The Creation of a Model for Ex Vivo Development of Postoperative Adhesions

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Abstract

Background: Postoperative adhesions occur in the overwhelming majority of patients after laparotomy and laparoscopy. The clinical consequences of adhesions can be devastating. Adhesions are believed to contribute to infertility and are the most common cause of small bowel obstruction and abdominal pelvic pain. Our objective is to develop a model for ex vivo development of postoperative adhesions. **Methods:** Peritoneal tissue strips were obtained from Sprague Dawley rats and were abraded by scraping the tissue surface with a scalpel. Tissues were incubated in a special medium, and visible adhesion bands were assessed. Experiments were performed in a university setting. **Results:** Adhesion bands were observed as early as 24 hours after abrasion. No adhesion bands were observed in the control tissue strips. **Conclusions:** This is a model system for the creation of actual adhesive bands utilizing an ex vivo culture of peritoneal strips.

Keywords

adhesions, abrasion, ex vivo model, peritoneum

Introduction

There is a major need for model systems for the assessment of the efficacy of potential adjuvants for the prevention of postoperative adhesion development. Much can be learned by cell culture systems of fibroblasts from normal peritoneum and adhesions, and specifically, it is possible to determine whether such agents can limit, prevent, or reverse acquisition of the adhesion phenotype.¹⁻⁴ As valuable as such systems are for early screening because of their relative low cost and potential for near simultaneous testing of multiple variants, such systems are limited by being isolated cell cultures.

Most typically, the next model system utilized (or historically, the first model system) is in vivo animal models.⁵ However, preclinical development of models for the determination of the efficacy of antiadhesion adjuvants is greatly limited by difficulty in achieving cost efficiency, reproducibility, and predictiveness of clinical outcomes. The latter is particularly difficult because of selective publication of animal and clinical studies, which demonstrate benefit of the tested adjuvant but rarely described agents or models that were not efficacious, as well as marked variability of parameters such as length and degree of tissue injury in models nonetheless given the same name and/or utilizing the same scoring system.⁵

In this study, we provide the initial description of an alternative model for the assessment of efficacy of adjuvants for the prevention/reduction of postoperative adhesion development. Namely, we describe a model system for the creation of actual adhesive bands utilizing an ex vivo culture of peritoneal strips.

Material and Methods

Media Preparation

To prepare 1 L of the media, 9.43 g of minimum essential medium (MEM) powder was dissolved in 900 mL water, and then 3.0 g glucose, 1.5 mg methionine, 5.2 mg leucine, and 72.5 mg lysine-HCl were added so that there was 4, 0.1, 0.1, and 1 times the usual concentrations of these substances found in the regular MEM. Additionally, 2.2 g of sodium bicarbonate (NaHCO₃), 10.0 mL of GIBCO MEM vitamin solution (Cat. No. 320-1120), 10.0 mL of GIBCO (Life Technologies, Inc., Grand Island, NY) nonessential amino acids solution (Cat. No. 320-1140), and 200.0 IU insulin were added to the MEM followed by the adjustment of the pH to 7.1 and 7.3. The volume was brought up to 1.0 L with water, and then 100 mL

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aliquots were filter sterilized (0.2 μ m) into autoclaved 500-mL medium bottles. Stock solutions of the MEM were stored at 4°C.

Animal Surgery and Tissue Collection

All animal procedures were approved by the Wayne State University Institutional Animal Care and Use Committee, Animal Welfare Assurance number A3310-01. Strips of tissue, including the peritoneum (0.5 $\times$ 1.0 cm), from 10 Sprague Daley female rats (200-250 g; Harlan, Indianapolis, Indiana) were cut from normal parietal peritoneal tissue from the anterior abdominal wall lateral to the midline incision. On average, peritoneum ranges from 80 to 100 μ m and was thus not separated from the abdominal tissue. Blood samples (0.5 mL) were obtained, from the same animal from which the tissue strips were obtained, by cardiac puncture and allowed to clot for 10 to 15 minutes before use. Strips were thoroughly washed with phosphate-buffered saline (Invitrogen, Carlsbad, California). Peritoneum on the tissue strips was abraded by evenly scraping the surface in 1 direction 10 times at a length of 0.5 cm inward from the end of the strip with a sharp, size 10 scalpel. Peritoneal strips without abrasion with and without blood clots were used as controls. Strips were folded in half, and ends were sutured with 4.0 vicryl sutures (Ethicon, Somerville, Massachusetts) to prevent the unfolding of the strip and to keep the peritoneal surfaces facing one another. Strips were immediately placed in a 100-mm cell culture dish with MEM media (GIBCO-BRL, Grand Island, Nebraska). Tissue strips were incubated in a regular cell culture incubator at 37°C and 5% CO₂ for up to 72 hours. The MEM media were supplemented with leucine, lysine, methionine, and sodium bicarbonate (NaHCO₃).

Results

Culture of peritoneal strips with surface approximated by sutures in media alone did not generate development of adhesive bands ($n = 2$). Similarly, placement of a blood clot between the approximated tissue surfaces in the absence of tissue abrasion also failed to generate the development of adhesive bands ($n = 2$).

