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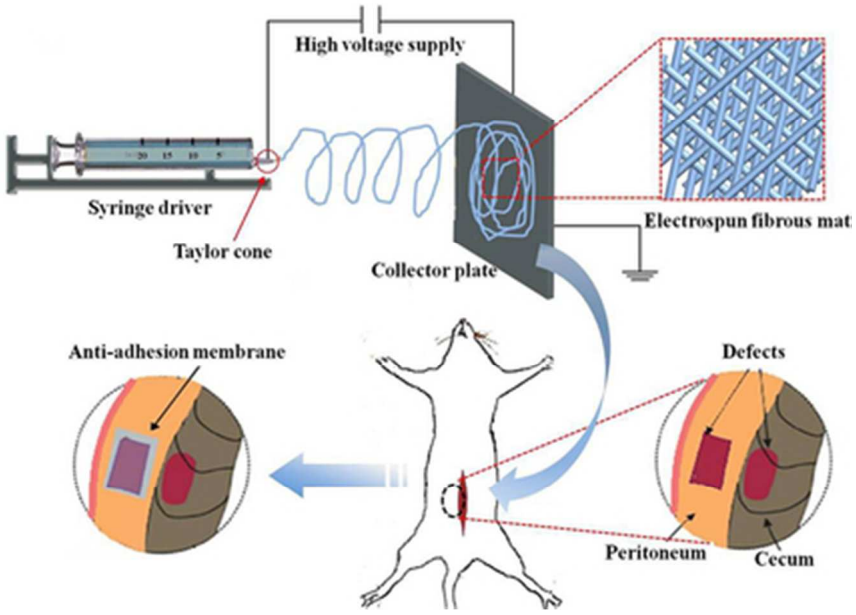

Schematic of the electrospinning set-up and the application of electrospun PCL membrane onto a peritoneal wall defect of a rat. It is truly interesting that the electrospun PCL membranes with various molecular weights also behave distinctly in the prevention of surgery induced-adhesions, which finally helped acquire the well-suited candidates as the anti-adhesion biomaterial films.
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Molecular weight-modulated electrospun poly(ε-caprolactone) membranes for postoperative adhesion prevention†

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Electrospun biodegradable polymer membranes can effectively serve as the barriers to prevent the postoperative intestinal adhesion. Previous work largely focused on utilizing different electrospun variables to regulate the membrane properties, but paid very limited attention to the straightforward influences of raw material characteristics. In the present work, the physical and physiological properties of electrospun poly(ε-caprolactone) (PCL) membranes with varying viscosity-average molecular weights (Mη = 40,000, 80,000 and 120,000 g/mol) were explored for the first time. Interestingly, the typical properties of electrospun films, such as morphological structure, mechanical properties, degradation kinetics and anti-adhesion effect, were revealed to be substantially dependent on the molecular weight. By clear contrast, PCL sample with viscosity-average molecular weight of 80,000 g/mol exhibited the best performance, including a regular fibrous morphology, superior tensile strength and Young's modulus of 1.13 and 8.41 MPa, respectively, which was presumably ascribed to the means of chain entanglements and interactions. Of most importance, no cytotoxicity was traced in the electrospun PCL membranes as revealed from the cell culture test, meanwhile a significant reduction of postoperative adhesion was observed. Because of the above excellent merits, the electrospun PCL membranes can be regarded as the excellent candidates for the anti-adhesion applications.

1. Introduction

Intestinal adhesion is a common complication occurring in most of patients after abdominal surgery. It usually renders a serious second surgery, as well as the chronic debilitating pain,[1] functional obstruction,[2] and female infertility.[3] To prevent the postoperative adhesion, some beforehand approaches, such as pharmacological inhibition[4] and barrier prevention,[5, 6] have been developed, of which placing a physical barrier between the injured site and the adjacent tissues is the most promising technique.[7] To date, various bioresorbable matrices have been reported to prevent adhesion, such as hyaluronic acid (HA),[8] injectable hydrogels (*e.g.*, thermosensitive Pluronic®, Poloxamer),[9] Interceed™ (Gynecare, Somerville, NJ),[10] Seprafilm™ (Genzyme, Cambridge, MA).[7] Unfortunately, some intrinsic defects block their clinical applications.[11] For instance, HA disappeared from the injured site soon after used, so as to weaken its efficacy.[12] Blood infiltration makes Interceed™ invalid in preventing adhesions,[13] thus surgeons must ensure that all blood is cleared from the surgical field prior to use. Seprafilm™ was found to present handling difficulties because of its brittleness and stickiness,[14] and it would undesirably cause adhesion in the presence of bacterial peritonitis in a murine model.

