

TATION PAGE

2



to average 1 hour per response, including the time for reviewing instructions, searching existing data sources, gathering the collection of information. Send comments regarding this burden estimate or any other aspect of this collection of information, including suggestions for reducing this burden, to Washington Headquarters Services, Directorate for Information Operations and Reports, 1215 Jefferson Davis Highway, Suite 1204, Arlington, VA 22202-4302, and to the Office of Management and Budget, Paperwork Reduction Project (0704-0186), Washington, DC 20503.

12b. DISTRIBUTION CODE

DTIC
ELECTE
APR 19 1994
C D

DTIC QUALITY INSPECTED 3



Use of Polypentapeptides of Elastin to Prevent Postoperative Adhesions: Efficacy in a Contaminated Peritoneal Model

LYNNE D. HOBAN, D.V.M.,^{*1} MARISSA PIERCE,^{*} JERRY QUANCE, D.V.M.,^{*} ISAAC HAYWARD, D.V.M.,^{*} ADAM MCKEE, D.V.M.,^{*} D. CHANNE GOWDA, PH.D.,[†] DAN W. URRY, PH.D.,[†] AND TAFFY WILLIAMS, PH.D.^{*}

^{*}Naval Medical Research Institute, 8901 Wisconsin Avenue, Bethesda, Maryland 20889-5055; and [†]Bioelastics Research, Ltd., 1075 South Thirteenth Street, Birmingham, Alabama 35205

Submitted for publication August 11, 1992

We investigated the use of a sheet of polypentapeptide of elastin as a physical barrier to adhesion formation in a contaminated peritoneal wound model. A total of 88 rats were studied with random assignment of animals to three study groups; control (29), polypentapeptide steam sterilized (30), and polypentapeptide gas sterilized (29). Animals were anesthetized and a laparotomy was conducted to reveal the cranial portion of the ileum. The abdominal wall muscle peritoneum was excoriated until hemorrhage was noted. In sham animals, there was no physical barrier placed between bowel loop and the abdominal wall. In two of the study groups, the polypentapeptide sheet was placed directly over the excoriated area. The intestinal loop was then loosely secured to excoriated area with 2-0 nylon (stay suture) which was tied subcutaneously in all groups. Four puncture wounds were made with a 20-gauge hypodermic needle in the bowel that was apposed to the excoriated peritoneal musculature which allowed leakage of intestinal contents and contamination. On Day 7 postsurgery, the animals were anesthetized and the stay suture was removed. On Day 14, all animals were sacrificed and adhesions were graded. The incidence of significant adhesions was 28% for the barrier group versus 90% for control animals ($P < 0.05$). The results of this study indicate that the polypentapeptide of elastin sheet is an effective physical barrier in this surgically induced contaminated wound model. © 1994

Academic Press, Inc.

INTRODUCTION

Despite the advances in modern surgical techniques, postoperative intra-abdominal adhesions are a common sequela of pelvic and abdominal operations [1, 2]. Abdominal adhesions consist of fibrofatty tissue that interconnects loops of bowel or solid organs such as the liver, spleen, or intestines. It is estimated that 67 to 93% of

mechanical small-bowel obstructions are caused by intraperitoneal adhesions [1, 2]. The presence of dense adhesion makes reoperation technically challenging because of the loss of intra-abdominal landmarks. Operations in this situation are often associated with increased bleeding, inadvertent enterotomy, and subsequent soilage of the operative field. Adhesions are a major cause of morbidity, including short-gut syndrome, chronic pain, and female infertility [3, 4]. Many substances have been employed in the prevention of adhesions, including heparin [5], surgical barriers [6], corticosteroids [7], antihistamines [8], nonsteroidal anti-inflammatory drugs [9], fibrinolytics [10], sodium carboxymethylcellulose [11], chondroitin sulfate [12], procoagulants [9], proteolytics [13], and dextran [14]. To date, these agents have not been proven to be consistently effective, especially in the presence of inadequate hemostasis and bowel soilage.

