopy images were superimposed on the DIC images so the newly formed collagens in the neotissue, identified using the corresponding antibody (anti-collagen I or III), appeared as red immunofluorescence, and the native collagens forming the prostheses appeared translucent.

Collagen I expression

Three months after implant, Surgisis showed moderate collagen I expression in the neoformed tissue that replaced the prosthetic material. This expression had increased slightly at 180 days postimplant. Tutomesh, however, only showed weak collagen I expression at both time points.

CollaMend and Permacol showed similar moderate staining for collagen I at 90 days, increasing slightly at 180 days. Staining was restricted to the fibrous connective tissue surrounding the prosthesis and to a few zones of tissue infiltrating the material. Most collagen I expression was detected for Strattice at both implant times compared to the other implanted meshes. At 90 days, labeling for this collagen type was observed in areas inside the mesh that had been infiltrated by the neoformed tissue. By 180 days, the fluorescence intensity had increased inside the prosthesis (Fig. 4).

Collagen III expression

90 days after implant, large areas of neoformed tissue appeared in the Surgisis meshes. These areas showed expression for collagen type III, which fell slightly at 180 days (Fig. 5). The Surgisis meshes showed the highest expression of this type of collagen at both time points compared to the remaining biomeshes tested. Remarkably, the prosthetic material could be seen to vanish as the time after implant increased. Weak collagen III expression was observed for Tutomesh in small connective tissue zones and around the white adipocytes. In the CollaMend, Permacol and Strattice implant groups, similar collagen III expression patterns were produced. Maximal expression was observed in the capsule and host cells infiltrating the meshes, in which cell nuclei could be seen at both time points (Fig. 5).

Macrophage response. Immunohistochemistry

The crosslinked meshes showed greater macrophage numbers than the non-crosslinked meshes at both 90 and 180 days. Macrophage counts at 90 days indicated higher numbers for the Permacol implants representing around 25% of total cell numbers. Thus, counts for Permacol were significantly higher than the counts recorded for the remaining implant groups. Macrophage

numbers for CollaMend were also significantly higher than those observed for Strattice and Surgisis. Hence, Strattice showed a similar inflammatory reaction to Surgisis which was significantly reduced compared to that induced by Permacol, CollaMend and Tutomesh.

At 180 days, Permacol continued to show higher numbers of labeled macrophages with significant differences emerging only with respect to the non-crosslinked meshes. CollaMend also showed significant differences when compared to Tutomesh and Strattice. Thus, as for the 90-day time point, a significant reduction in the inflammatory response was detected for Strattice relative to the response shown by the two crosslinked biomeshes (Permacol and CollaMend) and Tutomesh.

When the macrophage response to each biomesh was compared between the two time points, a significant reduction in macrophage counts was observed except for the Surgisis meshes, which induced a discrete yet stable inflammatory response over the entire study period (Fig. 6).

Discussion

Natural biomaterials, or bioprostheses, whose ultimate goal is to achieve good host tissue ingrowth while minimizing the tissue reaction by remodeling host tissue, are today a good alternative for use in contaminated abdominal surgery for which synthetic prostheses are contraindicated (Hiles et al., 2009).

The use of biomaterials in tissue-engineering pursues the regeneration of host tissue at the repair site (Bellows et al., 2007). Thus, once implanted, a biomaterial will promote the formation of new connective tissue, a process that involves the stimulation of growth factors and synthesis of extracellular matrix elements.

So far the literature lacks convincing data to support the occurrence of tissue remodeling. Moreover, the quality and strength of the tissue that replaces a biologic prosthesis have not yet been well defined. There is also much controversy as to how and when the complete

