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Efficacy of Polypropylene Mesh Coated with Bioresorbable Membrane for Abdominal Wall Defects in Mice

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Incisional hernias due to trauma, infection, or tumor are a common abdominal wall defect. Repair of these defects when autogenous tissue is insufficient or inadequate often results in abdominal tissue adhesion. These adhesions often lead to complications such as intestinal obstruction and enterocutaneous fistula. Previous reports have shown that application of prosthetic materials, such as polypropylene mesh and anionic polysaccharides, has been effective in reducing the amount of tissue adhesion. However, some tissue adhesion still occurs with application of these materials when previously described methodologies are used. We evaluated the efficacy of a novel surgical approach that combines the application of Sepramesh, a coated polypropylene mesh, and Seprafilm, composed of anionic polysaccharides (both products from Genzyme, Cambridge, MA), in the repair of abdominal wall hernias. We report that combined application of these 2 materials in a "sandwich technique," by placing the peritoneum between the Seprafilm and Sepramesh, further reduces (and in some instances prevents) tissue adhesion after abdominal wall defects in mice. Moreover, our combined treatment markedly decreased tissue inflammation after hernia repair.

Incisional hernia is the most common wall defect induced by trauma, infection, or tumor of the abdominal wall in humans and animals.[1,2,8] Abdominal wall repair is more secure with the implementation of prosthetic materials than by suture alone.[5,18] For many years polypropylene mesh has been used for hernia repair because of its ease of handling, low cost, and superior tensile strength at the mesh–tissue interface, compared with those of other materials.[9] However, when autogenous tissue is insufficient or inadequate, repair of abdominal wall defects with polypropylene mesh often leads to visceral adhesion. These adhesions can cause serious complications, such as intestinal obstruction and enterocutaneous fistula.[4,17] Repair of the defect and prevention of subsequent adhesions are a clinical concern when reconstruction is accomplished by patching the defect with prosthetic materials.

A bioresorbable membrane, Seprafilm (Genzyme, Cambridge, MA), which is composed of anionic polysaccharides of sodium hyaluronate and carboxymethylcellulose, has been reported to reduce adhesion formation after midline closures in humans and animals.[4,9,13,15] Seprafilm may produce this effect through the biochemical action of hyaluronic acid.[17] More recently the coated polypropylene mesh known as Sepramesh (Genzyme, Cambridge, MA), which has a protective layer of Seprafilm on its visceral side, also has been shown to reduce the level of adhesion when the mesh is laid over the viscera and fixed to the peritoneum. The mesh thereby provides a physical barrier between potentially adhesiogenic tissues and facilitates proper regeneration of mesothelial tissues.[3,8,13]

An earlier study in rats found that application of both Sepramesh and Seprafilm was effective in reducing the adhesion of polypropylene mesh to underlying viscera. However that study did not evaluate the influences of these materials on inflammation formation resultant of trauma.[12]

In our present study in mice, we evaluated the efficacy of Sepramesh, inserted in a "sandwich technique" between injured peritoneum and abdominal muscles and with Seprafilm on the visceral side, in reducing adhesion formation and tissue inflammation as compared with those of either prosthetic material implemented separately. We pursued 2 aims: 1) to verify repair of abdominal deficiencies by Sepramesh and Seprafilm, in combination and individually, and 2) to quantify the visceral adhesion and tissue inflammation after abdominal repair by combined application of Seprafilm and Sepramesh. We believe that application of both prosthetic materials in a sandwich technique (Seprafilm–peritoneum–Sepramesh) decreases adhesion and inflammation compared with that of Sepramesh and Seprafilm alone and that previously reported for polypropylene mesh.

Materials and Methods

Animals. We allocated 27 6-month-old healthy male BALB/c (Harlan Sprague Dawley, Indianapolis, IN) albino mice into 3 treatment groups. These animals were maintained in conventional static polycarbonate cages under a 12:12-h light:dark lighting cycle in a facility accredited by the Association for the Assessment and Accreditation of Laboratory Animal Care, International, and were fed standard rodent chow (Purina chow 5001 diet, Ralston Purina, St. Louis, MO) with tap water ad libitum. The study was approved by our institutional animal care and use committee.

