Prevention of Postoperative Adhesions in the Rat by In Situ Photopolymerization of Bioresorbable Hydrogel Barriers

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***Objective*: To assess the efficacy of a novel resorbable hydrogel barrier for preventing postoperative adhesions in animals.**

***Methods*: A hydrogel barrier was formed in situ by photopolymerizing a solution of a macromolecular prepolymer in buffered saline using long-wavelength ultraviolet light. Two models in the rat were evaluated. In a primary adhesion model, devascularization and serosal injury were performed on the uterine horns using bipolar electrocautery. The prepolymer solution was applied to the horns and illuminated to form the barrier. On the seventh postoperative day, the fraction of the length of the horns involved in adhesions was scored, as was the quality of the adhesions. In a readhesion model, adhesions were formed as described and were surgically lysed on the seventh day, then were treated subsequently with the barrier and scored after 7 additional days. Each group in both models consisted of seven animals per treatment condition. Four prepolymer concentrations were examined in the primary adhesion model, and the optimal one was examined in the readhesion model.**

***Results*: A conformal hydrogel barrier coating was formed upon in situ photopolymerization and adhered to the treated tissues. No residual hydrogel barrier was observed 7 days after application of the optimal gel concentrations. In the primary adhesion model, the mean fraction of the horns involved in adhesions was reduced significantly, from 76% in controls to 10% ($P < .0001$), and treatment with a 10% solution of prepolymer was determined to be optimal ($P = .025$). In the readhesion model, surgical lysis of adhesions alone did not reduce adhesions significantly (from 86% to 79%; $P = .3$), whereas lysis with barrier treatment did (79% to 28%; $P = .002$).**

Conclusions*: In situ photopolymerization allowed the formation of adherent, conformal barriers, which demonstrated high efficacy in the prevention of adhesion formation and reformation in animals. This efficacy and ease of use warrant clinical evaluation. *(Obstet Gynecol 1994;83:59–64)

Several barrier materials—liquid, solid, and gel—have been investigated for reducing the occurrence of postoperative adhesions.[1–3] Liquid means have focused primarily upon viscous solutions of macromolecules, such as 32% dextran 70[4] and chondroitin sulfate.[5] Hyaluronic acid solutions have demonstrated efficacy when applied before, but not after, peritoneal injury in a rat cecal abrasion model.[6] The mechanism of action may be related in the reduction of injury.[7] When pre-treatment cannot be accomplished, such as in lysis of adhesions, hyaluronic acid treatment has not demonstrated efficacy.[8]

A solid barrier of oxidized regenerated cellulose, Interceed TC7 (Ethicon, Inc., Somerville, NJ), has been in clinical use for some years. This material is enzymatically degraded over several weeks.[9] In a randomized clinical study of lysis of adhesions in women with bilateral pelvic sidewall adhesions, the incidence of adhesions was reduced.[10] However, limitations in the biocompatibility of this material have been noted, eg, in macrophage recruitment[11] and de novo adhesion formation in the mouse.[12] An expanded polytetrafluoroethylene surgical membrane, GoreTex surgical membrane (W. L. Gore and Associates, Inc., Flagstaff, AZ),

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has been used to reduce adhesion formation, and this material has been shown to lack such limitations in biocompatibility.[11,12] Good efficacy was noted in a uterine horn defect model,[13] but this was decreased in a model involving serosal denudation and devascularization.[14]

A gel barrier has also been investigated. Solutions of Poloxamer 407 (BASF Wyandotte Corp., Parsippany, NJ) are gelled in situ by warming from room temperature to 37C. In a serosal injury model in rats, adhesions were reduced by 58%.[15] In a golden hamster model of devascularization with serosal injury on the uterine horn, the mean adhesion score was reduced from 4.5 to 2.2 by the application of a 35% Poloxamer 407 solution.[16] In a rabbit model of lysis of adhesions, the mean adhesion score was reduced from 5.1 to 2.7.[16] In a rabbit model of lysis of adhesions using an optimized solution of Poloxamer 407, called FlowGel (Mediventures, Inc., Grosse Point Park, MI), the mean adhesion score was reduced from 5.6 to 1.3.[17]

