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Surface structure change properties: Auto-soft bionic fibrous membrane in reducing postoperative adhesion

Shanshan Xu [a], Chenhong Wang [b], Ruoqing Mao [c,d], Xiaoyu Liang [c], Heran Wang [b], Zhenyu Lin [a], Jiangxue Li [c], Shilin Li [c], Jipeng Jiang [e], Tongshuo Zhang [f], Yongfu Ma [e], Yang Liu [e], Charles C. Han [a], Ying Liu [c,g,*]

[a] Institute for Advanced Study, Shenzhen University, Shenzhen, 518060, China
[b] State Key Laboratory of Polymer Physics and Chemistry, Joint Laboratory of Polymer Science and Materials, Beijing National Laboratory for Molecular Sciences, Institute of Chemistry, Chinese Academy of Sciences, Beijing, 100190, China
[c] CAS Key Laboratory for Biomedical Effects of Nanomaterials and Nanosafety & CAS Center for Excellence in Nanoscience, National Center for Nanoscience and Technology of China, Beijing, 100190, China
[d] State Key Laboratory of Veterinary Etiological Biology, National Foot and Mouth Diseases Reference Laboratory, Key Laboratory of Animal Virology of Ministry of Agriculture, Lanzhou Veterinary Research Institute, Chinese Academy of Agricultural Sciences, Lanzhou, Gansu, 730070, China
[e] Department of Thoracic Surgery, Chinese PLA General Hospital, Beijing, 100853, PR China
[f] Department of Clinical Laboratory and Pathology, Jiangsu Provincial Corps Hospital of Chinese People's Armed Police Force, Yangzhou, Jiangsu, 225003, PR China
[g] GBA National Institute for Nanotechnology Innovation, Guangdong, 510700, China

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ABSTRACT

Peritoneal adhesion is the most common adverse effect following abdominal surgery or inflammation. The occurrence in clinical trials has been successfully reduced using barriers. However, the shortcomings of frequently used adhesion barriers, such as rapid degradation rate of gel barrier and inadequate operation ability of solid barrier, cannot be ignored. In this study, a fibrous membrane with an ECM-like structure was prepared. The adhesion properties were reduced significantly by changing the surface structure. The fibrous membrane caused less inflammatory response and much less peripheral adhesion and intestinal obstruction compared to the casting film and the commercial film with smooth surface, though with the same components. Because of the auto-soft bionic structure and similarity in the mechanical modulus of the tissues, the fibrous membrane was more flexible when it adhered to the tissues, showed excellent effectiveness and biocompatibility. In addition to the rat and miniature pig models, a randomized, placebo-controlled, and multicenter clinical pilot study with 150 patients confirmed that because of its flexibility, biodegradability, and similarity to mechanical modulus and structure with tissues involved, the fibrous membrane served as a favorable implant for preventing post-operation adhesion.

1. Introduction

Adhesion is a frequent-occurring complication due to the fibrous bridge formed during the improper healing process after abdominal, tendon, epidural, intrauterine, or pericardium surgery. Nearly every patient has suffered adhesions provoking severe problems such as chronic pain, infertility, and even bowel obstruction, and subsequently requiring adhesion-loosening, which results in a tremendous public health burden [1,2]. Most surgeons have identified adhesion's clinical significance and suggested that effective anti-adhesion agents should be applied broadly, particularly for the high-risk surgeries [3].

At present, anti-adhesion strategies mainly include surgical optimization, barriers (solid, liquid/gel), and pharmacological therapies, and it turns out that barriers are more feasible and effective [3,4]. Fluid barriers offer hardly sufficient mechanical strength as a physical separation above the injured tissues are easily diluted with the blood or displace to other tissue cavities, which downgrade their barrier performances. In comparison, solid barriers are clinically the most successful adjuncts to

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* Corresponding author. CAS Key Laboratory for Biomedical Effects of Nanomaterials and Nanosafety & CAS Center for Excellence in Nanoscience, National Center for Nanoscience and Technology of China, Beijing, 100190, China.
E-mail address: liuy@nanoctr.cn (Y. Liu).

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effectively separate the traumatized peritoneal surfaces because of the better mechanical conditions, significantly prolonged retention time (≥7–10 days), organ temperature triggered shape memory, potentially drug loading with slow/targeted drug release properties, the same biocompatibility, and biodegradability as liquid/gel barriers. However, solid barriers, including SEPRAFILM® and INTERCEED®, the only two anti-adhesion products approved by the Food and Drug Administration (FDA) in the United States, are simply physical barriers with limited options of degradation timing and not targeting the cellular or molecular pathogenesis of adhesions.

Indeed, an ideal strategy of preventing adhesion should have good biocompatibility, biodegradability with controllable retention time, outstanding mechanical properties, controllable hydrophilicity/hydrophobicity balance for "bio-adhesion" and water interaction for later swelling/degradation/dissolution, extracellular matrix (ECM)-like structure and morphological stability, and well-controlled glass transformation process for specific shape memory function [4,5]. At the same time, the implant-mediated immune response coupled with the wound-healing process determines the outcome of implantation. The implant may affect the wound healing through its interaction with the immune cells, which rapidly infiltrate and adhere to the implants, such as neutrophils, dendritic cells, monocytes, and macrophages [4]. Neutrophils are the first innate immune cells to arrive at the site of injury, and monocytes and macrophages subsequently arrive at the injury site, and the activity peaks at 4–7 days after injury [6]. Macrophages dominate the following inflammation phase, in which the inflammatory stimuli persist at the implantation site. The introduction of an implant can interfere with the wound-healing process, owing to macrophage implant interaction [6].

Electrospun scaffolds have played a significant role in regulating cell behaviors, tissue regeneration, and realizing nondestructive embedding and controllable targeted release. Fibrous membranes, based on biodegradable polymers, simulate ECM and demonstrate noticeable softness; they have remarkable biomedical applications, such as substrates for tissue regeneration and bioactive agent carrier [4]. As a well-known polymer, poly (lactic-co-glycolic acid) (PLGA), with controllable degradation rate and non-irritating delivery to tissues, has been approved by the FDA as an implant for years. Using a simple electrospinning approach, this old material can still lead to a new strategy [7], mainly used as a drug delivery system or tissue scaffold [8].

In this study, PLGA and an amphiphilic polymer PLA-b-PEG (polylactic acid-block-polyethylene glycol) were electrospun into the fibrous membrane with an ECM-like structure (fibrous and porous matrices with a high surface area), as shown in Scheme 1. Specific PLA-b-PEG content contributed to the better initial swelling and targeting glass transition temperature to body temperature and increased the stability of the porous membranes, which are all the compulsive requirements for industrialization. The casting film consisting of the same composition and a commercial film consisting of only PLGA were set as control groups to investigate the influence of physical structure mainly. The rat and miniature pig models performed detailed pre-clinical evaluations on product operability, effectiveness, and safety. A randomized, placebo-controlled, and multicenter clinical pilot study consisting of two parallel treatment groups was designed and conducted in 4 medical centers in China to determine whether a fibrous membrane was suitable for reducing postoperative peritoneal adhesion. The effective use of the fibrous membrane in clinical practice would demonstrate itself as an excellent implanted barrier and provide new insight into old polymers used as a tissue scaffold or drug carrier.

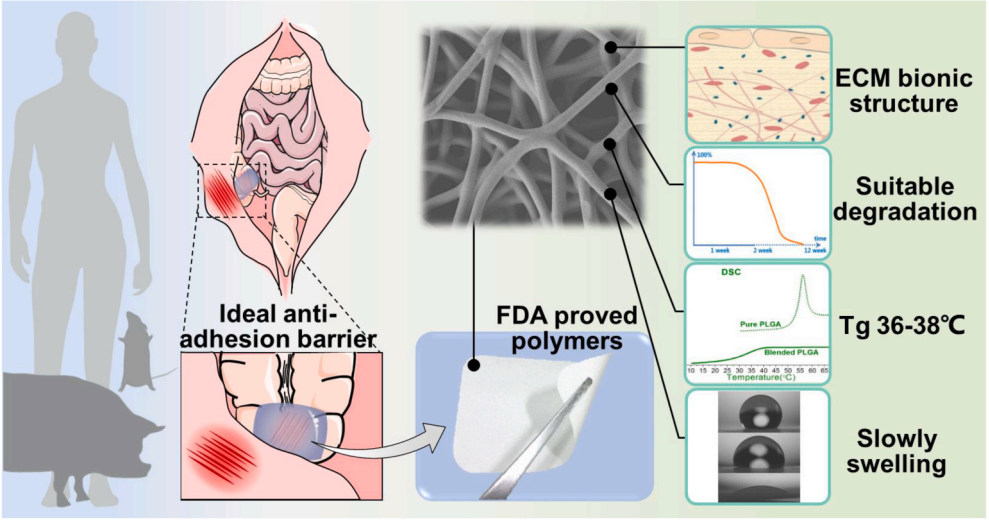
2. Materials and methods

2.1. Components of electrospun membranes

The fibrous membranes with different thicknesses (50, 60, 100, 120, 160, or 200 μm) were prepared. Two copolymers PLGA (Mw = 60,000, (D, L) LA/GA = 75/25) and PELA (Mw = 10,000, PEG-b-PLA, (D, L) LA/EG = 50/50) were purchased from Jinan Daigang Biological Technology Co. LTD. The molecular weights were measured using standard polystyrene calibration by size exclusion chromatography (SEC) in THF (tetrahydrofuran) solvent. The mixture of PLGA and PELA was dissolved in a mixed solvent of N, N-dimethylformamide (DMF), and acetone (DMF:acetone = 5:5, volume ratio). The weight ratio of PLGA:PELA was 85:15. The total polymer concentration in the solution was 50w/v% for electrospinning and 5w/v% for casting.

