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Application of Polycaprolactone as an Anti-Adhesion Biomaterial Film

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Abstract: Adhesions are unavoidable consequences of surgery and other trauma. How to prevent the adhesions remains a big issue in healthcare system. The objective of this study is to test the efficacy of polycaprolactone (PCL) films as physical barriers in reducing postoperative intra-abdominal adhesions in the rat cecum-abdominal wall model. PCL is quite cheap compared with the agents recently used in the market. The fabrication method is also very easy to perform. Scanning electron microscope (SEM) showed multiple pores over upper and bottom surfaces but too small to permit cells to migrate from one surface onto another surface. Those pores were proven to be not interconnected. The PCL film did not show any evidence of cytotoxic effects as it did not induce any significant increase in cytoplasmic lactate dehydrogenase release from the NIH3T3 cells that it came in contact with. In animal studies, the PCL films led to fewer adhesions than Seprafilm in rat adhesion model. PCL films were efficacious in reducing postoperative intra-abdominal adhesion formation in rat cecum-abdominal wall models. **Key Words:** Polycaprolactone—Adhesion—Barrier.

Adhesion formation is usually associated with tissue trauma, ischemia, foreign-body reaction, infection, and hemorrhage (1), which means that these conditions may lead to the problems of adhesion formation. Although adhesion is a physiologically inevitable and important part of wound healing, undesirable postsurgical adhesions can cause serious complications including pain, functional obstruction, and harder second surgeries (2,3).

To reduce postsurgical adhesion formation, fibrinolytic agents, anticoagulants, anti-inflammatory agents, and antibiotics have been used (4). However, these agents alone cannot prevent adhesion formation effectively because clearance occurs too rapidly.

Recently, a variety of bioresorbable anti-adhesion barriers have been developed. Such products, approved by the US Food and Drug Administration, included Interceed (Johnson & Johnson, New Brunswick, NJ, USA), Seprafilm (Genzyme Corporation, Cambridge, MA, USA), and Intergel (Lifecore, Chaska, MN, USA) (5). None of them are fully satisfactory when used in clinical practice, and their high cost is another problem.

Polycaprolactone (PCL) is a biodegradable polyester with a low melting point of around 60°C and a glass transition temperature of about –60°C. PCL has potential applications for bone and cartilage repair and holds certain advantages over other polymers such as polylactic acid. These advantages are that: (i) it is more stable in ambient conditions; (ii) it is significantly less expensive; and (iii) it is readily available in large quantities (6).

PCL is derived by chemical synthesis from crude oil. The first step in biodegradation is hydrolysis of the amorphous phase followed by enzymatic degradation. Complete biodegradation of polycaprolactone takes place after 2 months (7).

Because of the friendly cost, easy fabrication, lasting long enough before degradation, and being commonly used in the market, PCL is chosen to be applied to prevent the postsurgical adhesion. In this article, PCL film is fabricated by solvent casting, and the characteristics of the PCL film are identified by scanning electron microscope (SEM) for surface

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morphology, contact angle for hydrophilicity, and lactate dehydrogenase (LDH) assay for cytotoxicity. Animal studies were performed to compare the effect of PCL film, Seprafilm, and a control group.

MATERIALS AND METHODS

Preparation of PCL film

In this study, a 3% (W/W) PCL (Aldrich, Germany, MW 80,00) was prepared by dissolving the PCL in ethyl acetate (Walko, Osaka, Japan), stirring it with an electric magnetic bar, and heating it to 50°C. After the PCL completely dissolved, the solution (600 μL) was poured onto the surface of 1.5 × 1.5 cm cover glass and evaporated at 45°C, 1 atm for 3 days to obtain the dry film. The films were sterilized by ultraviolet radiation.

SEM of PCL film

For SEM imaging, the PCL films were sputter coated with Au and the images were obtained from JSM-5600 (JEOL, Tokyo, Japan) at an acceleration voltage of 15 kV. Both surfaces of the films were checked simultaneously.

Hydrophilicity test

Contact angle was used to measure the hydrophilicity of the film to water. A drop of water was put onto the surfaces of slide glass, PCL film, and Seprafilm. A digital camera placed perpendicular to the water drop was used to take a picture of the film. The larger the angle, the less hydrophilic the film was assumed.