To overcome the drawbacks of these anti-adhesion materials, membranes based on biodegradable and biocompatible polyesters, such as poly(ε-caprolactone) (PCL), polylactide (PLA) and poly(lactide-*co*-glycolide) (PLGA), have been designed and prepared. Among them, PCL shows a great potential to prevent the postsurgical adhesion and holds certain advantages over other biopolymers. For instance, it is significantly less expensive, readily available in large quantities, more stable in ambient conditions and lasting long enough before *in vivo* degradation.[14] However, the conventional processing of PCL, such as film casting and spin coating, often imposes several limitations in membranes properties. Ramakrishna *et al.* found that the PCL films fabricated by solvent casting were not suitable for anti-adhesion because they were too brittle to be handled.[3] In addition, it was demonstrated that PCL mats prepared by a spin coater were nonporous,[15] which was unfavorable for the transport of nutrients and the wound healing. In clear contrast to these processing technologies, electrospinning not only endows the membranes with excellent properties of mechanical flexibility, variable pore size and large surface area, but also presents less complications in eliminating tissue adhesions.[16] On the basis of these advantages, electrospinning was employed in the present study for the preparation of anti-adhesion barriers.

It is generally believed that an ideal electrospun membrane should have sufficient mechanical property, appropriate degradation rate, as well as good *in vivo* stability for preventing tissue adhesions.[15, 17] In the existing literatures, many researchers focused on utilizing different processing variables to regulate above performances, such as applied voltage, tip to collector distance and feeding rate,[18, 19] but

paid very limited attention to the effect of material characteristics on membrane properties. Molecular weight of polymers largely determines physical and biological properties of fibrous mats by means of chain entanglements and interactions, *etc*.[20] However, to the best of our knowledge, no investigation once reports the molecular weight dependence of the electrospun PCL membrane properties. On the context, with the goal of satisfying more specific application requirements of anti-adhesion membranes, the objective of this study is to reveal the influence of molecular weight on the properties of PCL anti-adhesion barriers, including morphology, mechanical property, thermal behavior, degradation kinetic and cytotoxicity. It is truly interesting that the electrospun PCL membranes with various molecular weights also behave distinctly in the prevention of surgery induced-adhesions, which finally helped acquire the well-suited candidates as the anti-adhesion biomaterial films.

2. Materials and methods

2.1. Materials

PCL was provided by Changchun SinoBiomaterials Co., Ltd. (Changchun, P. R. China). According to the previously reported literatures related to electrospun PCL,[21, 22] molecular weight of 80,000 g/mol is usually selected to electrospun for its excellent behaviors. Excessive or extremely low molecular weight will result in the formation of the unstable fibrous structure. Here, three viscosity-average molecular weights, *i.e.*, M_η = 40,000, 80,000 and 120,000 g/mol, were determined as representatives with regard to their significantly different apparent performances. Chloroform and methanol (analytical grade) were purchased from Sinopharm Chemical Reagent Co., Ltd. (Shanghai, P. R. China) and used without further purification.