2.2. Animals

To evaluate the postoperative adhesion ability of the fibrous membranes with different thicknesses, a total of 95 adult Sprague-Dawley rats (280–300 g) were used. After the treatment using the fibrous membranes with different thicknesses (50, 60, 100, 120, 160, or 200 μm), 40 rats were sacrificed on day 10 (Acute phase). After the treatment with different thicknesses (50, 120, or 200 μm), 30 rats were sacrificed



Scheme 1. The design concept of the fibrous membrane. The FDA proved that polymers were the targeted choice to form an ideal anti-adhesion barrier similar to ECM structure by optimizing the fiber diameter and porosity and tuning to degrade after 2 weeks. The amphiphilic polymer contributes to the hydrophilicity and Glassy transition temperature, resulted in excellent operation ability.

after 1 month of treatment (Subchronic phase), and the other 25 rats were sacrificed after 6 months of treatment (Chronic phase). After anesthetizing the rats, a transperitoneal approach was used to expose the cecum and opposing abdominal wall, and 2 cm^2 of the cecum and abdominal wall were scraped with sandpaper until the serosal surface was disrupted and hemorrhaged but not perforated. The impaired intestinal wall was covered with the fibrous membranes with different thicknesses, respectively. Approximately 6 cm^2 (the length was 3 cm and the width was 2 cm) of each membrane was used. Rats in the negative control group were not blanketed with any anti-adhesion membranes (Negative control group). Then, the abdominal cavity was closed using 1-0 silk sutures.

And then, a total of 133 adult Sprague-Dawley rats (280–300 g) and 25 miniature pigs were used as other recipients *in vivo*. 88 rats were sacrificed 10 days after treatment (Acute phase), and the other 45 rats were sacrificed 6 months after treatment (Chronic phase). Rats were divided randomly into 5 sub-groups (7 sub-groups in acute stage with two additional commercial groups of SEPRAFILM® and INTERCEED®), with one group of normal control without receiving any treatment (Normal control group), while the remaining rats were taken into laparotomy. As mentioned above, the impaired intestinal wall was covered with a commercial membrane (Positive control group), casting film (Casting film group), electrospun fibrous membrane (Fibrous membrane group), respectively.

25 miniature pigs were randomly divided into 5 groups, with one group of normal control not receiving any treatment, while the remaining 20 pigs underwent laparotomy; 6 cm^2 (the length was 3 cm and the width was 2 cm) of the cecum and abdominal wall were scraped with sandpaper. Each membrane used was approximately 96 cm^2 (the length was 12 cm and the width was 8 cm). After the abdominal cavity was closed, the miniature pigs were injected with penicillin subcutaneously twice a day (40,000 U/Kg).

The intestinal obstruction stage, average adhesion level, and average adhesion tenacity were calculated using the grading system synthesized from the previous researches (Supporting Information-Methods, Table S1) and examined blindly by 3 independent observations.

2.3. Patients

150 patients (74 women and 76 men) with a mean age of 50 years (18–70 years) were prospectively included in this study. They all received open abdominal surgery for the first time, voluntarily participated in this clinical trial, and signed an informed consent form. The 150 patients were randomly divided into two groups with 75 patients each: Patients whose surgical injury was not blanketed with any anti-adhesion membranes (Control group); and patients whose surgical injury was covered with the absorbable microfiber anti-adhesion electrospun membranes (Test group). The observation period for each case in this clinical trial was set as 12 weeks. Furthermore, the expected overall duration was about 6–8 months.

2.4. Statistical analysis

All the scores of adhesion level, adhesion tenacity and intestine obstruction were summarized in tables and compared between every 2 groups using SPSS 25.0 (IBM, USA) with Kruskal-Wallis and Fishier exact test. All the tests were two-sided, and a p-value less than 0.05 was considered a significant difference, and a p-value less than 0.01 was considered a very significant difference. Other results were presented as mean ± standard deviation (SD). Differences between groups were determined by Student's t-test, with values of $p < 0.05$ considered to be significantly different and $p < 0.01$ considered to be very significantly different.

For trial results, descriptive statistical analysis was performed. Qualitative indicators were described in the frequency table and percentage. Quantitative indicators were described as mean, standard deviation, median, lower quartile (Q1), upper quartile (Q3), maximum and minimum.

Inter-group comparison of clinical use performance and efficacy indicators: $\chi 2$ test, Fisher exact probability method, Wilcoxon rank-sum test, and Wilcoxon signed-rank test were used for qualitative data. Group t-test or paired t-test was used for quantitative data in the normal distribution, and Wilcoxon rank-sum test and Wilcoxon signed-rank test were used for quantitative data, not in a normal distribution. Except for the superiority test for the test of hypothesis "incidence of postoperative tissue adhesion", the two-sided test was used for all other tests. Test statistics and corresponding p-value were calculated. $p \leq 0.05$ was considered statistically significant.

Safety analysis: normal/abnormal changes in vital signs and laboratory examination before and after treatment were described. $\chi 2$ test and Fisher exact probability method were used to compare incidences of adverse events, serious adverse events, and adverse reactions between the two groups.

3. Results

3.1. Preparation and characterization of the auto-soft fibrous membrane

The PLGA/PLA-b-PEG electrospun fibrous membrane with an ECM-like structure (fibrous and porous matrices) was prepared and optimized. The commercial products most commonly used in the clinic were purchased as the positive control, including a PLGA commercial film (20 μm, PLGA), SEPRAFILM® (20 μm, crosslinked carboxymethylcellulose with Hyaluronic acid) and INTERCEED® (60 μm, oxidized cellulose). There was no significant difference in the peripheral adhesion or integral obstruction incidence among the three commercial products (Table 1). In order to ensure the composition consistency with the fibrous membrane, the PLGA commercial film (20 μm) was selected as a positive control in subsequent experiments. At the same time, the casting control group (20 μm) was set up as the intermediate control to maintain similar thickness with the PLGA commercial film and the same composition with the fibrous membrane (Fig. 1A and B).

The porous structure made fibrous membrane with a larger elongation ratio at break (Fig. 1C) and lower glass transition temperature (Tg) (approximately 37 °C) than the commercial film, which made it more flexible. Though the tensile strength is lower than the casting and commercial products, this is not the compulsive requirement of an anti-adhesion barrier, especially under the structure design of adherence to the tissue without suture. The casting and commercial film had the flat surfaces and smooth cross-sections (Fig. 1D–a to f). In a few minutes, water droplets during a static water contact angle (CA) analysis (Fig. 1D inserts) could swell the fibrous membrane and casting film but not the commercial film in few minutes. The fibrous membrane with approximately 120 μm thickness was composed of randomly oriented fibers, with a diameter ranging from 1 to 2 μm and a three-dimensional open-pored structure similar to the ECM. The LA/GA ratio of fibrous membrane contributed to the proper degradation time (Fig. 1E), that remained barrier structure within 2 weeks and gradually released the degradation products, while the commercial product is highly overloaded to more than 6 months. The proper flexibility, wettability, ductility, and structure of the implanted products are the crucial properties that improve the operation ability and tissue compatibility. After adhering to a cup filled with 37 °C water (Fig. 1A) or the patient's wound (Fig. 1F), the fibrous membrane became auto-soft and was easy to stick to the surface.

3.2. The fibrous membranes effectively prevent tissue adhesion in vivo

The preclinical evaluation was performed in rat and pig models by implanting samples between the damaged cecum and peritoneal wall defects. The fibrous membranes with different thicknesses (50, 60, 100, 120, 160 or 200 μm) and the same chemical composition (PLGA/PLA-b-

Table 1
Death time, adhesion level, and adhesion tenacity of the rat models in the acute phase.

Group		Normal Group (n = 12)	Seprafilm® Group (n = 12)	Interceed® Group (n = 12)	Fibrous Membranes (n = 13)	Casting Film (n = 13)	Commercial Film (n = 13)	Negative Group (n = 13)
Alive in each group		12	12	11	13	12	11	10
Death Time	<1d	0	0	0	0	0	1	1
	1d-3d	0	0	0	0	0	1	0
	>3d	0	0	1	0	1	0	2
Adhesion Level	0	12	7	5	9	6	8	2
	1	0	3	2	1	1	1	0
	2	0	0	0	0	2	0	1
	3	0	2	4	3	3	2	7
Adhesion Tenacity	0	12	7	5	9	6	8	2
	1	0	3	4	3	3	1	0
	2	0	2	1	1	1	0	0
	3	0	0	1	0	2	2	8
Intestinal Obstruction	0	12	5	6	12	10	7	6
	1	0	6	4	1	1	1	2
	2	0	1	1	0	1	3	2

PEG) have different anti-adhesion effects. The membrane with a small thickness (50 or 60 μm) can not prevent the cells from growing through the pores and play the role of the tissue scaffold. Moreover, the membranes with the larger thickness (160 or 200 μm) can be a solid barrier, while shows a higher occurrence rate of the intestine obstruction because of the severe foreign body reaction (Figs. S1 and S2). Therefore, we choose the fibrous membrane of 120 μm as the object in the following experiment.