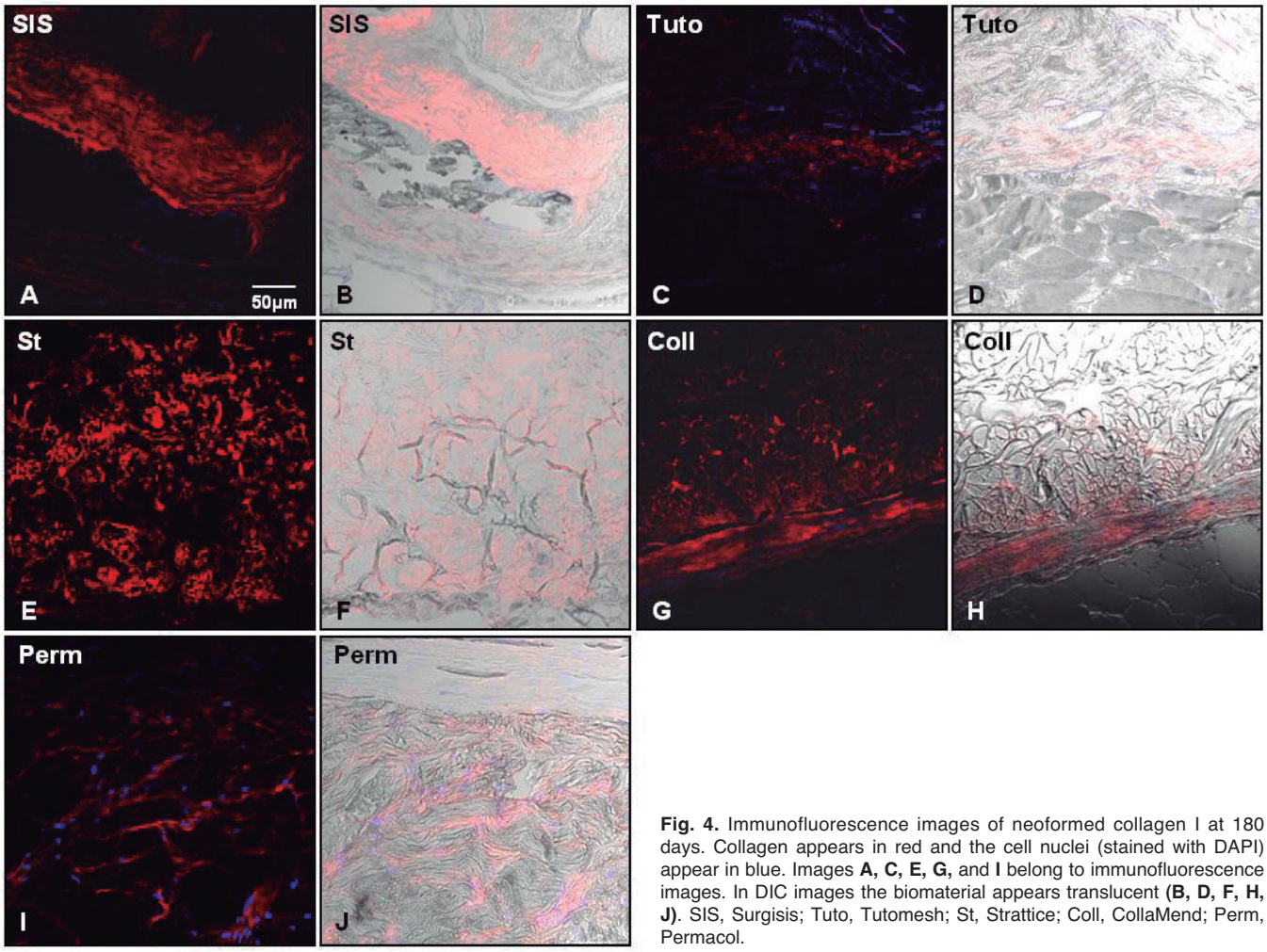

Fig. 4. Immunofluorescence images of neoformed collagen I at 180 days. Collagen appears in red and the cell nuclei (stained with DAPI) appear in blue. Images **A, C, E, G,** and **I** belong to immunofluorescence images. In DIC images the biomaterial appears translucent **(B, D, F, H, J)**. SIS, Surgisis; Tuto, Tutomesh; St, Strattice; Coll, CollaMend; Perm, Permacol.

replacement of the original prosthetic material occurs (Gaertner et al., 2007).

The technique used in this study, immunofluorescence confocal microscopy combined with differential interference contrast imaging (Pascual et al., 2012), enabled us to differentiate between the collagen that makes up the implanted mesh and the host collagen that forms part of the neoformed connective tissue that gradually invades and replaces the mesh.