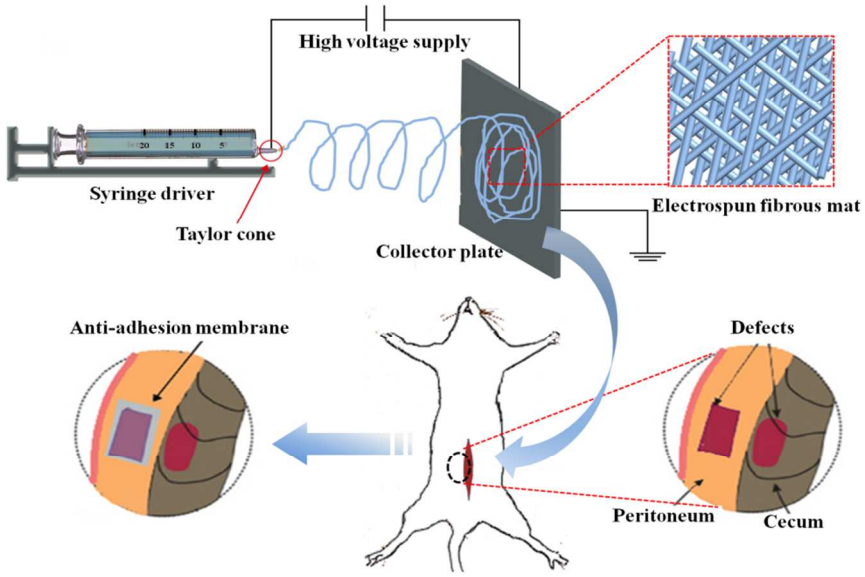

Fig. 1 Schematic of the electrospinning set-up and the application of electrospun PCL membrane onto a peritoneal wall defect of a rat.

2.2. Preparation of electrospun PCL membranes

A schematic diagram of the electrospinning device for manufacturing nonwoven PCL membranes is shown in Fig. 1. It consists of an infusion pump, high voltage power supply and a grounded target. Positive electrode is connected with a copper wire combined with the nozzle, while negative electrode is attached to the grounded collector wrapped with aluminum foil. Electrospun PCL membranes were fabricated by the following steps. Firstly, PCL was dissolved in two types of solvents, chloroform and the mixing solvent with chloroform/methanol (6:1, v/v), in which the PCL concentrations were 10 and 12% (w/w). To guarantee good dissolution, the PCL solution was held at room temperature overnight and stirred for 1 h before electrospinning. Secondly, PCL solution was loaded in a 10 mL syringe, which was fixed at approximately 10° from horizontal, in order to minimize the falling drop at the end of capillary tip.[23] An electrical field of 15 kV was applied by a high voltage power supply, and the injection rate of solution was set as 0.1 mm/min. PCL fabrics were collected on a grounded aluminum sheet kept at a distance of 14 cm from the needle tip. For the sake of briefness, the electrospun PCL membranes with different molecular weights (*i.e.*, 40,000, 80,000 and 120,000 g/mol) will be referred to as PCL-40k, PCL-80k and PCL-120k, respectively.

2.3. Morphology observation

The surface morphology of all the electrospun PCL membranes was observed by a field emission scanning electron microscopy (SEM, Inspect-F, FEI, Finland), operating in high vacuum and with an accelerating voltage of 20 kV. Samples were dried under vacuum, mounted on metal stubs, and sputter-coated with gold-palladium for 30 − 60s. Average diameters of the fibers were calculated based on SEM images using image analysis software of Nano Measurer 1.2.

2.4. Thermal behaviors

The melting behaviors of electrospun PCL membranes were measured by TA Q2000 V7.3 differential scanning calorimeter (DSC). The calibration was performed with indium and all tests were carried out in ultra-pure nitrogen as purge gas. Samples (about 5.0 mg) enclosed in aluminum pans were heated from −80 to 100 ºC at a scanning rate of 10 ºC/min. Melting points were determined from the melting curves as peak temperatures. The fractional crystallinity (X_c) was calculated by using measured enthalpy change (ΔH) value of the

sample and the melting enthalpy of PCL perfect crystal, which was taken as 136.0 J/g in this study.[24]

2.5. Mechanical properties

For mechanical performance tests, the electrospun fibrous membranes were cut into the strips (0.5 × 4.0 cm^2 in size and approximately 0.15 mm in thickness) before tested. Uniaxial tensile tests were performed using an Instron-4502 machine in ambient atmospheric condition (25 ºC and 50% relative humidity), in which a cross-head speed of 5.0 mm/min was applied. From the stress-strain curves, Young's modulus, tensile strength and elongation at break of membranes were obtained. At least three samples were tested for each type and the results were averaged.