In order to evaluate the superior efficiency of 120 μm fibrous membrane, we purchased the commercially available products most commonly used in the clinic, including commercial film (20 μm, PLGA), SEPRAFILM® (20 μm, crosslinked carboxymethylcellulose with Hyaluronic acid) and INTERCEED® (60 μm, oxidized cellulose). The fibrous membrane showed much better flexibility, hygroscopicity, and tacking ability than the casting film and commercial film (Fig. 2A, Table S2). Tissue adhesions were also examined by direct observation, and the extent of adhesion was graded and evaluated with statistical analysis with calculating the *p*-value for every 2 groups in acute and chronic periods (Fig. 2B and C, Tables 1 and 2). The industry standard SEPRAFILM® and INTERCEED® showed the similar preventing adhesion results in the acute stage, but more severe intestinal obstruction (Table 1). Most of the negative animals (those that underwent surgery without placing any samples) showed the dense adhesions. Fibrous membranes can prevent the tissues from adhesion effectually and casting film and commercial film *in vivo* and decrease adhesion tenacity. Gross lesions in the rats of the commercial film group included intestinal distension, and the intestinal wall became significantly thinner and nearly transparent, likely due to marked atrophy of the villi (Fig. 2D). In terms of adhesion in untreated animals at 6 postoperative months, the cecal smooth muscle was fused to the striated muscle of the abdominal wall; no adhesion and recovered cecum were observed in animals treated with the fibrous membrane (Fig. 2E). Fibrous membranes could also prevent the tissue from adhering effectively as well as casting film and commercial film in the pig model (Fig. 3, Table 3). Due to their surface roughness, the fibrous membranes were at their original positions, without visible moving or shifting aside. In approximately 75% of the casting films (9/12), the distance of migration in the abdominal cavity was <2 cm, while none of the commercial films migrated less than 2 cm (Table S2). Similar results were observed in pig models (Table S3), indicating the excellent position stability of fibrous membranes, without sutures, which was closely relevant to the excellent anti-adhesion.

3.3. The fibrous membranes present proper degradation rate in vitro and in vivo

In the design and development of anti-adhesion materials, the degradation behavior is also of great importance. As a solid barrier for preventing post-operation adhesion, a fast degradation rate of fewer than 7 days would affect the efficiency and safety of the anti-adhesion membrane [1,2,6]. Degradation of in vitro polyester process is based on the hydrolytic reaction and mostly follows the heterogeneous degradation mechanism. The degradation rate of PLGA materials is mainly determined by Lactide (LA)/Glycolide (GA) ratio. Furthermore, the PLGA materials with the same LA/GA ratio and similar molecular weight showed a similar degradation rate [9–11]. Apparent morphology deficiency (Fig. 1D–g to l) and weight loss (Fig. 1E) only start when the molecular weight of the sample is reduced to a critical value, and the soluble oligomers are dissolved. The percentage of the LA segments in the commercial film was more than 95%; therefore, minor weight loss and few surface changes were observed after 18 weeks of incubation. The PLGA/PLA-b-PEG blends retained their weight and structure in the first 4 weeks, and then both lost their weight gradually due to the better wettability from the introduction of PLA-b-PEG and the faster hydrolysis of the GA segments compared to the LA segments, which is the same as the previous study [12–14]. The PLGA/PLA-b-PEG blended fibrous membrane's degradation rate and commercial film *in vivo* was accelerated, as shown in Fig. 1D-m to r, but it still kept the barrier structure at 4 weeks post-implantation.

3.4. The fibrous membrane show protective effects against acute inflammation in vitro and in vivo

The degraded products of anti-adhesion materials were reported to interact with intra-abdominal tissue, cells, or peritoneal fluid, and caused further systematic reactions. The inflammatory reaction might determine the extent of adhesion formation [15]. Pro-inflammatory cytokines and signaling cascades participate in the pathophysiology of the peritoneal adhesion, including pro-inflammatory molecules, cytokines, chemokines, and cell signal molecules, which trigger inflammation and respond to injury or infections (Fig. 4).

Pro-inflammatory molecules, such as interleukin-1 (IL-1)β, IL-2, IL-6, IL-10, IL-13, IL-18, tumor necrosis factor-alpha (TNF-α), are mainly secreted by monocyte-macrophages after tissue injury and inflammation, which can help fibroblast proliferation, modulate fibrin degradation and collagen deposition and stimulate releasing adhesion molecules to promote the adhesion between epithelial cells and inflammatory cells [16–18]. IL-1 plays a significant role in inflammatory responses and refers to IL-1α and IL-1β, which are produced by the endothelial cells or monocytes/macrophages. IL-1β, IL-6' and TNF-α of patients with significantly increased peritoneal adhesion. IL-1α is one marker of innate activation [19]. Other cytokines, such as granulocyte-macrophage colony-stimulating factor (GM-CSF) [20], IL-5

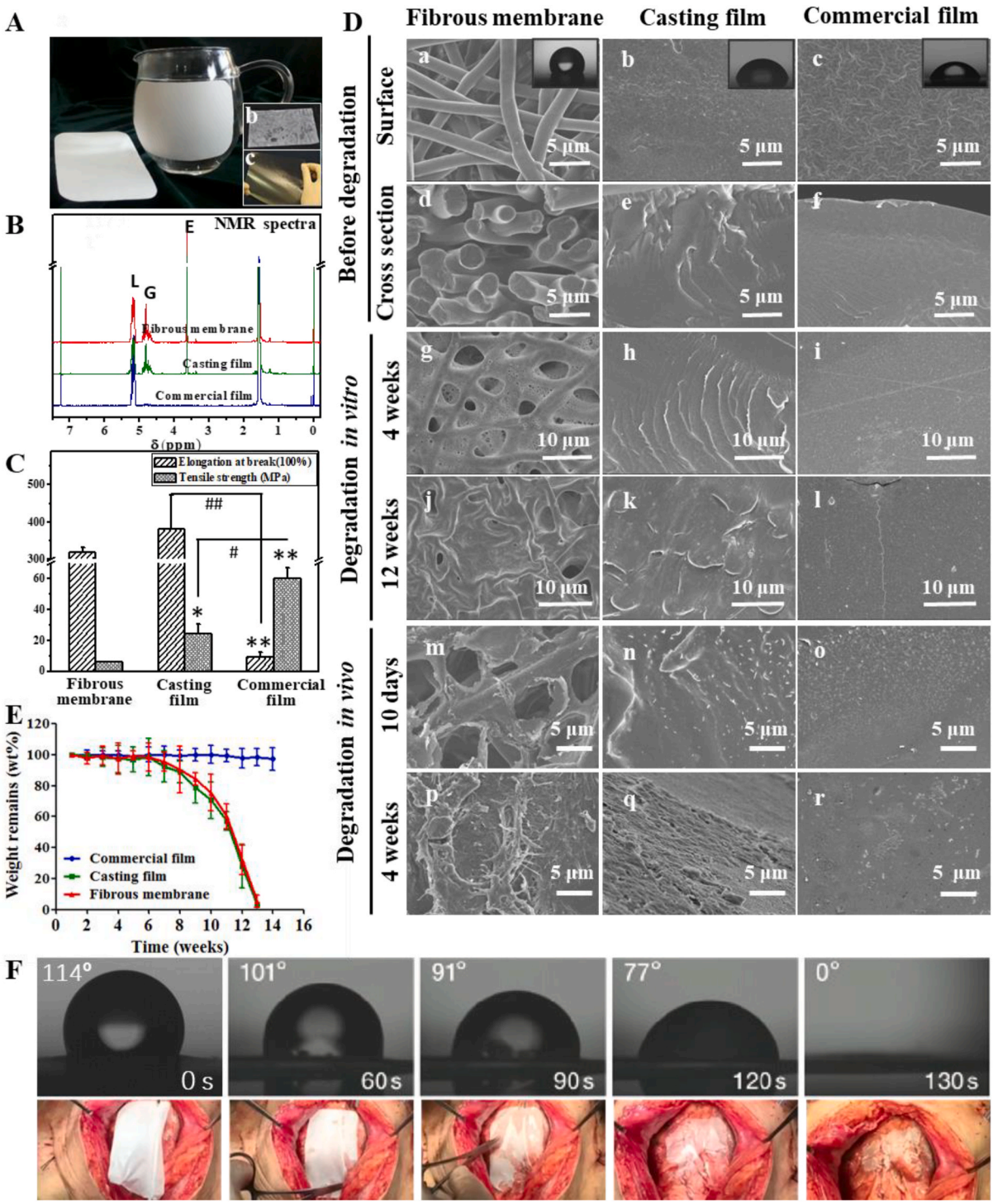


Fig. 1. Characterization of the fibrous membrane, casting film, and commercial film. A, Morphology of the fibrous membrane (a), casting film (b), and commercial film (c). B, ^{1}H NMR spectra. C, Mechanical properties (elongation at break and tensile strength). D, SEM images and wettability. a, b and c are the surfaces. The inserts show the static contact angles of the corresponding scaffold. d, e, and f are the cross-section. g to r are the degradations of fibrous membrane, casting film and commercial film. g to l are the degradation in vitro at 37 °C in PBS buffer solution after 4 weeks (g, h, i) and 12 weeks (j, k, l). m to r are the degradation changes *in vivo* after 10 days (m, n, o) and after 4 weeks (p, q, r). E, The degradation curves in vitro at 37 °C in PBS buffer solution. F, Flexibility and hygroscopicity of fibrous membrane on the patient's wound.

