# *In Vivo* Performance of Antibiotic Embedded Electrospun PCL Membranes for Prevention of Abdominal Adhesions

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**Abstract: The aim of this study was to prepare nonwoven materials from poly(ε-caprolactone) (PCL) and their antibiotic containing forms by electrospinning, so as to prevent postsurgery induced abdominal adhesions in rats. ε-Caprolactone was first polymerized by ring-opening polymerization, and then it was processed into matrices composed of nanofibers by electrospinning. A model antibiotic (Biteral®) was embedded within a group of PCL membranes. In the rat model, defects on the abdominal walls in the peritoneum were made to induce adhesion. The plain or antibiotic embedded PCL membranes were implanted on the right side of the abdominal wall. No membrane implantation was made on the left side of the abdominal wall that served as control. Macroscopical and histological evaluations showed that using these barriers reduces the extent, type, and tenacity of adhesion. The antibiotic embedded membranes significantly eliminated postsurgery abdominal adhesions, and also improved healing.** © 2006 Wiley Periodicals, Inc. J Biomed Mater Res Part B: Appl Biomater 81B: 530–543, 2007

**Keywords: abdominal adhesion; animal model; electrospinning; nonwoven membranes; biodegradable nanofibers; poly(ε-caprolactone)**

## INTRODUCTION

Abdominal adhesions are defined as pathological fibrotic bands developed between any surfaces in the peritoneal cavity.[1] Adhesion formation is a well-known complication of abdominal surgery, which not only renders future operations more difficult but also is the most common cause of small bowel obstruction, female infertility, and chronic debilitating pain.[2,3] Adhesion formation is estimated to occur in over 90% of all abdominal surgical procedures.[4] Trauma, foreign bodies, ischemia, and infections are major factors associated with the formation of postsurgery adhesions. Histological studies of experimental adhesion formation have demonstrated that adhesions result from the normal peritoneal wound healing response following surgery. It begins with tissue inflammation and fibrin deposition within an inflammatory exudate. The organization of fibrin with fibroblast invasion and collagen formation are followed by maturation of collagen forming a bridge between peritoneal surfaces.[5] Fibrous bands and newly formed capillaries remain at the site and these structures form the permanent fibrotic adhesions.[1]

A wide variety of approaches have been demonstrated in animal models and clinical practice to reduce or prevent adhesion by improving surgical procedures and using antiadhesion materials.[6–8] The use of fibrinolytic agents such as anticoagulants, anti-inflammatory agents, and antibiotics has also been invistigated.[9–11] One of the techniques that has been studied extensively and has demonstrated the most promising results is placing a physical barrier between the injured site and the adjacent tissues to prevent adhesion. With this barrier technique, surgically traumatized surfaces are kept covered during mesothelial regeneration, thus preventing adherence of adjacent structures and reducing adhesion formation.[12] It was shown that commercially available synthetic polymeric materials such as silicon and polytetrafluoroethylene sheets can effectively reduce/prevent adhesion.[13] However, such materials are nondegradable, and need to be removed by a second surgery, which is not desirable. It has been reported that a variety of polymer solutions such as hyaluronic acid,[14]

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dextran,[15] polyvinylpyrolidone, carboxymethylcellulose,[16] and polyethylene glycol[17] have been used as potential agents to prevent adhesion; however, their effectiveness is low in most cases.[9] Hyaluronic acid has been considered a good antiadhesive agent. However, it disappears from the injured site very quickly, which limits its efficacy as an adhesion preventative therapy.[18] Products using carboxymethylcellulose have also been reported to prevent adhesion formation in experimental models.[19,20] Seprafilm® is a bioresorbable membrane barrier composed of sodium hyaluronate and carboxymethylcellulose. Interceed® is a knitted fabric composed of oxidized regenerated cellulose. Although both Seprafilm® and Interceed® barriers have been shown to be safe and effective in all human clinical trials, their use does not eliminate adhesions in all patients.[8,13] Seprafilm® is claimed to have limitations in application and handling difficulties because of its poor mechanical properties within the surgical field. Similarly, the efficacy of Interceed® may be significantly reduced in the presence of blood, which is very frequently associated in the surgical setting.[21–23]

Besides the limitations of the existing commercial products, use of membranes is thought to be the most effective method for adhesion prevention. There are many ongoing developmental studies to design novel biomaterials that have less complications and high efficiencies. It is generally agreed that an antiadhesive membrane should be designed to stay at the injured site during the postoperative wound healing phase and should be absorbed by biodegradation, and also should have the following characteristics: mechanically strong for better handling and flexiblity, and should be biocompatible in general sense (nontoxic, nonallergic, nonmutagenic, noncancerogenic, etc.).[12,24]

Poly(ε-caprolactone) (PCL), a semicrystalline biodegradable polyester, belongs to poly(α-hyroxy acids) family. Its copolymers with lactides and glycolide are getting increasing attention because of their controllable biodegradation rates in the desirable ranges (usually slower) and also more suitable and tailor-made mechanical properties (usually more flexible and softer) for some applications such as long-term drug delivery and tissue repair and regeneration.[25–29]

One novel method of processing of biodegradable polymers including poly(α-hydroxy acids) is electrospinning, which is a unique method that produces polymer fibers with diameters usually in nanoscale and nonwoven fibrous structures composed of these fibers.[30–32] The processing of biomaterials by conventional means (such as film casting and foaming) often imposes several limitations in the optimization of their final properties. The PCL cast films are not suitable for cell scaffolding because they are not porous and do not allow cell ingrowth. In addition, the cast films can be too brittle to be handled. In contrast, electrospun nonwoven materials have small and controllable pore size, high porosity, and high surface area; therefore, they can be used in a wide variety of biomedical applications, such as for scaffolds in tissue engineering.[33–36]

In our recent related studies, we have first synthesized PCL with desirable molecular weight that can be electrospun into nonwoven membranes formed of PCL nanofibers. Details of polymers synthesis/characterization, and preparation/properties of the electrospun membranes were provided in our previous article.[37] Here, we attempted to use these membranes and their antibiotic embedded forms as mechanical barrier between surgically damaged surfaces to prevent post-surgery induced abdominal adhesions in rats.

## MATERIALS AND METHODS

### Membrane Preparation

ε-Caprolactone (Aldrich, Germany) was dried on a molecular sieve for about 24 h. The catalyst, stannous octoate (Sigma, USA), and other agents were analytical grade and used as received. The polymerization system and procedure has been described in detail elsewhere.[38] Briefly, polymerization was performed in a glass reactor under nitrogen atmosphere for 24 h at 120°C. The monomer/catalyst ratio was 1700:1 (mol/mol) in the homopolymerization of ε-caprolactone. Low-molecular-weight residuals were removed by a dissolution-precipitation method in which chloroform and methanol were used as the solvent and precipitant, respectively.

The number and weight average molecular weights ($M_n$ and $M_w$) and polydispersity index were determined by gel-permeation chromatography (Shimadzu, LC 10A, Japan) in chloroform at ambient temperature. Molecular weight of the polymer was determined relative to narrow molecular weight polystyrene standards.

