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Original research

Rapid reperitonealization and wound healing in a preclinical model of abdominal trauma repair with a composite mesh

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HIGHLIGHTS

- Peritoneal healing following implanted mesh in a surgical model of adhesion formation.
- Neoperitoneum formation and mesothelial cell presence crucial to adhesion prevention.
- Mesothelial cells were observed along composite mesh surface 3 days post-implantation.
- Complete coverage by neoperitoneum was observed at 7 days post-implantation.
- In part, the neoperitoneum may be formed by free-floating mesothelial cells.

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ABSTRACT

Purpose: Peritoneal tissue healing is characterized by the simultaneous repopulation of mesothelial cells and the formation of neoperitoneum. Despite the common use of mesh products for abdominal wall repair, there are few investigations of how these materials may impact the peritoneal healing process. Here, we utilized an animal model of abdominal trauma to specifically investigate the peritoneal healing process in conjunction with a composite (poliglecaprone 25-coated polypropylene) mesh.
Methods: Abdominal wall injury was simulated in New Zealand White rabbits and peritoneal tissue was covered with composite mesh and fixed with peripheral sutures. Animals were sacrificed at regular intervals (up to 28 days) for macroscopic and microscopic evaluation.
Results: Mesothelial cells were consistently identified on the surface of the central areas of the implanted mesh as early as 3–5 days after implantation. From day 7 onward, the entire mesh surface was covered by neoperitoneum which matured over the remaining study intervals. Fibroblast ingrowth of the mesh was apparent by day 5 and increased over time, concurrent with fragmentation of the film on the composite mesh.
Conclusions: These results suggest that composite mesh products used for abdominal wall repair do not significantly delay mesothelial repopulation. Study results also support the hypothesis that mesothelial cells involved in healing are derived, at least in part in this model, from free-floating precursor cells located within the peritoneal cavity.
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1. Introduction

The peritoneum is a specialized membrane consisting of a layer of loose connective tissue and a continuous layer of mesothelial cells [1]. The peritoneum is rich in blood vessels, lymphatics and mesenchymal cells and is connected to the underlying tissue by subserous tissue which contains elastin and fat cells (primarily in the greater omentum). The peritoneum provides physical separation of the abdominal contents from the body wall and minimizes friction of the abdominal viscera.

The healing process of the peritoneum differs significantly from that of the skin and other epithelial surfaces. Unlike the skin, which

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heals from the edges of the wound and involves granulation and contraction processes, the entire surface of traumatized peritoneum is simultaneously repopulated with mesothelial cells. It is widely accepted that the time required for regeneration of the mesothelial layer is approximately five to six days [2,3]. However, the source of cells involved in re-epithelialization remains controversial. Some investigators have suggested that undifferentiated precursor cells in the subserosal connective tissue populate the site of injury [2,4,5]. Other investigators have suggested that free-floating mesothelial cells implant on and contribute to the repopulation of an injured peritoneal surface [6–9].

Although a large variety of synthetic mesh products with absorbable barrier components are now available, data is limited with these devices regarding early peritoneal healing. In the present study, we investigated the process of peritoneal healing over time following tissue injury in a preclinical model using a composite polypropylene mesh with a transparent coating (poliglecaprone 25) designed to minimize the formation of adhesions. This was a descriptive evaluation with no treatment comparison; therefore, the absolute minimum number of animals was utilized to support the study.

2. Materials and methods

2.1. Animals and care

All animal protocols were approved by the appropriate institutional animal care and use committees and followed the guidelines established by the Care and Use of Laboratory Animals (NIH publication 85-23), and met or exceeded the Johnson & Johnson Animal Care and Use Guidelines.

2.2. Evaluation of early tissue integration

Implantation, necropsy, and sample collection were conducted at an independent contracting facility (North American Science Associates, Incorporated, Northwood, OH) strictly following the approved protocol and the standard operating procedures. The necropsies and tissue collection were conducted by the surgeon who implanted the mesh. One of the authors, who served as the study pathologist (TM) was present at the necropsies, but did not contribute to the evaluations or the resulting macroscopic report. All components of the signed report were reviewed and discussed by all of the authors with additional review of the macroscopic and microscopic findings.