[18], IL-12p70, IL-17A [21]' and vascular endothelial growth factor (VEGF), were also played essential roles after tissue injury and inflammation. Chemokines are the proteins that attract and activate leukocytes and play a potential role in mediating inflammation [22], including chemokine (C-X3-C motif) ligand 1 (CX3CL1 or fractalkine) [23], C–C motif chemokine ligand 5 (CCL5 or RANTES, the expression levels of regulated and normal T cell expressed and secreted) [21], leptin [23], macrophage Inflammatory Protein 2 (MIP-2) [21], CCL3 (macrophage inflammatory protein 1-alpha, MIP-1α) [24], interferon γ (IFN-γ) [25], granulocyte colony-stimulating factor (G-CSF) [26], Lipopolysaccharide-induced CXC chemokine (LIX) [27], Eotaxin (CCL11) [21]. Moreover, the anti-inflammatory (IL-4) level in the

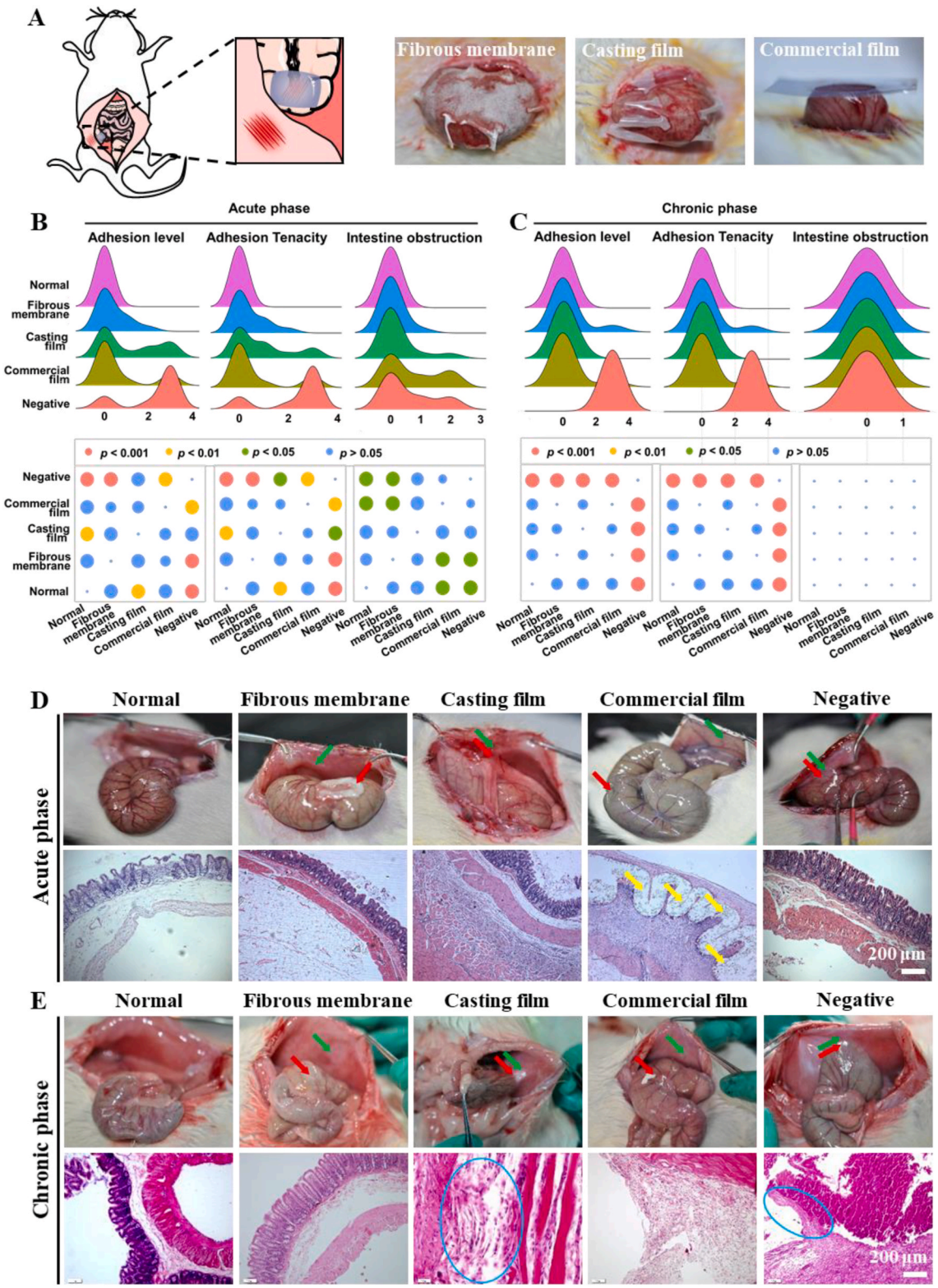


Fig. 2. The fibrous membranes effectively reduced the peripheral adhesion and intestinal obstruction *in vivo*. A, Flexibility, hygroscopicity, and tacking ability of fibrous membrane, casting film, and commercial film. B and C, Adhesion level, adhesion tenacity and intestinal obstruction of rats in acute (B) and chronic periods (C). D and E, The operation and HE staining of adhesions during the acute (D) and chronic periods (E), with the abdominal wall defect indicated by the green arrows, the defected cecum indicated by the red arrows, the intestinal distension indicated by the yellow arrows and the adhesive muscles indicated by the blue circles. (For interpretation of the references to colour in this figure legend, the reader is referred to the Web version of this article.)

Table 2
Death time, adhesion level, and adhesion tenacity of rat models in the chronic phase.

Group		Normal Group (n = 9)	Fibrous Membrane (n = 9)	Casting Film (n = 9)	Commercial Film (n = 9)	Negative Group (n = 9)
Alive in each group		9	9	9	9	6
Death Time	<1d	0	0	0	0	0
	1d-3d	0	0	0	0	1
	>3d	0	0	0	0	2
Adhesion Level	0	9	8	8	8	0
	1	0	0	1	0	0
	2	0	0	0	0	0
	3	0	1	0	1	6
Adhesion Tenacity	0	9	8	8	8	0
	1	0	0	1	0	0
	2	0	0	0	0	0
	3	0	1	0	1	6
Intestinal Obstruction	0	9	9	9	9	6
	1	0	0	0	0	0
	2	0	0	0	0	0

fibrous membrane group was higher than that of the negative group, which tends to be closer to the normal value. Inflammation relief was most significant in the fibrous membrane group, which was related to its auto-soft characteristic. The casting film and the commercial film were much harder than the fibrous membrane.

In peritoneal lavage fluid (PLF), the changes of the pro-inflammatory molecules, lymphokines, chemokines, and cell signal molecules were not significant. VEGF in the anti-adhesion implant groups was higher than in the control group, which explains that the implants might play an anti-apoptotic effect on endothelial cells and increase vascular permeability. TNF-α level in the negative group was significantly higher than the other groups, while the fibrous membrane, commercial film, and casting film groups had no significant difference compared with the normal group. The level of IL-6 in the negative and commercial film groups was higher than the normal, casting film, and fibrous membrane groups. The level of IL-6 in the fibrous membrane group was higher than that of the normal group, but considerably lower compared to the negative and commercial film groups.

Based on the above findings, surgery was an important cause of peritoneal adhesion formation, which caused severe inflammation. Using different treatments, the casting films and fibrous membranes or commercial film can decrease the adhesions and inflammation. The casting films and fibrous membranes can ultimately help the tissue recover from surgery-induced damage. PLGA/PLA-b-PEG fibrous membranes exhibited potent broad-spectrum anti-inflammatory activity.

3.5. The degradation product of the fibrous membranes does not affect macrophages

Macrophages are essential effectors and regulator cells that phagocytize foreign materials, secrete cytokines, and are involved in the entire immune response process [28]. Many macrophages were adhesive on commercial film and casting film, and most dead cells were detected on the commercial film. Fewer macrophages affixed themselves to the surface of the fibrous membrane (Fig. 5A). Judging from the physical properties, films with better hydrophilic surfaces resulted in enhanced cell adhesion. However, an overly hydrophilic surface was unsuitable for the protein attachment, which is necessary for cell adhesion [29]. Thus, it was necessary to maintain hydrophilic and hydrophobic balance in the material surface. Judging from the chemical composition, PLGA/PLA-b-PEG was non-toxic and demonstrated good biological characteristics, which degraded lactic and glycolic acids, natural by-products of several metabolic processes in the body. Hence, it was proven that PLGA/PLA-b-PEG fibrous membrane satisfied the application's feasibility, validity, and safety requirements.

Biodegradable materials can be degraded into numerous monomers or oligomers in the peritoneal cavity. The concentration of the degraded product in blood circulation was demonstrated to be very low *in vivo* using the radio-labeled materials. The gradual degradation of ^{125}I labeled PLGA material was detected, as well as the stable distribution in different kinds of tissues (<1 μg/mL) [10]. Moreover, the concentration of the degradation products in serum was only at the level of ng/mL using the PLGA materials labeled with ^{14}C [11]. Macrophages undergo "the frustrated phagocytosis" by releasing various substances, such as cytokines, reactive oxygen species, and proteolytic enzymes to degrade and engulf the materials [30,31]. For further analysis of the direct interaction of PLGA/PLA-b-PEG casting film/fibrous membrane to macrophages, their degradation product was used to evaluate their effects on macrophages' proliferation, growth, and morphology.

