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Efficacy of Adhesion Barriers

Resorbable Hydrogel, Oxidized Regenerated Cellulose and Hyaluronic Acid

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OBJECTIVE: To compare a novel resorbable hydrogel barrier with two previously studied barriers, oxidized regenerated cellulose and hyaluronic acid, for the prevention of postoperative adhesions.

STUDY DESIGN: Two models were employed in the rat uterine horn, one of adhesion formation after devascularization and serosal injury and one of adhesion reformation after adhesiolysis.

RESULTS: In the devascularization model, hydrogel treatment reduced the mean extent of adhesion formation from 73% in the control group to 13% (P < .005). Hyaluronic acid pretreatment reduced the extent of adhesion formation to 44% (P < .05), while oxidized regenerated cellulose failed to reduce formation (P > .25). In the adhesiolysis model, treatment with the hydrogel reduced the mean extent of adhesion formation from 87% in the control group to 20% (P < .005). Neither the oxidized regenerated cellulose nor the hyaluronic acid treatments lowered the extent of adhesion formation from the control group (P > .25). The hydrogel barrier was observed to be resorbed over a five-day period and remained adherent to the tissue during resorption.

CONCLUSION: Resorbable hydrogel barriers are highly effective in the reduction of adhesion formation and reformation in the rat. This is probably due to the good biocompatibility and retention of these materials upon the site of application. (J Reprod Med 1996;41:149–154)

Keyword: adhesions.

Introduction

The formation of postoperative adhesions continues to limit the success of many abdominal and pelvic surgical procedures. During surgery the mesothelium suffers injuries, resulting in the release of a serosanguinous exudate that may form fibrin. Healthy tissue will quickly resorb this fibrin, but damaged, ischemic mesothelium is unable to do so due to down-regulation of tissue plasminogen activator secretion.[1-3] This fibrinous exudate may then become ingrown with fibroblasts, which secrete collagen to form a permanent scar bridge. A method of preventing adhesions has been to use a physical barrier, in the form of a solid, gel or viscous liquid, between the

The combination of high biocompatibility and retention upon the site of application over a four- to five-day period may account for the high efficacy [of PEG].

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site of surgical manipulation and other organs. This method has shown some promise, but none of the barrier materials has yet proven unequivocally successful in reducing both adhesion formation after initial surgical trauma and adhesion reformation after adhesiolysis. The purpose of this investigation was to evaluate a novel adhesion prevention barrier material based on a resorbable polyethylene glycol (PEG) hydrogel[4] side by side with other materials either under investigation or currently in clinical use for adhesion prevention.

It may not be possible to limit injury in adhesiolysis performed by cautery or dissection.

Oxidized regenerated cellulose has been in clinical use for a number of years. This material is applied to tissue as a solid fabric and then swells to form a gel that is enzymatically degraded over several weeks.[5] While oxidized regenerated cellulose reduced the incidence of adhesions in a randomized clinical study of the lysis of adhesions in women with bilateral pelvic sidewall adhesions,[6] there have been concerns regarding its biocompatibility. Oxidized regenerated cellulose has been reported to elicit increased numbers of macrophages in the peritoneal fluid[7] and to cause *de novo* adhesion formation in mice.[8]

Hyaluronic acid solutions are viscous liquids that may act as a barrier to adhesion formation. Hyaluronic acid solutions reduced adhesion formation when applied prior to but not following manipulations in a rat uterine horn injury model.[9] Hyaluronic acid pretreatment probably reduces adhesions by lessening trauma to the tissues.[9] Hyaluronic acid was shown to be ineffective in an adhesiolysis model,[10] presumably due to the inability to pretreat the surface to be lysed.

We recently reported on the use of a novel, resorbable barrier material that is applied to tissue as an aqueous solution of a macromer and then converted to a covalently cross-linked hydrogel upon exposure to long-wavelength ultraviolet light.[4] This material consists of a PEG chain, molecular weight 8,000 d, that has been copolymerized at each end with an average of five degradable lactic acid groups and further capped at each end with a reactive acrylate group. The synthesis and characterization of this material have been described elsewhere.[11] This hydrogel has been found to be highly effective in the prevention of postoperative adhesions in a rat uterine horn model with devascularization and serosal injury and in a rat uterine horn adhesiolysis model.[4]

In the current study we compared the PEG-based hydrogel to oxidized regenerated cellulose and hyaluronic acid pretreatment in rat uterine horn models for primary adhesion formation and adhesion reformation following lysis of adhesions. Additionally, rats were treated with the PEG-based hydrogel labeled with fluorescent tracers and opened at varying postoperative durations to assess the rate of degradation and the extent of retention upon the treatment site in the rat.