Ideally, a biomesh rather than rapidly degrading should remain stable until it is gradually fully incorporated in the host tissue. To delay the absorption process, the crosslinks in the triple-helix comprising the collagen molecule need to be efficient, otherwise mechanical firmness will be compromised. Some authors have shown that the degradation of the mesh collagen occurs in parallel to host tissue regeneration (Huang et al., 1998). In a rat biomesh implant model, Liang et al. (2004) observed that crosslinking affected mesh degradation and the tissue regeneration pattern. Thus, the depth of cell infiltration into the acellular mesh tissue decreased with the extent of crosslinking, such that this factor determines the degradation rate of the acellular biomaterial and its tissue regeneration pattern. Also, if the implanted biomesh was non-crosslinked, infiltration by inflammatory cells was accompanied by mesh reabsorption and the scaffold was rapidly degraded such that there was insufficient time for tissue remodeling.

This was observed in the present study in that the thinner non-crosslinked biomeshes (Surgisis and Tutomesh) were rapidly absorbed and had completely disappeared between 90 and 180 days post-implant. In agreement with our observations at 3 months, Clarke et al. (1996) reported the complete absence of non-crosslinked Surgisis meshes 4 months after their use to repair abdominal wall defects in dogs. These findings have prompted some authors to recommend a cautious approach when Surgisis is used in high-tension zones such as the abdominal wall because of the weakness of the repair zone (Claerhout et al., 2008).