Cytotoxicity test

The cytotoxicity of the anti-adhesion film was determined from the amounts of cytoplasmic LDH released by the cells incubated with the polymeric films under investigation. LDH is a soluble cytosolic enzyme that is released into the culture medium following the loss of membrane integrity resulting from either apoptosis or necrosis. LDH activity, therefore, can be used as an indicator of cell membrane integrity and serves as a general means to assess cytotoxicity resulting from chemical compounds or environmental toxic factors. The increase of the LDH activity in culture supernatant is proportional to the number of lysed cells. First, PCL film and Seprafilm were separately immersed in the cell culture medium in 48-well tissue culture plates. Aliquots of 400 μL NIH3T3 cell suspensions were added to each well at a density of 4×10^4 cells/mL, and incubated at 37°C. After a designated incubation period, the medium was aspirated and centrifuged at $250 \times g$ for 10 min. Supernatant (100 μL) was taken from each well, mixed with 100 μL of the reagent (LDH-Cytotoxicity Assay Kit, BioVision, Mountain View, CA, USA) and then incubated in a 96-well plate for 30 min at room temperature. The absorbance of the reaction mixture at 490–500 nm was measured using a microtiter plate reader with reference wavelength at 600 nm. The amounts of LDH released from the cells cultured on a 48-well plate were used as the background release, whereas those amounts released from the cells lysed with 1% Triton X-100 were used as the positive control. The cytotoxicity (%) of the sample was calculated by the following equation:

$$\text{Cytotoxicity (\%)} = ([\text{test sample} - \text{background release})/(\text{positive control} - \text{background release}]) \times 100\%:$$

For each experimental value, four independent experiments were conducted.

Animal experiments

A total of 30 Wistar rats (200–250 g) were used for this study and divided into three groups. The animals were anesthetized with Zoletil-50 (Vibrac Lab, Carros, France) (1 mg/100 g). The abdomen was swabbed with 70% alcohol and iodine. A 3-cm-long vertical midline incision was made, and the distal 3 cm of the cecum and opposing abdominal wall were scraped with a scalpel blade carefully until the serosal surface was disrupted and hemorrhaged, but not perforated. The denuded peritoneal wall was then covered with either PCL (1.2 cm × 1.2 cm) or Seprafilm (1.2 cm × 1.2 cm). The PCL film was fixed to the serosa with two 4/0 Vicryl stitches (Vicryl, West Somerville, NJ, USA). Rats in the control group were not covered with any anti-adhesion film. The denuded cecum and opposing peritoneal wall were maintained in all animal groups with two nonoccluding loops of 4/0 polypropylene suture placed 2 cm apart. The purpose of the sutures was to approximate the abraded areas and to fix the floppy rat cecum. Care was taken in preventing puncture of the cecal wall with these sutures. After completion of the procedure, the abdomen was closed in a double layer using 4/0 polypropylene in a continuous fashion.

After 1 week, the rats were sacrificed by carbon dioxide asphyxiation. Adhesions were scored in a blinded manner according to the method of Zuhlke et al. (8) (Table 1), whereby grade 0 means no adhesions and grade 4 means firm extensive adhesions that are only dissectable with sharp instruments and almost unavoidable organ damage.

Statistical analysis

All quantitative results were obtained from triplicate samples. Data were expressed as the means.

TABLE 1. *Postoperative adhesions grading scale*

0	No adhesions
1	Filmy, fibrin adhesions, easily removed by blunt dissection (mild)
2	Fibrous adhesions, easily dissected (moderate)
3	Thick fibrous adhesions, dissectable (severe)
4	Thick fibrous adhesions, not dissectable without damage to the adherent tissue (very severe)

Statistical analysis was carried out using unpaired Student's *t*-test. A value of $P < 0.05$ was considered to be statistically significant.

RESULTS

SEM for PCL

There were multiple pores noted in the PCL film. The size of the pores om the undersurface is around 5–15 μm in length and 2–4 μm in width. The size of the pores on the upper surface is around 2–30 μm in length and 2–8 μm in width (Fig. 1). These pores are not interconnected, which is proved by pouring some amount of water onto the film, and the underneath paper does not get wet.

Hydrophilicity test

According to the contact angle measurement, Seprafilm is quickly hydrated and becomes flattened on the glass surface. The contact angle of water drop on glass is 88° and that of PCL film is 97.5°. PCL film is more hydrophobic than glass. The water contact angle reflects the superficial property of a material. Hence, the existence of hydrophilic–hydrophobic domains on a material surface will have great influence on its value.