In the present study, we evaluated the adhesion-preventing potential of a novel resorbable hydrogel formed in situ by photopolymerization from a solution of a prepolymer in saline. The prepolymer, the synthesis and characterization of which have been reported elsewhere,[18] is a functional block copolymer consisting of a central chain of poly(ethylene glycol), with oligomeric blocks of lactic acid at each end of the poly(ethylene glycol) central chain and an acrylate end-cap at each end of the lactic acid oligomer. Long-wavelength ultraviolet light is used to gel the liquid precursor in situ, forming an adherent, conformal gel barrier which is resorbed by hydrolysis in vitro and in vivo. In the present study, two models were evaluated in the rat: 1) prevention of adhesions after devascularization and serosal injury, in which the concentration of the macromer was optimized; and 2) prevention of readhesion after lysis of adhesions, using only the optimal precursor concentration from the other model.

Materials and Methods

The block copolymer prepolymer was based on poly(ethylene glycol) of molecular weight 8000 d, extended at both ends with oligomers of lactic acid with an average of five repeats per end, and further acrylated at both ends. It was synthesized and purified as described elsewhere.[18] This material was provided by Focal, Inc. The prepolymer was stored as a dry powder and was dissolved in sterile physiologic HEPES buffered saline (10 mmol/L) in the hours before use at the concentration desired for the studies, ranging from 5% w/v to 20%. The long-wavelength ultraviolet photoinitiator 2,2-dimethoxy,2-phenyl acetophenone (Aldrich Chemical Co., Milwaukee, WI) was dissolved in N-vinyl pyrrolidinone (Aldrich) at a concentration of 600 mg/mL, and 1.5 μL of this solution was added to each 1 mL of the prepolymer solution in buffered saline to achieve a final concentration of 900 parts per million initiator.

Polymerization to convert the solution to a gel was accomplished by illumination with a hand-held long-wavelength ultraviolet lamp with a maximum intensity at 365 nm (Black Ray, UVP, Inc., San Gabriel, CA). Conversion to a gel occurred within approximately 7 seconds of exposure at an irradiance of approximately 10 mW/cm^2; exposures at this intensity upon the hand are imperceptible over durations of several minutes. All exposures in vivo were performed for 20 seconds to ensure complete gelation.

We used a devascularization model of the rat uterine horns similar to that employed by others.[14,16,17,19] Specifically, female Sprague-Dawley rats, 200–250 g, were anesthetized with pentobarbital (50 mg/kg intraperitoneally), and a midline laparotomy was performed. The uterine horns were exposed, and the vasculature of the arcade of the horns was systematically cauterized using bipolar electrocautery; the most proximal and most distal large vessels on each horn were not cauterized. Following this, the antimesenteric surface of each horn was cauterized at two spots 1 mm in diameter, each separated by 2 cm and each pair centered along the length of each horn. Following injury, the animals were randomly assigned to any of the treatment or control groups. The investigator performing the injury (SMC) was blinded to the ultimate fate of the animal. In the treatment group (n = 7), 0.5 mL of macromer solution was applied to each horn and was gelled by exposure to long-wavelength ultraviolet light at an irradiance of 10 mW/cm^2 for 20 seconds on the front and back sides. Control animals (n = 7) were untreated. The horns were replaced in the peritoneal cavity, and the musculoperitoneal layer was closed with continuous 4-0 Vicryl sutures (Ethicon, Inc.). The skin was closed with 9-mm staples.

Adhesions were scored in two ways on the seventh postoperative day. The length of the horns involved in adhesions was measured, and the fraction of the total length of the horns was calculated; this score is referred to as the extent of adhesions. The nature of the adhesions was also scored on a qualitative scale, with 1 representing filmy adhesions that were easily separated by hand, and 2 representing dense adhesions that could only be separated by sharp instrument dissection. The score for grade pertained to the quality of the adhesions formed, regardless of the total area covered; thus, if a filmy adhesion formed over only 10% of the length of the uterine horns, the grade score

for that animal would be 1. Scoring was performed following sacrifice by CO_2 asphyxiation. After scoring, representative tissue samples were collected and fixed in 2.5% glutaraldehyde for microscopic analysis. Scoring and tissue sample collection were performed blind. The data were analyzed by nonparametric analyses, namely Kruskal-Wallis test for overall significance and ranking, and Mann-Whitney *U* test for comparisons between groups.