Our results support this weakness in that our

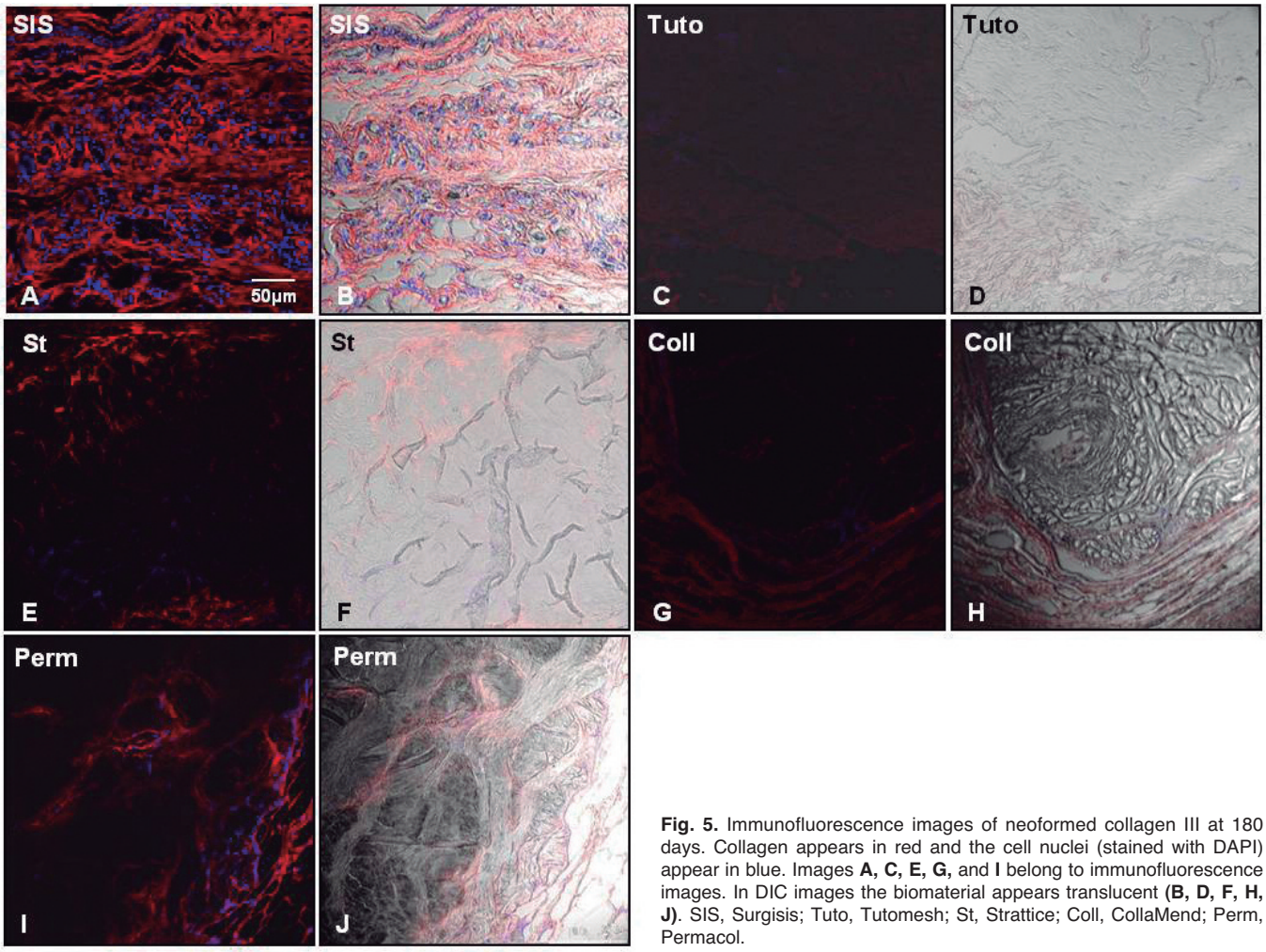

Fig. 5. Immunofluorescence images of neoformed collagen III at 180 days. Collagen appears in red and the cell nuclei (stained with DAPI) appear in blue. Images **A, C, E, G,** and **I** belong to immunofluorescence images. In DIC images the biomaterial appears translucent **(B, D, F, H, J)**. SIS, Surgisis; Tuto, Tutomesh; St, Strattice; Coll, CollaMend; Perm, Permacol.