2.6. *In vitro* degradation

The degradability of PCL membranes were carried out in a vial containing a small piece of electrospun PCL film (*ca.* 3.0 mg) and 10.0 mL of phosphate buffered saline (PBS, 0.1 M, pH 7.4) with 2.0 mg of α-chymotrypsin. The vial was then incubated at 37 ºC with a constant reciprocal shaking at 75 rpm. The incubation media were replaced daily to maintain the bioactivity of enzyme, and the degradation tests were lasted for a period of 6 days. At predetermined time intervals, the samples were carefully removed, rinsed with distilled water, lyophilized and weighted. Measured weights of the samples were normalized against their initial mass to illustrate the fraction of weight loss, which was calculated using the following equation: $Weight\ loss = (W_0 - W_t)/W_0 \times 100\%$, where W_0 *and* W_t were the weights of membranes before and after degradation for a specific time interval, respectively. The weight loss averaged for three specimens was recorded.

2.7. Cytocompatibility of electrospun PCL membranes

The proliferations of cells on the electrospun PCL membranes were evaluated by the live/dead and CCK-8 assays. The electrospun PCL membranes were sterilized by ultraviolet-irradiation for 0.5 h on each side. L929 cells (a mouse fibroblast cell line) were cultured in Dulbecco's modified Eagle's medium (DMEM) containing 10% (v/v) fetal bovine serum (FBS; Gibco BRL, 30 Gaithersburg, MD, USA) at 37 °C, 5% (v/v) carbon dioxide.

Live/dead assays: L929 cells were seeded onto the electrospun PCL membranes in 24-well tissue culture plates (TCPs) at a seeding density of 2.0×10^4 cells per well. After incubation for 1, 3, 5 or 7 days, the culture media were removed, and the cells were rinsed with PBS thrice. Subsequently, 20.0 μL of PBS containing calcein-acetoxymethyl ester (calcein AM; 2.0 μg/mL) and propidium iodine (PI; 3.0 μg/mL) was added into each well and incubated at 37 °C for 30 min, and then the stained cell constructs were examined using fluorescence microscopy.

CCK-8 assays: The quantitative number of L929 cells was evaluated by adding Cell Counting Kit-8 (CCK-8, Dojindo, Japan) solution to each well. 1.0 mL of CCK-8 solution in complete DMEM (10%, v/v) was added to each well at predetermined time intervals. After incubation for 4 h, the absorbance at 450 nm was measured with a Bio-Rad microplate reader (Model 550, Hercules, CA, USA). The absorbance at 600 nm was used for baseline correction. The relative cellular proliferations were calculated with respect to the result of the first day.

2.8. *In vivo* evaluation of electrospun PCL membranes

Rat cecum abrasion surgical model: All the animal experiments adhered to the principles of Jilin University of Medicine and the National Institutes of Health. A total of 36 Sprague-Dawley rats (weight: 300 to 400 g) were randomly divided into 6 groups, and a rat model of sidewall defect-bowel abrasion described by Chang *et al.* was employed to assess the anti-adhesion potential of the electrospun PCL membranes,[25] as shown in Fig. 1. Briefly, animals were anesthetized with intraperitoneal injection of chloral hydrate (30.0 mg/kg). The defects (1 × 1 cm^2) were respectively created in the cecum and opposite abdominal wall by removing the peritoneum with scalpels. Afterwards, the electrospun PCL membranes with thickness of 0.15 mm were immersed in PBS for 10 minutes, and applied to defects before the abdomens were closed. For comparison, control animals did not receive any intervention.

Macroscopic evaluation: The animals were sacrificed at 14 days after surgery, grading scale was scored at the handled site on the basis of the macro views to evaluate the level of adhesion: score 0, no adhesions; score 1, thin and filmy adhesions that can be easily separated with blunt dissection; score 2, moderate adhesions with freely dissection plane; score 3, severe adhesions with fibrosis difficult dissection plane.[26] The scores were evaluated by a double-blind process.

Histological analysis: The tissues were dissected after sacrifice and rinsed with PBS, and were fixed in 4% paraformaldehyde (w/v) and embedded in paraffin. 5 μm thick transverse sections were cut and stained by hematoxylin and eosin (H&E) and Masson's trichrome staining. As for Masson's tritchrome staining, the collagen was stained as green, nuclei were stained as blue-brown, cytoplasm, muscle fibers and blood cell were stained as red.

