FERTILITY AND STERILITY
Vol. 55, No. 3, March 1991

The use of an amniotic membrane graft to prevent postoperative adhesions*

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Grafts of trypsin-treated, gamma-irradiated human amniotic membranes were used to cover injured uterine horns of nulliparous female rabbits to prevent adhesions. In this study, the gradual integration of the membranes into the serosal layer of the uterus, together with marked neovascularization, was observed. By the 30th postoperative day, the grafts had been completely integrated, with little evidence of rejection and no evidence of infection at the graft sites. Of 30 uterine horns treated with membrane grafts, only 4 (13.4%) showed any adhesion formation at or among the graft sites. All of the 24 untreated controls showed adhesion formation at the site of injury. Furthermore, whatever adhesions were found in membrane-treated horns could be graded as thin and filmy, accounting for <10% of the surface area of the graft, whereas the controls showed dense, thick adhesions covering 50% to 100% of the injured areas. We conclude that these specially prepared amniotic membranes are safe and effective in dramatically reducing postoperative adhesion formation in this animal model. Fertil Steril 55:624, 1991

The presence of pelvic or abdominal adhesions is known to be a major cause of infertility in the human female. Adhesions may result from a great number of medical conditions or from surgical intervention.[1,2] Illness leading to adhesion formation includes pelvic inflammatory or other pelvic or abdominal inflammatory processes, resulting either from infection or endometriosis. The surgical procedures required by these and other pathological conditions, e.g., cysts, tumors, may also result in adhesion formation. Adhesions may, in turn, be associated with infertility by causing occlusion of the fallopian tubes or by interfering with tubal-ovarian function, inhibition of ovum pick-up being the best example. It is postulated that the formation of adhesions evolves from trauma to serosal surfaces followed by release of a fibrinogen-rich exudate and subsequent deposits of fibrin. If the fibrin fails to lyse and becomes organized, adhesion formation may result. This leads either to thick or filmy adhesive bands that may bridge the pelvic organs or tissues or to the dense fixation of these structures to each other.

Through the years, a great number of natural and synthetic graft materials have been employed in an effort to reduce adhesion formation on traumatized surfaces but with only marginally successful results. Natural materials have included peritoneum, omentum, fat, and amnion, as well as amnion plus chorion.[3-10] Synthetic materials, including polyvinyl alcohol film and tantalum foil, were used in the past and, more recently, barriers consisting of Gelfilm and Gelfoam paste (Upjohn Co., Kalamazoo, MI); Surgicel (Johnson and Johnson, New Brunswick, NJ); and Silastic (Dow-Corning, Midland, MI); as well as meshes of Gore-Tex (Gore-Tex, Gore, TX) and Interceed (Johnson and Johnson) have been employed.[6,11-17] The newer materials have led to more promising results. In the present study, macroscopic and microscopic peri-

Received May 21, 1990; revised and accepted October 4, 1990.
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toneal adhesions to an amniotic membrane graft were studied in a rabbit model to determine if these specially processed membranes could reduce adhesion formation when placed over experimentally damaged tissues.

MATERIALS AND METHODS

Amniotic Membrane Grafts

Amniotic membranes were harvested from freshly delivered human placentas taken at the time of cesarean section. The amniotic membranes were manually separated from the chorion and washed in distilled water. The clean membranes were first treated by soaking for 3 hours in a 10% solution of trypsin. Subsequently, they were irradiated with gamma radiation to sterilize them in the following manner. First, the entire membrane underwent an 8-hour 20-minute irradiation at 60,000 rads/h for a total dose of 500,000 rads. The membranes were then cut into smaller squares of approximately 2 × 2 cm and reradiated at 60,000 rads/h for a total of 33 hours and 20 minutes, equivalent to a two-million rad dose. The small squares thus prepared were frozen at −70°C in distilled water to maintain them until they were used (within 4 weeks). Just before use, the membranes were thawed at 22°C. No antibiotics were used during this process.