Cytotoxicity test

Material cytotoxicity of a test specimen is measured by the release of LDH from cells incubated with it. Results from such a cytotoxicity test for the PCL film and Seprafilm are compared in Fig. 2. As shown in the figure, the cytotoxicity indices are around 20% for both membranes after 6 and 24 h of direct contact with NIH3T3 cells, the Seprafilm films, and PCL films. When compared with positive control, there is no statistical difference in the release of LDH, indicating that both PCL and Seprafilm are nontoxic to the cells.

Animal study

Tissue adhesion between the cecum and the peritoneum was examined on the 7th day after surgery. For the control group, adhesion of the cecum to the peritoneal wall was found in all rats operated with a score between 2 and 4 (mean is 3.1) (Fig. 3 and Table 2). For the Seprafilm group, three out of 10 showed adhesion with score between 0 and 2 (mean was 0.4). For the PCL film group, just one out of 10 showed adhesion with score between 0 and 1 (mean is 0.1).

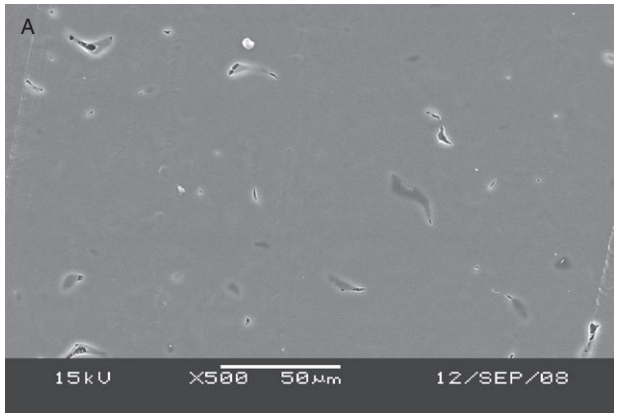

FIG. 1. (A) SEM for bottom surface of PCL film showed that there were small pores around 5–15 μm in length and 2–4 μm in width. (B) SEM for upper surface of PCL film showed that there were rougher appearance and slightly bigger pores, 2–30 μm in length and 2–8 μm in width.

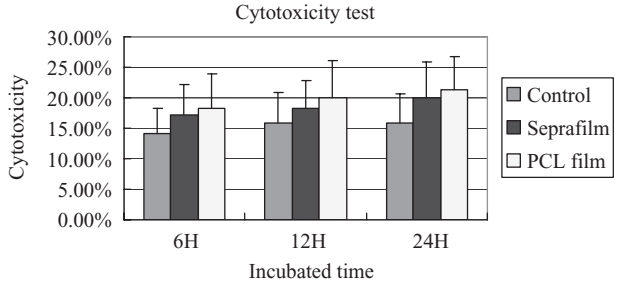

FIG. 2. Cytotoxicity of Seprafilm and PCL film. The LDH assay indicated the cytotoxicity of the films. Data represented the mean value of the tests.