Firstly, we carried out the experiments related to degradation *in vivo*, and detected the concentration of the degraded monomers or oligomers, Lactide (LA) and Glycolide (GA), in the whole blood and peritoneal fluid using GC-MS at different time points, whose detection limit is 1 μg/mL (1 ppm). As shown in Fig. S3, the concentration of the degradation products in the whole blood and peritoneal fluid from all the groups is less than 1 μg/mL (1 ppm). And then, the degradation fluid was obtained from the fibrous membrane after immersion in PBS for 5 months. Moreover, all of the oligomers and monomers have been released. The original concentration was 2.5 mg/mL, and then to be diluted to the different concentrations (320 μg/mL, 160 μg/mL, 32 μg/mL, 16 μg/mL, and 3.2 μg/mL, which are all much higher than the local concentration *in vivo*). The degradation product did not influence cell proliferation and growth in certain concentration ranges (i.e., <32 μg/mL, which was much higher than the concentration of degradation product *in vivo*, Fig. 5B and C), while degradation products stimulated macrophages to secrete IL-1β, IL-6, and TNF-α (Fig. 5D, E and F). The changing trends of expression were in agreement with the *in vivo* results. With an increase in degraded product concentration, the cell toxicity level increased gradually, which implied that an unnecessary inflammation and influence on wound healing might be caused by the fast release of the degraded product. The intestinal obstruction level of SEPRAFILM® and INTERCEED® showed a significant difference from the fibrous membrane and casting film, which should be caused by the fast degradation product releasing these hydrophilic components with around 10 days degradation time. Therefore, the controlled degradation of implantation includes suitable structure maintaining capability and relatively slow release of degradation products. The fibrous membrane is well-controlled, designed, and qualified compared to hydrophilic film or gel, which would be absorbed in a few days.

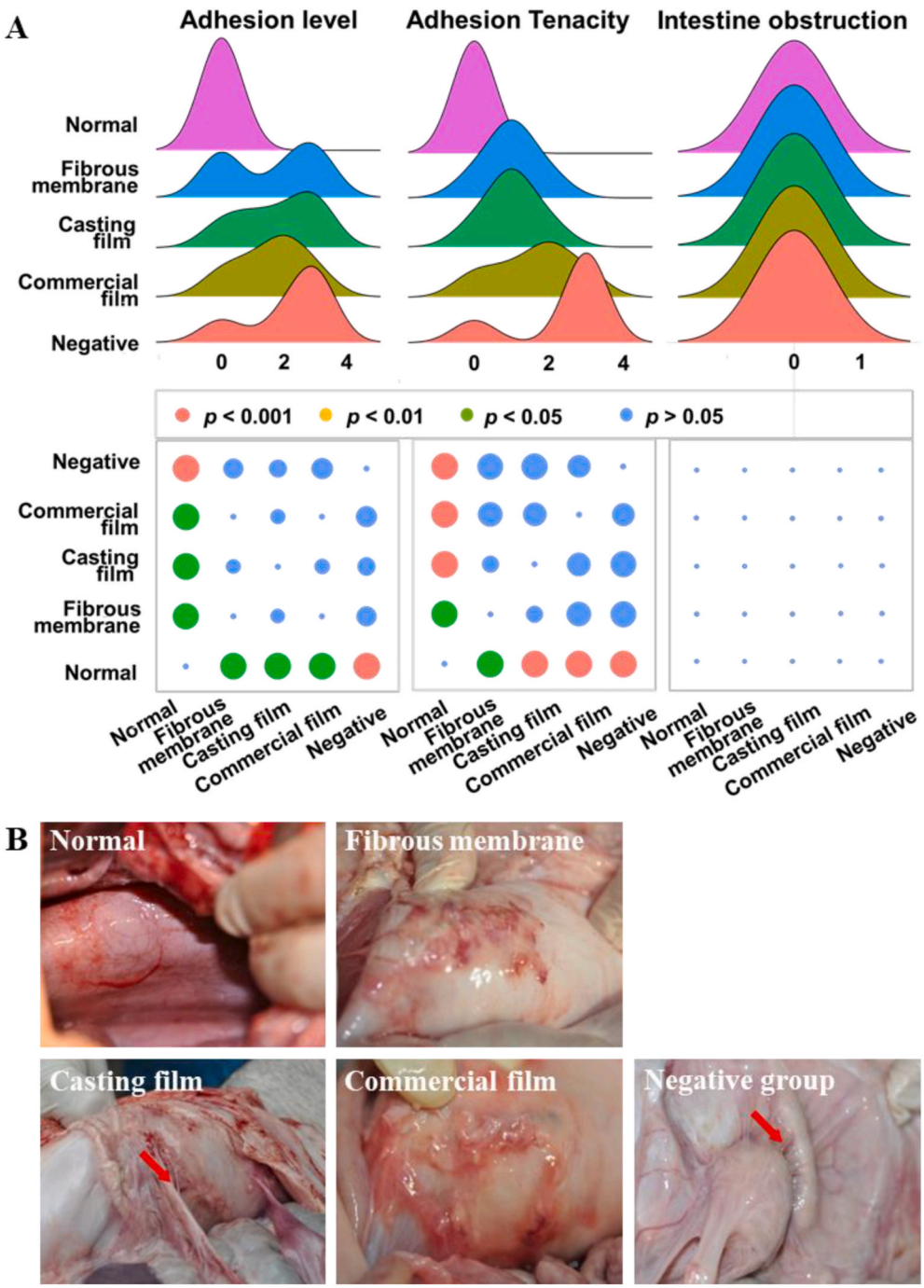


Fig. 3. The fibrous membranes effectively reduced the adhesion tenacity in a pig model. A, Adhesion level, adhesion tenacity and intestinal obstruction. B, Photos in operation. Red arrows represented the adhesion. (For interpretation of the references to colour in this figure legend, the reader is referred to the Web version of this article.)

3.6. The fibrous membranes demonstrate much lower toxicity than commercial film in vivo

Safety is critical for the implantable medical devices, such as the bodyweight, pathological changes, toxicity, and immune responses, to prolong life and improve the quality of life. From the pathological changes of liver and spleen, in agreement with the organ coefficient results, the commercial film had much higher toxicity than the fibrous membrane and casting film (Fig. S4, Table S4-S5). Hepatocytes were arranged irregularly in liver tissues of the commercial film group without a characteristic lobular pattern associated with different degrees of liver atrophy. Decreased splenic pulp, thickened trabeculas, and congested blood vessels were observed in the spleen tissues. The liver and spleen coefficients and the histopathological micrographs were restored to normal levels with prolonged exposure times (Fig. S5). Serum blood urea nitrogen (BUN) and creatinine (Cr) levels were used to determine the cause of acute renal injury or dehydration, and alanine transaminase (ALT) and aspartate transaminase (AST) measurements were used to evaluate hepatic damage. Injury caused by surgical manipulation affected the liver function. Fibrous membrane brought considerable relief from liver toxicity than commercial film (Table S6). It was likely that the serious peritoneal adhesion caused the enrichment of serum creatinine in the casting film group, which is consistent with the pathological changes. All biomarkers detected in other groups returned

Table 3
Death time, adhesion level, and adhesion tenacity in the pig models.

Group		Normal Group (n = 5)	Fibrous Membranes (n = 5)	Casting Film (n = 5)	Commercial Film (n = 5)	Negative Group (n = 5)
Alive in each group		5	5	5	5	5
Dead Time	<1 d	0	0	0	1	1
	1 d–3 d	0	0	0	1	0
	>3 d	0	0	1	0	2
Adhesion Level	0	5	2	1	1	1
	1	0	0	1	1	0
	2	0	1	1	2	1
	3	0	2	2	1	3
Adhesion Tenacity	0	5	1	1	1	1
	1	0	3	3	1	0
	2	0	1	1	2	0
	3	0	0	0	1	4