Surgical Procedure

Study animals consisted of nulliparous female New Zealand rabbits, each weighing at least 3.5 kg to ensure adequate size of the pelvic organs. Six rabbits were assigned to groups A through C and 18 to group D. At the time of surgery, the rabbits were anesthetized with a mixture consisting of ketamine (10 mg/kg), promazine (1 mg/kg), and xylozine (6 mg/kg). The abdomen was shaved, subjected to sterile prep, and draped. Sterile microsurgical techniques under the operating microscope were employed, as previously described by Badaway et al.[3] Experimental injuries consisted of a series of incisions through the serosal and muscularis layers of the uterine horn extending into the endometrial cavity, with frequent avulsion of the mucosa. The cuts, 1 cm long and spaced 5 mm apart, were created with microscissors. Gross hemostasis was obtained with bipolar electrocautery. Membrane grafts, approximately 1 × 2 cm in size, were sutured into place over the lesion in a single layer using multiple interrupted sutures of 7-0 polyglactin (Fig. 1). The maternal side of the membrane was placed against the injury, and the fetal side faced into the abdominal cavity. The glistening fetal surface can easily be distinguished from the matte maternal side, even after freezing and thawing. This orientation was chosen in the expectation that the rougher side of the membrane would incorporate itself better to the injury site, and the smoother surface would afford better protection against adhesion formation. After surgery, the abdomen was closed in three layers and a sterile dressing left in place for 72 hours. All animals received procaine penicillin at a dosage of 50 mL/kg intramuscularly four times daily × five doses postoperatively and were maintained in a vivarium at 27°C with 40% to 70% humidity and given food pellets and water ad libitum.

Figure 1 Uterine horn showing incision sites covered with amniotic membrane sutured into place.

Description of Experimental Groups

Animals were randomly assigned to three groups of 6 animals each and a fourth group of 18 animals. Each rabbit was subjected to two surgical procedures, the initial laparotomy including the designated operative procedure and a second-look laparotomy to evaluate the effects of the experimental intervention. The groups were as follows: group A (n = 6) was the background control group. The ab-

Table 1 Comparison of Results in Membrane-treated Versus Untreated Uterine Hours After Experimental Injury

Group	Horn	Surgical procedure	No. with adhesions	Types of adhesions	Amount of surface affected
					%
A (n = 6)	Right	None	0		
	Left	None	0		
B (n = 6)	Right	Incisions	6 (100)[b]	Dense, thick	75 (50 to 100)
	Left	None	0		
C (n = 6)	Right	Incisions + membrane	0		
	Left	Membrane only	1 (17)[a]	Filmy, thin	10
D (n = 18)	Right	Incisons + membrane	3 (17)[a]	Filmy, thin	10 (0 to 20)
	Left	Incisions + sutures	18 (100)[a]	Dense, thick	75 (50 to 100)

[a] Values in parentheses are percents. Right versus left horn: Fisher's exact test, in each case $P < 0.05$.

domen in this group was opened, exposed to no specific injury or treatment, and closed. Group B (n = 6) was the model control group in which controlled injuries, as described above, were made on one uterine horn of each animal. The contralateral horn was not injured, and no therapeutic interventions were made on either horn. Group C (n = 6) was the first treatment group in which injuries were carried out on one uterine horn as in group B, and then both injured and noninjured horns were covered with membrane grafts held in place with microsutures. Group D (n = 18) formed the second treatment group in which both uterine horns were experimentally injured in a similar manner. One horn was then treated by suturing a membrane into place, and the contralateral horn was treated with interrupted, hemostatic microsutures of 7-0 maxon.

Thirty days after the initial laparotomy and surgical intervention, each animal was reoperated on. Adhesions were photographed and evaluated relative to their presence or absence and percentage of surface included and graded as to adhesion quality (thin, thick, filmy, or dense). All initial surgery was completed by one team of surgeons (R.L.Y., J.M.C.), and subsequent evaluation of all results by the second team (B.A.M., G.Z.) in blinded fashion. Additionally, all specimens underwent standard histologic examination. Permanent sections were created from paraffin blocks, and hematoxylin and eosin staining was used on the sections. Statistical analysis was performed using Fisher's exact test, with significance accepted at $P < 0.05$.

RESULTS

All results are summarized in Table 1. The background control group plus model control group B confirmed the validity of the model by demonstrating that there were no background adhesions from laparotomy alone and that the experimental injury was sufficient to cause dense adhesions in 100% of the cases if untreated. These included surface adhesions as well as loop-to-loop adhesions leading to severe tortuosity of the involved horn (Fig. 2). It was further noted that there was no crossover of these adhesions to the uninjured contralateral horn ($P = 0.002$).

Experimental group C showed no significant difference ($P = 0.5$) in adhesion formation on sites of membrane grafts placed over injured versus noninjured uterine horns. Thin, filmy adhesions were found in only one case on the noninjured horn and

Figure 2 Right and left horns demonstrating tortuosity secondary to dense adhesions on the right horn (*below*) and absence of adhesions on the membrane-treated left horn (*above*).