Statistical analysis: All data were expressed as mean ± standard deviation, statistical software SPSS 13.0 was used to analyze the data by one-way analysis of variance; $p < 0.05$ were considered significantly different.

3. Results and discussion

3.1. Physical properties of electrospun PCL membranes.

The solvent type and solution concentration are the crucial parameters to affect the sizes and distributions of electrospun fibers, thereby, determine the bulk properties of the resultant fabrics.[27] To choose the optimum solvent system, various solution compositions and concentrations are examined to fabricate PCL fibers. The representative SEM photographs, average diameters and frequency distributions of electrospun PCL fibers prepared from different polymer solutions are shown in Fig. 2. It can be seen that the fibers from each group are randomly arrayed as a porous membrane and seemingly smooth with no beads in the fibrous structure. With regard to pure chloroform system, the fiber diameter obviously rises from 6.80 to 8.81 μm as the polymer concentration increases to 12 wt.%. It presumably results from the less effective stretch of molecular chains in the situation of high concentration, leading to the formation of thicker fibers.[28] On the contrary, the mixture of methanol into chloroform decreases the miscibility of solvent with PCL, so as to result in the formation of thinner fibers. According to the previous literature, the toughness of thicker-fiber membranes is much greater than the thinner-fiber mats.[29] Besides, the increased fiber diameter results in a moderate increase in Young's modulus. These results indicate that pure chloroform along with solution concentration of 12 wt.% is the best solution system among the predesigned solvent types and solution concentrations. Therefore, the fiber membranes

reported below were fabricated using this solution system.

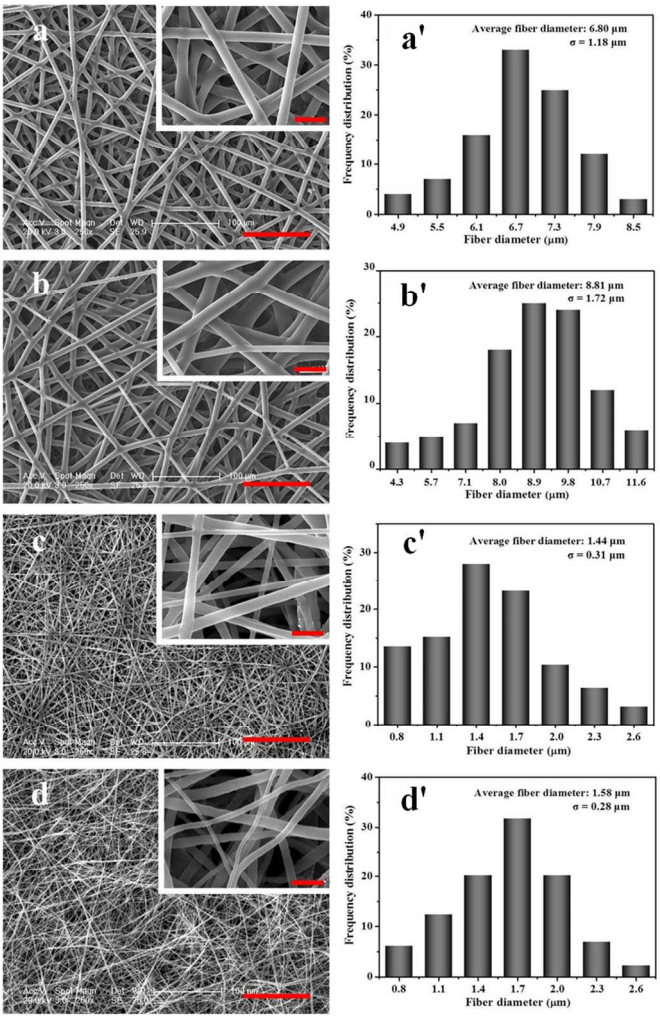

Fig. 2 Representative SEM micrographs, average diameters and frequency distributions of the electrospun PCL membranes prepared from four types of solutions ($M_{\eta,\ PCL}$ = 80,000 g/mol): pure chloroform system, 10 wt.% PCL solution (a and a′); pure chloroform system, 12 wt.% PCL solution (b and b′); mixed chloroform/methanol system, 10 wt.% PCL solution (c and c′); mixed chloroform/methanol system, 12 wt.% PCL solution (d and d′). Scale bar represented 20 and 100 μm in the enlarged and original micrographs, respectively.

