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NEW TECHNOLOGY

Dried human amniotic membrane as an antiadherent layer for intraperitoneal placing of polypropylene mesh in rats

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Abstract
Background Intraabdominal peritoneal onlay polypropylene (PP) mesh repair of incisional hernia has the potential risk of adhesions, bowel obstructions, and intestinal fistulae. Fresh or cryopreserved human amniotic membrane (HAM) has been tested as an antiadherent layer in animals, with excellent outcomes. However, it has disadvantages: it is difficult to handle, and it is expensive to store. Another processing method is available: drying in a laminar flow hood and gamma irradiation. Because this method impairs the membrane's cell viability, it may affect its antiadherent properties. However, such properties may also result from the collagen matrix and its basement membrane, which remain after drying. The aim of the present study was to asses dried irradiated HAM in adhesion prophylaxis in rats.
Methods Twenty-four female rats were randomized into two groups. In the first group (control group), PP meshes were placed in the intraabdominal space, and in the second group (treatment group), PP meshes coated with HAM were used. Animals were killed on day 30 after surgery. Adhesions and parietal prosthetic incorporation were assessed macroscopically and expressed as the average percentage of the covered area. The portion of the abdominal wall was then resected for histological testing.
Results The treatment group had a significantly higher percentage of adhesions and parietal incorporation compared with the control group ($p = 0.003$). Histological testing showed a higher inflammatory response in the treatment group, with an intense foreign body reaction.
Conclusions Dried irradiated HAM does not prevent adhesion formation in intraabdominal peritoneal onlay PP mesh repair in rats. Any use of this biomaterial in adhesion prophylaxis must be undertaken respecting graft cell viability as much as possible.

Keywords Antiadhesives · Hernia repair · Human amniotic membrane · Intraperitoneal mesh placement · Polypropylene

The repair of abdominal wall defects has greatly improved since the development of prosthetic materials that can reinforce the impaired tissues. Recurrence in incisional hernia surgery has decreased up to 46 % without using prosthetic material to 10 % when using it, thus becoming the procedure of choice [1–4].

Today, polypropylene (PP) is the most widely used prosthetic material because of its low cost, excellent tissue ingrowth, and high infection resistance; it is also inert, noncarcinogenic, and easy to handle [1–6]. However, it is far from being the ideal mesh because it can cause an excessive inflammatory reaction, and in direct contact with viscera (when implanted in the intraabdominal space), it can cause adhesions, bowel obstructions, intestinal fistulae, or some combination of these [5–9].

Another type of mesh, composite, has been developed in which an antiadhesive coating is added, allowing

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intraabdominal peritoneal onlay mesh repair and laparoscopic approach of the abdominal wall surgery. These products have been tested in several studies, with various outcomes. Extended polytetrafluoroethylene meshes are also available. They have very low rates of adhesion formation but poor tissue ingrowth and high rates of seroma and infection that may require explantation [1, 3, 6, 10–17].

Human amniotic membrane (HAM) is a collagen avascular matrix with a basement membrane and a monolayer of epithelial cells without major histocompatibility complex antigens. It has many therapeutic uses, such as ocular coverage procedures, dermal substitute for skin ulcers, and treatment of deep burns. The advantages of this biomaterial include immunologic tolerance and antibacterial properties; it is also useful in promoting epithelial regeneration and inhibition of scarring because it contains pluripotent mesenchymal and epithelial cells. In addition, amniotic membrane is cheap, easy to obtain, and raises no ethical problems [18–25]

In the last decade, HAM has been demonstrated to prevent intraabdominal adhesions in rats and can be used as a coating film in PP meshes [26–30]. In these studies, HAM was used either in a fresh or cryopreserved state, which makes it difficult to handle; it has expensive and complex storage conditions; and it is cumbersome to use in widespread procedures such as incisional hernia repair. Furthermore, even if donors are serologically tested for infectious disease and the membranes are treated with antibiotics, when fresh grafts are used, a potential minimal risk of disease transmission is present.

Another processing, sterilization, and storage method of HAM is available: drying in a laminar flow hood and gamma irradiation [23]. The final product is a dried membrane that is easy to handle, store, and transport, without the potential risks for infection. Its use would allow a massive application of this biomaterial in abdominal wall hernia surgery.