The second model tested readhesion after lysis of adhesions. Adhesions were formed by the procedure described above, and on the seventh postoperative day, the adhesions were surgically lysed at laparotomy. The adhesions were carefully separated by sharp instrument dissection, and hemostasis was achieved by bipolar cautery. The extent and grade of adhesions were scored at lysis. Following lysis, the rats were randomly assigned to the treatment or control group (n = 7 for each). At treatment, the surfaces of the horns were treated with prepolymer as described above, and the animals were closed. On the seventh day following lysis, the extent and grade of adhesions were scored blindly as described above. The data were analyzed by nonparametric analyses.

Tissue samples were prepared for light microscopic examination by fixation in 2.5% glutaraldehyde for at least 12 hours. The samples were dehydrated in a graded ethanol series, exchanged with xylene, embedded in paraffin, sectioned to a thickness of 5 μm, and mounted on glass slides. Staining was by Masson trichrome.

Results

Gelation of the liquid prepolymer solution was complete within 20 seconds of illumination, both in vitro and in vivo. This was determined by noting the time to resistance to the motion of a probe placed in the liquid during long-wavelength ultraviolet illumination. The gel had properties typical of a chemically crosslinked gel, in that it was not capable of flow or of dissolution under water. The texture of the gel was similar to that of polyacrylamide gels used in electrophoresis. Gelation in situ resulted in adhesion to the tissue. Adhesion to the tissues was not quantified in these studies, but rather was noted qualitatively by the difficulty encountered in attempting to remove the gel from the tissue with a probe.

The extent and grade of adhesions on the uterine horns were measured at laparotomy on the seventh postoperative day. There was no evidence of residual gel in the animals treated with 5, 10, or 15% solution. Some residual particles were observed with the 20% solution. Table 1 shows the extent and grade of adhesions in the control animals and in those treated with 5, 10, 15, or 20% solutions of prepolymer. The extent of adhesions was reduced from 76% in the control group to 10% in the group treated with 10% prepolymer solution. The score for extent was reduced significantly for each treatment relative to control ($P < .0001$ by Kruskal-Wallis), and the score for treatment with 10% solution was reduced significantly relative to all other treatments ($P = .025$ relative to the nearest group, treatment with 15% prepolymer solution, by Mann-Whitney *U* test).

Table 1. Adhesion Extent and Grade 7 Days After Primary Uterine Horn Injury

No. of animals	Concentration of macromer	Extent of adhesions* (mean ± SD)	Grade of adhesions† (0–2)
7	Control	76 ± 9‡	1, 2, 2, 2, 2, 2, 2
7	5%	22 ± 4	1, 1, 1, 1, 1, 1, 2
7	10%	10 ± 6§	0, 1, 1, 1, 1, 1, 1
7	15%	18 ± 6	1, 1, 1, 1, 1, 1, 1
7	20%	26 ± 9	1, 1, 1, 1, 1, 2, 2

* Mean percentage of the length of the horns involved in adhesions.
† Quality of the adhesions present in each animal. 0 = no adhesions; 1 = filmy adhesions; 2 = fibrous adhesions.
‡ $P < .001$ *vs* all treatment values by Kruskal-Wallis test.
§ $P = .025$ *vs* all other treatments by Mann-Whitney *U* test.

Light microscopic histologic examination of the uterine horns on the seventh postoperative day showed a normal morphology at the surface of the horns injured and treated by in situ photopolymerization of hydrogel (Figure 1a), with slightly increased collagen deposition, compared with the horns from normal animals, not injured or treated (Figure 1b). By contrast, the horns from the animals injured and not treated demonstrated adhesions, eg, the intimate connection with the mesentery shown in Figure 1c.

