Study on Prevention of Postoperative Abdominal Adhesions with PLGA Nanofiber Membrane

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Abstract: Objective: To evaluate the effectiveness of poly (lactic-co-glycolic acid) (PLGA) nanofiber membrane on prevention of postoperative abdominal adhesion. Methods: PLGA nanofiber membrane was prepared by high-voltage electrospinning technique. The effectiveness of the membrane in prevention of postoperative abdominal adhesions was characterized with rat abdominal adhesion models. Results: PLGA nanofiber membrane was prepared successfully by electrospinning technique. Scanning electron microscopy (SEM) observation showed that the average diameter of PLGA fibers was approximately 800 nm, and the membrane had microporous structures. Mechanical tests showed that the tensile strength of PLGA nanofiber membrane was 6.36 ± 0.39 MPa, which was significantly higher than the tensile strength of DIKANG absorbable medical film. The results of in vivo experiments showed that PLGA nanofiber membrane and DIKANG absorbable medical film could both reduce the degree of abdominal adhesions. The histological results showed that there was only a small extent of inflammatory cell infiltration in the PLGA group and the control group. The proliferation of connective tissue was reduced, and so was the degree of adhesion. Conclusion: PLGA nanofiber membrane can significantly reduce the incidence and severity of postoperative adhesions, and bodes well for future clinical applications.

1. Introduction
Postoperative abdominal adhesion is a surgical problem that has not been fully solved, and still has a high occurrence. Studies have shown that the rate of adhesion after abdominal surgery could be as high as 90% [1]. Once adhesion occurs, it may induce serious complications, such as small bowel obstruction (SBO), female infertility, and chronic pain, significantly affecting the patient’s health and quality of life [2]. Meanwhile, postoperative adhesion will complicate secondary surgical processes, which further brings a series of adverse consequences, including prolonged surgery, unplanned enterotomy, and switching from laparoscopic surgery to laparotomy, thus inconveniencing the patient both clinically and financially [3, 4]. At present, there are three main approaches to prevent postoperative adhesion: improved surgical techniques, drugs that inhibit fibrin formation and reduce inflammation, and physical isolation [5]. Physical isolation is the favored clinical approach, and there are two types of physical isolation products that are commonly used: anti-adhesion gels and anti-adhesion membranes [6, 7]. The difficulty...
of anti-adhesion gel remaining in the desirable position may have limited its clinical application, whereas anti-adhesion membrane is more widely used to physically isolate the wound from other tissues or organs, and effectively prevent the formation of postoperative adhesion \cite{8, 9}. There are mainly two types of anti-adhesion membranes that are clinically available: Seprafilm (made from sodium hyaluronic acid and carboxymethyl cellulose) and polylactic acid-based anti-adhesion membranes. The Seprafilm system lasts approximately a week in vivo \cite{10}, resulting in insufficient prevention of adhesion. The polylactic acid-based membrane, however, has inferior mechanical strength caused by irradiation sterilization, and is difficult to suture. Additionally, the polylactic acid-based membrane also appears to be too dense for the exchange of oxygen and nutrients at the wound, which is unfavorable for tissue repair \cite{11}. Therefore, clinical needs exist for the development of a porous anti-adhesion membrane with satisfactory mechanical properties.

PLGA is biocompatible, biodegradable, easy to process, and controllable in performances. It is widely used in biomedical applications such as tissue engineering scaffolds, drug-carrying microspheres, and surgical sutures. It is the only medical polymer material approved by the U.S. Food and Drug Administration (FDA) for injection \cite{12}. Electrospinning is a common method to prepare nanofiber membranes. The nanofiber membranes prepared by electrospinning have high porosity, high specific surface area, and good flexibility, which makes them suitable to be applied to the wound surfaces \cite{13}. In this study, PLGA was used to prepare the ultra-fine nanofiber membrane by electrospinning, and the performance of the membrane was compared with that of a commercially available product (DIKANG absorbable medical film). With the rat abdominal adhesion models, the anti-adhesion effects of PLGA nanofiber membrane was assessed, providing preliminary results for future clinical applications.