Health monitoring. All the animals were monitored for ecto- and endoparasites and bacterial and viral infections such as cilia-associated respiratory bacillus, *Clostridium piliforme*, corona virus, *Mycoplasma pulmonis*, paravirus, and Sendai virus both in-house and by the University of Missouri Research Animal Diagnostic Laboratory (RADIL; Columbia, MO). The reports were negative for infection.

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Table 1. Tenacity and extent of adhesion formation after abdominal wall repair with Sepramesh and Seprafilm

Treatment	Tenacity[a]		Extent[b]	
	Individual scores	Group median (range)	Individual scores	Group median (range)
Sepramesh only (group A)	0, 0, 0, 1, 1, 1, 1, 1, 1	1 (0–1)	0, 0, 0, 0, 1, 1, 1, 2, 2	1 (0–2)
Sepramesh + Seprafilm (group B)	0, 0, 0, 0, 0, 0, 0, 1, 1	0 (0–1)	0, 0, 0, 0, 0, 0, 0, 1, 1	0 (0–1)
Seprafilm only (group C)	1, 1, 2, 2, 2, 2, 2, 2, 3	2 (1–3)	1, 1, 2, 2, 2, 2, 2, 3, 4	2 (1–4)

For both parameters, values for group C were significantly ($P < 0.05$) higher than those for groups A and B.
[a]Scoring system: 0, no adhesion; 1, adhesion readily fell apart; 2, adhesion lysed with traction; 4, required sharp dissection before adhesion gave way.
[b]Scoring system (percentage of abdominal wall involved): 0, none; 1, ≤ 25%; 2, 26% to 50%; 3, 51% to 75%; 4, ≥ 76%.

Table 2. Inflammation after abdominal wall repair with Sepramesh and Seprafilm

Treatment	Inflammation[a]	
	Individual	Group median (range)
Sepramesh only (group A)	0, 0, 0, 0, 1, 1, 1, 1, 1	1 (0-1)
Sepramesh + Seprafilm (group B)	0, 0, 0, 0, 0, 0, 0, 1, 1	0 (0-1)
Seprafilm only (group C)	0, 1, 1, 1, 2, 2, 2, 3, 3	2 (0-3)

Values for group C were significantly ($P < 0.05$) higher for group C than groups A and B.
[a]Scoring system: 0, no inflammation; 1, mild inflammatory reaction with giant cells, scattered lymphocytes, and plasma cells; 2, moderate reaction with giant cells and increased mixed lymphocytes, with plasma cells, eosinophils, and neutrophils; 3, severe inflammatory reaction with microabscesses.

Preoperative treatment. Food was withheld from mice for 8 h prior to surgery. Anesthesia was induced with 5% isoflurane in a gas anesthesia chamber and maintained with 2.5% isoflurane by nosecone mask. The abdominal skin was shaved, cleansed with disinfectant soap, prepared with 70% isopropyl alcohol and 1% povidone–iodine solution, and draped in a sterile fashion. Each mouse was positioned in dorsal recumbency on an isothermal pad (Deltaphase, Braintree Scientific, MA) for the duration of surgery.

Surgical treatment. A 2-cm midline laparatomy was performed, skin flaps were raised, and a 1- × 1.5-cm rectangular, full-thickness defect consisting of fascia muscles and peritoneum was created in the abdominal wall, according to an established model for herniation.[1,9,14] To mimic the effects of trauma and to elicit the subsequent inflammatory response induced by trauma, a portion of small bowel serosa underlying the abdominal defect was scratched gently using sterile gauze.

Treatment groups. The mice were divided into 3 groups of 9 animals each (groups A, B, and C). In group A, the abdominal wall defect was repaired only with Sepramesh (Genzyme, Cambridge, Mass). In group B, the defect was repaired with combined application of Sepramesh and Seprafilm (Genzyme, Cambridge, Mass) and in group C, Seprafilm alone was used to repair the induced defect. A negative control group was unnecessary because some tissue adhesion was expected; groups A and C served as positive controls for the study.