Electrospun PCL membranes were fabricated according to procedures previously described.[37] Briefly, the membrane was prepared by using a PCL solution in a mixture of chloroform and dimethylformamide (DMF), with a PCL concentration of 13 g/100 mL and a DMF content of 70%. For the process of electrospinning, polymer solution was placed in a glass Pasteur pipette. The copper probe of the high voltage generator was inserted into the capillary. The grounded aluminum sheet was positioned opposite to the tip of the capillary at a distance of 10 cm. An electrical field of 13 kV was applied by a high voltage power supply (CPS, 2594). The fluid jet was ejected from the capillary. As the jet accelerated toward the grounded collector, the solvent evaporated and the polymer nanofibers were deposited on the collector in the form of a nonwoven fabric, that is, the PCL membrane.

### Membrane Characterization

The fiber morphology of the electrospun nanofibrous structures were investigated with a scanning electron microscope (SEM) (LEO, Supra 35VP). The SEM images were taken after the deposition of a conductive gold coating on the electrospun films with a sputter coater (Emitech, K950X, USA).

For mechanical tests, a Universal Test Machine (Lloyd Instruments, LR 5K Serensworth Fareham, UK) was used. The specimens that were cut from the nonwoven matrices ($0.5 \times 5$ cm$^2$ in size and $\sim 25$ μm in thickness) were uti-

lized in the tests in which a crosshead speed 5 mm/min at room temperature was applied.

### Drug Loading and *In Vitro* Release

The antibiotic that was used in this study was a commercial product, that is, Biteral® (Roche, France), which was a drug solution (in an ampule) containing 500 mg active substance (i.e., ornidazole) in 3 mL (900 mg absolute alcohol and 1600 mg propylene glycol) as reported in its prospectus. 0.15 mL drug solution (containing 25 mg ornidazole) was taken with an injector from the ampule and was dropped slowly (evenly distributed) on the each electrospun nonwoven membrane specimen ($2 \times 3$ cm$^2$ in size and $\sim 25$ $\mu$m in thickness). Note that because of their unique structure, the electrospun membranes absorbed the whole drug solution.

To obtain *in vitro* release kinetics, each drug-loaded electrospun membrane specimen was put in one flask containing 10 mL distilled water. Two milliliter solution was taken from the medium (and 2 mL fresh water was added) every 3 h in the first 12 h and then every 6 h for total 36 h. The drug released within the medium was measured spectrophotometrically at 270 nm. Five parallel studies were conducted to obtain the average release data.

### Animal Model and Surgical Protocols

Twenty seven Wistar-Albino female rats weighing between 250 and 300 g were used. All rats were fed food and water *ad libitum*. They were maintained in a temperature and humidity controlled environment at the Animal Research Center of Hacettepe University. The following study was conducted after receiving permission/approval from the Animal Ethical Committee of the University (Approval number: B.30.2.HAC.0.01.00.05, Approval date: April 1, 2004).

Sterile surgical technique was applied throughout the study. The electrospun nonwoven membranes produced in the previous step were cut into specimens ($2 \times 3$ cm$^2$ in size and approximately 25 $\mu$m in thickness) and sterilized by gamma radiation (2.5 Mrad). Animals were anesthetized by intraperitoneal injection with a mixture of ketamine HCl (Parke Davis, 50 mg/mL, Taiwan) and Rompun (Bayer, 2%, Germany). An area (about 15 cm$^2$) in the abdomen of the test animal was shaved and disinfected with Baticon solution (Droksan, 10%, Turkey). A longitudinal incision ($\sim 6$ cm long) was made on the midline of the abdominal wall using a blade (No. 11), and both abdominal walls (right and left side) were reflected and similar adhesion models were made on each of the abdominal walls. A $2 \times 3$ cm$^2$ template was put on the internal surface (on the peritoneum) of the abdominal wall, and the peritoneum was injured by creating vertical and horizontal lines, which formed about 10 small rectangles with roughly the same size, using a No. 11 blade. A tweezer was covered with a gauze, and the outer surfaces of the internal organs exactly seeing this defected abdominal wall area were abraded/brushed gently to trigger the adhesion process between these defected surfaces. The plain or antibiotic embedded PCL membrane was fixed with 6-0 prolene suture at four corners on the right side of the abdominal wall to cover the injured area. The left side of the abdominal wall was defected in a similar procedure but left as it is (no material was put), which served as control. Then, the middle line incision was closed using 4-0 silk suture.

### Macroscopical Evaluations

The test animals were sacrificed at the selected time intervals (after 14, 30, 45, 60, and 90 days of postimplantation), and the abdominal cavity was opened and the injured sites were first observed for the incidence of adhesion by naked eye. The adhered sites on the injured area were marked with a black marker. The tissue specimens ($2 \times 3$ cm$^2$) were surgically removed from both the membrane-treated (the tissue with the membrane) and the control (only the tissue) sites. The removed sites ($2 \times 3$ cm$^2$) were put onto milimetric papers to obtain the size of the marked area. The percent of adhesions were then calculated from these papers using the following data: the sum of the adhered area and the total specimen area. The extent, type, and tenacity of the adhesions were graded according to the adhesion scale described by Haney et al.[13]

A statistical analysis was done by using the macroscopical observations data with the Pearson's Chi-Square test. A $p$ value $< 0.05$ was considered significant.

### Histological Evaluations

Tissue specimens removed from the injured areas were fixed in 10% phosphate buffered formalin (pH 7.0) at room temperature, rinsed in buffer, and dehydrated in a graded series of ethanol before embedding in paraffin. Five- to seven-micrometer-thick sections were cut with a rotary microtome (Microm, HM 360, Germany). Hematoxylin and Eosin, and Mallory Trichrome stained sections were investigated for overall morphology, adhesion, and tissue response to the biomaterial. The stained sections (a minimum of 10 sections obtained from different levels of each tissue) were examined by at least two independent and blinded investigators with a Leica DMR microscope (Germany). The images were captured via Leica DC500 digital camera (Germany). Histological findings were evaluated and scored in two subcategories; consisting of cell and tissue morphology of capsule and the surrounding tissue components of the capsule, according to An and Friedman.[39] Mean of score of two independent investigators were taken. The scoring system is summarized in Table I. According to this system, tissue response that takes place in implant surrounding site (the capsule and the surrounding connective tissue) consisting of an acute and/or chronic inflammatory process was evaluated with its cellular content. The capsular fibroblastic layers were counted. Inflammatory cells (macrophages, polymorphonuclear leukocytes, lymphocytes, and plasma cells) locations, presence of giant cells, and the blood vessels were separately eval-

**TABLE I. Histological Scoring System Consists of Two Categories: 1st for the Capsule and 2nd for the Surrounding Tissue**

| | | Scores | | | |
|---|---|---|---|---|---|
| Parameters | | 4 | 3 | 2 | 1 |
| Capsule localization | | Capsule on two sides present | Capsule on one (lower) side present | Capsule on one (upper/dermis) side present | No capsule present |
| Capsule formation | | Dense | Loose fibroadipose or loose adipose | Loose fibroelastic | No capsule present |
| Capsule cellular features | Fibroblast thickness | More than 30 layers | 10–30 layers | 0–10 layers | 0 layer |
| | Fibroblast contacting surface | | | No | Yes |
| | Acute/chronic inflammatory process | | | Chronic | Acute |
| | Severity of inflammatory process | Severe | Moderate | Mild | None |
| Inflammatory cells location | Inflammatory cells location | End and middle | Middle | End | None |
| | Macrophages contacting surface | | | No | Yes |
| | Giant cells contacting surface | | | No | Yes |
| | Polymorphonuclear leucocytes contacting surface | | | No | Yes |
| | Plasma cells contacting surface | | | No | Yes |
| | Blood vessels present | | | No | Yes |
| Capsule surrounding tissues | Acute/chronic inflammatory process | | | Chronic | Acute |
| | Severity of inflammatory process | Severe | Moderate | Mild | None |
| | Macrophages | | | No | Yes |
| | Giant cells | | | No | Yes |
| | Polymorphonuclear leucocytes | | | No | Yes |
| | Plasma cells | | | No | Yes |
| | Blood vessels present | | | No | Yes |

uated. Total tissue response was scored and expressed as means ± standard deviations.