The effect of molecular weight on the physical properties of electrospun PCL membranes is shown in Fig. 3. As shown in Fig. 3A, the molecular weight of PCL takes a significant role in determining the fiber morphologies of electrospun membranes. As for the PCL-40k samples, a beads-on-string structure is obtained, which refers to the coexistence of beads and fine fibers. When the molecular weight is doubled, the PCL-80k samples assume well-defined fibers. While, given a further increase of M_η up to 120,000 g/mol, the membrane is composed of an uneven fibrous structure with a broad diameter distribution. It was proposed that morphology and diameter of the resultant fibers were dependent on the viscosity of the solvent.[30] To unravel the origin of the relationship between M_η of PCL and the morphology of electrospun nonwoven, the rheological behavior of each polymer solution is examined (Fig. S1, ESI†). At the lowest M_η (40,000 g/mol), the viscosity of PCL solution is less than 2.5 Pa s, where the stretched chains are extremely prone to relax and form beaded fibers driven by the surface tension.[31] For the PCL-80k samples, the solution viscosity increases significantly for the existence of entangled network, which prevents the jets from breaking up rather than splitting into filaments.[32] However, in terms of the PCL-120k sample, a strong shear-thinning phenomenon results in the instability of the jet of polymer solution,[33] thus an inhomogeneous morphology is observed. It is fairly concluded that the molecular weight of PCL plays a crucial factor in controlling the fiber morphology, and M_η of 80,000 g/mol is the most suitable for its regular fibrous structure.

Fig. 3B shows the DSC scans of virgin and electrospun PCL with different M_η. The melting temperature and crystallinity of electrospun PCL are evidently below those of the virgin PCL regardless of molecular weight, wherein the reduction of melting point and crystallinity are about 8 °C and 15%, respectively. According to Zong *et al.*, the stretched chains had insufficient time to arrange themselves into suitable crystalline lattices due to the rapid solidification process during electrospinning,[34] indicating that the electrospun fibers suffered from a retarded crystallization.[35] Furthermore, the crystallinity of electrospun PCL exhibits a down-trend with molecular weight, which drops from 60.2 to 47.6% as M_η rises from 40,000 to 120,000 g/mol. Such drastic decrease can be explained by the fact that higher molecular weight means more chain entanglements and weaker mobility, which may hinder crystalline growth.

Mechanical properties of the electrospun fiber mats are extremely important from the surgical handling point of view.[36] Fig. 3C illustrates the stress-strain curves of electrospun PCL nonwoven mats. In the case of PCL-40k samples, the beads-on-string structure of electrospun membrane is hard to maintain its integrity so that the tensile test could not be performed. It was once reported that the beads on the fiber surface reduced the cohesive force between fibers of the nonwoven mat and ultimately gave rise to the weak mechanical performance.[37] Fig. 3C indicates that the high molecular weight PCL-based electrospun membrane is a little stronger but less flexible than the low molecular weight one. As M_η varies from 80,000 to 120,000 g/mol, the ultimate strength and Young's modulus show an increase from 1.13 to 2.25 MPa and 8.41 to 8.77 MPa, respectively, while the elongation at break of the film decreases drastically to only 63%. Such a remarkable fall in flexibility is mainly ascribed to the following two aspects. On one hand, the highly entangled PCL chains cannot make a response quickly to the change of external force.[38] On the other hand, some thin fibers become the weak spots that impair the deformation resistance of electrospun membrane.[39] On the basis of the above results, PCL-80k membrane is considered to reach a desirable balance of strength, modulus and flexibility.