Unfortunately, the antiadherent properties of the HAM are likely to lie either in the amniotic epithelium or in the properties that derive from its stem cells—that is to say, in the presence of vital cells that will be lost in the drying and irradiation process. However, it is also possible that the HAM simply acts as a transitory barrier (because of the smooth surface on its amniotic side) until the reperitonealization process is complete, with no need for vital cells.

The aim of the present was to assess adhesion formation after abdominal wall hernia repair using PP coated with dried irradiated HAM compared to PP alone in rats. We also looked at the parietal integration of both products.

Materials and methods

Twenty-four female rats of the native "M" line (Medical Sciences School, National University of Rosario, Argentina) weighing 235–260 g were used. At arrival, the animals were 10 weeks old. They were kept according to the international principles of laboratory animal care. Water and food were provided ad libitum. Surgery was performed in the experimental operating rooms at the Medical Sciences School. The experimental protocol was reviewed and authorization was given by the Science and Methods Department of our institution.

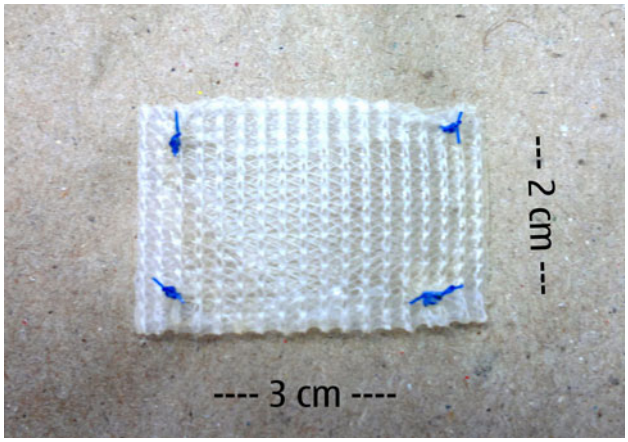

Fig. 1 Dried irradiated HAM

Dried irradiated HAM

The dried irradiated HAM was supplied by Biotar Enterprises (Rosario, Argentina, under ISO 9001). The membrane was collected from serologically negative tested donors under extreme aseptic conditions from planned, uneventful cesarean sections. Immediately after surgery, the placenta was cleared of blood clots with sterile saline solution, and the amnion membrane was removed from the chorion by blunt dissection under a laminar flow hood. Pieces of the membrane 4 × 3 cm in size were cut and overlayed in order to obtain a double membrane, then sutured with the epithelial side down to a 3 × 2 piece of PP (Prolene, Ethicon) folding the ends so that the edges of the mesh were covered. Finally, the membrane was dried in a laminar flow hood for 24 hours, then packed and irradiated with 25 kGy of gamma radiation (Ionics Institute, Campana, Argentina) (Fig. 1).

Study design

The rats were randomized into two groups of 12 animals each, both with an observation period of 30 days. In the control group, PP meshes were implanted in the intraabdominal space. In the treatment group, PP meshes coated with dried irradiated HAM were implanted in the intraabdominal space with the epithelial side directed to the intestine.

Surgical procedure

All animals were anesthetized by an intraperitoneal injection of ketamine (Cost, Fada Pharma; 1 mL/kg of body weight) and diazepam (Daiv, Fada Fharma). No shaving was done. Skin disinfection was performed. A midline incision of approximately 4 cm was made. Meshes were placed in the intraabdominal peritoneal space and fixated to the abdominal wall by six transparietal stitches of PP 4–0 (Prolene, Ethicon), one in each corner and the other two in the lateral midpoint. Omentectomy was not performed. The abdominal wall incision was closed with a running suture of PP 4–0. Skin closure was performed with a subcuticular running suture of the same material.

Animal death

All animals were killed on day 30 after surgery via an intraperitoneal pentothal (Bensulf, Fada Fharma) overdose injection. A midline skin incision was made, and subcutaneous fat tissue was dissected to the flanks, entering into the abdominal cavity through the left side and exposing the anterior abdominal wall and the viscera.