2. Materials and Methods

2.1. Materials

PLGA (LA: GA = 85:15, η = 3.1 DL / g) was purchased from Corbion Trading (Shanghai) Co. Ltd. (China). 2,2,2-trifluoroethanol (purity: 99.5%) was purchased from Shanghai Aladdin Bio-Chem Technology Co. Ltd. (China). DIKANG absorbable medical films (made of polylactic acid) were purchased from Chengdu DIKANG Pharmaceutical Co. Ltd. and adult SPF grade Sprague-Dawley rats were purchased from Guangdong Medical Laboratory Animal Center.

2.2. Methods

2.2.1. Preparation of PLGA nanofiber membrane

1 g of PLGA was dissolved in 10 ml of 2,2,2-trifluoroethanol for 24 hours to achieve spinning solution with a concentration of 10% wt/v. The spinning solution was then electrospun with a voltage of 15 kV, a flow rate of 2 ml / h, and a distance of 20cm between the needle and the receiving roll. The prepared PLGA nanofiber membrane was dried in vacuum at 40 °C for 24 hours to remove residual solvent, and subsequently irradiated with 25 kGy for sterilization.

2.2.2. Physical characterizations

The morphology of PLGA nanofiber membrane and DIKANG absorbable medical film were observed by cold field emission scanning electron microscope (SEM, Hitachi S-520), and the distribution of fiber diameter was calculated with ImageJ.

In order to characterize the mechanical properties of the anti-adhesion membranes, a universal testing machine (UTM6103, Shenzhen Suns Technology) was used. Tests followed the protocol of GB/T 1040.3-2006. Test samples were cut into 10 mm × 60 mm strips with 5 samples for each group. The testing machine had a load cell of 200 N, and the tests were carried out with a clamp distance of 30 mm and a stretching rate of 0.6 mm/min. The ultimate tensile strengths were recorded.
2.2.3. Surgical procedure
18 healthy female Sprague-Dawley rats (weighing 200-250g each) were divided into three groups (n=6): PLGA group, control group, and blank group.

All rats were anesthetized by abdominal injection of 0.6 ml of 2% sodium amobarbital. Subsequently, the rats were sheared and sterilized at the abdomen areas, and then placed on sterile stainless steel operating table. On every rat, a 5 cm incision was made along medioventral line and a lesion of 1×1 cm² was then created in the abdominal wall muscle tissue with a scalpel. Dry sterilized gauze was then used to rub the serous layer of cecum (to cause bleeding but not perforation) at the site opposite to the lesion, so as to create another 1×1 cm² lesion on the cecum. After hemostasis, PLGA nanofiber membranes and DIKANG absorbable medical films were sutured to abdominal walls for PLGA group and control group respectively. For blank group, no intervention was performed. Finally, incisions were closed and routine postoperative nursing was provided.

On the 14th day after operation, rats were killed by intraperitoneal injection of sodium pentobarbital (120 mg / kg). Abdominal cavity was opened carefully with a U-shape incision. The implant was identified, and the adhesion was observed and graded. The adhesion tissues were separated and stored in paraformaldehyde solution.

2.2.4. Macroscopic observation
Macroscopic observation was performed on the 14th day after operation. Degree of adhesion was evaluated and graded according to the scoring system developed by Martin-Cartes [14], as is shown in Table 1. Scores were given based on Quantity of Adhesion and Quality of Adhesion.

| Score | Criterion                                                                 |
|-------|---------------------------------------------------------------------------|
| 0     | no adhesions                                                              |
| 1     | adhesions covering no more than 25% of the surface of the implant          |
| 2     | adhesions covering between 25 and 50% of the surface of the implant        |
| 3     | adhesions covering between 51 and 75% of the surface of the implant        |
| 4     | adhesions covering between 76 and 100% of the surface of the implant       |
| 0     | no adhesions                                                              |
| 1     | light adhesions that instantly come free without damaging surrounding tissues |
| 2     | adhesions that could be easily released                                   |
| 3     | adhesions that need to be released by blunt dissection                    |
| 4     | firm adhesions only released by sharp dissection                          |

2.2.5. Histological observation
The adhesion tissues from the PLGA group and the control group were resected and fixed in 10% paraformaldehyde solution. Specimens were dehydrated, embedded in paraffin, and sectioned to thickness of 4 μm. They were then mounted on slides, and stained with hematoxylin and eosin (HE) for examination of presence of fibrous connective tissues and inflammatory cell infiltration.