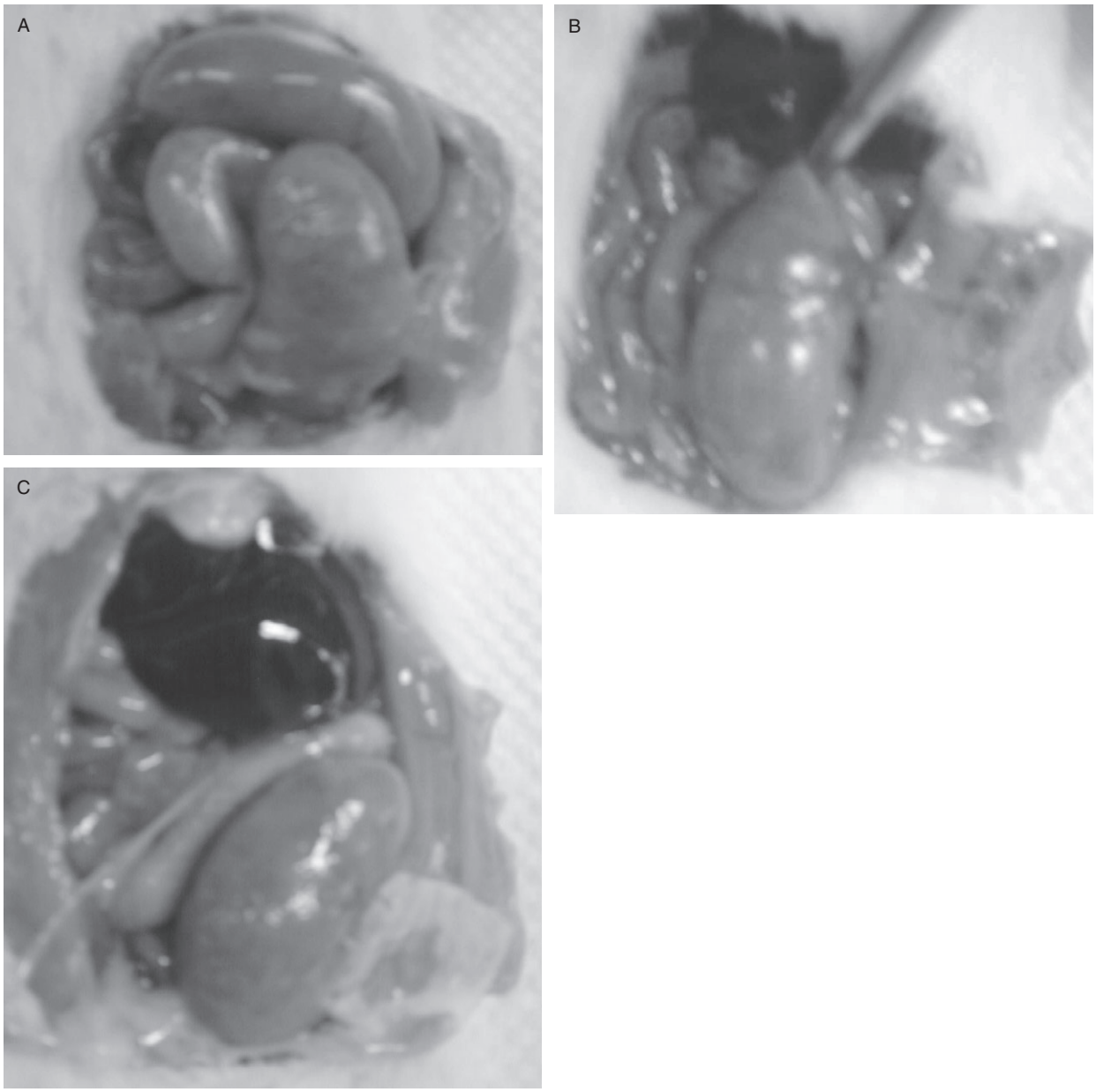

FIG. 3. (A) Adhesion between cecum and abdominal wall in the control group. (B) One week after abrasion and placement of Seprafilm, a small area of adhesion was found. (C) One week after abrasion and placement of PCL, no adhesion was found and the film became thinner.

Student's *t*-test shows that the adhesion prevention score (Fig. 4) *P* value is 0.23 between the Seprafilm and the PCL film group, and is less than 0.0001 between both the Seprafilm and the control group and the PCL film and the control group. It means that there is statistical significance between Seprafilm and control group, PCL film and control group, but not significant between Seprafilm and PCL film group.

TABLE 2. *Scoring of postsurgical tissue adhesion of the rats operated*

Score	0	1	2	3	4	Mean	SD
Control	0	0	2	5	3	3.1	0.707
Seprafilm	7	2	1	0	0	0.4	0.699
PCL film	9	1	0	0	0	0.1	0.316

SD, standard deviation.

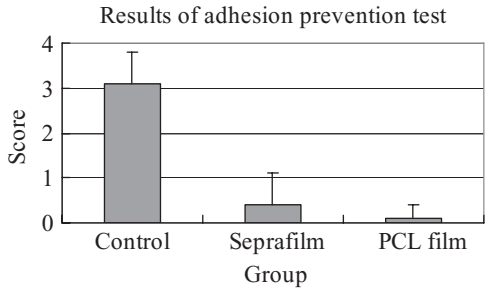

FIG. 4. Results of the adhesion prevention test. Mean adhesion scores of the PCL film, Seprafilm, and control groups. Data are expressed as means and standard error ($n = 10$). It shows $P < 0.05$ in Seprafilm, PCL film versus the control group, 0.23 for the Seprafilm and PCL film group as determined by Student's *t*-test.

DISCUSSION

A peritoneal injury invokes an inflammatory response from the serosal surface with the concurrent loss of the mesothelium (9). Increased permeability of the blood vessels in the traumatized tissues due to the release of prostaglandin E2 and histamine produces an outpouring of serosanguineous exudates rich in inflammatory cells. This exudate coagulates within a period as short as 3 h. Normally, the majority of fibrinous attachments so formed are lysed within a few days of development (9,10). If they persist for 3 days or longer, fibroblastic proliferation may occur within them, causing adhesion formation. So, this study considered 7 days as the result of whether adhesion formed or not.