Adhesions were assessed macroscopically and expressed as the percentage of the mesh surface covered by adhesions. The organs involved in the process (i.e., greater omentum, liver, small bowel) was determined and also recorded as a percentage. Criteria such as adhesion strength and traction resistance were not applied because we consider them subjective and difficult to quantify in such small surfaces. The percentage of the mesh completely incorporated to the abdominal wall was also noted. Finally, the portion of the abdominal wall that included the implant was resected and placed in formaldehyde for future histological testing.

Histological testing

All the samples were stained with hematoxylin and eosin and examined microscopically at $\times 10$. The degree of inflammatory response around the PP fibers and the HAM tissue was assessed.

Statistical analysis

Data were expressed as average percentage and standard deviation. Statistical comparison was done by the Wilcoxon test. Statistical results were considered significant at $p < 0.05$.

Results

One rat in each group died in the immediate postoperative period. Because no justifying cause was noted, deaths were attributed to anesthetic complications.

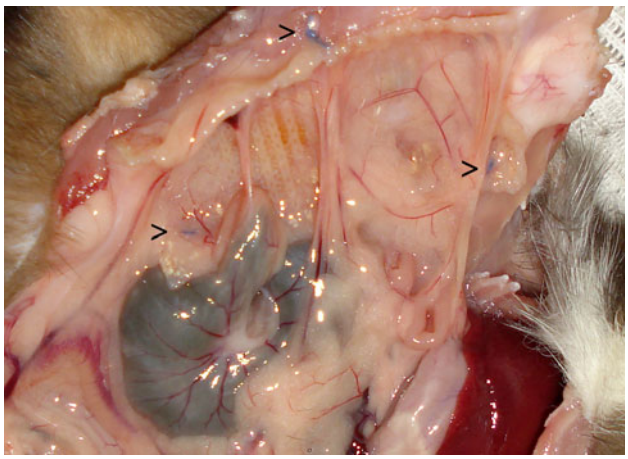

Fig. 2 Control group. Almost all the mesh surface is covered with adhesions. The edges of the mesh and the fixating stitches were the most critical areas (>). The mesh is not completely incorporated into the abdominal wall

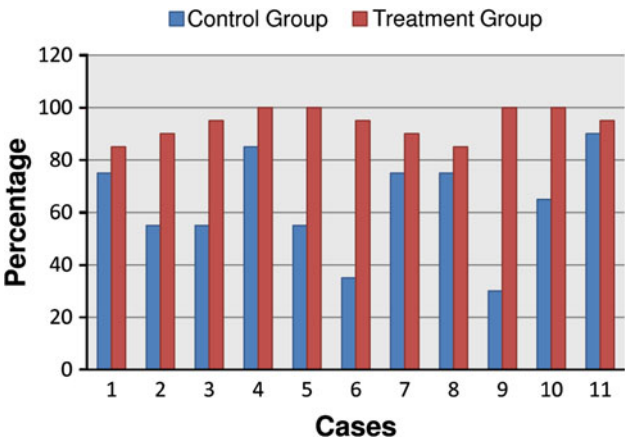

Fig. 3 Adhesion formation, expressed as a percentage, in the control and treatment groups. The average for the control group was 63.18 with a standard deviation of 19.27 and for the treatment group was 94.09 and 5.83, respectively. The differences were statistically significant ($p = 0.003$)

No infections, seromas, or dehiscence were noted.

Control group

The average percentage of the mesh surface covered with adhesions was 63.18 % (standard deviation [SD] 19.27 %), with 4, 6.25, and 53.58 % contributed by the liver, small bowel, and greater omentum, respectively. Most adhesions were centered in the edges of the implant, mainly over the fixing stitches.

The average percentage of the mesh completely incorporated to the abdominal wall was 35 % (SD 20.44 %), highlighting that the incorporated zones were centered in the stitches and spread radially (Figs. 2, 3).