2.2.6. Statistical analysis
Quantitative data were presented as mean±S.E., and one-way analysis of variance (ANOVA) was performed for comparisons of multiple groups. P-values <0.05 were considered statistically significant.

3. Results

3.1. Morphology
PLGA nanofiber membranes were successfully fabricated by using electrospinning technique. The membranes exhibited white color, and flexible and smooth texture. SEM micrograph of PLGA nanofiber membrane was shown in Fig 1a. It could be seen that the membrane consisted of randomly aligned nanofibers, and was highly porous. The average diameter of the nanofibers was approximately 800 nm (see Fig 1b). In comparison, Fig 1c shows the SEM micrograph of DIKANG absorbable medical film. Its average fiber diameter was approximately 2000 nm (see Fig 1d).

Fig 1. SEM micrographs and fiber diameter distribution of PLGA nanofiber membrane and DIKANG absorbable medical film: a. SEM micrograph of PLGA nanofiber membrane; b. Fiber diameter distribution of PLGA nanofiber membrane; c. SEM micrograph of DIKANG absorbable medical film; d. Fiber diameter distribution of DIKANG absorbable medical film

3.2. Mechanical properties
Fig 2 listed the tensile strengths of PLGA nanofiber membranes and DIKANG absorbable medical films. We could see that PLGA nanofiber membranes had considerably higher tensile strengths (6.36 ± 0.39 MPa) than DIKANG absorbable medical films (3.15 ± 0.19 MPa).

Fig 2. Tensile strengths of PLGA nanofiber membranes and DIKANG absorbable medical films

3.3. In vivo experiment results
All three groups of rats survived the experiment with normal wound healing and dietary behaviors. It could be seen from Fig 3a and 3b that PLGA nanofiber membrane and DIKANG absorbable medical film were both still present after 14 days, suggesting they had been successfully serving as physical barriers during this period.
Fig 3. Macroscopic images of each group on 14th day after operation: a. PLGA group; b. control group; c. blank group

In terms of Quantity of Adhesion, it could be seen from Fig 3c that the blank group had the largest area of adhesion and the PLGA group had the smallest. Fig 4a further listed the scores of Quantity of Adhesion for each group. The blank group had the highest score of 4.0±0. The PLGA group had a lower score (1.2±0.8) than the control group (1.3±1.0), although not statistically significant. "Scores of Quality of Adhesion were also given to all groups and were listed in Fig 4b. Both categories of scores suggested that PLGA nanofiber membrane and DIKANG absorbable medical film could substantially reduce the degree of adhesion.

Fig 4. Comparison of adhesion scores of all groups: a. Quantity of Adhesion; b. Quality of Adhesion

Fig 5 showed the histological micrographs of the PLGA group and the control group. We could see that, in both groups, there was only a small amount of connective tissue and inflammatory cell infiltration, confirming reduced adhesions.

Fig 5. Histological micrographs of: a. PLGA group (×100); b. PLGA group (×250); c. PLGA (×400); d. control group (×100); e. control group (×250); f. control group (×400)

3.4. Discussion
Currently, surgeons are constantly improving their surgical techniques to reduce the incidence of postoperative adhesions, including minimizing the size of incisions, repeated and timely hemostasis, etc. These measures, however, will not prevent the creation of wounds in surgeries and hence cannot completely eliminate adhesions [6]. Researchers have also been studying the mechanisms of adhesions and have developed drugs to prevent adhesions, such as non-steroidal anti-inflammatory drugs, streptokinases, fibrinolytic agents [15]. These drugs have shown certain anti-adhesion functions, but
may have negative effects on wound healing [7]. Therefore, physical isolation, which separates wound and surrounding tissues until the wound repair is completed, has become the most promising method to prevent postoperative adhesions.