Nine healthy female New Zealand White (NZW) rabbits were acclimated to Elizabethan (E) collars prior to study onset. In each animal, a ventral midline incision was made from just caudal of the xiphoid to just cranial of the pubis. An abdominal wall peritoneal defect approximately 2 cm × 4.5 cm was created along one side of the animal as far caudal and lateral as possible using sharp dissection. The peritoneal defect was abraded to induce consistent punctate bleeding between defect sites and animals. The defect site was completely covered by a 3 cm × 5 cm piece of mesh (ETHICON PHYSIOMESH™, ETHICON, Inc., Somerville, NJ). Mesh was attached to the abdominal wall using size 4-0 Prolene Polypropylene Suture (ETHICON, Inc., Somerville, NJ) in a simple continuous pattern around the periphery. Once the implant procedure was completed for one side of the animal, the same procedure was conducted on the contralateral side. The laparotomies were closed using standard surgical technique. Rabbits were fitted with E-collars and monitored twice daily for the first five days post-surgery and then daily until assigned termination intervals. Two animals were sacrificed at 3, 5, and 7 days post-implantation and one animal was sacrificed at 14, 21, and 28 days post-implantation. Adhesion formation and macroscopic alterations, if present, were recorded at necropsy. The implant sites with adjacent tissues were collected and immersed in 10% neutral buffered formalin (NBF). Implantation, necropsy, and sample collection were conducted at an independent contracting facility, North American Science Associates, Incorporated, Northwood, OH. Once adequately fixed, samples were submitted to Veterinary Pathology Services, Incorporated, Mason, OH, for trimming, soft resin-based tissue processing, and staining with hematoxylin and eosin (HE). Soft-resin histology processing was utilized to reduce artifacts associated with paraffin fixation techniques. Following the protocol and standard operating procedures, a single section through the short axis was carefully trimmed from each tissue sample. The short axis was used to minimize the potential disruption of the implant site during the trimming process. This was particularly critical for the very early study intervals where the mesh was just beginning to become integrated with the adjacent tissues. Each trimmed section underwent standard soft-resin histologic processing. Soft-resin processing was chosen to limit the tissue processing artifacts that were observed in paraffin-based tissue sections from previous studies and because such processing does not suffer the loss of resolution that is observed with hard-resin tissue sections.

Stained tissue sections were submitted to the study pathologist who prepared an independent report directly to the contract laboratory. The entire section from each implant site was examined (four sections available for 3-, 5-, and 7-day study intervals and two tissue sections for the 14-, 21-, and 28-day study intervals); only the findings from the central area of the tissue section that were devoid of processing artifacts were reported and used for images. This method of evaluation limited any potential "edge effects" associated with the healing processes observed and avoided areas that were too disrupted by the collection and processing techniques to be of value. The microscopic evaluation focused upon the assessment of the tissue response, ingrowth, and integration into the mesh. The amount of tissue response present in the tissue sections was described using standard pathology terminology defined as follows: minimal-tissue response just discernible, mild-tissue response becoming a prominent feature of the tissue section, moderate-the tissue reaction was a prominent component present and biologically relevant, and severe-tissue response was a dominant component present and considered a significant adverse finding. Tissue ingrowth (defined as the amount of immature or mature fibrous tissue that penetrated into and through the site of mesh implantation) was described as none, poor (inconsistent or limited penetration of the article by individual cells or fine strands of fibroplasia or fibrous tissue), fair (multifocal-to-diffuse penetration of the article by individual cells or fine bands of fibroplasia or fibrous tissue), good (consistent deep penetration of the article by bands of fibroplasia or fibrous tissue) or excellent (article completely penetrated bands of fibroplasia or fibrous tissue). The tissue integration associated with the mesh was subjectively described as none, minimal (article–tissue interface consisting primarily of inflammatory cells or fluid accumulation), fair (multifocal areas where the article–tissue interface consisting mostly of fibroplasia or fibrous tissue admixed with low levels of inflammatory cells), good (article–tissue interface consisting mostly of fibroplasia or fibrous tissue) or excellent (article completely integrated with tissue by fibroplasia or fibrous tissue).

2.3. Evaluation of adhesion formation

Fifteen NZW rabbits had similar surgical procedures as described above except the defect sites were just less than 2.0 cm × 4.5 cm to facilitate complete coverage by 2.0 cm × 4.5 cm pieces of mesh, and the adjacent cecum was abraded using dry

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