Physical barrier and coating products have been investigated more intensely in the past several years [11, 15]. The newest physical barrier product is Interceed (TC7), an oxidized regenerated cellulose, which is self-adhering and absorbable [16]. When properly utilized, Interceed (TC7) reduced the extent and severity of post-surgical adhesions in a clinical trial [16]. However, the use of Interceed (TC7) in the presence of frank infection is contraindicated. For best results, the manufacturer recommends that complete hemostasis be established prior to the instillation of Interceed (TC7) since the effectiveness of this product is reduced when it is saturated with blood.

This study was undertaken to evaluate the effectiveness of a polypentapeptide of elastin (polymer) as a physical barrier to adhesion in a contaminated abdominal wound in rats. These protein-based polymers of elastin were engineered to be biocompatible. The polymer is composed of a series of peptides which have been cross-linked by γ -irradiation to form elastomeric sheets measuring approximately 2×2 in.

Preliminary studies indicated that the polymer Val-Pro-Gly-Val-Gly [poly(VPGVG)], which exhibits ex-

¹ To whom correspondence should be addressed.

cellent biocompatibility [17], would serve as a physical barrier to adhesions in the presence of bowel soilage and hemorrhage. The emphasis of this study was to utilize the polymer in a simulated abdominal trauma model with resulting hemorrhage and contamination.

MATERIALS AND METHODS

Peptide Synthesis

The chemical synthesis and characterization of poly(GVGVP) has been extensively reported elsewhere [18, 19]. It is to be emphasized that considerable care is required to purify and characterize the intermediates that are used in the preparation of the pentamer and in the pentamer itself, which is then polymerized to make the high-molecular-weight polymers. Small impurities, including racemization, which can occur as a side reaction during the synthesis, can produce significantly different properties in the final polymer. The synthesis were verified by ^{13}C NMR spectra. The presence of all requisite peaks and the absence of extraneous peaks is required to verify the synthesis. This is done not only with the final product, but also with the pentamer building blocks.

On raising the temperature, aggregation occurs with settling to form the coacervate phase. The aggregation is monitored by the onset of turbidity. The temperature for onset of turbidity provides a critical assay for the quality of the synthesis. The correct temperature for the onset of turbidity for poly(GVGVP) is $25.5 \pm 1^\circ\text{C}$.

Preparation of the Cross-Linked Matrix

Poly(GVGVP) is dissolved in pyrogen-free water at a concentration of 250 mg/ml. The high polymer poly(GVGVP) is soluble in water below 25°C . The solution is then placed in a mold and centrifuged with the temperature maintained at 10°C below the transition. Aggregation is induced by raising the temperature to 10°C above the transition over a period of 1 hr and then centrifuging for 4 more hours. The coacervate phase is then checked for uniformity. If it does not have any irregularities such as bubbles, it is then γ -irradiated with a 20 MRad dose of cobalt-60 radiation to form the cross-links which result in an insoluble matrix. The molds are then opened in a laminar flow hood using sterile conditions and placed in a tube containing sterile water. The tube is then sealed until prepared for implantation.

All of the polypentapeptide material was sterilized by either heat or gas. Heat sterilization was accomplished by autoclaving in sterile saline for 20 min at 120°C . The polymer was secured in gauze and a gas-permeable sealed bag for ethylene oxide sterilization. Once sterilized, all polymer specimens were incubated at 37°C for 20 min in 0.9% NaCl to allow for rehydration of the sheets prior to placement in the study animals.

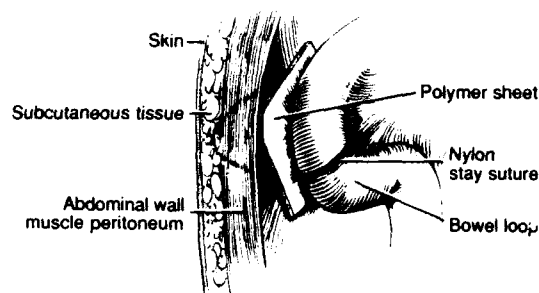


FIG. 1. Schematic diagram of surgically implanted polypentapeptide of elastin.