Materials and Methods

Female Sprague-Dawley rats weighing between 225 and 250 g (14 weeks old) were used. The studies were approved by the Animal Use Committee, University of Texas, Austin. Rats were fed standard rat chow and water *ad libitum*.

A rat model of devascularization and serosal injury was used to assess efficacy in the prevention of primary adhesions. Anesthesia was induced by intraperitoneal injection of pentobarbital (50 mg/kg), 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 cautery. The most proximal and most distal large vessels on each horn were not cauterized. In addition, two 1-mm-diameter spots, separated by approximately 2 cm, on the antimesenteric surface of each horn were cauterized. Rigorous hemostasis was achieved in all animals. Following injury (by S.M.C.), the rats were randomly assigned to groups: control or treatment with oxidized regenerated cellulose or PEG-based hydrogel (seven rats per group). Animals in the hyaluronic acid treatment group were assigned prior to injury, and the surgeon was not blind to this pretreatment due to its nature.

Hyaluronic acid (2 mL) was instilled into the peritoneal cavity as a sterile 0.4% solution in physiologic HEPES-buffered saline (10 mM) prior to tissue manipulation. After the uterine horns were isolated but prior to injury, an additional 1 mL was applied directly to each horn. Oxidized regenerated cellulose (Interceed TC7, Ethicon, Inc., New Brunswick, New Jersey) was applied as a single layer and was then slightly wetted with sterile

physiologic HEPES-buffered saline to enhance adhesion to the tissue. The PEG macromer (supplied by Focal, Inc., Lexington, Massachusetts) was applied to the tissue as a research formulation consisting of a 10% wt/vol solution in sterile physiologic HEPES-buffered saline containing 900 parts per million of the long-wavelength ultraviolet photoinitiator 2,2-dimethoxy,2-phenyl acetophenone (Aldrich, Milwaukee, Wisconsin) dissolved in N-vinyl pyrrolidone (Aldrich, 0.15% final concentration in the macromer solution). The uterine horns were then exposed to long-wavelength ultraviolet light using a hand-held long-wavelength ultraviolet lamp (BlackRay, UVP, Inc., San Gabriel, California) emitting with maximum intensity at 365 nm and irradiance of 10 mW/cm^2. Tissue was exposed to light for 20 seconds on each side. The horns were replaced in the peritoneal cavity following treatment. The musculoperitoneal layer was closed with continuous 4-0 Vicryl suture (Ethicon), and the cutaneous layer was closed with 9-mm staples.

Adhesions were scored on the seventh postoperative day. Rats were killed by CO_2 asphyxiation, and adhesions were evaluated as the percentage of the length of the horn involved in adhesions and the severity of the adhesions present. The percentage was determined by measuring the total length of the horns and the length involved in adhesions using a ruler. A severity grade of 1 was given to all filmy adhesions that could easily be separated by hand, and a grade 2 was given to all dense adhesions that required dissection with sharp instruments for separation. Scoring was performed blind to treatment groups (by S.M.C.). Statistical analysis of the extent of adhesions was by the Kruskal-Wallis test with *post hoc* comparisons between groups, and that of the severity of adhesions was by the χ^2 test.

A rat model of adhesion reformation after lysis of adhesions was also used to assess efficacy. Adhesions were formed (by S.M.C.) as described above prior to assignment to treatment groups. On the seventh postoperative day the adhesions were surgically lysed by laparotomy (by S.M.C.). The extent and severity of adhesions were scored prior to lysis (by S.M.C.). Rigorous hemostasis was achieved prior to treatment using bipolar cautery. Following lysis, the animals were randomly assigned to groups: control, oxidized regenerated cellulose and PEG hydrogel (seven animals per group). Animals were assigned to the hyaluronic acid pretreatment group prior to adhesiolysis, as in the model above. Treatment was performed as described above except that in the hyaluronic acid group, an additional 1 mL was applied to each horn after lysis to ensure coverage of areas that had been involved in adhesions. Seven days after lysis, the rats were killed by CO_2 asphyxiation, and the adhesions were blindly scored, as described above (by S.M.C.). Statistical analysis of the extent of adhesions was by the Kruskal-Wallis test with *post hoc* comparisons between groups and that of the severity of adhesions by the χ^2 test.