PLGA is a biocompatible and biodegradable material with extensive clinical applications. The ratio of glycolic acid (GA) and lactic acid (LA) in the polymer chain could be adjusted so as to give PLGA variable mechanical properties and degradation rates. This study used PLGA to fabricate nanofiber membranes by electrospinning, and the resultant membranes were satisfactorily smooth and pliable for surgical application. The membrane has an average fiber diameter of ~800 nm and accordingly a pore size of ~2000 nm. This gives the membrane a larger specific area than the commercially available anti-adhesion product (with average fiber diameter of ~2000 nm and pore size of ~800 nm) and is likely to facilitate the exchange of oxygen and nutrients at the wound, providing a desirable microenvironment for tissue regeneration. Moreover, reports have shown that similar fibrous structures could mimic the extracellular matrix (ECM) of tissues and promote cell proliferation and hence wound repair [16, 17].

Mechanical property is crucial for the clinical performances of anti-adhesion membranes as it determines the intactness of the physical barrier [18]. The tensile strength of PLGA nanofiber membrane is higher than that of the commercial product, which would make it easier to remain intact during the surgical procedure, and to endure stresses caused by movements throughout the recovery period.

It has been found that the high-occurrence period of abdominal adhesion is 7-15 days after operation [19]. Therefore, anti-adhesion membranes should be designed to last for at least two weeks. The PLGA nanofiber membrane and the DIKANG absorbable medical film evaluated in this study both remained intact after 14 days, meeting the requirements of the intended application. It was worth noticing that the PLGA nanofiber membrane exhibited slight area shrinkage, indicating the onset of biodegradation. This is probably due to the higher specific area and hence the increased contact between the nanofibers and body fluid [20]. Rats in neither group have exhibited any fever, death, or severe inflammatory reactions, indicating desirable biocompatibility of both implants.

Severe adhesion was found on every single rat in the blank group, confirming the prevalence of postoperative adhesions in clinical scenarios. On contrast, the PLGA group and the control group had significantly lower degree of adhesion judging from macroscopic observation, which was further confirmed by the minimal connective tissue and inflammatory cell infiltration in histological analysis. This is because the physical barrier formed by the implants is able to hinder the participation of exogenous fibroblasts and inflammatory stimuli in the healing process, while promoting the proliferation of endogenous myofibroblasts and endothelial cells, as well as ECM synthesis [21]. Therefore, it is believed that the PLGA nanofiber membrane has a therapeutic efficacy comparable to that of the commercial product, which bodes well for its future clinical applications.

3.5. Conclusions
PLGA nanofiber membrane fabricated by electrospinning has desirable specific area and mechanical property, and has equal performance to the commercial product in reducing postoperative adhesion. Therefore, it is a promising candidate material for future anti-adhesion membranes.

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Highlights
In this study, PLGA nanofiber membrane was prepared by electrospinning technology, and its performance was compared with that of DIKANG absorbable medical membrane. At the same time, the anti-adhesion effect of the two kinds of biofilm was evaluated by rat abdominal adhesion model. The feasibility of PLGA nanofiber membrane as the anti-adhesion membrane was analyzed by animal experiment and histological system, so as to provide more theoretical basis for subsequent application.
References

[1] D. Menzies. Postoperative adhesions: Their treatment and relevance in clinical practice: Ann R Coll Surg Engl. Vol.75 (1993), p.147-153.

[2] J. Becker. Prevention of postoperative abdominal adhesions by a sodium hyaluronate-based bioresorbable membrane: a prospective, randomized, double-blind multicenter study: J Am Coll Surg. Vol.183 (1996).

[3] F.R. Dijkstra, M. Nieuwenhuijzen, M.M. Reijnen, et al. Recent clinical developments in pathophysiology, epidemiology, diagnosis and treatment of intra-abdominal adhesions: Scand J Gastroenterol. Vol.233 (2000), p.52.

[4] R.P.G. Ten Broek, M.W.J. Stommel, C. Strik, et al. Benefits and harms of adhesion barriers for abdominal surgery: a systematic review and meta-analysis: Lancet, Vol. 383 (2014), p.48-59.