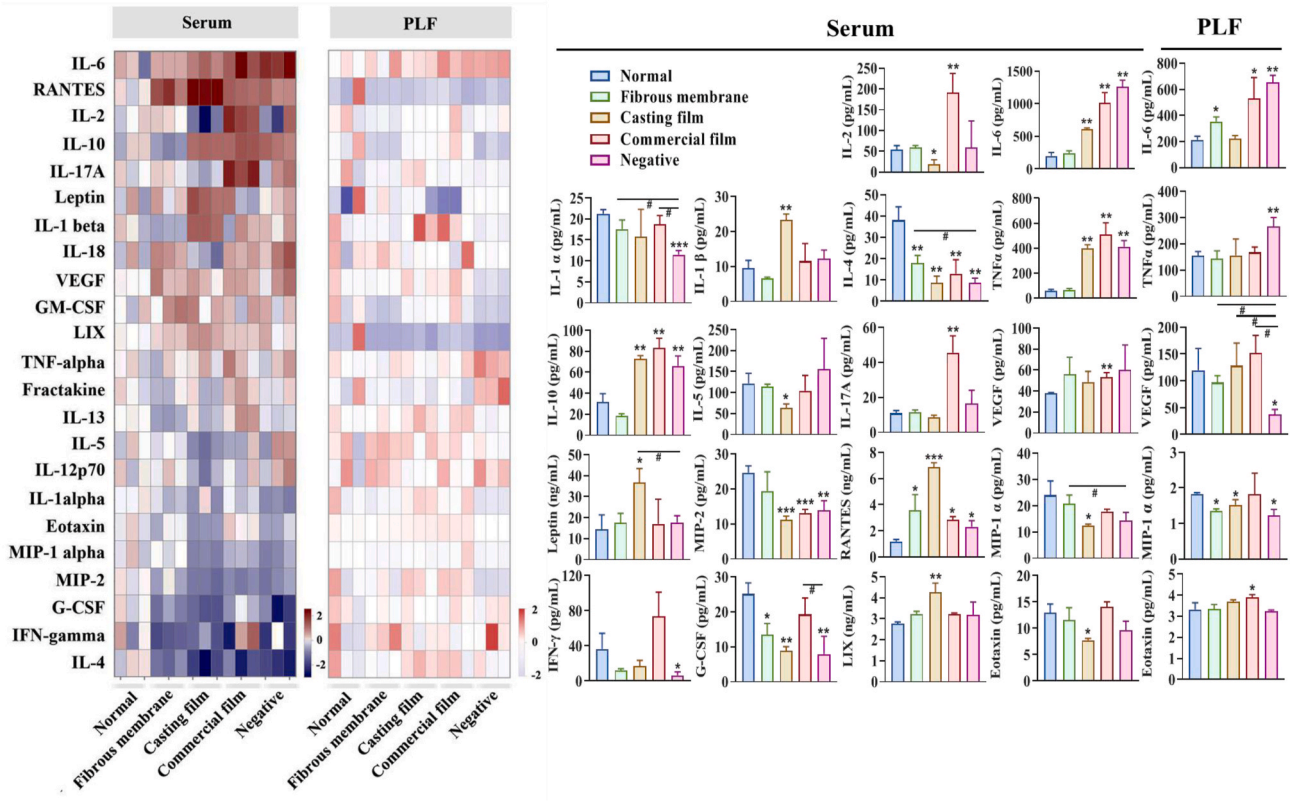


Fig. 4. The fibrous membranes reduced the inflammation induced by peripheral surgery. Multiple cytokine/chemokine expression levels in PLF and serum were detected, including EGF, Eotaxin, Fractalkine, G-CSF, GM-CSF, GRO/KC/CINC-1, IFNγ, IL-1α, IL-1β, IL-2, IL-4, IL-5, IL-6, IL-10, IL-12 (p70), IL-13, IL-17A, IL-18, IP-10, Leptin, LIX, MCP-1, MIP-1α, MIP-2, RANTES, TNFα, and VEGF.

to normal levels after 6 months (Table S7).

The white blood cell (WBC) count and percentage of granulocyte and monocytes in the negative group were significantly higher than those in the normal group because of the injury caused by surgical manipulation, which in the fibrous membrane group were lower than commercial film and negative groups ($p < 0.05$). The fibrous membrane significantly relieved inflammation and resulted in much slighter inflammation than the commercial film. Most of the inflammatory indicators returned to normal levels after 6 months (Table 4). Malondialdehyde (MDA) and superoxide dismutase (SOD) activity are well-known biomarkers that evaluate oxidative damage [32]. As shown in Table 5, a significant increase in the MDA level and a significant reduction in the SOD level were observed in the casting film or commercial film groups compared to the normal group, similar to the negative group (both acute and chronic phase) because of the injury caused by surgical manipulation ($p < 0.05$). The fibrous membrane possessed better biological compatibility and successfully inhibited the oxidative damage, which might be related to the ECM-like structure of fibrous membranes, which presented less foreign body reaction than the commercial film and casting film. Nitric oxide (NO) is a critical reactive oxygen species (ROS) and function molecule released from activated macrophages [33]. Similar to MDA, NO levels significantly increased after surgical injury. The casting films and fibrous membranes reduced the NO levels, while the commercial film had no effect. As time passed, the commercial film demonstrated the capability to reduce the NO levels. Alkaline phosphatase (AKP) activity in both commercial film and negative groups decreased significantly compared to the normal group, which would be reduced measurably in severe and widespread liver damage ($p < 0.05$).

Moreover, its activity in both the fibrous membrane and casting film was similar to the normal control. These results were consistent well with pathological appearances. The fibrous membrane possessed better biological compatibility and successfully relieved the organ damage.

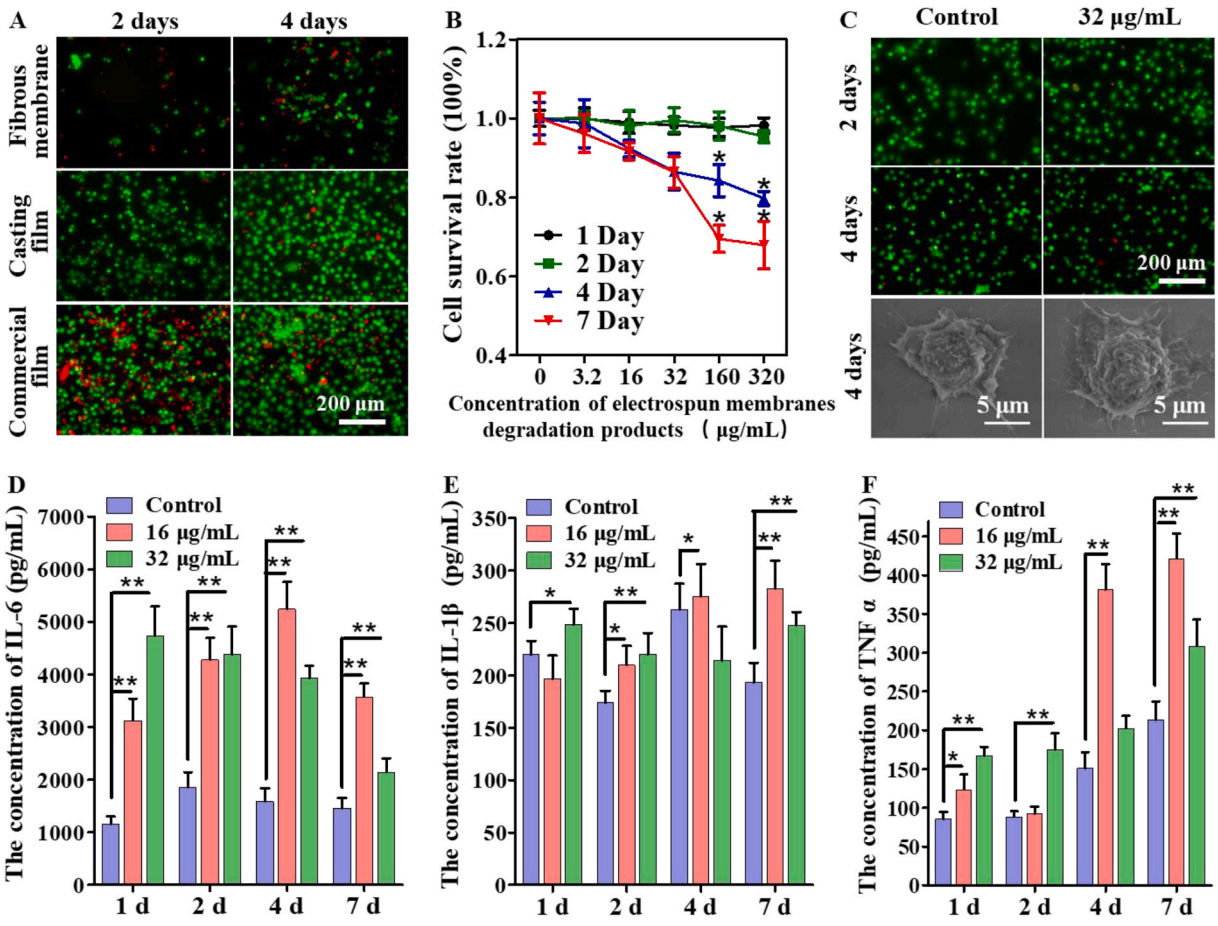


Fig. 5. The degradation product of the fibrous membranes does not affect macrophages. A, The cell growth and morphology on the fibrous membranes, casting film, and commercial film were observed using a live-dead cell staining kit. B and C, After being cultured with the degradation fluid of the fibrous membrane, the cell survival rate was detected using CCK-8 (B). The cell growth and morphology were observed using a live-dead staining kit and ESEM (C). The concentrations of IL-1β (D), IL-6 (E), and TNF-α (F) in the culture supernatant were determined using ELISA after 1 day, 2 days, 4 days, and 7 days.

Table 4
Hematologic examinations of rat models in the acute and chronic phase.