A statistical analysis was done by using the surrounding tissue and capsule formation scores with the Pearson's Chi-Square test. A $p$ value $< 0.05$ was considered significant.

## RESULTS

### Membrane Properties

On the basis of our previous studies, we selected one of the nonwoven PCL membranes that we produced from PCL with different molecular weights[37] and used it in our present study as a barrier material to prevent abdominal adhesions. $M_w$, $M_n$, and polydispersity index ($M_w/M_n$) of the PCL synthesized and used for preparation of this material (obtained by gel-permeation chromatography) are 84387, 51172, and 1.64, respectively. This homopolymer was dissolved in a mixture of chloroform (30%) and DMF (70%), with a concentration of 13 g/100 mL, and nonwoven membranes were prepared by electrospinning with an applied voltage and tube tip-collector distance of 13 kV and 10 cm, respectively. The mechanical properties, namely, elongation at break, ultimate strength, and Young modulus of this fibrous material (with a thickness of 25 $\mu$m) were 69.0%, 16.6 MPa, and 3.7 MPa, respectively. Figure 1(A,B) gives representative images (SEM and optical micrographs, respectively) of this membrane.

### Drug Loading and *In Vitro* Release

In the present study, 25 mg ornidazole ("Biteral®") was loaded in each membrane specimen by following a very simple and effective method as described before. Then, drug release from the electrospun membranes was followed by measuring the drug concentration within the medium (i.e., distilled water), spectrophotometrically. From the release data obtained spectrophotometrically, "percent release" was calculated, which was the amount of the drug release at certain

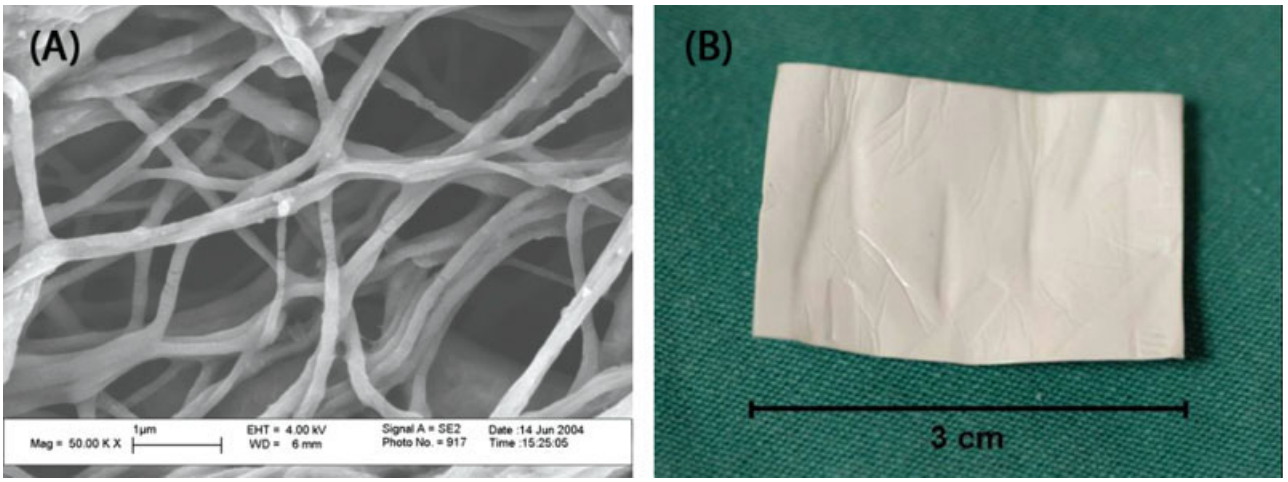


**Figure 1.** Representative images of the nonwoven electrospun PCL membrane: (A) a SEM micrograph and (B) an optical micrograph. [Color figure can be viewed in the online issue, which is available at www.interscience.wiley.com.]

time divided by the total amount (25 mg) multiplied by 100. The average values of five parallel studies and standard deviations are given in Figure 2. As seen here, about 80% of the drug was released in 3 h, and the release was completed almost in 18 h.

### Membrane Performance by Macroscopical Evaluation

In our animal model, defects were created by using $2 \times 3$ cm$^2$ templates in two sites in the abdominal cavity. The electrospun membranes were implanted in one site, and the other injured area was left as control. The adhesions, both at the control and membrane-implanted sites in the abdominal cavity of the experimental rats, were first evaluated macroscopically as described in the previous sections. The extent, type, and tenacity of the adhesions were graded on a scale to demonstrate the adhesion prevention efficacy of the membranes. The adhesions were investigated after 14, 30, 45, 60, and 90 days of postimplantation. The grades of adhesions for the control site and the sites in which the plain and antibiotic embedded PCL membranes were implanted are summarized in Table II, as the collection of this several time points. Some selected images of

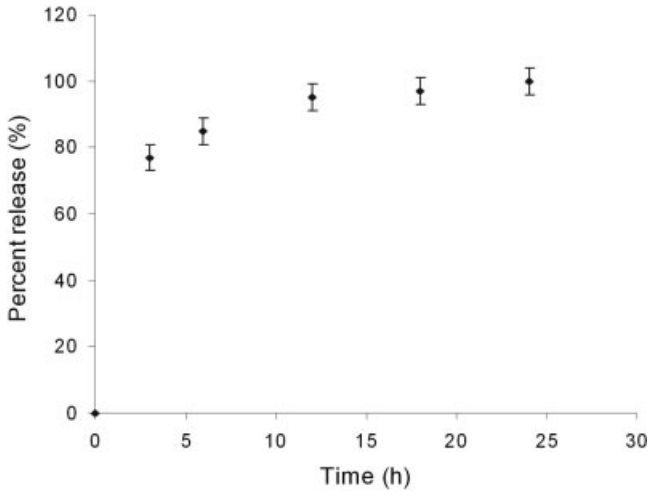


**Figure 2.** *In vitro* release of ornidazole from electrospun membranes.

**TABLE II. The Extent, Type, and Tenacity of the Adhesions on the Control Site and the Sites in Which the Plain and Antibiotic Embedded PCL Membranes Were Used**

| Grade | Control (%) ($n = 27$)[a] | Plain PCL (%) ($n = 14$)[a] | Antibiotic Embedded PCL (%) ($n = 13$)[a] |
|---|---|---|---|
| Extent (adhesion area) | | | |
| No adhesion | 40.7 | 14.3 | 46.1 |
| ≤25% | 37.0 | 64.3 | 38.5 |
| 25–50% | 18.5 | 21.4 | 15.4 |
| 50–75% | 0.0 | 0.0 | 0.0 |
| ≥75% | 3.8 | 0.0 | 0.0 |
| Type | | | |
| No adhesion | 40.8 | 14.3 | 46.1 |
| Filmy, transparent, avascular | 0.0 | 0.0 | 0.0 |
| Opaque, translucent, avascular | 0.0 | 14.3 | 15.4 |
| Opaque, capillaries present | 18.4 | 42.8 | 38.5 |
| Opaque, larger vessels present | 40.8 | 28.6 | 0.0 |
| Tenacity | | | |
| No adhesion | 40.7 | 14.3 | 46.1 |
| Adhesions fall apart | 0.0 | 14.3 | 7.8 |
| Adhesions lysed with traction | 3.7 | 50.0 | 46.1 |
| Adhesions sharply dissected | 55.6 | 21.4 | 0.0 |

[a] $n$, the number of the sites that were evaluated.