[5] Wang W, Ma C, Wu Q, et al. Gynecology. Clinical efficacy and safety of adhesion prevention measures in preventing postoperative abdominopelvic adhesions: a Meta-analysis: Progress in Obstetrics & Gynecology (2016).

[6] Liu B, Sheng M, Zhang Y, et al. Research progress on anti-adhesive products in post peritoneal surgery: Journal of Pharmaceutical Practice (2016).

[7] J.J. Stangel, J.D. Nisbet, H. Settles, Formation and prevention of postoperative abdominal adhesions: Journal of Reproductive Medicine, Vol.29 (1984), p.143-156.

[8] Song Z, Zhang Y, Shao H, et al. Effect of xanthan gum on the prevention of intra-abdominal adhesion in rats: International Journal of Biological Macromolecules (2019).

[9] P.N. Zawaneh, D. Putnam. Materials in Surgery: A Review of Biomaterials in Postsurgical Tissue Adhesion and Seroma Prevention: Tissue Engineering Part B Reviews, Vol.14 (2008), p.377-391.

[10] Chen JP, Chen SH, Chen CH, et al. Preparation and characterization of antiadhesion barrier film from hyaluronic acid-grafted electrospun poly(caprolactone) nanofibrous membranes for prevention of flexor tendon postoperative peritendinous adhesion: Int J Nanomedicine. (2014), p.4079-.

[11] C.I.W. Lauder, G. Garcea, A. Strickland, et al. Abdominal Adhesion Prevention: Still a Sticky Subject: Digestive Surgery, Vol.27 (2010), p.347-358.

[12] Zhao L, He CG, Cen L, et al. Preparation and cytocompatibility of PLGA scaffolds with controllable fiber morphology and diameter using electrospinning method: J Biomed Mater Res B Appl Biomater, Vol.87 (2008), p.26-34.

[13] J.E. Ko, Y.G. Ko, W.I. Kim, et al. Nanofiber mats composed of a chitosan-poly(d,l-lactic-co-glycolic acid)-poly(ethylene oxide) blend as a postoperative anti-adhesion agent: Journal of Biomedical Materials Research Part B Applied Biomaterials, Vol.105 (2016).

[14] J. Martín-Cartes, S. Morales-Conde, J. Suárez-Grau, et al. Use of hyaluronidase cream to prevent peritoneal adhesions in laparoscopic ventral hernia repair by means of intraperitoneal mesh fixation using spiral tacks: Surgical Endoscopy, Vol.22 (2008), p.631-634.

[15] N.F. Inagaki, F.F. Inagaki, N. Kokudo, et al. Cell-based therapy for preventing postoperative adhesion and promoting regeneration after hepatectomy: Journal of Hepato-Biliary-Pancreatic Sciences, Vol.22 (2015), p.524-530.

[16] D. Bhavsar, D. Shettko, M. Tenenhaus. Encircling the Tendon Repair Site with Collagen-GAG Reduces the Formation of Postoperative Tendon Adhesions in a Chicken Flexor Tendon Model: Journal of Surgical Research, Vol.159 (2010), p.0-771.

[17] Liu X, Tong L, Fang J et al. In vivo wound healing and antibacterial performances of electrospun nanofibre membranes: Journal of Biomedical Materials Research Part A, Vol.94 (2010), p.499-508.

[18] Liu L, Yuan F, Zhang HH, et al. Evaluation of surgical anti-adhesion products to reduce postsurgical intra-abdominal adhesion formation in a rat model: PLOS ONE, Vol.12 (2017), p.e0172088
[19] Zhu Y, Li WL, Mao CQ et al. Experimental study on protection of tetramethylpyrazine nanoparticles on rat peritoneal mesothelium cells: China Journal of Modern Medicine (2015).

[20] Dong Y, Liao S, Chan CK, et al. Degradation Behaviors of Electrospun Resorbable Polyester Nanofibers: Tissue Engineering Part B Reviews, Vol.15 (2009), p.333-351.

[21] Liu HH, Zhang Y, Wu W, et al. Repair of tendon injury and prevention of adhesion: Journal of Clinical Rehabilitative Tissue Engineering Research, Vol.13 (2009), p.9946-9949.