In an additional set of experiments, fluorescent beads, 1 μm in diameter (Polysciences, Warrington, Pennsylvania), were added to the macromer solution. Ten female Sprague-Dawley rats weighing 225–250 g were anesthetized and their abdomens opened as before. The uterine horns were isolated, and the macromer solution was applied and polymerized as before. Two animals were killed at each time point: 0, 1, 3, 5 and 7 days. Tissue samples were

Table I *Adhesion Extent and Grade Seven Days Following Devascularization Uterine Horn Injury*

Treatment	Extent of adhesions % (S.D.)[a]	Significance of extent relative to control[b]	Grade of adhesions (0–2[c])	Significance of grade relative to control[d]	No. of animals
Control	72.8 (11.3)		1,2,2,2,2,2,2		7
PEG hydrogel	12.5 (6.1)[e]	P<.005	1,1,1,1,1,1	P<.001	6
Oxydized regenerated cellulose	82.9 (11.6)	P>.25	1,2,2,2,2,2,2	P=1.0	7
Hyaluronic acid	44.3 (20.3)	P<.05	1,1,1,1,1,2,2	P<.01	7

[a]Mean fraction of the length of the horns involved in adhesions.
[b]By Kruskal-Wallis test with *post hoc* comparisons between groups.
[c]A grade of 1.0 corresponds to a filmy adhesion that can be separated easily by hand. A grade of 2.0 corresponds to a dense adhesion requiring sharp instruments for dissection.
[d]By χ^2 test.
[e]P<.01 relative to the next highest score, treatment with hyaluronic acid prior to injury, by Kruskal-Wallis test.

Table II *Adhesion Extent and Grade Seven Days Following Sharp Instrument Lysis of Adhesions Formed on the Uterine Horns*

Treatment	Extent of adhesions % (S.D.)[a]	Significance of extent relative to control[b]	Grade of adhesions, 0–2	Significance of grade relative to control[c]	No. of animals
Control	87.1 (7.6)		2,2,2,2,2,2,2		7
PEG hydrogel	20.4 (16.4)	P<.005	1,1,1,1,1,1,1	P<.001	7
Oxydized regenerated cellulose	97.1 (7.6)	P>.25	2,2,2,2,2,2,2	P=1.0	7
Hyaluronic acid	75.0 (15.8)	P>.25	2,2,2,2,2,2,2	P=1.0	7

[a]Mean extent (overall for all groups) at the time of lysis was 72%; there were no statistical significance differences in the mean scores between groups (P>.7).
[b]By Kruskal-Wallis test with *post hoc* comparison between groups.
[c]By χ^2 test.

taken from each uterine horn and then frozen in liquid nitrogen. The tissue was sectioned while frozen to a thickness of 5 μm and mounted on glass slides. The tissue was observed by fluorescence microscopy to visualize the bead-containing gel to permit measurement of its thickness using a video image processor (Hammamatsu Photonics, Bridgewater, Connecticut). It was necessary to employ a histologic process that did not involve dehydration since the hydrogel barrier consists mostly of water (approximately 90%).

Results

Adhesions were found in all the rats, and the control rats consistently had dense, vascularized adhesions involving most of the horn length. One rat in the devascularization model, PEG hydrogel group, died in the postoperative period, presumably of anesthesia complications. This animal was not replaced.

The adhesion formation scores for the four groups in the devascularization model are shown in Table I. Application of the PEG hydrogel reduced adhesion formation by 83% relative to controls, which is consistent with our prior studies.[4] Pretreatment of the tissues with 0.4% hyaluronic acid reduced adhesion formation by 39% relative to the controls, while treatment with oxidized regenerated cellulose did not reduce adhesion formation. The severity of adhesions that formed in the hyaluronic acid pretreatment group was reduced, but that in the oxidized regenerated cellulose treatment group was not. The PEG hydrogel reduced the severity of the adhesions from a grade of mostly 2 in controls (mostly dense) to all 1 (all filmy). There was no evidence of PEG hydrogel on the seventh postoperative day, while a yellowish mass was observed in five animals that were later determined to be in the group treated with oxidized regenerated cellulose.