Surgical Procedure

A total of 88 rats were studied with random assignment of animals to three study groups as follows: sham-operated ($n = 29$), polypentapeptide steam sterilized ($n = 30$), and polypentapeptide gas sterilized ($n = 29$). Animals were anesthetized with isoflurane administered via nose cone. A midline abdominal incision was made through the skin and muscle tissue. The cranial portion of the ileum was located. The abdominal wall muscle peritoneum was excoriated in a small area using a No. 15 scalpel blade until hemorrhage was noted. At this point, the animals were divided into their respective groups. In the control animals, there was no physical barrier placed between bowel loop and the abdominal wall. In the study groups, the polypentapeptide was placed directly over the excoriated area. The intestinal loop was then loosely secured to the excoriated area with 2-0 nylon (stay suture) which was tied subcutaneously in all groups. In the case of the polypentapeptide, the stay suture was placed directly through the polypentapeptide sheet to insure proper placement. Four puncture wounds were made with a 20-gauge hypodermic needle in the bowel that was apposed to the excoriated peritoneal musculature. Intestinal contents were then milked through the puncture wounds in the bowel which contaminated the area. There was hemorrhage as a direct result of the puncture wounds. There were no attempts to achieve hemostasis. The abdominal incision was closed in a continuous pattern with 2-0 nylon and the skin with wound clips (Fig. 1). Each animal was given 3 cc of lactated Ringers solution subcutaneously prior to recovery from anesthesia. Animals had free access to food and water postrecovery.

On Day 7 postsurgery, the animals were anesthetized briefly with isoflurane as before. The stay suture was surgically removed through a skin incision, exposing the subcutaneously tied stay suture. The nylon suture was cut and withdrawn. The skin was closed with wound clips. At Day 14, animals were euthanized with carbon dioxide via inhalation and abdominal adhesions were assessed.

Adhesions were graded according to the parameters outlined in Table 1, which are a modification of the

TABLE 1
Scoring Criteria for Adhesions

Grade	Description	Classification
0	No adhesions	Insignificant adhesions
1	Single band of adhesion composed of omental fat between omentum and abdominal wall offering no resistance to separation.	
2	Involving omental fat and intestines, with a fibrous band of adhesion tissue between viscera and abdominal wall. Moderate force required for separation.	Significant adhesions
3	Abscessed adhesion involving omental fat, abdominal wall, intestines with fibrous connective tissue proliferation. Sharp dissection needed for separation.	

grading system presented by Nair *et al.* [20]. Comparisons were made by contingency tables between treatment groups, $P < 0.05$.

RESULTS

Several differing amino acid sequences were evaluated (unpublished data). Their success was limited by factors such as fragility and effectiveness in preventing adhesions. The polymer [poly(VPGVG)] formulation was the most promising, as it was easy to manipulate, durable, and effective.

Table 2 summarizes the results of the study. Significant adhesions were noted in 90% of the control rats studied. Since all control animals had at least one area of adhesion formation, we were satisfied with the method utilized for the creation of adhesions in this model. The incidence of significant adhesion was 28% for the polymer group versus 90% for control animals ($P < 0.05$). There was no significant difference in polypentapeptide effectiveness based on sterilization techniques. Gas sterilization resulted in insignificant adhesions for 80% of the animals ($P < 0.05$). Steam-sterilized polymer provided protection for 56% of the animals ($P < 0.05$).

Five animals had grade 2 adhesions via a defect in the polymer. These defects probably resulted from an inadvertent tear during the placement of the stay suture. In these instances, the adhesion was strictly confined to the

tear in the polymer. These animals were included in the study in the grade 2 category.

The polymer was completely encapsulated in the grade 3 adhesions. Dissection through the matted bowel and fibrous connective tissue revealed an encapsulated polymer sheet. The polymer was not adhered to any of the tissues comprising the adhesion and in fact it could be easily removed with tissue forceps. The incidence of abscessation did not vary between control and polymer-treated groups.

During the course of the experimental design, four animals died from bowel strangulation within 24 hr as a complication of the surgical method. These animals were not included in the study.