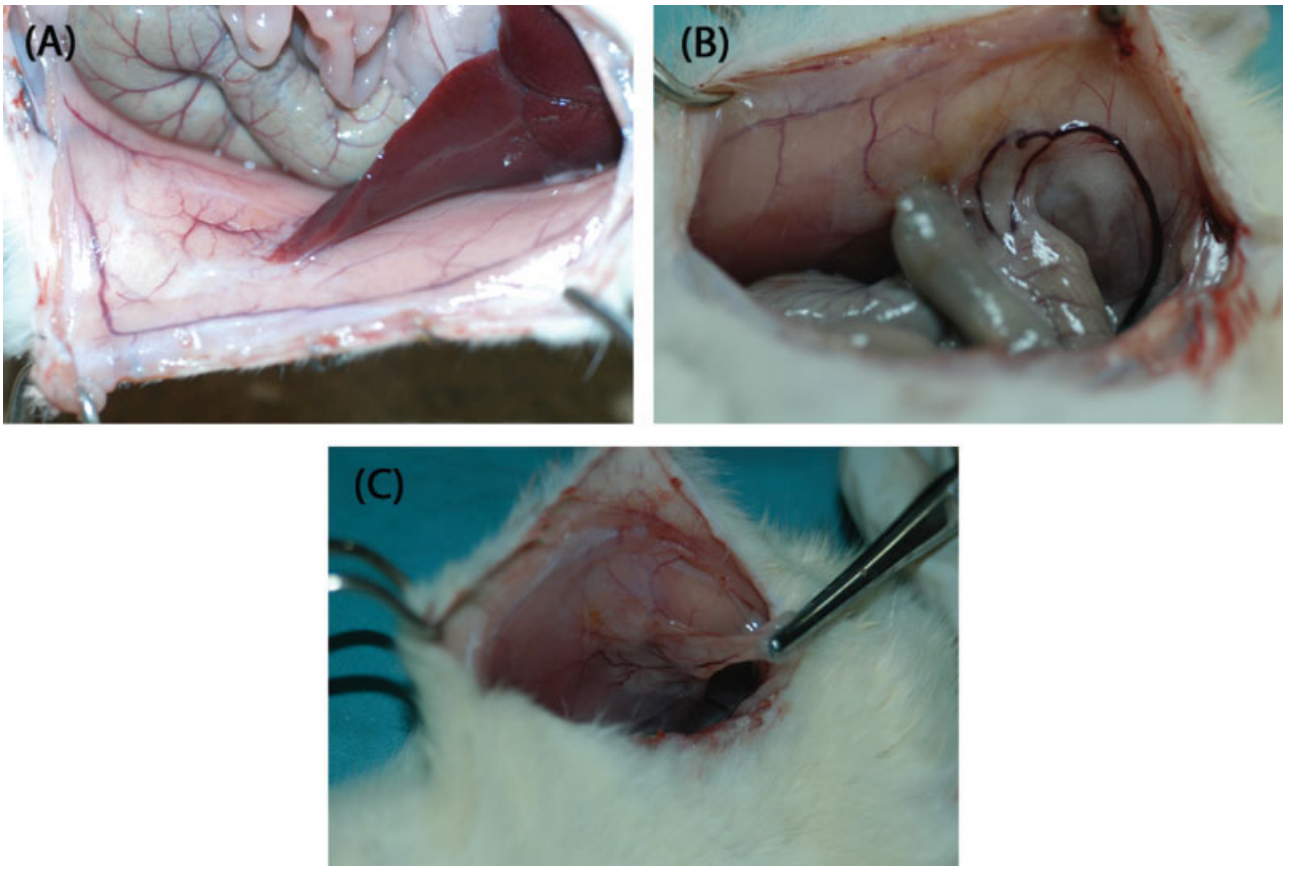


**Figure 3.** Representative images taken from the control site: (A) adhesion of liver; (B) adhesion of intestines; and (C) adhesion of omentum. [Color figure can be viewed in the online issue, which is available at www.interscience.wiley.com.]

the control and PCL membrane implanted sites are given in Figures 3 and 4, respectively.

Figure 3 shows that adhesions in the control sites involve mainly the omentum; however, in some of the cases, adhesions of intestine and liver were also observed. These adhesions were all over the dissected area and usually involved blood capillaries (18.4%) and especially larger blood vessels (40.8%) (Table II). The extent of adhesion was more than 75% in the 3.8% of the cases. Most of the present adhesions (55.6%) at the control site had a high strength that could be sharply dissected. In the sites in which the plain PCL membranes were used, almost 64.3% of the cases adhesion area was less than 25%. It is important that in most of the cases these were in the sutured sites (see e.g., in Figure 4), and the rest of the area was quite clean. In contrast to the control site, the adhesions were associated mostly with capillaries (42.8%); however, in the case of about 28.6%, there were also some larger vessels formed. Almost 50% of the cases adhesions were lysed with traction. But, 21.4% of the cases, adhesions were quite strong and therefore they were only separated by sharp dissections. The type and tenacity of antibiotic embedded PCL membranes group were significantly different; in 46.1% of the cases there was no adhesion. Only in the case of 38.5%, vascular formation was observed, but it was as capillaries (no large size vessel formation). Adhesions were quite weak and separated easily by traction. The type and tenacity results of both the plain and antibiotic embedded PCL membranes exhibited significant difference from the control group ($p < 0.05$), but the results of the extent of adhesions were not significantly different from the control group. However, it should be carefully noted that the adhesions observed with the membranes (with or without antibiotic loading) were mainly around the sutures.