In the readhesion model, the extent and grade of adhesions were measured on the seventh day following lysis. We examined only the optimal formulation from the study of primary adhesions, ie, gel formed in situ from 10% prepolymer solution. On the seventh day following lysis, there was no evidence of residual gel. The results are shown in Table 2. The extent of adhesions was not significantly different between the groups at the time of lysis of adhesions ($P = .3$ by Mann-Whitney *U* test). Surgical lysis of adhesions alone was ineffective in reducing the extent of adhesions ($P = .3$ by Mann-Whitney *U* test, comparing extent in controls 7 days after lysis to that at the time of lysis). By contrast, surgical lysis of adhesions and treatment with 10% prepolymer polymerized in situ to

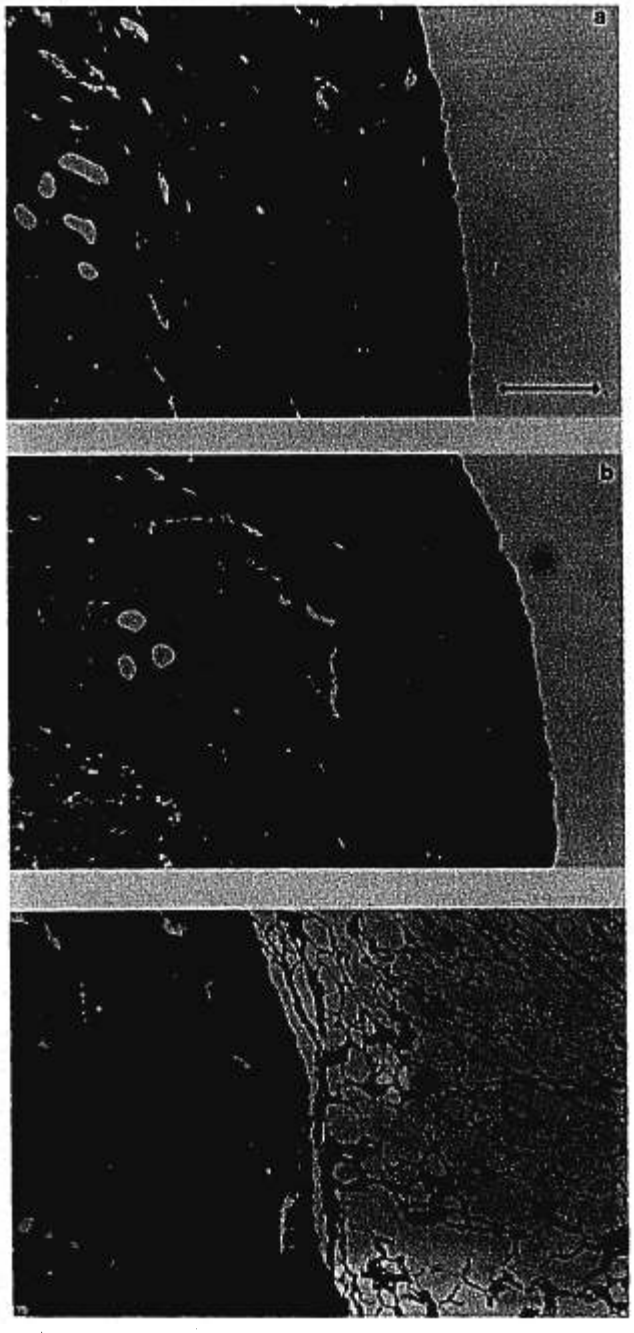

Figure 1. Histologic cross-section of the uterine horns showing the tissue near the surface of the horn: (a) healed horn, injured and treated with 10% prepolymer solution and gelled in situ; (b) normal horn, neither injured nor treated; and (c) adhered horn, injured and not treated (control), showing intimate adhesion to the mesentery. Staining was by Masson trichrome, and collagen is indicated by blue-green staining. The horns were examined 7 days following injury. Length bar = 75 μm.

form a hydrogel barrier were effective in reducing both the extent and the grade of adhesions 7 days following lysis. The extent was reduced from 79% to 28% (P = .002) and the grades were reduced from 2 (mostly fibrous) to 1 (all filmy).

Table 2. Adhesion Extent and Grade 7 Days After Lysis of Adhesions and Treatment With Gel Formed From 10% Prepolymer

No. of animals	Condition at scoring	Extent of adhesions* (mean ± SD)	Grade of adhesions† (0–2)
	Controls		
7	Before adhesion lysis	86 ± 10‡	1, 2, 2, 2, 2, 2, 2
7	7 d after lysis	79 ± 11	1, 2, 2, 2, 2, 2, 2
	Treated group		
7	Before adhesion lysis	79 ± 7§	1, 2, 2, 2, 2, 2, 2
7	7 d after lysis	28 ± 5	1, 1, 1, 1, 1, 1, 1

* Mean percentage of the length of the horns involved in adhesions.