Surgeons and healthcare professionals developed several methods for minimizing tissue injury in order to minimize the formation of adhesions. However, even an experienced surgeon using advanced techniques may not be able to prevent the formation of adhesions following surgery without the aid of an adhesion barrier. Consequently, many surgeons have come to rely upon adhesion barriers for adhesion prevention following abdominal and pelvic surgery. An adhesion barrier is a medical implant that can be used to reduce abnormal internal scarring (adhesions) following surgery by separating the internal tissues and organs while they heal. Prior to the availability of adhesion barriers, adhesions were documented to be an almost unavoidable consequence of abdominal and pelvic surgery.

Adhesion barriers, such as Seprafilm, are films that are applied between layers of tissues at the end of a surgery before the incision site is closed. Seprafilm is a clear, sticky film composed of chemically modified sugars, some of which occur naturally in the human body. It sticks to the tissues to which it is applied and is slowly absorbed into the body over a period of 7 days. While in place, Seprafilm acts as a physical barrier that separates traumatized tissue surfaces so that they do not adhere to one another while the tissue surfaces heal (10–12). Seprafilm should not be wrapped around an intestinal anastomosis as such usage may result in increased anastomotic leak-related events. The drawbacks of Seprafilm are its expensive price, especially when applied to a wide area.

According to the contact angle measurement, Seprafilm is quickly hydrated and flattened on the glass surface. The contact angle of PCL film is 97.5°. During the animal study, PCL film is adhered to the denuded serosa, but is still easy to be moved away. This is important when undergoing endoscopic surgery. Seprafilm fell into pieces as soon as it was rehydrated. So, it is not feasible to relocate the Seprafilm if the initial placement is not as desired (13).

Under SEM, there were small pores around 5–15 μm in length and 2–4 μm in width over the bottom surface of PCL film, and slightly bigger pores, 2–30 μm. in length and 2–8 μm in width over the upper surface of PCL film. These pores have some characteristics. First, these pores are small enough to prevent the cells from migrating to another surface and prohibit adhesion formation. Second, although PCL is hydrophobic, in practical appliance, PCL film has some adherence ability to the wet surface. This may be attributed to multiple pores of the films just like a honeycomb pattern increases the bio-adhesive ability of poly(lactide) film (14).

PCL has been widely used in tissue engineering. When used in prevention of adhesion formation, there are several reasons to dictate the successful application. The surface of PCL is hydrophobic and does not have any physiological activity, which makes it unfavorable for cell growth when it comes into contact with living body (15).

Pore interconnections within tissue-engineering scaffolds are essential to allow, at one level, nutrient supply, metabolite dispersal, and cell signaling. However, cell migration and colonization of the matrices requires an additional level of control over macropore size, geometry, and connectivity (16).

To attest to the interconnectivity of PCL film, a piece of tissue paper was put under PCL film, and an adequate amount of blue colored water was poured onto it. After 1 h, the tissue paper still did not get wet. This could prove that the pores of the PCL film in this study were not interconnected.

Several technologies have been used to make PCL porous such as gas foaming (17), salt leaching (18), solvent casting (18), and their combination (19). These methods could be tried to increase bio-adhesive ability.

For security, two stitches in diagonal order were used to fix the PCL film to the serosa. This, of course, will create another problem; the stitches may cause some adhesions. Fortunately, it seldom resulted in a big problem during this study. To improve the bio-adhesive ability without stitches seems to be very important.

Further attempts to correlate cell adhesion and the physicochemical properties of biomaterials reveal that multiple factors, including surface electronic charge, surface topography, rigidity, and hydration extent of materials, contribute to their interaction with cells (20). The interaction could also be cell-type specific.

The prevention of adhesion in PCL film is better than that of Seprafilm in this study. This may be caused by the much more delayed degradation of PCL film which remains in a whole piece during this period. Application of PCL film as anti-adhesion barrier is quite promising. If proper bio-adhesive ability and long enough degradation time could be achieved at the same time, it will become the best suitable adhesion prevention material.

CONCLUSION

This study presents the PCL film, an easily fabricated, cheap biodegradable material, as a good anti-adhesion barrier. The long lasting character of this film acts better than the Seprafilm. If it can be kept in place, it will function well to separate both sides of the eluded surface. As the price is friendly, most patients should be able to afford to use it. Further work to improve the bio-adhesive ability based on PCL should be mandatory.

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