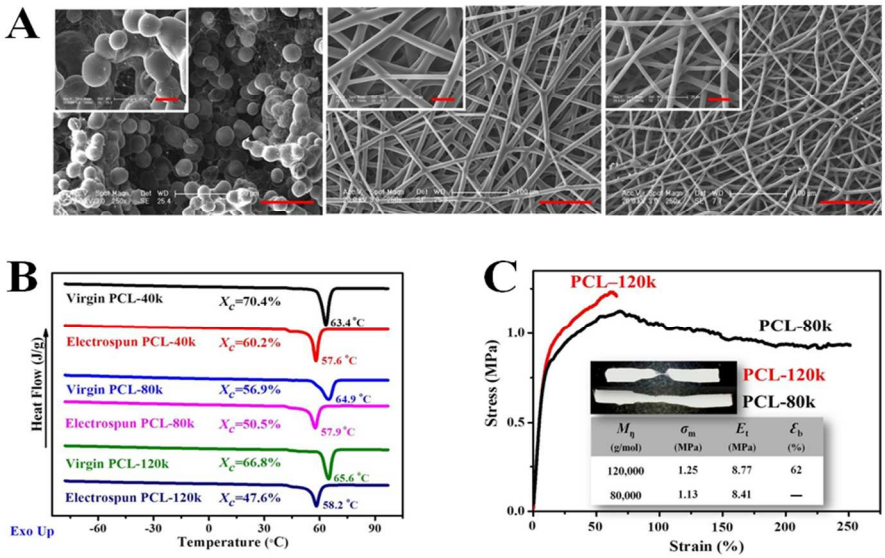

Fig. 3 Representative SEM micrographs of electrospun PCL membranes with different molecular weights: M_η = 40,000 (a), 80,000 (b) and 120,000 g/mol (c) (A). Scale bar represented 20 and 100 μm in the enlarged and original micrographs, respectively; DSC scans of virgin and electrospinning PCL of different M_η (B); Stress-strain curves of electrospun PCL-80k and PCL-120k nonwoven mats (C).

The *in vitro* biodegradation of electrospun PCL membranes in terms of weight loss is performed during a period of 6 days, as depicted in Fig. 4. The biodegradation rate has a steep decline with increasing molecular weight of PCL. For PCL-40k samples, the weight loss of membrane is 67.5% after 6 days incubation. In clear contrast, PCL-80k and PCL-120k samples exhibit relatively slow biodegradation kinetics, whose weight loss is even less than 10% under the same conditions. It is manifested that the membrane with low M_η is more sensitive to the enzymatic biodegradation, which is probably ascribed to the molecular flexibility. A more flexible molecular chain could adapt to conformation better than a more rigid one for an enzyme-catalyzed biodegradation,[40] thus an increase in enzymatic biodegradation rate is presented.

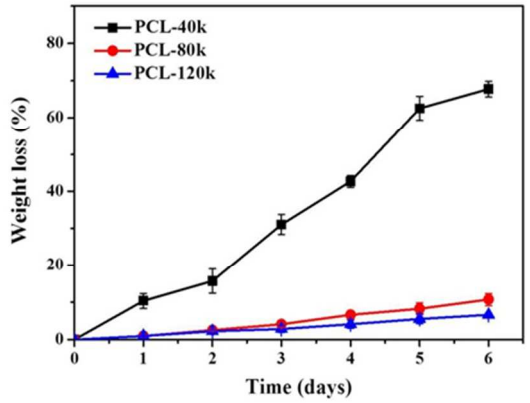

Fig. 4 Effect of molecular weight on the enzyme-catalyzed biodegradation properties of electrospun PCL membranes. Data were presented as mean ± standard deviation (n = 3).