### Histological Evaluation

Histological evaluations were done on the specimens as described in Animal Model and Surgical Protocols. Note that during the processing of the specimen, the polymer membrane was dissolved, leaving empty spaces on tissue sections. Evaluations were done according to the scoring technique given in Table I, and the results obtained are presented in Tables III and IV. Table III gives the results of total 14 animals sacrificed at different times (14–90 days), which were carrying the plain PCL membranes in which evaluations of the control site (Ctrl) were also presented for comparison. Table IV exhibits the results of 13 animals treated with the antibiotic embedded PCL membranes in a similar fashion. In these two tables, "$n$" shows the number of animals evaluated in each group. The "mean" is the average for these three,

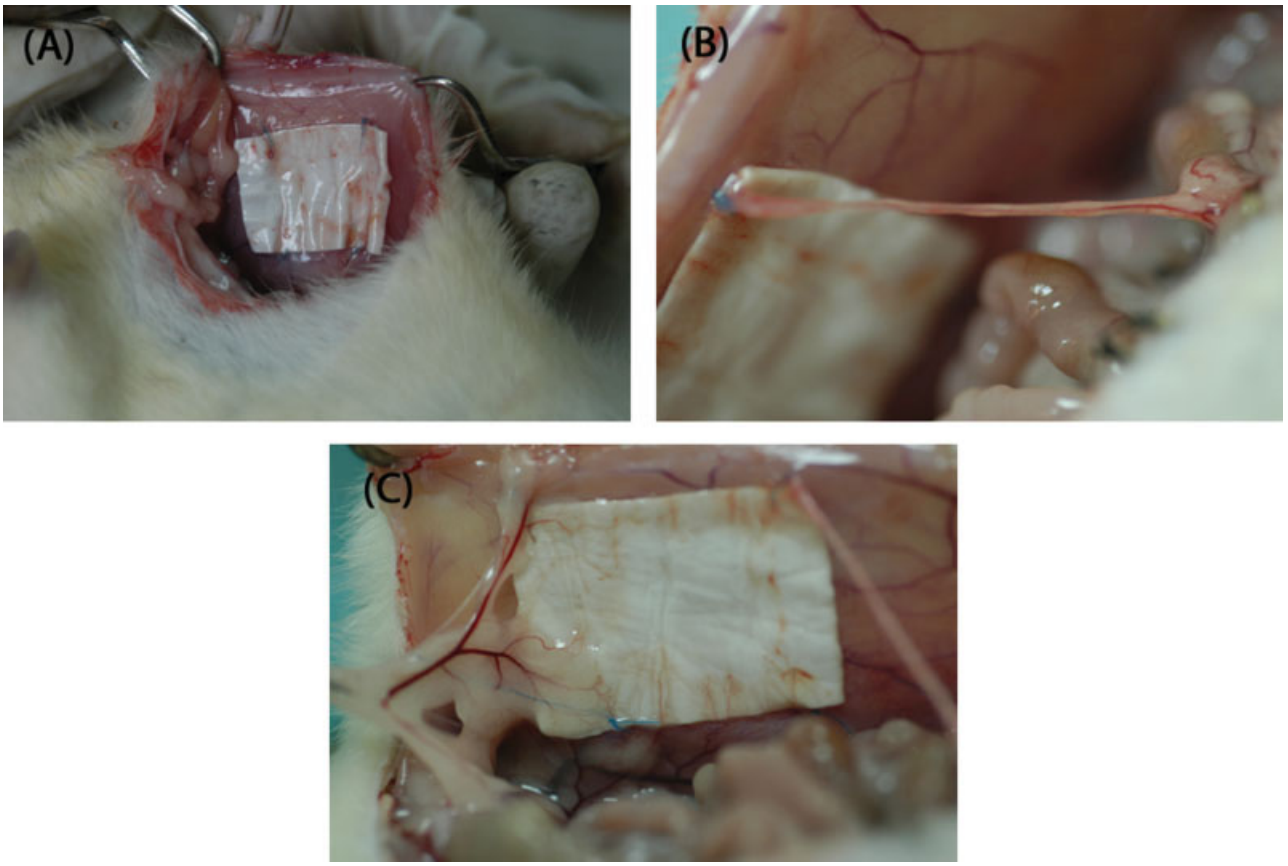


**Figure 4.** Representative images taken from the sites where PCL membranes were used: (A) no adhesion; (B) adhesion of omentum to the suture; and (C) adhesion of omentum. [Color figure can be viewed in the online issue, which is available at www.interscience.wiley.com.]

where the "standard deviations" as "±" were also given. Scorings were done for two categories, 1st and 2nd, which are for the capsule and surrounding tissue, respectively. Note that the control group has a constant score of 3 for the 1st category, because no PCL membrane was implanted and therefore no capsule formation occurred in these control sites of the animals.

As seen in both tables, the scores for the 1st category were high around 20–24, comparing with 2nd category, which were around 6–8. Scores were a little higher for the plain PCL membranes. Also, higher scores were obtained for the control sites in all cases. As a general tendency, the scores decreased with time, which means that tissue reaction toward the implant subsided in time. Parallel to the tissue reaction decrease, a general recovery in tissues was observed in all groups; the recovery of the antibiotic embedded implants shows better results than the others.

A series of images of histological sections taken at low magnifications to present the overall picture of the implantation site are given in Figure 5(A–O). High magnification micrographs showing the cellular details of connective tissue layer consisting of capsule and capsule surrounding tissue adjacent to the implant are presented in Figure 5(P–R).

Some important observations seen on these pictures can be summarized as follows: A moderately thick fibrous capsule was present around the polymer membrane in all cases at all time points. Both type of membranes (PCL and aPCL) were continuous with the surrounding tissue. However, no significant ingrowth of connective tissue cells into the polymer membranes was observed in any of the groups until the 60th day. Some scattered fibroblasts accompanied by phagocytic cells and few fibers were observed in the pores of the membranes on the 90th day, as seen in Figures 4(N) and 5(M).

The capsules were always rich in blood vessels and consist of several layers of spindle shaped fibroblasts and mononuclear phagocytes that were surrounding the PCL membranes at both sides [Figure 5(A,B,D,E,G,H,J,K,M,N,P,Q,R)]. The capsules were relatively thicker near the abdominal wall comparing with the peritoneal side [Figure 5(A,B,E,G,H,J,K,M,N)]. Phagocytes, lymphocytes, and to some extent polymorphonuclear leucocytes were present adjacent to the PCL membranes on day 14, 30, and 45; however, this inflammatory process was subsided later [Figure 5(P–R); Tables III and IV].

Neither necrosis nor foreign body reaction was noted in any of the samples at any time point. The thickness of the soft tissue that covered the membrane decreased from day 45 to 90, consisting of 3 to 10 loose fibroblast layers [Figure 5(P–R); Tables III and IV]. Some occasional phagocytic cells were observed in these fibroblastic layers, especially in the plain membrane implanted group on day 90.