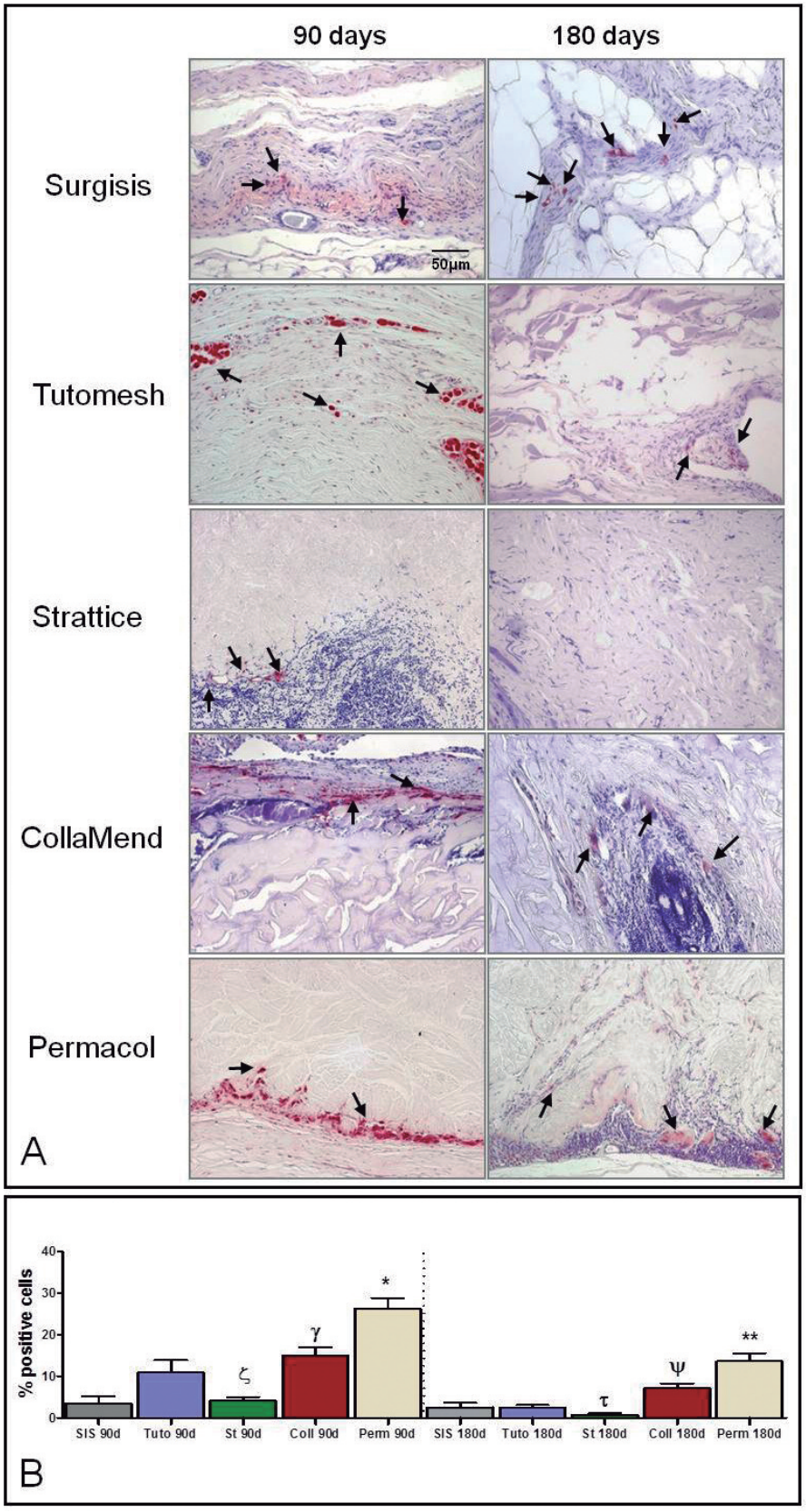

Fig. 6. A. Immunohistochemical labeling of rabbit macrophages (red color, arrows) using the RAM-11 monoclonal antibody. **B.** Percentage of positive cells in the different protheses after 90 and 180 days postimplantation. * p<0,05 vs the rest of groups of study at 90 days; γ p<0,05 vs SIS and Str at 90 days; ζ p<0,05 vs Tuto at 90 days; ** p<0,05 vs Str, Tuto and SIS 180 days; ψ p<0,05 vs Strattice and Tuto at 180 days; τ p<0,05 vs Tuto at 180 days; SIS, Surgisis; Tuto, Tutomesh; St, Strattice; Coll, CollaMend; Perm, Permacol.

immunofluorescence study revealed larger amounts of collagen III in the long term. This is the immature and less resistant form of collagen involved in remodeling at the expense of the mature form (type I). The rapid replacement of the native implant with poorly structured host connective tissue would explain the diminished mechanical resistance reported for Surgisis in the literature (Claerhout et al., 2008).

Contrary to our observations for Surgisis, the crosslinked CollaMend and Permacol biomeshes induced both collagen I and III deposition in the newly formed tissue. Thus, although tissue remodeling was slow, the mechanical and structural support provided led to the formation of a better quality connective tissue without compromising biomechanical strength.

Some authors (Mulier et al., 2011) argue that crosslinked materials may be more durable during tissue remodeling, as suggested by the gradual thinning and weakening of the non-crosslinked Strattice beyond 3 months of implant. However, we found that the long term behavior of this biomesh was similar to that of the similar thickness yet crosslinked meshes like CollaMend and Permacol, since over time the Strattice implants were significantly more stable and showed greater cell infiltration and deposits of neoformed collagen, indicating clear benefits in terms of tissue incorporation.

Consistent with our results, others have described the similar behavior of CollaMend and Strattice (Butler et al., 2010) and have observed no negative effects on the tensile strength of the repair zone when used in abdominal reconstruction (Campbell et al., 2011; Ngo et al., 2011). In clinical practice, Strattice has been used as an effective adjunct in abdominal wall reconstruction, acting as reinforcement during component separation in a wide variety of indications and showing low patient complication rates (Patel et al., 2012).

It has been proposed (Sandor et al., 2008) that the biological signals that promote remodeling are lost in biomeshes with extensive crosslinking. Deficient signaling may inhibit host fibroblast infiltration into the material and angiogenesis within the matrix. This could explain the lack of recellularization of some crosslinked meshes, in agreement with reported data (Petter-Puchner et al., 2008).

A recent study using a rat model (de Castro Brás et al., 2012) has shown the rapid rate of matrix remodeling of non-crosslinked matrices. These authors argue that crosslinked prostheses like Permacol are safer for ventral hernia repair, since wound healing was found to parallel host tissue incorporation and tissue strengthening. Other experimental abdominal ventral hernia repair models in large animals (Stanwix et al., 2011) have also revealed the ideal properties of acellular porcine dermal products (Permacol) for ventral hernia repair.

The inflammatory reaction induced by the different biomeshes has been scarcely addressed in the literature. We observed a significant drop in macrophage counts from days 90 to 180 postimplant in all our study groups with the exception of the animals implanted with Surgisis. This finding is inconsistent with the report of elevated macrophage numbers persisting for 6 months in a study conducted in primates using cross and non-crosslinked porcine derived biologic meshes (Surgisis, Permacol and CollaMend) (Sandor et al., 2008).

Among the different biomeshes examined here, the two crosslinked materials (Permacol and CollaMend) were found to elicit the greatest foreign body reaction at both study time points, differing significantly from the reaction induced by the non-crosslinked meshes. This observation contradicts the findings of a study in which a severe inflammatory and immune response to non-crosslinked materials was observed in a rodent model of abdominal wall repair (de Castro Brás et al., 2012).