DISCUSSION

The pathogenesis of adhesion formation has been described [1, 9, 21, 22]. Adhesions are the most common cause of intestinal obstructions in the industrialized nations. Greater than 80% of these adhesions are the result of previous abdominal surgery [21, 23]. Postmortem examinations reveal that at least 67% of people who have had a laparotomy developed adhesions as a direct result of this procedure. This figure rose to 93% for patients who experienced two or more procedures [1].

Much of the postoperative adhesion prevention work has been conducted utilizing models suited for the investigation of infertility. These models are designed to minimize hemorrhage and contamination. Therefore, most of the adhesion preventing substances tested to date are not effective when there is bleeding and bowel soilage. Studies evaluating synthetic, absorbable barriers to adhesion formation include Poloxamer 407, Surgicel, and Interceed (TC7) [6, 16]. While Surgicel provided minimal protection against adhesions, Interceed (TC7) has been proven to reduce the incidence and severity of postoperative adhesions. However, the efficacy of Interceed (TC7) was significantly reduced in the presence of blood and is contraindicated in the presence of frank infection [16]. While it has been demonstrated that Poloxamer 407 may have some hemostatic properties, the effectiveness in a contaminated setting has not been studied to date.

TABLE 2

Grading of Adhesions on Postmortem Examination

Grade	% Adhesions		
	Control	Gas	Steam
0	0	59 (17)*	40 (12)*
1	10 (3)	21 (6)*	16 (5)*
2	62 (18)	10 (3)*	13 (4)*
3	28 (8)	10 (3)*	30 (9)*

Note. (N) refers to the number of rats studied.

* $P < .05$ compared to control.

Minimal work has been done in the area of adhesions secondary to severe abdominal trauma or insult, especially when the abdominal cavity is contaminated with intestinal contents and blood. This model was designed with this principal in mind. The polypentapeptide of elastin was effective in this contaminated peritoneal wound. The incidence of significant adhesion was 28% for the polymer group versus 90% for control animals.

Exposure to heat results in a *cis-trans* isomerization change of the proline peptide in the poly(VPGVG) formulation [19]. However, this steam-induced alteration of the polymer configuration did not alter the adhesion prevention effectiveness of polymer in this model. There was not a significant difference in the effectiveness based on the sterilization technique.

The sheet form tested in this investigation was not self-adherent and not absorbed within 6 months (unpublished observations). At the end of the 2-week period the polymer was found floating freely within the peritoneal cavity. There was no gross or histological evidence of inflammation associated with the presence of the polymer. By these criteria, it would appear that the polymer is inert with respect to the abdominal cavity.

The polypentapeptide in sheet form has also been examined in a rabbit strabismus surgery model in which two compositions were compared: poly(VPFVF) and poly[3(VPGVG), (VPGFG)] [24]. Neither significant inflammation nor significant scarring occurred with either of the compositions, one placed surrounding the superior rectus muscle beneath the conjunctiva, where scarring always occurred in the controls. No fibrous membrane formed around poly(VPGVG) in a 2-month period whereas a fibrous membrane did form around poly[3(VPGVG), (VPGFG)] within 2 weeks. These initial studies showed poly(VPGVG) to be promising for the prevention of adhesions in the rabbit strabismus model with the current objective being to enhance the rate of degradation [25].

While the efficacy of the polymer sheets in this contaminated peritoneal model has been addressed, the practicality has not been discussed. Part of the problem in adhesion prevention is the fact that the surgeon can not accurately predict all areas of adhesion formation. This fact will limit the success of any physical barrier sheet. Another obstacle to the use of this formulation of polymer is that it is not self-adhering. Sutures were used to hold the sheets in the proper position. The presence of suture material acting as a nidus to adhesion formation has been well documented.