		Normal	Fibrous membrane	Casting film	Commercial film	Negative
Acute phase	White blood cell count (10^9 cells/L)	5.39 ± 1.18	9.78 ± 4.28$^{\&}$	7.82 ± 3.76$^{\&}$	10.49 ± 6.22	18.92 ± 6.42*
	Percentage of granulocyte (%)	3.33 ± 2.08	5.33 ± 3.05	5.67 ± 1.15	8.00 ± 1.00*	10.50 ± 3.36*
	Percentage of monocyte (%)	19.00 ± 1.41	42.38 ± 19.39$^{*,\ \&,\#}$	38.96 ± 8.94$^{*,\ \&,\#}$	88.00 ± 19.27**	81.67 ± 4.93**
	Percentage of lymphocyte (%)	71.55 ± 8.55	65.11 ± 7.94	73.53 ± 5.42	65.63 ± 8.57	66.75 ± 8.82
	PLT (10^9/L)	844.80 ± 237.86	962.00 ± 112.17	1167.6 ± 227.66*	891.33 ± 159.43	944.00 ± 214.56
Chronic phase	White blood cell count (10^9 cells/L)	3.79 ± 0.78	3.60 ± 0.12	3.71 ± 0.60	3.99 ± 1.28	4.53 ± 1.20
	Percentage of granulocyte (%)	2.53 ± 0.68	2.42 ± 0.38	2.08 ± 0.49	2.50 ± 0.41	2.35 ± 0.47
	Percentage of monocyte (%)	18.25 ± 6.08	42.33 ± 7.50*	30.24 ± 5.24*	51.25 ± 8.69*	63.00 ± 26.51*
	Percentage of lymphocyte (%)	69.37 ± 9.36	70.92 ± 7.43	69.23 ± 6.39	72.45 ± 6.42	56.88 ± 6.90
	PLT (10^9/L)	831.33 ± 201.62	847.60 ± 70.34	867.10 ± 113.58	728.25 ± 83.75	624.67 ± 218.03

* and ** represent a significant difference compared with the normal group, $^{*}p < 0.05$, $^{**}p < 0.01$.
$^{\&}$ and $^{\&\&}$ represent a significant difference compared with the negative group, $^{\&}p < 0.05$, $^{\&\&}p < 0.01$.
$^{\#}$ and $^{\#\#}$ represent a significant difference compared with the commercial film group, $^{\#}p < 0.05$, $^{\#\#}p < 0.01$.

The LDH activity in the negative group significantly increased compared with that of the normal control, which demonstrated acute damage induced by abdominal surgery ($p < 0.01$) because LDH activity was measured as an indicator of tissue damage and cytotoxicity. When cells and tissues are injured, LDH will be released from cytosol and present in the peritoneal lavage fluid. The LDH activity of the casting films and fibrous membranes group ($p < 0.05$) and commercial film group ($p < 0.01$) were significantly decreased compared to the negative control group. In addition, the casting films and fibrous membranes showed a higher ability to reduce the LDH activity than the commercial film. After long-term exposure, the casting films and fibrous membranes can ultimately help tissue recover from surgery-induced damage, with their LDH activity similar to the normal group. However, the LDH activity in the PLF of the commercial film group was still as high as the negative group, which indicated that commercial film cannot help to recover tissue injury.

Table 5
The levels of SOD, NO, MDA, AKP, and LDH in PLF of rat models in the acute and chronic phase.

		Normal	Fibrous membrane	Casting film	Commercial film	Negative
Acute phase	MDA (nmol/mL)	1.01 ± 0.17	1.11 ± 0.22	1.27 ± 0.36	1.75 ± 0.16*	1.64 ± 0.21*
	SOD (U/mg prot)	132.01 ± 19.74	120.76 ± 10.56	91.09 ± 11.87*	102.64 ± 22.00	84.32 ± 5.7*
	NO (μmol/g prot)	2.75 ± 0.66	2.53 ± 1.41	2.62 ± 0.95	5.98 ± 1.31*	4.66 ± 0.92*
	AKP (U/g prot)	53.99 ± 5.42	46.11 ± 5.98	52.96 ± 6.79	19.22 ± 3.71**	41.98 ± 6.58*
	LDH activity (U/g prot)	1566.15 ± 239.39	2766.83 ± 377.40$^{*, \&, \#}$	2165.80 ± 209.83$^{*, \&, \#}$	3645.02 ± 172.90**	3257.02 ± 101.64**
Chronic phase	MDA (nmol/mL)	1.56 ± 0.07	1.53 ± 0.06	1.75 ± 0.09	1.62 ± 0.24	4.63 ± 0.35**
	SOD (U/mg prot)	120.52 ± 16.04	109.12 ± 17.69	101.78 ± 9.01	82.24 ± 10.07*	44.59 ± 7.10**
	NO (μmol/g prot)	2.01 ± 0.40	2.47 ± 0.70	2.15 ± 0.58	2.53 ± 0.32	1.85 ± 0.25
	AKP (U/g prot)	24.69 ± 5.36	31.08 ± 5.46	29.58 ± 4.15	30.75 ± 5.68	22.82 ± 4.06
	LDH activity (U/g prot)	1149.41 ± 359.89	1620.91 ± 914.76$^{\&\&, \#\#}$	1318.17 ± 168.05 $^{\&\&, \#\#}$	4200.30 ± 1178.32**	4362.39 ± 553.17**

* and ** represent a significant difference compared with the normal group, $^{*}p < 0.05$, $^{**}p < 0.01$.
& and && represent a significant difference compared with the negative group, $^{\&}p < 0.05$, $^{\&\&}p < 0.01$.
# and ## represent a significant difference compared with the commercial film group, $^{\#}p < 0.05$, $^{\#\#}p < 0.01$.

3.7. The fibrous membranes effectively prevent tissue adhesion in the clinical trial

A total of 150 patients were included in the study database (n = 75 in each treatment group). Furthermore, the study duration was 12 weeks (Fig. 6). The demographic characteristics were homogeneous between the groups (Table S8). In general, the incidence of postoperative tissue adhesion was the primary evaluation indicator. The incidence of adhesion in the test group was lower than the control group in both FAS (full analysis set) and PPS (per-protocol set) populations (Table 6). The results supported the premise that PLGA/PLA-b-PEG absorbable anti-adhesion fibrous membrane could prevent postoperative tissue adhesion in humans. Thus, there were 12 cases of adhesion for the FAS population, including 10 mild cases and 2 moderate cases among 73 subjects in the test group. The incidence of adhesion was 16.4%, and there were 25 cases, including 21 mild cases and 4 moderate cases among 73 subjects in the control group. The incidence of adhesion was 34.2%. For the PPS population, there were 12 cases of adhesion, including 10 mild cases and 2 moderate cases among 71 subjects in the test group. The incidence of adhesion was 17.0%, and there were 25 cases, including 21 mild cases and 4 moderate cases among 72 subjects in the control group. The incidence of adhesion was 34.7%.

It is most important to evaluate the extent or severity of the postoperative adhesion development (Table 7), severity of abdominal and pelvic pain (Table 8), and occurrence of abdominal and pelvic effusion (Table 9). They are secondary evaluation indicators to assess the efficacy of PLGA/PLA-b-PEG absorbable anti-adhesion fibrous membrane. The extent of adhesion in the test group was less severe than that of the control group in both FAS and PPS populations. For the FAS population, there were 12 cases of adhesion, including 10 mild cases and 2 moderate cases among 73 subjects in the test group. Furthermore, there were 25 cases of adhesion, including 21 mild cases and 4 moderate cases among 73 subjects in the control group. For the PPS population, there were 12 cases of adhesion, including 10 mild cases and 2 moderate cases among

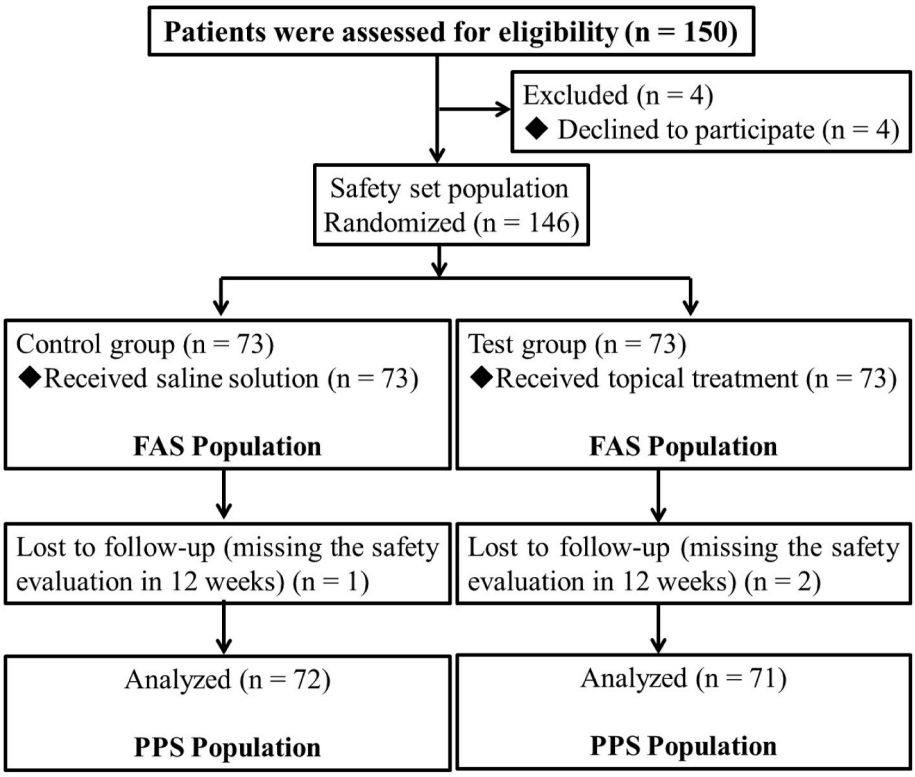


Fig. 6. Flow diagram of the clinical trial progress. Safety Set (SS): Randomized patients who took the treatment as well as all of the evaluations; Full Analysis Set (FAS): randomized patients who met all selection criteria; Per Protocol Set (PPS): FAS patients, who participated in the treatment and provided significant statistical data.

Table 6
Comparison of the frequency and rate of postoperative peritoneal adhesion formation.

Variable	Full analysis set (FAS)				Per-protocol set (PPS)			
	Test n = 73	Control n = 73	Z	p	Test n = 71	Control n = 72	Z	p
Number of adhesion	12	25	−0.21	<0.0001	12	25	−0.21	<0.0001
Rate of adhesion (%)	16.4	34.2			17.0	34.7		

Table 7
Comparison of the severity of postoperative tissue adhesion development.