The adhesion formation scores for the four groups in the adhesiolysis model are shown in Table II. Prior to lysis, all groups had statistically indistinguishable mean scores for the extent and severity of adhesions present ($P>.7$ for all comparisons, mean extent for all groups = 72 ± 16%, mean ± SD). Surgical adhesiolysis alone was ineffective in reducing either the extent ($P>.5$) or grade ($P>.8$) of adhesion formation. Treatment with the PEG-based hydrogel decreased the extent of adhesion formation by 77% relative to the controls. Treatment with neither hyaluronic acid nor with oxidized regenerated cellulose reduced adhesion formation in this model.

The hydrogel barrier containing fluorescent beads was observed to degrade over a five-day period and to remain adherent to the tissue throughout the degradation period. Prior to degradation the thickness of the hydrogel barrier was found to be 176 ± 44 μm (mean ± SD). After degrading for one day, the barrier was thinned to 102 ± 15 μm. At three days the gel was 32 ± 9 μm thick and still formed a continuous barrier. After five days in the peritoneal cavity the hydrogel was present only in very thin patches having a mean thickness of 4.9 ± 0.9 μm.

Discussion

The present study was undertaken to permit comparison of a resorbable hydrogel barrier developed in our laboratory[4,11] with other barrier materials that have been previously studied, side by side in the same models. Two models were examined: a primary adhesion model of devascularization with serosal injury and one of adhesion formation after lysis of preformed adhesions. Meticulous hemosta-

sis was achieved in both models; it was a requirement for the use of oxidized regenerated cellulose in the comparison group.

Treatment with oxidized regenerated cellulose was ineffective in both models examined. Similar results have been found in rat uterine horn models.[12,13] In both the present study and one by Pagidas and Tulandi,[12] pockets of residual oxidized regenerated cellulose were found. Other groups have found moderate reductions in adhesion formation attributable to the application of oxidized regenerated cellulose in rabbit models.[14,15] It is possible that the lack of efficacy of oxidized regenerated cellulose in the present models is due to a species difference. The bioincompatibility of oxidized regenerated cellulose, observed as increased numbers of peritoneal macrophages and *de novo* adhesion formation in mice treated with oxidized regenerated cellulose,[7,8] may also limit its efficacy.

Treatment with hyaluronic acid reduced adhesion formation in this study in the devascularization model but not in the adhesion reformation model. The lack of efficacy of hyaluronic acid in preventing adhesions after adhesiolysis has been previously reported.[10] These findings may be due to differences in the pathogenesis of healing and adhesion formation in the adhesion reformation model.[16] Coating tissues with viscous solutions of macromolecules, such as hyaluronic acid, has been found to cause local accumulation of plasminogen activator by preventing its diffusion from the mesothelium into the abdominal cavity.[17] Plasminogen activator secretions in the tissue exposed by adhesiolysis may be too low for this mechanism to be effective in the adhesion reformation model. Moreover, pretreatment with viscous solutions of macromolecules is thought to protect the tissue and limit injury.[9] It may not be possible to limit injury in adhesiolysis performed by cautery or dissection.

The apparent high levels of efficacy of the resorbable, PEG-based hydrogel, in the models examined, may be due to several features. Previous studies from our laboratory have shown that exposure to long-wavelength ultraviolet light under the conditions of this study had no influence on adhesion formation.[18] PEG has been used to limit cellular recognition and adhesion due to its high degree of hydrophilicity and nonionic character—for instance, rendering deendothelialized arteries nonthrombogenic[19] and preventing immune recognition of liposomes.[20] The barrier conforms to the tissues and remains adherent to them during degradation, which occurs over approximately five days. The hydrogel is degraded independently of macrophage action,[11] in contrast to homopolymers of lactic acid (e.g., suture materials); the lactic acid in the PEG-based hydrogel accounts for only approximately 0.8% of the hydrated hydrogel material's weight. The degradation products, primarily polyethylene glycol and small amounts of lactic acid and polyacrylic acid, are nontoxic and noninflammatory and are rapidly cleared.[11] The combination of high biocompatibility and retention upon the site of application over a four- to five-day period may account for the high efficacy in these models.

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