† Quality of the adhesions present in each animal. 0 = no adhesions; 1 = filmy adhesions; 2 = fibrous adhesions.

‡ P = .3 *vs* controls 7 days after lysis and treated group before lysis by Mann-Whitney U test.

§ P = .002 *vs* treated group after lysis by Mann-Whitney U test.

Discussion

The pathophysiology of adhesion formation, including the time courses of adhesion formation and remesothelialization, has been reviewed.[2,20–23] When adhesions form, mature collagen bundles can be observed after 5 days.[24] When adhesions do not form, remesothelialization occurs by the fifth day following injury.[23] Thus, we sought to develop a barrier that would remain in place for approximately 5 days.[18]

The poly(ethylene glycol)-based hydrogel was evaluated in two models for pelvic adhesions, one involving primary adhesions and one following lysis of adhesions. The concentration of prepolymer was first optimized in the primary adhesion model, examining prepolymer concentrations of 5 to 20%. The maximal efficacy occurred at a prepolymer concentration of 10%, possibly because of both the physical characteristics and the resorption rate of the gel. Gels made from higher concentrations of prepolymer (at least 20%) are stiffer and may be slightly less biocompatible. Moreover, they degrade more slowly than those made from more dilute prepolymer solutions because of higher crosslink densities. Thus, the optimum concentration may reflect a combination of rate of degradation, mechanical properties, and biologic response to the resulting gels. Adhesions were reduced by 87% relative to controls in the primary adhesion model, and by 64% relative to controls in the more severe model of readhesions following lysis. In all cases, residual gel was not present on the treated tissues, ie, the transient

presence of the gel determined the ultimate and longer-term healing outcome.

Other approaches with gels formed in situ have been examined, most notably with Poloxamer 407 gels.[15–17] There are considerable differences between the properties of the gels in our study and the properties of previously described gels. Both are formed in situ from liquid precursor solutions. The Poloxamer 407 gels are held together by physical bonds and are relatively loose. For example, Poloxamer 407 gels are capable of flow, and a gel formed upon a tissue can be readily removed under a stream of running water. The photopolymerized gels in our study are held together by covalent chemical bonds; they are incapable of flow and are not readily removed by washing. Future studies should compare the two disparate approaches to gel formation to elucidate any advantages in efficacy from the longer residence upon the tissues.

It is difficult to obtain adhesion of a barrier on one surface, but nonadhesion on the other. Because the barrier is delivered to the tissues as a liquid and is subsequently gelled in situ, it is readily conformable to irregularly shaped tissues, even on the cellular length scale. Gelation upon a tissue resulted in gel adhesion to the tissue, presumably because the liquid prepolymer solution penetrated into crevices upon the surface of the tissue, resulting in mechanical interdigitation. If the gel was polymerized before contact with the tissues, adhesion did not occur, as this initial flow was not possible.

The synthesis, properties, and degradation of the family of materials used in this study have been described.[18] The barrier material is a hydrogel, consisting of approximately 90–95% water in its equilibrium swollen state, and is quite flexible and permeable to even moderately large proteins.[25] The hydrogel's constituents and its degradation products are materials already in use in the body: poly(ethylene glycol), lactic acid and its oligomers, and oligomers of acrylic acid. The acrylate crosslinking chemistry has been used to polymerize medical materials in man, eg, in dental restorative resins[26] and in bone cements.[27] The light used to initiate crosslinking is long-wavelength ultraviolet light (365 nm), which is not mutagenic.[28] The photoinitiator used, 2,2-dimethoxy,2-phenyl acetophenone, has low toxicity, with an oral LD_{50} greater than 6 g/kg in rats.[29]

Future studies will be necessary to determine the duration of residence of this barrier upon the surface of the tissue and the time course of remesothelialization. Investigations should compare this new adhesion barrier to those already tested and assess the extent of protection from adhesion formation at longer durations following surgery.

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