Variable	Full analysis set (FAS)			Per-protocol set (PPS)		
	Test n = 71 (lost 2)	Control n = 72 (lost 1)	p	Test n = 71	Control n = 72	p
No adhesion (%)	61 (83.56)	48 (65.75)	0.0146	59 (83.10)	47 (65.28)	0.0163
Mild adhesion (%)	10 (13.70)	21 (28.77)		10 (14.08)	21 (29.17)	
Moderate adhesion (%)	2 (2.74)	4 (5.48)		2 (2.82)	4 (5.56)	
Severe adhesion (%)	0 (0.00)	0 (0.00)		0 (0.00)	0 (0.00)	

71 subjects in the test group. Moreover, among 72 subjects in the control group, there were 25 cases of adhesion, including 21 mild cases and 4 moderate cases. There was no difference in abdominal and pelvic pain intensity and effusion between the two groups in both FAS and PPS populations.

4. Discussion

The formation of postoperative peritoneal adhesions is mainly based on the interplay of the fibrinolytic system, extracellular matrix, and inflammatory system [6,34]. The design and development were optimized to satisfy the following strictly three crucial factors (i.e. feasibility, validity, and safety). Firstly, to ensure the quality and output, we choose raw materials from well-researched, biodegradable, and biocompatible polymers that have been used in clinical applications for a long time [35]. The PLGA with a certain LA/GA ratio and molecular weight can target the required degradation time [14]. No burst releasing of the degradation product during the tissue repairing process might result in an unpredictable influence on wound healing.

Moreover, the implants could maintain the stable morphology and complete the barrier structure [6]. Both SEPRAFILM® and INTERCEED® were absorbed in 1–2 weeks, same as the continuous sheet of mesothelial cells was restored [36,37]. In addition, their fast-releasing degradation would also induce the immune system to overreact, which causes a significant intestinal obstruction. Secondly, the non-woven membrane was prepared by electrospinning technology, a mature, inexpensive, simple, and stable technology, to present a bionic ECM-like structure with high specific surface area and easily regulated morphology [4]. Thirdly, the gradual swelling and Tg at approximately

Table 8
Comparison of the severity of abdominal and pelvic pain.

Variable	Full analysis set (FAS)			Per-protocol set (PPS)		
	Test	Control	P	Test	Control	p
5 days later			0.4947			0.6175
Number of case (lost)	73 (0)	73 (0)		71 (0)	72 (0)	
No pain (%)	0 (0.00)	0 (0.00)		0 (0.00)	0 (0.00)	
Mild pain (%)	60 (82.19)	63 (86.3)		59 (83.10)	62 (86.11)	
Moderate pain (%)	13 (17.81)	10 (13.7)		12 (16.90)	10 (13.89)	
Severe pain (%)	0 (0.00)	0 (0.00)		0 (0.00)	0 (0.00)	
Intensive pain (%)	0 (0.00)	0 (0.00)		0 (0.00)	0 (0.00)	
Intolerable pain (%)	0 (0.00)	0 (0.00)		0 (0.00)	0 (0.00)	
3 weeks later			0.7998			0.6883
Number of case (lost)	73 (0)	72 (1)		71 (0)	72 (0)	
No pain (%)	16 (21.92)	18 (25.00)		15 (21.13)	18 (25.35)	
Mild pain (%)	55 (75.34)	51 (70.83)		54 (76.06)	50 (70.42)	
Moderate pain (%)	2 (2.74)	3 (4.17)		2 (2.82)	3 (4.23)	
Severe pain (%)	0 (0.00)	0 (0.00)		0 (0.00)	0 (0.00)	
Intensive pain (%)	0 (0.00)	0 (0.00)		0 (0.00)	0 (0.00)	
Intolerable pain (%)	0 (0.00)	0 (0.00)		0 (0.00)	0 (0.00)	
6 weeks later			/			/
Number of case (lost)	71 (2)	72 (1)		71 (0)	72 (0)	
No pain (%)	71 (100.00)	72 (100.00)		71 (100.00)	72 (100.00)	
Mild pain (%)	0 (0.00)	0 (0.00)		0 (0.00)	0 (0.00)	
Moderate pain (%)	0 (0.00)	0 (0.00)		0 (0.00)	0 (0.00)	
Severe pain (%)	0 (0.00)	0 (0.00)		0 (0.00)	0 (0.00)	
Intensive pain (%)	0 (0.00)	0 (0.00)		0 (0.00)	0 (0.00)	
Intolerable pain (%)	0 (0.00)	0 (0.00)		0 (0.00)	0 (0.00)	
12 weeks later			/			/
Number of case (lost)	71 (2)	72 (1)		71 (0)	72 (0)	
No pain (%)	71 (100.00)	72 (100.00)		71 (100.00)	72 (100.00)	
Mild pain (%)	0 (0.00)	0 (0.00)		0 (0.00)	0 (0.00)	
Moderate pain (%)	0 (0.00)	0 (0.00)		0 (0.00)	0 (0.00)	
Severe pain (%)	0 (0.00)	0 (0.00)		0 (0.00)	0 (0.00)	
Intensive pain (%)	0 (0.00)	0 (0.00)		0 (0.00)	0 (0.00)	
Intolerable pain (%)	0 (0.00)	0 (0.00)		0 (0.00)	0 (0.00)	

Table 9
Comparison of the occurrence of abdominal and pelvic effusion.

Variable	Full analysis set (FAS)			Per-protocol set (PPS)		
	Test	Control	P	Test	Control	p
5 days later			0.3256			0.2840
Number of case (lost)	73 (0)	73 (0)		71 (0)	72 (0)	
No effusion (%)	70 (95.89)	66 (90.41)		69 (97.18)	66 (91.67)	
Effusion (%)	3 (4.11)	7 (9.59)		2 (2.82)	6 (8.33)	
3 weeks later			0.1169			0.1752
Number of case (lost)	73 (0)	72 (1)		71 (0)	72 (0)	
No effusion (%)	70 (95.89)	65 (89.04)		69 (97.18)	65 (90.28)	
Effusion (%)	3 (4.11)	8 (10.96)		2 (2.82)	7 (9.72)	
6 weeks later			/			/
Number of case (lost)	70 (3)	72 (1)		69 (2)	72 (0)	
No effusion (%)	70 (100.00)	72 (100.00)		69 (100.00)	72 (100.00)	
Effusion (%)	0 (0.00)	0 (0.00)		0 (0.00)	0 (0.00)	
12 weeks later			1.0000			1.0000
Number of case (lost)	70 (3)	69 (4)		69 (2)	69 (3)	
No effusion (%)	70 (100.00)	68 (99.29)		69 (100.00)	68 (99.29)	
Effusion (%)	0 (0.00)	1 (0.71)		0 (0.00)	1 (0.71)	

37 °C made the PLGA/PLA-b-PEG fibrous membrane with better flexibility, hygroscopicity, and tacking ability than the casting film or commercial film with a smooth surface, which also contributed to the fact that there is no need for sutures during the surgery, thereby significantly reducing the operation time.

As a well-designed product, its destination should be applied in the clinic, which requires stable manufacture quality and approval by FDA. It has been recognized that the complex approval procedure by the FDA of most countries makes the application prospects of new material seem unpractical or unfeasible due to the time duration and cost involved. Our results confirmed that familiar and old materials could be used to solve new problems through rational designing and processing to tailor the structure and morphology of the matrix, while still providing a great application potential. The pre-clinical study was based on a simple principle among solid barriers, which had the same degraded products and no chemical modification to ensure that no new functional groups or active agents were introduced to cause potential adverse responses. Though the adhesion level, tenacity, and intestinal obstruction did not significantly differ among the groups, fibrous membrane tends to reduce the severe adhesion (lower level 3 adhesion occurrence percentage). Insignificant adverse reactions were observed during the entire clinical trial.

5. Conclusion

PLGA/PLA-b-PEG fibrous membrane with ECM-like structure was found to cause only slighter acute inflammatory responses, much less intestinal obstruction, and decreased peripheral adhesion, compared to the casting film with the same components and the commercial film. As a soft solid bio-absorbable barrier, the fibrous membrane can be used as a favorable implant because of its flexibility, biodegradability, and similarity to the mechanical modulus of the involved tissues. These advantages led to success in a clinical trial, which also indicated the reduction of severe adhesion and excellent safeness.Old materials are still worthy of further research, and the critical issue is to optimize all necessary parameters through the rational control of the physical processes of fabricating the device or membrane.

CRediT authorship contribution statement

Shanshan Xu: Conceptualization, Methodology, Writing – review & editing. **Chenhong Wang:** Data curation, Writing – original draft, preparation. **Ruoqing Mao:** Visualization, Investigation. **Xiaoyu Liang:** Data curation, Validation. **Heran Wang:** Visualization, Investigation. **Zhenyu Lin:** Data curation, Validation. **Jiangxue Li:** Data curation, Validation. **Shilin Li:** Data curation, Validation. **Jipeng Jiang:** Data curation, Validation. **Tongshuo Zhang:** Data curation, Validation. **Yongfu Ma:** Data curation, Validation. **Yang Liu:** Reviewing. **Charles C. Han:** Writing – review & editing. **Ying Liu:** Writing – original draft, Writing – review & editing.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.bioactmat.2021.10.040.

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