In a recent study (Bryan et al., 2012), by measuring the production of reactive oxygen species by leukocytes activated by the implant of a biologic material, small intestinal submucosa (Surgisis) was found to be more proinflammatory than dermis, with the observation of significantly reduced ROS production by human leukocytes in contact with Strattice.

In the non-crosslinked Strattice, the main immune response activator associated with xenografts, galactose-alpha 1,3 antigen, has been enzymatically removed (Sandor et al., 2008; Connor et al., 2009). This could explain why when used to repair a ventral hernial defect in non-human primates, Strattice does not induce an intense immune response (Connor et al., 2009). In effect, in our study Strattice was found to elicit the least intense macrophage response.

Our study is not without its limitations. In our experience although the rabbit model has provided excellent results in terms of tissue repair and immune response, its behavior is difficult to be translatable to human clinical practice.

In conclusion, the findings of our study indicate that:
a) the relatively thin non-crosslinked collagen bioprostheses examined here (Tutomesh/Surgisis) were quickly and easily degraded. These implants disappeared in the long term and induced the formation of a poorly-structured neotissue that could compromise the mechanical properties of the repair site.
b) the crosslinked meshes emerged as good candidates for hernia repair. However, our results suggest the need for further improvement such as modifying the extent of crosslinking to enhance their host tissue incorporation.
c) the thicker, more compacted non-crosslinked Strattice implant showed the best balance between host tissue incorporation and prosthetic degradation. This resulted in optimal tissue behavior during the repair process along with a minimal foreign-body reaction in the long term. The good performance of thicker non-crosslinked bioprostheses such as Strattice requires confirmation in future studies.

Acknowledgements. This study was supported by the spanish Ministry of Science and Technology, through research proyects DPI 2011-27939-C01-01 and C02-02

References

Abraham G.A., Murray J., Billiar K. and Sullivan S.J. (2000). Evaluation of the porcine intestinal collagen layer as biomaterial. J. Biomed. Mat. Res. 51, 442-452.

Badylak S.F., Kropp B., McPherson T., Liang H. and Snyder P. (1998). Small intestine submucosa: A rapidly resorbed bioscaffold for augmentation cytoplasty in a dog model. Tissue Eng. 4, 379-387.

Bellón J.M., Buján J., Contreras L.A. and Carrera-San Martín A. (1996). Comparison of a new type of polytetrafluoroethylene patch (MycroMesh) and polypropylene prosthesis (Marlex) for repair of abdominal wall defects. J. Am. Coll. Surg. 183, 11-18.

Bellón J.M., Contreras L.A., Buján J., Palomares D. and Carrera-San Martín A. (1998). Tissue response to polypropylene meshes used in the repair of abdominal wall defects. Biomaterials 19, 669-675.

Bellows C.F., Albo D., Berger D.H. and Awad S.S. (2007). Abdominal wall repair using human acellular dermis. Am. J. Surg. 194, 192-198.

Bryan N., Ashwin H., Smart N., Bayon Y., Scarborough N. and Hunt J.A. (2012). The innate oxygen dependant immune pathway as a sensitive parameter to predict the performance of biological graft materials. Biomaterials 33, 6380-6392.

Butler C.E. (2006). The role of bioprosthetics in abdominal wall reconstruction. Clin. Plast. Surg. 33,199-211.

Butler C.E., Burns N.K., Campbell K.T., Mathur A.B., Jaffari M.V. and Rios C.N. (2010). Comparison of cross-linked and non-cross-linked porcine acellular dermal matrices for ventral hernia repair. J. Am. Coll. Surg. 211, 368-376.

Campbell K.T., Burns N.K., Rios C.N., Mathur A.B. and Butler C.E. (2011). Human versus non-cross-linked porcine acellular dermal matrix used for ventral hernia repair: comparison of in vivo fibrovascular remodeling and mechanical repair strength. Plast. Reconstr. Surg. 127, 2321-2332.

Catena F., Ansaloni L., Gazzotti F., Gagliardi S., Di Saverio S., D'Alessandro L. and Pinna A.D. (2007). Use of porcine dermal collagen graft (Permacol) for hernia repair in contaminated fields. Hernia 11, 57-60.

Claerhout F., Verbist G., Verbeken E., Konstantinovic M., De Ridder D. and Deprest J. (2008). Fate of collagen-based implants used in pelvic floor surgery: a 2-year follow-up study in a rabbit model. Am. J. Obstet. Gynecol. 198, 94-96.