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**TABLE III. Histological Evaluation for the Animals With the Plain PCL Membranes**

| | Plain PCL and Control | | | | | | | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | 14 days | | | | 30 days | | | | 45 days | | | | 60 days | | | | 90 days | | | |
| Animal Group/ | PCL | | Ctrl | | PCL | | Ctrl | | PCL | | Ctrl | | PCL | | Ctrl | | PCL | | Ctrl | |
| Categories | 1st | 2nd | 1st | 2nd | 1st | 2nd | 1st | 2nd | 1st | 2nd | 1st | 2nd | 1st | 2nd | 1st | 2nd | 1st | 2nd | 1st | 2nd |
| 1 | 24 | 8 | 3 | 9 | 21 | 7 | 3 | 7 | 20 | 6 | 3 | 6 | 19 | 6 | 3 | 7 | 19 | 6 | 3 | 6 |
| 2 | 23 | 7 | 3 | 9 | 22 | 8 | 3 | 7 | 20 | 6 | 3 | 7 | 20 | 8 | 3 | 6 | – | – | – | – |
| 3 | 22 | 7 | 3 | 10 | 21 | 7 | 3 | 8 | – | – | – | – | 19 | 6 | 3 | 6 | – | – | – | – |
| 4 | 22 | 7 | 3 | 10 | 22 | 7 | – | – | – | – | – | – | – | – | – | – | – | – | – | – |
| Mean ± SD | 22.8 ± 1.0 | 7.3 ± 0.5 | 3.0 ± 0.0 | 9.5 ± 0.6 | 21.5 ± 0.6 | 7.3 ± 0.5 | 3.0 ± 0.0 | 7.3 ± 0.6 | 20.0 ± 0.0 | 6.0 ± 0.0 | 3.0 ± 0.0 | 6.5 ± 0.7 | 19.3 ± 0.0 | 6.7 ± 1. 2 | 3.0 ± 0.0 | 6.3 ± 0.6 | 19.0 | 6.0 | 3.0 | 6.0 |
| *n* | 4 | | 4 | | 4 | | 3 | | 2 | | 2 | | 3 | | 3 | | 1 | | 1 | |

**TABLE IV. Histological Evaluation for the Animals With the Antibiotic Embedded PCL Membranes**

| | Antibiotic Embedded PCL and Control | | | | | | | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | 14 days | | | | 30 days | | | | 45 days | | | | 60 days | | | | 90 days | | | |
| Animal Group/ | PCL | | Ctrl | | PCL | | Ctrl | | PCL | | Ctrl | | PCL | | Ctrl | | PCL | | Ctrl | |
| Categories | 1st | 2nd | 1st | 2nd | 1st | 2nd | 1st | 2nd | 1st | 2nd | 1st | 2nd | 1st | 2nd | 1st | 2nd | 1st | 2nd | 1st | 2nd |
| 1 | 22 | 6 | 3 | 8 | 19 | 6 | 3 | 9 | 18 | 6 | 3 | 8 | 18 | 5 | 3 | 6 | 18 | 6 | 3 | 6 |
| 2 | 21 | 6 | 3 | 11 | 18 | 6 | 3 | 8 | 18 | 5 | 3 | 7 | 18 | 5 | 3 | 6 | – | – | – | – |
| 3 | 20 | 7 | 3 | 9 | 18 | 5 | 3 | 7 | 18 | 5 | 3 | 7 | 18 | 5 | 3 | 7 | – | – | – | – |
| 4 | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – |
| Mean ± SD | 21.0 ± 1.0 | 6.3 ± 0.6 | 3.0 ± 0.0 | 9.3 ± 1.5 | 18.3 ± 0.6 | 5.7 ± 0.6 | 3.0 ± 0.0 | 8.0 ± 1.0 | 18.0 ± 0.0 | 5.3 ± 0.6 | 3.0 ± 0.0 | 7.3 ± 0.6 | 18.0 ± 0.0 | 5.0 ± 0.0 | 3.0 ± 0.0 | 6.3 ± 0.6 | 18.0 | 6.0 | 3.0 | 6.0 |
| *n* | 3 | | 3 | | 3 | | 3 | | 3 | | 3 | | 3 | | 3 | | 1 | | 1 | |

The antibiotic embedded electrospun PCL membranes prevented abdominal adhesion to some extent. Note that histologic samples were obtained only from the adhesive regions if present. Adhesion generally involved mainly the omentum [Figure 5(D–F,I,K,N,O)], sometimes intestines and liver [Figure 5(C,H)], in both control and implant groups. The antibiotic embedded and plain membranes and their fibrous capsule were integrated into a thick fibroelastic and/or sometimes loose adipose connective tissue at the abdominal side. In control groups, the abdominal wall side similarly consisted of a thick fibrous capsule. Loose to adipose surrounding tissue directly attached to the peritoneal sheets of the abdominal muscles and to omental components at its external and internal sides, respectively.

Subacute inflammation was observed on day 14 [Figure 5(A, B,P); Tables III and IV]. Macrophages, lymphocytes, and small blood vessels were prominent around the membranes. From day 30 to 90, inflammatory cells were gradually replaced by fibroblasts, adipocytes, and collagen fibers; but vessels remained [Figure 5(D,E,G,H,J,K,P–R); Tables III and IV]. On day 90, a thin to moderate capsule continuous with a well-vascularized loose connective tissue was observed around the membranes, and inflammation almost subsided [Figure 5(M,N); Tables III and IV]. Chronic mononuclear phagocytic cell infiltrate locus was noted at the surrounding connective tissue, especially in the plain PCL membranes on day 30, 60, and even 90 (Tables III and IV).

## DISCUSSION

### Using Drug with Electrospun Membranes

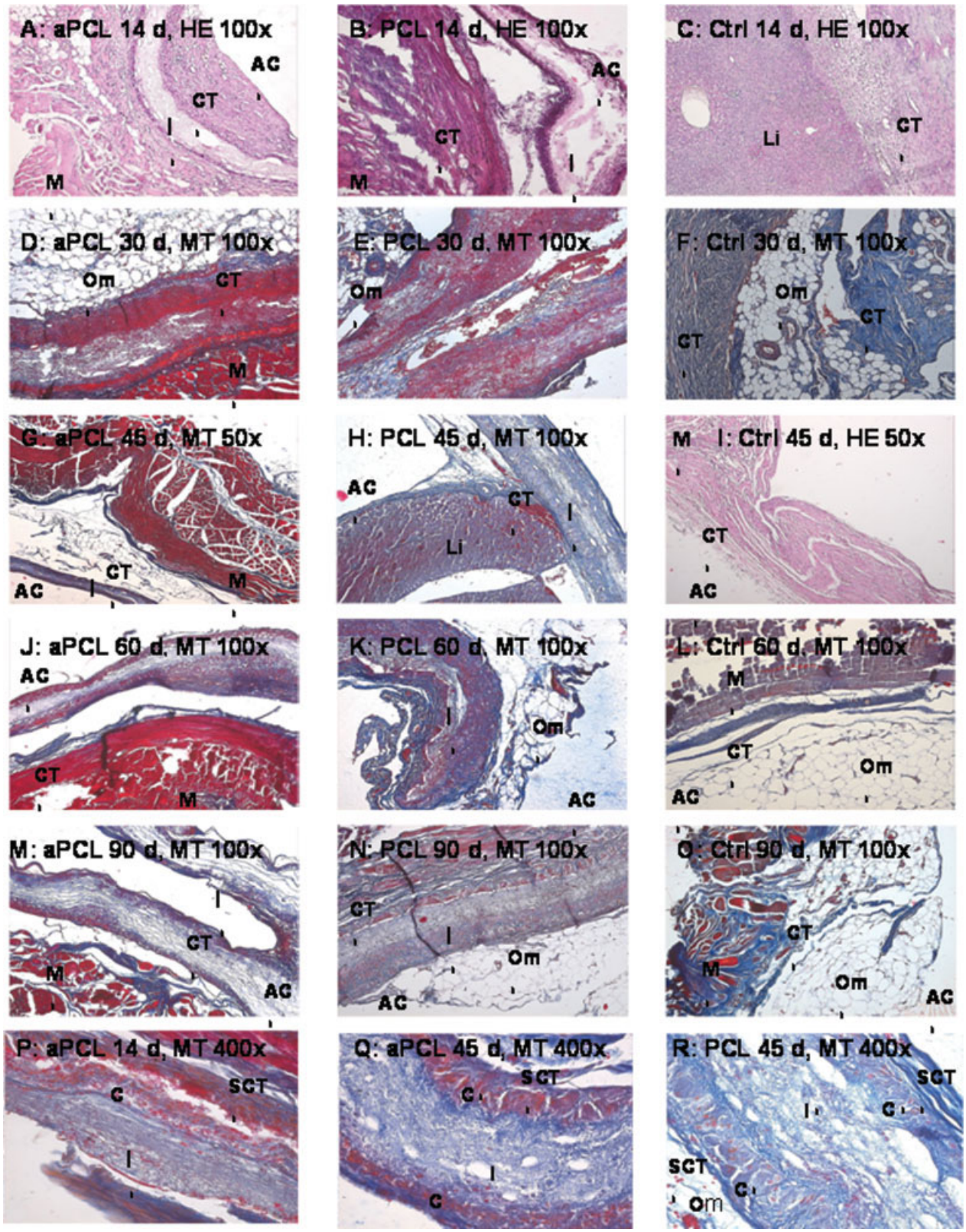
Intra-abdominal infection and abscess are frequently caused by microorganisms of gastrointestinal origin.[40] Effectiveness of several antibiotics used either systemically or in intraperitoneal irrigation fluids in preventing postoperative intra-abdominal adhesions has been studied by several groups.[41–47] It seems that the effects of the antibiotics may be related to the type of bacteria taking role in the pathogenesis of adhesions. Bacteria can accelerate and intensify adhesion formation by several means: (i) by disturbing the balance between fibrin deposition and fibrinolysis; (ii) by secreting enzymes that decrease the level of tissue plasminogen activators and increase the concentration of the related inhibitors; and (iii) by secreting inflammatory exudates and substances that limit blood, which causes ischemia and the migration of inflammatory cells.[47–50] Therefore, using combinations of antibiotics would be a better approach. However, the effects are still not clear and deserve further studies.