For group A animals, a 1.5- × 2-cm piece of Sepramesh was cut, soaked in 0.9% saline solution, placed intraperitoneally, and fixed to the cut margins of peritoneum by using 4-0 nylon with a taper-cut needle in a simple interrupted pattern, with sutures positioned 0.25 cm apart. For group B, the abdominal wall defect was repaired by laying Seprafilm intraperitoneally over the underlying viscera and then suturing Sepramesh at the 4 corners of the mesh with 4-0 nylon and a taper-cut needle between the muscles and peritoneum by using a sandwich technique (viscera–Seprafilm–peritoneum–Sepramesh–muscle–skin).[10,12] Mice in group C received Seprafilm deep to the abdominal wall defect, and then the skin was closed in the same manner as for the other groups by using 4-0 nylon in an interlocking pattern.

Postoperative treatment and observation. All animals were given 5 ml warm lactated Ringer solution subcutaneously before complete recovery from anesthesia. After recovery, the mice were allowed water ad libitum and were returned to full feed gradually over the next 24 h. Each mouse received, in a total volume of 0.05 ml, cephazoline (2.5 mg/kg) and buprenorphine (0.03 mg/kg) intramuscularly once daily for 3 d. Each mouse also was monitored daily for signs of pain, incisional swelling, or drainage. Mice in all 3 groups were euthanized 28 d after mesh repair.

Scoring of adhesion and inflammation. The abdominal incision, peritoneal cavity, and all abdominal organs were evaluated for adhesion and any other abnormality according to established protocols.[1,8] Adhesion scoring was conducted by a pathologist who was blinded regarding treatment group. The presence of adhesions between bowel and Seprafilm or Sepramesh was assessed by sectioning the defect surface into 3 fields. Adhesion was characterized according to its tenacity (score, 0 to 3) and extent (score, 0 to 4; Table 1) of the adhesion. Adhesion tenacity grading reflected the amount of force required to sever the adhesion, whereby 0 indicates no abdominal tissue adhesion, and 3 denotes the greatest adhesion, which required sharp dissection to sever it. Adhesion extent was evaluated visually after the visceral and abdominal wall areas covered by the prosthetic materials were divided into quadrants. An extent score of 0 denotes no adhesion was present in any of the quadrants, and a score of 4 indicates that all 4 quadrants of the visceral and abdominal areas had some adhesion.

The same pathologist who evaluated adhesion tenacity and extent also assessed tissue inflammation after abdominal wall repair. The extent of inflammation was assessed using a semiquantitative system (score, 0 to 3; Table 2), whereby 0 denotes no inflammation present, and 3 indicates severe inflammatory reaction with microabscesses.

Histologic evaluation. Two samples (mesh and tissue) were removed from each animal, fixed in 10% neutral buffered formalin, and sent to a commercial veterinary pathology laboratory (Antech Diagnostics, Irvine, CA) as well as stained with hematoxylin and eosin and examined under light microscopy in-house.

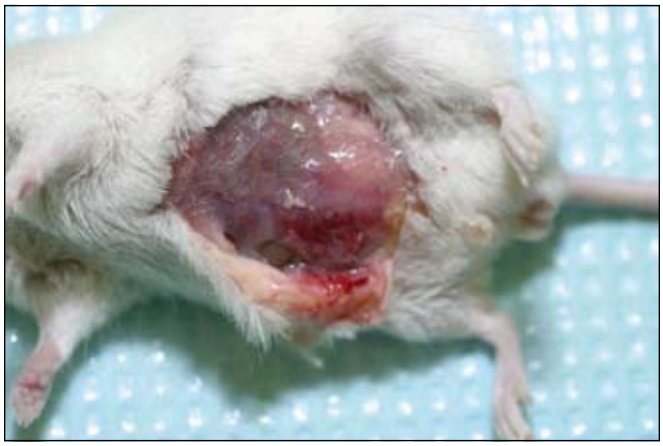

Figure 1. Abdominal wall repair in mice treated with Seprafilm only (group C). Extensive and tenacious abdominal adhesions are apparent.

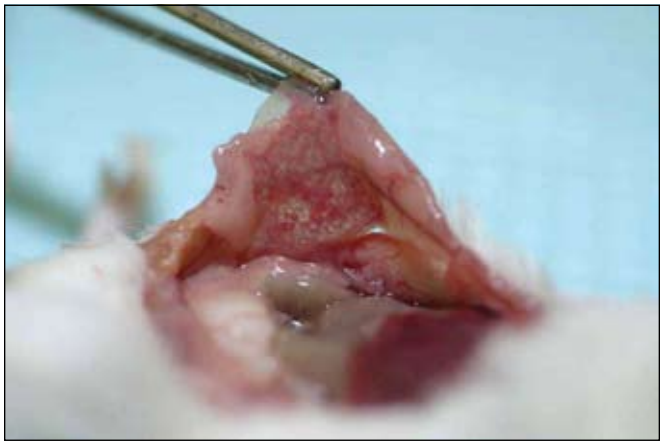

Figure 2. Abdominal wall repair in mice treated with both Sepramesh and Seprafilm (group B). The severity of adhesion is considerably less that for either group A or C.