We are currently investigating the possibility of obtaining liquid and foam forms of the polypentapeptide of elastin. These formulations may have more potential applications than do the polymer sheets. Liquid and foam can be evenly dispersed within the abdominal cavity and act as a bath to keep abdominal organs separated. Like the polymer sheets, the liquid and foam formulations will also be acting as a physical barrier by

maintaining the separation of the serosal surfaces. Unlike the polymer sheets, an exact location of the adhesion formation would not be necessary, thereby making these formulations more useful in the prevention of generalized adhesions. If the serosal surfaces are kept separated during the first 72 hr postoperatively, adhesion formation will be significantly reduced [28]. Currently we are working with increasing the viscosity of these forms in an effort to slow absorption and maintain separation for the 72-hr period.

In conclusion, it appears that the polypentapeptide of elastin is an effective physical barrier to surgically induced adhesions in this animal model. This model also provided a source of bleeding and frank contamination to further assess the capabilities of the polymer in a trauma setting. The polymer may potentially be very useful in the prevention of postoperative intra-abdominal adhesions in contaminated wounds. Further studies are needed to completely evaluate the possible applications of this polypentapeptide of elastin in the area of wound healing and adhesion prevention.

ACKNOWLEDGMENTS

Naval Medical Research and Development Command, Work Unit No. 63706N MM33C30.008-1005. The opinions and assertions contained herein are the private ones of the writers and are not to be construed as official or reflecting the views of the Department or the Naval Service at large. This work was supported in part by Contracts N00014-89-C-0282 and N00014-90-C-0265 from the Department of the Navy, Office of Naval Research. The authors express their gratitude to Joan Stephens and Edd Olds for their efficient editorial assistance. Appreciation is also expressed to Dr. Betty Cox for her technical assistance. The experiments reported herein were conducted according to the principles set forth in the "Guide for the Care and Use of Laboratory Animals," Institute of Laboratory Animals Resources, National Research Council, DHEW, Pub. No. (NIH) 78-23.

REFERENCES

1. Weibel, M. A., and Majno, G. Peritoneal adhesions and their relation to abdominal surgery. *Am. J. Surg.* **126**: 345, 1973.
2. Menies, D., and Ellis, H. Intestinal obstruction from adhesions—How big is the problem? *Ann. R. Coll. Surg. Engl.* **72**: 60, 1990.
3. Gordji, M. Pelvic adhesions and sterility. *Acta Eur. Fertil.* **6**: 279, 1975.
4. Lehmann-Willenbrock, E., Mecke, H., and Riedel, H-H. Sequelae of appendectomy, with special reference to intra-abdominal adhesions, chronic abdominal pain, and infertility. *Gynecol. Obstet. Invest.* **29**: 241, 1990.
5. Davidson, M. M. Systemic administration of heparin and dicumarol for postoperative adhesion. *Arch. Surg.* **59**: 300, 1979.
6. Steinleitner, A., Lambert, H., Kazensky, C., and Cantor, B. Poloxamer 407 as an intraperitoneal barrier material for the prevention of postsurgical adhesion formation and reformation in rodent models for reproductive surgery. *Obstet. Gynecol.* **77**: 48, 1991.
7. Replogle, R. L., Johnson, R., and Gross, R. E. Prevention of postoperative intestinal adhesion with combined promethazine and dexamethasone therapy: Experimental and clinical studies. *Ann. Surg.* **163**: 580, 1966.