In the present study, we decided to use an antibiotic to investigate if there is a synergetic effect of using a barrier matrix with an antibiotic to reduce/prevent abdominal adhesions. The antibiotic that was selected as a model drug in this study is Biteral®. The active material is "ornidazole," 5-nitroimidazole derivative of "metronidazole," which is quite effective against several strains of intestinal microorganisms, and therefore is widely used in abdominal surgery in patients. In most cases, infections occur during the surgery. Hence, it is important to have the drug in the wound area just after the surgery (even before) to prevent infections and abdominal adhesions triggered by these microorganisms in that site. Therefore, it is widely accepted that burst-release is an ideal drug release profile for several medical applications including prevention of postoperation-induced adhesion because most infections occur within the first few hours after surgery. This was our aim in this study.

Note that loading the drug within the nanofibers of the electrospun matrices during electrospinning is possible. Even in this case, similar burst-type release could be observed.[51] Electrospun matrices with much slower drug release rates can be prepared by changing the formulations and protocol of electrospinning.[52] However, loading any drug during electrospinning makes the procedure more complex, and each material containing a different type of drug is usually considered as a new material, which makes the commercialization procedure longer, and much expensive. In our approach, we suggest that materials should be prepared separately and then combined with any type of drug (of mixtures of drugs) just before use. This is a very simple and practical approach, but should be applied if only a burst-type release is desired.

Selection of the amount of loading onto each electrospun membrane was another important concern. We assumed that 1000 mg (recommended daily dose) of the drug is used for about 70-kg patient, which is a systemic dosage, and can be applied several times for several days depending on the degree of infection. The animals used in this study were about 250–300 g, and our approach allows local administration; therefore, we decided to use 0.15 mL of drug solution (containing 25 mg ornidazole).

**Figure 5.** Some selected pictures representing the histological observations: for the antibiotic embedded PCL membranes [(A), (D), (G), (J), (M), (P), and (Q)]; for the plain PCL membranes [(B), (E), (H), (K), (N), and (R)]; and the control sites [(C), (F), (I), and (O)]. The symbols in these figures are as follow: PCL: plain PCL membrane; aPCL: antibiotic embedded PCL membrane; Ctrl: control site; HE: hematoxylin and eosin; MT: mallory trichrome; CT: connective tissue; C: capsule; SCT: capsule surrounding connective tissue; M: abdominal wall muscle; AC: Abdominal cavity; Om: omentum; Li: Liver; and I: Implant. The date of the specimen that was taken from the animal (for instance 90 d which meant on the 90 day) and magnification (e.g., 100×) are also given in these images. Note that muscles and the cells nuclei were stained in red and connective tissue collagen fibers and residuals of the membrane in blue.


A: aPCL 14 d, HE 100x
AC
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C: Ctrl 14 d, HE 100x
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**Figure 5**

To obtain *in vitro* release kinetics, we put each drug-loaded electrospun membrane specimen in one flask containing 10 mL distilled water, and followed the drug release spectrophotometrically. This was of course not the best model of release study to mimic the situation *in vivo*, since we do not know the exact consumption rate of the drug in the abdominal cavity that would of course significantly control the drug release (because it is by diffusion). However, we thought that these tests would at least allow us to demonstrate if the release was fast enough or not, as we have expected/aimed.

As shown in Figure 2, the drug release was quite fast, like a typical burst-type release that was expected. Note that Zong et al. have reported similar release kinetics for mefoxin delivery with their electrospun materials, in which drug was loaded during electrospinning process.[51] They showed that most of the drug was released because of the concentration gradient in 3 h, and the release was completed in the first 48 h. They claimed that a large amount of drug molecules is aggregated on or near the fiber surface by electrospinning; therefore, they have reached burst-type release, similar to what we have observed in this study.

### Performances of Electrospun Membranes in Animal Model

Creating reproducible wounds/injuries on the abdominal wall to study surgical adhesions in animal models is an important consideration to obtain statistically meaningful results. There are several approaches in the related literature, not only one that are agreed on and applied. For instance, Zong et al. excised a $1 \times 1$ cm$^2$ of abdominal wall muscle to create a cecal wound. The celiotomy was then closed in two layers immediately (control), after a barrier was laid in between the cecum and the abdominal wall.[53] Wang et al. stripped a $3 \times 4$ cm$^2$ patch of parietal peritoneum corresponding to the cecal and also scraped an area of about $3 \times 4$ cm$^2$ at both sides of peritoneum along the abdomen incision.[54] Pestieau et al. first made a midline incision in the abdomen using a scalpel.[55] Then, they burned a 2-cm circle of parietal peritoneum located laterally 2 cm from the incision line and directly opposite the cecum by electrocoagulation until there was a uniform coagulum of abdominal surface. In our animal models, defects were created by using $2 \times 3$ cm$^2$ templates. Injuries as small rectangular shapes were created on the abdominal wall at both the control and membrane-implanted sites as described in the previous sections, which were quite reproducible, and we were very careful to reach high reproducibility.