Statistical analysis. Individual scores for adhesion and inflammation were compared between groups, with 1 group compared with the other 2. Significant differences ($P < 0.05$) were determined by one-way analysis of variance and the Kruskal–Wallis test.[8]

The Kruskal–Wallis test is a simple, non-parametric test to compare the medians of 3 or more samples drawn from identical populations. A P value of 0.05 was the cut-off value for rejection of the null hypothesis, indicating that there was a statistically significant difference between the medians.

Results

All the mice survived throughout the 28-d experiment. Individual and group scores for adhesion tenacity and extent are summarized in Table 1. All animals in group C (Seprafilm only) developed a high degree of adhesion between the abraded small bowel and peritoneum (Figure 1). Most of the animals in group A (Sepramesh only) developed little, if any, adhesion. However, the group with the most mice lacking adhesion was group B (Sepramesh and Seprafilm), in which epithelization of the inner surface of the mesh was completed without evidence of adhesion formation (Figures 2 and 3).

As expected, scores of adhesion extent and tenacity were significantly ($P < 0.05$) lower for group B mice compared with the other groups. Moreover, inflammation was least in group B, followed by group A and with group C having the highest level of inflammation. These differences in inflammation score between the surgical treatments were found to be statistically significant ($P < 0.05$; Table 2).

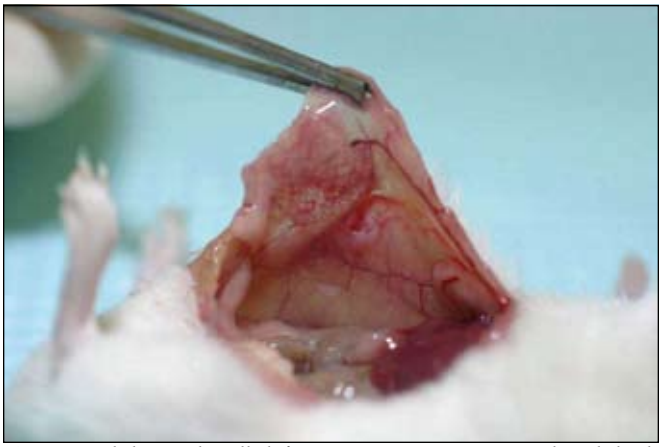

Figure 3. Abdominal wall defect recovery in mice treated with both Sepramesh and Seprafilm (group B). Neoperitoneal ingrowth was complete by day 28 postsurgery.

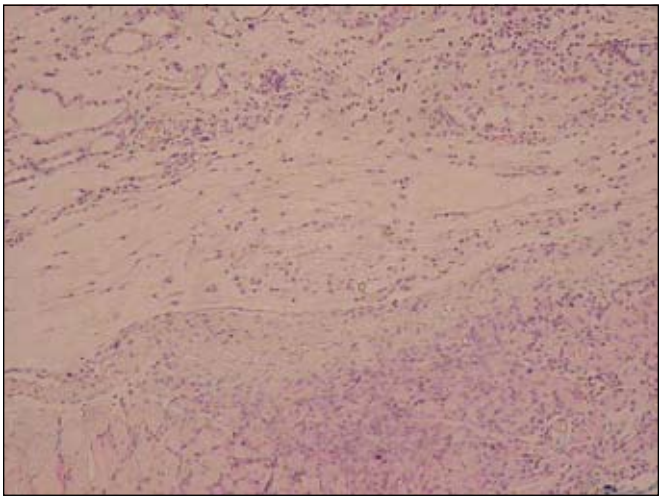

Figure 4. Histologic section of abdominal wall repair in mice treated with both Sepramesh and Seprafilm (group B). Dense fibrous connective tissue and epithelial macrophages are readily apparent by day 28 postsurgery, with no evidence of neoplasia or infection.