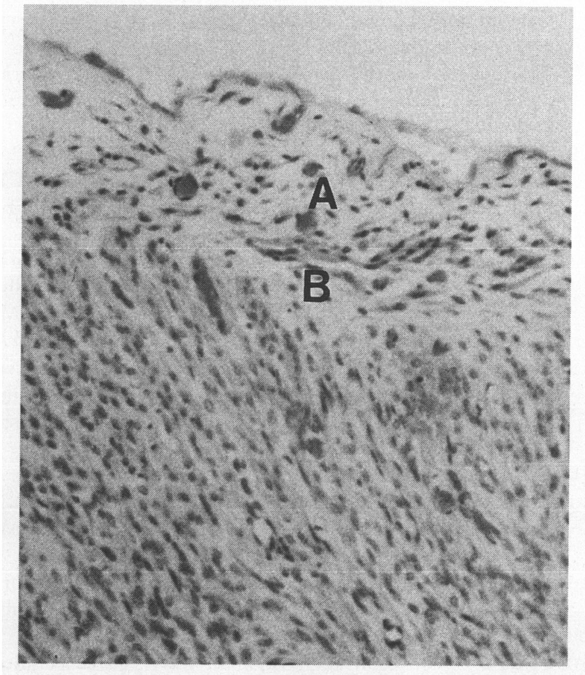

Figure 3 Photomicrograph of serosal surface of uterine horn covered by amniotic membrane (**A**) demonstrating both neovascularization of the membrane and a lack of significant inflammatory response in the serosa at the interface (**B**).

in no cases on the injured side. Finally, in experimental group D, membrane grafts significantly reduced the formation of adhesions as compared with those found at the site of hemostatic microsutures. Dense, thick adhesions over an average of 75% of the surface area of the sites of injury were noted on the horns treated with the sutures in 100% of the cases. In contrast, only 17% of the injured horns ($P = 0.0000003$) that had been covered with membrane grafts showed any adhesions, and these were thin, filmy, and covered only about 10% of the injured/grafted area. Histologic studies showed that the membranes were integrated with the serosal layer and showed neovascularization at the site of the graft. Minimal polymorphonuclear infiltration of the serosal surfaces was present, suggesting no significant immunological response (Fig. 3).

DISCUSSION

The use of human amnion as a surgical adjunct has a long history. An excellent review has been published by Trelford and Trelford-Sauder.[5] Amnion has been used to prevent pelvic and abdominal adhesions in a number of experimental animal models, as well as in human patients.[8–10] The natural membrane has been used in tubal surgery,[4] and there exists an extensive experience with its use in vaginal reconstructive surgery in women.[18,19] Other applications include repair of conjunctival defects,[20] reconstruction of the bile duct,[21] and prevention of meningocerebral adhesions after craniotomy.[6,7] Ongoing research at our own institution involves the use of amniotic membranes in plastic surgery, peripheral neurosurgery, as well as extensive use in urologic surgery. Its primary role in humans, however, has remained in the areas of burns, ulcers, other skin trauma, and in wound healing.[22–25]

No substantial literature exists describing its potential in preventing intra-abdominal adhesions in humans, although some progress has been made elsewhere in this area through the use of a number of synthetic agents. Badaway et al.[3] recently reported on the intra-abdominal application of amniotic membranes to prevent adhesions in the rat model. They noted little effect inhibiting adhesion formation on serosal surfaces but observed somewhat better results on the parietal peritoneum. The explanations that they offered for the lack of success involved problems with postoperative organ immobility and blood pooling, both of which may play a role in adhesion formation after human surgery.

Our own success with the rabbit model may be, in part, explained by the novel preparation of the membranes as described above. A study of the literature reveals almost as many different methods of preparing and storing the membranes as there are case or experimental reports. Our own previous poor results with glutaraldehyde-treated membranes (unpublished data), as well as equally unsatisfactory experience elsewhere with alcohol pretreatment and oven drying[7] or simple freezing in saline,[3] seem to indicate that the radiation retreatment after trypsin washing may have some advantage. The membranes thus prepared underwent adequate neovascularization and caused no significant inflammatory infiltration. This also seems to support a conclusion of no significant immunological reaction induced by the membranes, as also observed in the Badaway et al.[3] study.

The thinness and the exceptional compliant quality of the membranes made them extremely facile to use. Single layer application is mandatory in the pelvis and abdomen to prevent fibrosis formation within the membrane itself, a phenomenon we had observed previously in unreported experiments. Although synthetic grafts are applied without suturing, in our procedures, the membranes were fixed in place using microsutures. In the human, this adds the potential for concomitant use of liquid antiadhesive adjuvants, such as dextran. An added technical advantage to the use of amniotic membranes, as opposed, apparently, to the synthetic meshes, is the fact that they can be applied and sutured over surfaces not perfectly dry.

It now remains to test the membranes against the formation of adhesions involving the parietal peritoneum. The aim would be to improve the outcome of procedures involving extensive endometriosis or pelvic sidewall adhesions of the adnexal structures. This model has been difficult to establish,[2] but earlier results in the rat with more simply prepared membranes have already shown some promise.[3]

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