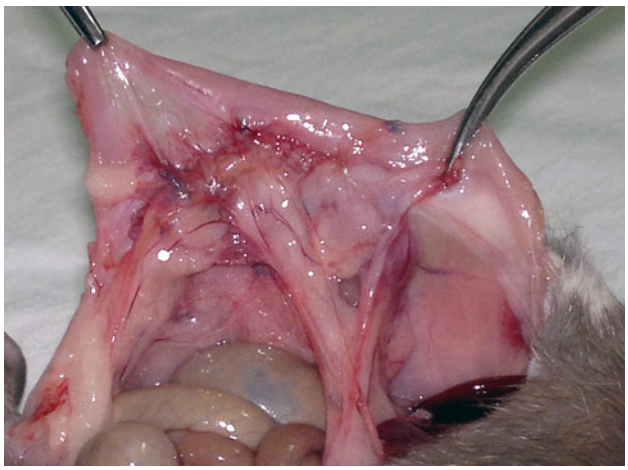

Fig. 4 Treatment group. All the mesh surface is covered with adhesions, with a remarkable inflammatory process intensely vascularized. The mesh is shrunken and completely incorporated into the abdominal wall

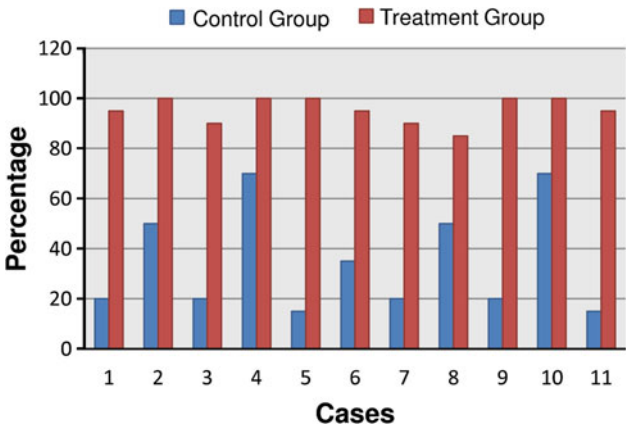

Fig. 5 Mesh incorporation, expressed as a percentage, in the control group and treatment groups. The average for the control group was 35 (SD 20.44) and for the treatment group was 95.45 (5.22). The differences were statistically significant ($p = 0.003$)

Treatment group

The average percentage of the mesh surface covered with adhesions was 94.09 % (SD 5.83 %), with 90 and 4 % contributed by the greater omentum and small bowel, respectively. With this amount of adhesions, we could not recognize differences in their distribution as seen in the control group.

The average percentage of the prosthesis completely incorporated to the abdominal wall was 95.45 % (SD 5.22 %). Meshes showed remarkable shrinkage (Figs. 4, 5).

Histological testing

In the control group, a mild chronic inflammatory response (mainly lymphocytes) around the PP fibers was noted, with occasional formation of lymphoid follicles, abundant edema, capillary ectasia, slight fibrin deposit, and scant collagen tissue formation (Fig. 6).

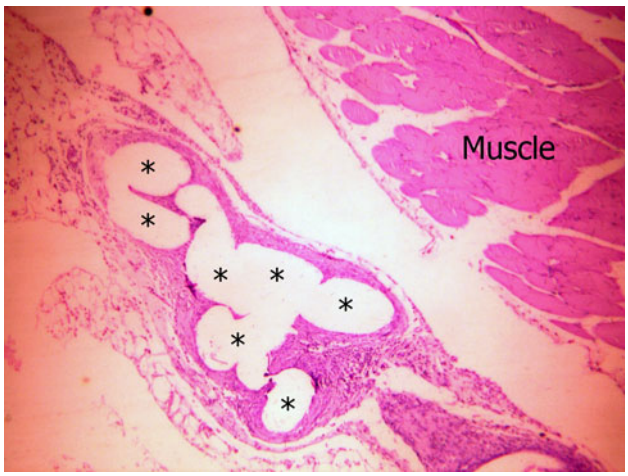

Fig. 6 Histological testing revealing a mild chronic inflammatory response (mainly lymphocytes) around the PP fibers (*), with no close contact of the implant with the overlying muscle (hematoxylin and eosin, original magnification ×10)