Necropsy of the viscera revealed no abnormalities. There was no evidence of neoplasia or infection in any of the 3 treatment groups. The microbiologic surveys showed no evidence of bacterial infections. On macroscopic evaluation, the Sepramesh in groups A and B was firmly attached to the muscles and peritoneum. Histologic analysis of the stained specimens revealed good fibroblast and collagen accumulation in the wound site, which resulted in well-formed scar tissue. This finding implies proper wound healing with resultant acceptance of Sepramesh repair of the ventral hernia in these groups (Figure 4).

Discussion

Peritonitis and adhesions are reported as the most common causes of death that occur after resection and anastomosis of the small intestine.[1,6,13] Adhesion is the consequence of peritoneal response to injury and inflammation.[2] Vasoactive substances released after peritoneal trauma increase vascular permeability and exudation of fibrinogen-rich plasma. Injury to tissue stimulates release of tissue thromboplastin and activation of coagulation cascade. As a result, large amounts of activated thrombin convert fibrinogen to fibrin, which in turn, is deposited

on peritoneal surfaces.[1-3,6]

For many years, several devices have been used to reduce postoperative adhesions.[7,10,11,13] Although most of these devices have noteworthy limitations, promising results have been reported for physical barriers. Seprafilm is one such barrier.[3,4,7] Seprafilm is a bioresorbable translucent adhesion barrier composed of 2 anionic polysaccharides, sodium hyaluronate and carboxymethylcellulose, that has been shown to be effective in limiting adhesion to surgical incisions when it is used alone or together with polypropylene mesh.[1-3,7-9] Hyaluronate membrane serves as a temporary bioresorbable barrier that separates opposing tissue surfaces—the physical presence of the membrane impedes adhesiogenic tissue while the normal tissue repair process takes place.[19,20] These effects of Seprafilm also may be due to the biochemical action of hyaluronic acid.[16]

In our study, we used Sepramesh, which is made of polypropylene monofilaments and coated on one side with Seprafilm, for the reconstruction of soft tissue deficiencies. When Seprafilm was laid over the abdominal viscera, with Sepramesh placed retroperitoneally and fixed between abdominal muscles in a sandwich pattern, the coated portion of the Sepramesh was completely covered by neoperitoneum by the end of the experiment (28 d after surgery). The uncoated side of the Sepramesh underwent a prompt fibroblastic response through the interstices of the mesh and complete tissue ingrowth (Figure 3). The scores for adhesion extent and tenacity were reduced significantly ($P < 0.05$) in the animals treated with both Sepramesh and Seprafilm (group B), and there were significant ($P < 0.05$) differences in adhesion scores between the 3 groups. The Kruskal–Wallis test is applied to all samples combined into a single group, and significant differences identified with this test denote differences within that group as a whole. The results shown in Tables 1 and 2 compare the medians of the 3 samples.

Our observations also suggest that the inflammatory response associated with trauma to the underlying viscera can be overcome by the anti-adhesinogenic properties of Seprafilm and the coated side of Sepramesh. This effect results in a significant reduction in the extent and severity of adhesions and inflammation between abdominal viscera and mesh with no evidence of impaired wound healing in mice.

The novelty of our methodology is the combined application in mice of Seprafilm positioned intraperitoneally over the underlying viscera with Sepramesh sutured between the muscles and peritoneum in a sandwich pattern. Felemovicius and colleagues[12] previously used both Seprafilm and Sepramesh for hernia repair in rats, but not in a sandwich pattern with the peritoneum between the 2 prosthetic materials. The incidence of visceral adhesion of polypropylene mesh to the underlying viscera in that earlier study was slightly higher than we found. Moreover, the earlier study did not evaluate the influence of the prosthetic treatments on tissue inflammation.

Our findings suggest that among the 3 treatment groups, the animals treated with both materials developed the least amount of tissue inflammation, possibly because of the chemical properties of hyaluronic acid.[16] The wound-healing process in the mice that received both Sepramesh and Seprafilm seemed to have the most potential for minimal adhesions as well as complete tissue regrowth after repair of an abdominal wall defect. We found that the combined use of Sepramesh and Seprafilm in a sandwich pattern to repair adnominal wall defects resulted in strong tissue repair of body wall defects, decreased use of suture material, and minimal intra-abdominal tissue adhesion after abdominal wall closure.

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