### Macroscopical Observations

The adhesions, both at the control and membrane-implanted sites, in the abdominal cavity of the experimental rats were first evaluated macroscopically to obtain the extent, type, and tenacity of the adhesions. As mentioned before, the adhesions were investigated after selected time periods up to 90 days of postimplantation. It should be noted that the macroscopical observations did not give clear idea about the progress of intra-abdominal adhesion formation and prevention. Therefore, in these macroscopical examinations, we have examined the results by pooling the observations obtained at different times. The progress of adhesion formation/prevention has been evaluated by only microscopical (histological) observations/tests given in the later part.

As demonstrated in detail in the previous section, we have observed severe and strong adhesions in the control sites, all over the dissected area, involving not only the omentum but also, in some cases, intestine and liver. We have observed blood capillaries and especially larger blood vessels in the control site. Covering the injured area with the plain PCL membranes reduced the overall adhesion profile. Interestingly, most of the adhesions were around the sutured sites, and the rest of the area was quite clean. However, even in this case, there were still blood vessels that were mostly as capillaries in contrast to the control site. Using the antibiotic with the barrier membrane significantly reduced the extent of adhesion and there were only capillaries. In addition, the adhesions were quite weak and, therefore, separated easily by traction. However, there were still adhesions around the sutures.

It should be noted that the electrospun PCL membranes produced in this study are hydrophobic, soft, elastic, very light like a butterfly wing, and comfortable but mechanically strong enough to handle; therefore, we were able to place them easily/tightly on the wound surface during surgical use because of their unique nonwoven nanofibrous structure.[37] However, by considering the experience of Zong et al. (who pointed out the shrinkage problem of hydrophobic PLGA electrospun membranes because of their nanofibrous structures, which lead displacement of the barrier from the abdominal wall) regarding electrospun membranes,[53] we decided to suture our PCL membranes to eliminate the risk of displacement that may occur in the long-term stay in the abdominal cavity of the animals. But the data discussed above clearly showed that this was not a very good choice, and created problems. It seems that in these kind of surgery these barriers should not be sutured, may be only dressed on the defected area by using some kind of tissue glue, or some other means.

The mechanism of postsurgical peritoneal adhesion formation and reformation remain poorly understood. The experimental evidence suggest that adhesions form between two surgically traumatized surfaces in natural apposition during the healing interval because it is more efficient to combine two sites of tissue repair into a single healing site, resulting in coalescing adhesions between peritoneal surfaces.[56] In theory, surgical barriers separate contacting peritoneal surfaces during healing to enable reconstitution of the surgically traumatized surfaces separately without coalescing adhesions. However, according to our results, when we compared the macroscopic observations for the plain and antibiotic embedded PCL membranes, it seems that placing only a physical barrier is not enough to prevent adhesion. This was also one of our initial concerns; therefore, we have used an antibiotic. The electrospun membranes were loaded (embedded) with the antibiotic with a very easy technique,

by dropping the antibiotic solution on the membranes just before the surgery.

Our macroscopic observations demonstrated that using antibiotics with this type of physical barrier (the electrospun membrane) is quite an effective way of treatment. It seems that the microorganisms may contaminate the wound area during the surgical operations and play a very important role in the adhesion process. In our case, the antibiotic molecules, which were loaded in the matrix by simple absorption, were released (diffuse out) quite easily from the matrix and create a burst effect in the abdominal cavity, which most probably prevented infections in the wounds locally in the earlier phase of healing process. Therefore, this fast release in the beginning of healing strongly affected the success of our barrier system. Zong et al. have also reached a similar conclusion in their animal studies with a very similar approach on postsurgical adhesions.[53]

### Histological Evaluations

Histological evaluations were done according to the scoring technique (see Table I). A series of images of histological sections have also been taken to present the overall picture of the implantation site. As we have also observed in our previous study, the PCL membranes maintain their integrity (both appearance and mechanical properties) in 90 days *in vivo* even if the $M_w$ drops from about 85,000 to 62,000.[37] During routine histological processing, the PCL membranes partly dissolve by well-shaped blocks from different regions. There were no cells or tissue components neighboring these regions. Note that if there were particle-form degradation products around, we would observe these particles that are almost always surrounded by reactive and regenerating cells.

Abdominal wall healing was slower in the control sites compared with the sites where the PCL membranes were used. Healing process improved with the antibiotic embedded PCL membranes. Both a decrease in the the tissue reaction and a general recovery in tissues were observed in all groups; the recovery of the antibiotic-containing implants showed better results than the others. These findings were in accordance with macroscopical observations. Statistical analysis done by using the surrounding tissue showed that only the membranes containing antibiotics were significantly different from the control group ($p < 0.05$). Since no PCL membrane was implanted to the control site, no capsule formation was observed in the control sites of the animals. The comparison of capsule scores of PCL and aPCL groups exhibited that there is a significance difference between these two ($p < 0.05$). These analyses clearly demonstrated that the antibiotic-containing PCL membranes are successful in the prevention of abdominal adhesions. Note once again that the undesirable events observed both in macroscopical and histological examinations were around the suturing area. Most probably, without suturing, much successful (statistically significant) improvements may be obtained even with the plain PCL membranes, as mentioned earlier.

## CONCLUSIONS

PCL, as a biocompatible and biodegradable polymer, is a very good candidate in the preparation of polymeric implants for several biomedical applications. It can easily be polymerized by ring-opening polymerization with different molecular weights and therefore with different *in vivo* degradation rates.[25–27,37,38] Nonwoven PCL membranes (or films) can easily be prepared with different thicknesses which can be formed from nanofibers with different diameters, therefore with different degradation rates.[30,31,33–35,37,53] Recently, we also synthesized PCL with different molecular weights and prepared a series of nonwoven membranes by electrospinning.[37] Here, we investigated their possible use as a physical barrier for prevention of adhesions after abdominal surgery. We thought that they will be a very suitable matrix for these types of biomedical applications because of their excellent physical properties, such as elasticity, softness, and lightness. Note that they were strong enough to be handled. One important advantage of these nonwoven matrices was their high absorption capacities. We were able to load antibiotics easily by simply dropping antibiotic solution on the membranes. Note that the others use more sophisticated methods for drug loading within the polymeric materials, such as they do electrospun polymer fibers with the antibiotic solutions.[53]

Both macroscopical and histological observations showed that the PCL membranes reduce abdominal adhesions and even if we observe some adhesions they were loose. Antibiotic loading significantly affected the type of adhesion and the tenacity. In the case of antibiotic-loaded PCL membranes, only capillaries were formed which were mostly on the edges of the membrane in which sutures were applied. The statistical analysis done both by using the macroscopical and histological observations exhibited that only the PCL membranes carrying antibiotic are significantly different from the controlled sites. However, we believe that this is due to suturing. These observations clearly demonstrated that using the plain PCL membranes changes the type and tenacity of adhesion in a positive direction and improves healing. The suturing should be eliminated in the use of synthetic barriers. Antibiotics should be included in the formulations. The use of membrane and antibiotic together synergistically affects the healing processes and makes the process better and faster, and reduces abdominal adhesions.

The biodegradation rate of our membranes is too slow comparing the healing rate in the abdominal cavity; therefore, we concluded that thinner PCL membranes formed of nanofibers with smaller diameters, and made of PCL with much lower molecular weights, should be used to match the healing rate *in vivo*. Our related studies in this direction for production of PCL membranes (and/or their copolymers with lactides) are under investigation before the clinical applications.

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