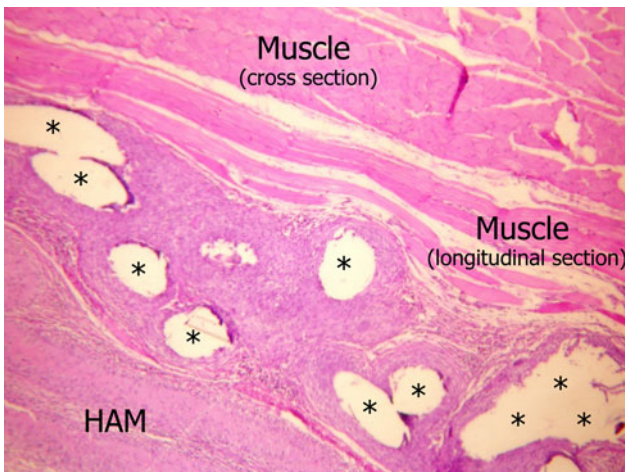

Fig. 7 Intense inflammatory foreign body-like process surrounding the PP fibers (*) and the double-layered HAM. Note the thickness of such response compared to the control group. The overlying muscle is in close contact with the mesh (hematoxylin and eosin, original magnification ×10)

In the treatment group, an intense chronic inflammatory response around the PP fibers and the double-layered HAM was noted, with foreign body giant cell reaction, abundant young fibrous tissue with numerous capillary vessels, and intense fibrous adherence to adjacent structures. Muscular tissue was closely attached over the parietal side of the mesh (Fig. 7).

Statistical analysis proved that the difference shown for both studied variables (adhesions and ingrowth) was statistically significant ($p = 0.003$), according to the Wilcoxon test.

Discussion

Adhesion prophylaxis has previously been noted with the use of fresh or cryopreserved HAM in rats. Rennekampff et al. [29] published the first trials and demonstrated that once the HAM was placed with its epithelial side directed to the intestine, it showed nearly no adhesions (0–3 %). However, when using a saline-stored graft, the adhesions were higher (33 %), although not as high as when the membrane was used with the chorionic side directed to the intestine. Szabo et al. [30] had a similar outcome with PP coated with fresh HAM or Seprafilm, but they did not specify the orientation of the membrane. In another experimental trial, Kesting et al. [26, 27] tested cryopreserved HAM in adhesion prophylaxis in comparison with PP, concluding in favor of HAM. Recently, Petter-Puchner et al. [28] successfully used cryopreserved HAM and pointed that the presence of vital pluripotent cells guarantees its antiadherent properties and counteracts the potential side effects of using human tissue in rats—that is, a xenograft. It is interesting how they confirm such viability by immunochemistry assays (mitochondrial activity markers) [31]. However, the cryopreservation method used by the authors has a HAM residual viability of less than 20 % at the thawing time [31]. This encouraged us to try dried HAM (which has no vital cells) on the hypothesis that the antiadherent properties lie in the structure of the collagen matrix and its basement membrane, which provides an excellent smooth surface (which remains present after the drying), rather than in the viability of epithelial and/or pluripotent cells.

The lack of another control group with fresh or cryopreserved HAM is a limitation of the present study because it decreases its ability to compare processing methods. However, the excellent outcomes achieved in the previous trials led us use only one control group (PP alone) because it made the design and interpretation of results less complex.

Unfortunately, in light of our negative results, and unlike what we expected, dried and irradiated HAM does not prevent adhesion formation in intraabdominal peritoneal onlay PP mesh repair in rats. Apparently, the drying and gamma radiation process makes HAM act as a foreign body and triggers an intense inflammatory response (clearly shown in the histological testing of the samples) that leads to extensive adhesion formation. This may explain the behavior of the parietal incorporation, which was grossly enhanced in the treatment group. In other words, the bigger the inflammatory response, the greater the parietal incorporation, shrinkage of mesh, and adhesion level. It seems that some degree of residual viability (even minimal) is necessary for keeping the adhesion properties.

The negative outcome of the present study reinforces the idea that any use of HAM as adhesion prophylaxis must be undertaken applying conservation and handling methods that respect graft cell viability as much as possible.

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Disclosures Franco Pomilio Di Loreto, Andrés Mangione, Ezequiel Palmisano, Juan Ignacio Cerda, María José Dominguez, Guillermo Ponce, Marianela Bernaus, Silvina Gaffuri, Guillermo Torresi and Sergio Bianco have no conflicts of interest or financialties to disclose.

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