Clarke K.M., Lantz G.C., Salisbury S.K., Badylak S.F., Hiles M.C. and Voytik S.L. (1996). Intestine submucosa and polypropylene mesh for abdominal wall repair in dogs. J. Surg. Res. 60, 107-114.

Connor J., McQuillan D., Sandor M., Wan H., Lombardi J., Bachrach N., Harper J. and Xu H. (2009). Retention of structural and biochemical integrity in a biological mesh supports tissue remodeling in a primate abdominal wall model. Regen. Med. 4, 185-195.

de Castro Brás L.E., Shurey S. and Sibbons P.D. (2012). Evaluation of crosslinked and non-crosslinked biologic prostheses for abdominal hernia repair. Hernia 16, 77-89.

Franklin M.E., Treviño J.M., Portillo G., Vela I., Glass J.L. and González J.J. (2008). The use of porcine small intestinal submucosa as a prosthetic material for laparoscopic hernia repair in infected and potentially contaminated fields: long-term follow-up. Surg. Endosc. 22, 1941-1946.

Gaertner W.B., Bonsack M.E. and Delaney J.P. (2007). Experimental evaluation of four biologic prostheses for ventral hernia repair. J. Gastrointest. Surg. 11, 1275-1285.

Hiles M., Record Ritchie R.D. and Altizer A.M. (2009). Are biologic grafts effective for hernia repair?: a systematic review of the literature. Surg. Innov. 16, 26-37.

Huang L.L., Sung H.W., Tsai C.C. and Huang D.M. (1998). Biocompatibility study of a biological tissue fixed with a naturally occurring crosslinking reagent. J. Biomed. Mater. Res. 15, 568-576.

Liang H.Ch, Chang Y., Hsu ChK., Lee M.H. and Sung H.W. (2004). Effects of crosslinking degree of an acellular biological tissue on its tissue regeneration pattern. Biomaterials 25, 3541-3552.

Menon N.G., Rodríguez E.D., Bymes C.K., Girotto J.A., Goldberg N.H. and Silverman R.P. (2003). Revascularization of human acellular dermis in full-thickness abdominal wall reconstruction in the rabbit model. Ann. Plast. Surg. 50, 523-527.

Mulier K.E., Nguyen A.H., Delaney J.P. and Marquez S. (2011). Comparison of Permacol™ and Strattice™ for the repair of abdominal wall defects. Hernia 15, 315-319.

Ngo M.D., Aberman H.M., Hawes M.L., Choi B. and Gertzman A.A. (2011). Evaluation of human acellular dermis versus porcine acellular dermis in an in vivo model for incisional hernia repair. Cell Tissue Bank 12, 135-145.

Oliver R.F., Hulme M.J., Mudie A. and Grant R.A (1975). Skin collagen allografts in the rat. Nature 258, 537-539.

Pascual G., Rodríguez M., Sotomayor S., Moraleda E. and Bellón J.M. (2012). Effects of collagen prosthesis cross-linking on long-term tissue regeneration following the repair of an abdominal wall defect. Wound Repair Regen 20, 402-413.

Patel K.M., Nahabedian M.Y., Gatti M. and Bhanot P. (2012). Indications and outcomes following complex abdominal reconstruction with component separation combined with porcine acellular dermal matrix reinforcement. Ann. Plast. Surg. 69, 394-398.

Petter-Puchner A.H., Fortelny R.H., Walder N., Mittermayr R., Ohlinger W. van Griensven M. and Redl H. (2008). Adverse effects associated with the use of porcine cross-linked collagen implants in an experimental model of incisional hernia repair. J. Surg. Res. 145, 105-110.

Sandor M., Xu H., Connor J., Lombardi J., Harper J.R., Silverman R.P. and McQuillan D.J. (2008). Host response to implanted porcine-derived biologic materials in a primate model of abdominal wall repair. Tissue Eng. Part A 14, 2021-2031.

Stanwix M.G., Nam A.J., Hui-Chou H.G., Ferrari J.P., Aberman H.M., Hawes M.L., Keledjian K.M., Jones L.S. and Rodriguez E.D. (2011). Abdominal ventral hernia repair with current biological prostheses: an experimental large animal model. Ann. Plast. Surg. 66, 403-409.

Accepted July 15, 2013