8. Horne, H. W., Clyman, M., Debrovner, C., Griggs, G., Kistner, R., Kosasa, T., Stevenson, C., and Taymor, M. The prevention of postoperative pelvic adhesions following conservative operative treatment of infertility. *Int. J. Fertil.* **18**: 109, 1973.
9. diZerega, G., and Hodgen, G. Prevention of postoperative tubal adhesions: Comparative study of commonly used agents. *Am. J. Obstet. Gynecol.* **136**: 173, 1980.
10. Koltai, J. L., and Gerhard, A. Intraperitoneal application of fibrinogen gluing in the rat for adhesions prophylaxis. *Prog. Ped. Surg.* **25**: 71, 1990.
11. Elkins, T. E., Bury, R. J., Ritter, J. L., Ling, F. W., Ahokas, R. A., Homsey, C. A., and Malinak, L. R. Adhesion prevention by solutions of sodium carboxymethylcellulose in the rat. I. *Fertil. Steril.* **41**: 926, 1984.
12. Oelsner, G., Graebe, R. A., Pan, S.-B., Haseltine, F. P., Barnea, E. R., Fakih, H., and DeCherney, A. H. Chondroitin sulphate: A new intraperitoneal treatment for postoperative adhesion prevention in the rabbit. *J. Reprod. Med.* **32**: 812, 1987.
13. James, D. C. O., Ellis, H., and Hugh, T. B. The effect of streptokinase on experimental adhesion formation. *J. Pathol. Bacteriol.* **90**: 279, 1965.
14. Soules, M. R., Dennis, L., Bosarge, A., and Moore, D. E. The prevention of postoperative pelvic adhesions: An animal study comparing barrier methods with dextran 70. *Am. J. Obstet. Gynecol.* **143**: 829, 1982.
15. Goldberg, E. P., Sheets, J. W., and Habal, M. B. Peritoneal adhesions: Prevention with the use of hydrophilic coatings. *Arch. Surg.* **115**: 776, 1980.
16. Interceed (TC7) Adhesion Barrier Study Group, "Prevention of postsurgical adhesions by INTERCEED (TC7), an absorbable adhesion barrier: A prospective, randomized multicenter clinical study. *Fertil. Steril.* **51**: 933, 1989.
17. Urry, D. W., Parker, T. M., Reid, M. C., and Gowda, D. Biocompatibility of the bioelastic materials, poly(GVGVP) and its g-irradiation cross-linked matrix: Summary of generic biological test results. *J. Bioact. Comp. Polym.* **6**: 263, 1991.
18. Urry, D. W., and Prasad, K. U. Syntheses, characterizations and medical uses of the polypentapeptide of elastin and its analogs. In D. F. Williams (Ed.), *Biocompatibility of Tissue Analogues*, Boca Raton, FL: CRC Press, 1985. Pp. 89-116.
19. Prasad, K. U., Iqbal, M. A., and Urry, D. W. Utilization of 1-hydroxybenzotriazole in mixed anhydride coupling reactions. *Int. J. Pept. Protein Res.* **25**: 408, 1985.
20. Nair, S. K., Bhat, I. K., and Aurora, A. L. Role of proteolytic enzymes in the prevention of post-operative intraperitoneal adhesions. *Arch. Surg.* **108**: 849, 1974.
21. Ellis, H. The causes and prevention of intestinal adhesions. *Br. J. Surg.* **69**: 241, 1982.
22. Ellis, H. The cause and prevention of postoperative intraperitoneal adhesions. *Surg. Gynecol. Obstet.* **133**: 497, 1971.
23. Ellis, H. Internal overhealing: The problem of intraperitoneal adhesions. *World J. Surg.* **4**: 303, 1980.
24. Elsas, F. J., and Urry, D. W. Synthetic polypeptide sleeve for strabismus surgery. *J. Pediatr. Ophthalmol. Strab.*, in press.
25. Urry, D. W., Gowda, D. C., Harris, C., Harris, R. D., and Cox, B. A. Development of bioelastic materials as biocompatible, transducible and degradable drug delivery matrices. *Polymer Preprints* **33**: 84, 1992.
26. Urry, D. W. Preprogrammed drug delivery systems using chemical triggers for drug release by mechanochemical coupling. *Sci. Eng.* **63**: 329, 1990.
27. Urry, D. W. Bioelastic materials as chemomechanically transducing ("Smart") matrices for drug delivery. In C. G. Gebelein, T. C. Cheng, and V. C. Yang (Eds.), *Cosmetic and Pharmaceutical Applications of Polymers*. New York: Plenum, 1991. Pp. 181-192.
28. Buckman, R. F., Woods, M., Sargent, L., and Gervin, A. S. A unifying pathogenetic mechanism in the etiology of intraperitoneal adhesions. *J. Surg. Res.* **20**: 1, 1976.

Accession For	
NTIS CRA&I	☒
DTIC TAB	☐
Unannounced	☐
Justification	
By	
Distribution /	
Availability Codes	
Dist	Avail and/or Special
A-1	20