In contrast, when blood clot was placed between the approximated edges following tissue abrasion, adhesive bands were identified at 24 hours, which at this early time point could already be dense and opaque, with some visible blood vessels formation (Figure 1; $n = 3$). Adhesive bands were also identified but at later time, at 48 hours, without the placement of the blot clot between the approximated edges following tissue abrasion ($n = 3$). All of the above-mentioned results were accurately reproduced in peritoneal strips obtained from all of the 10 rats used in this study. A single adhesive band was observed in abraded sites of all animals, with and without the placement of blood clots, with no observed differences in the surface area of involvement.

Figure 1. Ex vivo model of postoperative adhesions. A visible adhesion band between the 2 peritoneal surfaces can be seen.

Discussion

The ex vivo peritoneal strip adhesion model provides important information on the pathophysiology of adhesion development. First, it demonstrates that grossly visible adhesive bands can form within 24 hours and that at this early time point, it can already be dense and opaque (as opposed to filmy and transparent).⁶ Additionally, at this early time point, vessels can already be present, teleologically likely representing the tissue's attempt to establish vascular connections, by which to resupply oxygen and nutrients to the devascularized tissue.

Second, consistent with clinical teaching,⁷ the placement of a blood clot between peritoneal surfaces incited greater incidence, tissue block surface area involvement, and tenacity of the adhesion band(s) that developed. Thus, these findings are consistent with the concept of attempting to achieve hemostasis to minimize adhesion development. However, it should be noted that adhesions developed even in the absence of clot placement (and in a model where delayed bleeding at the surgical site or blood pooling from bleeding at other sites could not occur).

Third, based on our observations, the incidence, surface area of involvement, and tenacity of the adhesions that developed were greater following tissue traumatization (simulating tissue injury such as grasping with manipulation during the surgical procedure). These findings are consistent with the clinical observations of increased incidence of adhesion formation at sites of surgical procedures (type 1B sites) as opposed to sites that were not operated upon at prior procedures (type 1A de novo adhesions or so-called "incidental adhesions").⁸

This ex vivo peritoneal strip adhesion model additionally provides a potentially valuable approach to examine efficacy of postoperative adhesion adjuvants. Multiple strips can be obtained from each animal, thereby minimizing the number of animals needed for individual research projects as well as their associated costs. The multiple strips from each animal also allow for intra-animal paired comparisons of multiple different adjuvants as well as paired dose-response comparisons.

The latter may not be possible in in vivo trials with liquids or gels, which could affect surfaces throughout the abdominal cavity, or even with resorbable barriers in which peritoneal fluid would contain components of the resorbing material and thus exert potentially beneficial effects at sites distant from the location of placement.

Potential uses for this model in the future will be to assess the ability of adjuvants to reduce postoperative adhesion development. Such adjuvants could be devices such as barriers, drugs, or biologics (such as antisense, antibodies, small interfering RNAs, and adenoviral vectors) which could be added to the culture media or delivered in a barrier, which both has its own efficacy from physical separation of opposing surfaces, as well as prolonged delivery of the adjuvant to the sites of application (at the site of tissue injury/traumatization).

The model also provides the potential for study of the molecular biologic processes that lead to development of an adhesion or healing without it. Ex vivo specimens can be observed and harvested at desired time points or following identification of key end points (such as adhesion creation or vascularization) to more completely characterize molecular biologic manifestations of adhesions and the mechanism of impact of adjuvants on these processes. Additionally, this model is cost effective as multiple peritoneal strips can be harvested from 1 animal, thereby allowing testing of multiple arms of a study from each animal as opposed to each animal being assigned to only 1 arm.

Multiple in vivo models have been utilized to examine postoperative adhesion development and to assess the efficacy of adjuvants for the reduction of postoperative adhesions.^{9,10} For gynecological surgery models, a variety of species have been utilized including mice, rats, hamsters, rabbits, and pigs. Models have also utilized many intra-abdominal anatomical sites, including the ovaries, uterine horns, large bowel, and abdominal pelvic side walls. An additional variation is models for the initial creation of de novo (or new) adhesions, as opposed to models of adhesions reformation after adhesiolysis at a preceding surgical procedure.

There are many challenges to the creation and application of in vivo animal adhesion models.^{9,10} These include difficulty in creation of adhesions, such as exemplified by a study that required successive increasing amounts of tissue injury and manipulation in order to reproducibly create adhesions.¹¹ A second issue has been the ability of new research teams to adopt previously described models with variability in the success of creating adhesions. A third issue has been adoption of use of adhesion scoring systems by new investigative teams, with variation in grading in characteristics such as the extent, severity, and/or easability of separation of adhesions. Consequently, there has not been an ability to create an in vivo animal model of adhesion development, which has been accepted as the “gold standard” for animal in vivo adhesion models.

In summary, this article describes steps necessary for the establishment of a cost-effective, reproducible ex vivo model of adhesion development. There are several factors that may affect the characteristics of adhesions developed (incidence, extent, and severity), such as the force and length of the

abrasion and the quantity of blood that should be standardized for reproducibility of this model. Further studies will be needed in the future to determine the most appropriate timing and purposes of utilization of this model in the armamentarium of scientists attempting to develop approaches to prevent postoperative adhesion development.

Declaration of Conflicting Interests

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