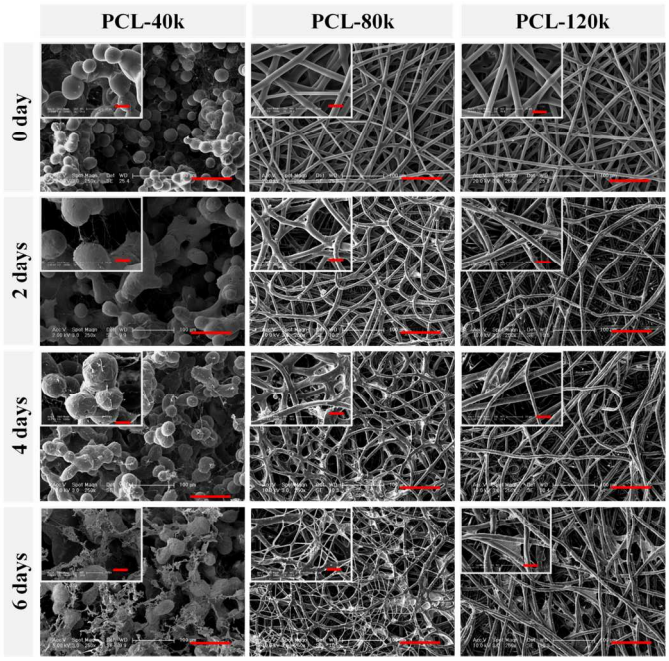

Fig. 5 Morphological changes of the electrospun PCL membranes with different molecular weights after incubation for 6 days. Scale bar represented 20 and 100 μm in the enlarged and original micrographs, respectively.

SEM observation is further performed to gain more intuitive evidence for revealing the relationship between *in vitro* biodegradation and molecular weight. The surface morphology of electrospun PCL membranes upon limited biodegradation is illustrated in Fig. 5. After 6 days' incubation, the PCL-40k sample displays a significant α-chymotrypsin-catalyzed biodegradation, as evidenced by the appearance of serious eroded surface and the destruction of droplets. On the contrary, the fabrics with M_η above 80,000 g/mol just revealed slight surface erosion, which still sustain their fibrous structure and only some tiny pores are observed. The

above morphological changes of films are well in line with the variation trend of weight loss, suggesting that the *in vitro* biodegradation rate of electrospun PCL membranes could be regulated by controlling the molecular weight. Such a tunable biodegradability is very useful in the prevention of post-surgical adhesion as it can satisfy more specific biomedical applications.

3.2 Proliferation of L929 Cells on Electrospun PCL Membranes

To evaluate the cytocompatibility of electrospun PCL membranes, the proliferations of L929 cells are examined by both live/dead staining and CCK-8 assays. Fig. 6 shows the fluorescence microscope photographs of L929 cells cultured on various electrospun nonwovens for 1, 3 and 5 days, where the live and dead cells are fluorescently labeled green and red, respectively. TCP is used as a control. The microimages reveal that the viabilities of cells cultured on membranes are comparable with control, where almost all the cells are alive and no obvious dead cells are traced. It implies that the fabricated PCL membranes exhibited excellent cellular compatibility and do not induce cytotoxic effects to fibroblasts. More interestingly, in comparison with PCL-40k samples, the PCL-80k and PCL-120k membranes present a higher cellular density, which is presumably ascribed to its regular porous structure and sufficient mechanical properties.[12]

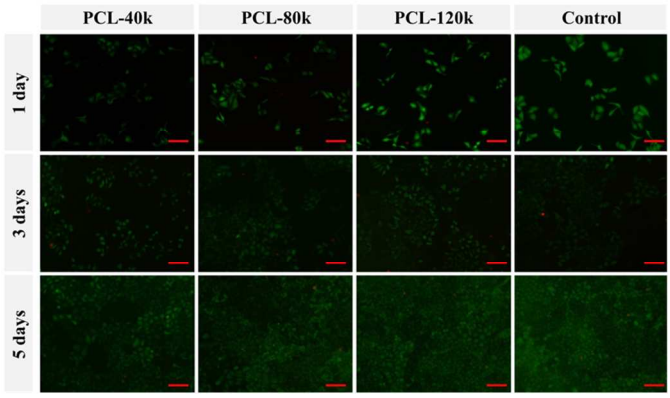

Fig. 6 Fluorescence micrographs of L929 cells on TCP and electrospun fabrics with different molecular weights after *in vitro* culture for 1, 3 and 5 days. The viable cells were stained green with calcein AM and dead cells were dyed red by PI. Scale bar represented 100 μm.

The *in vitro* proliferations of L929 cells on various electrospun PCL membranes are further quantitatively examined by CCK-8 tests, as shown in Fig. 7. The viabilities remain high during the proliferation course, which confirms the low toxicities of our fabrics towards L929 cells. Consistently with the fluorescence photographs, PCL membrane with molecular weight of 80,000 g/mol shows the most efficient cellular proliferation, and displays over 6 times increase of cellular number at 7 days in relative to that of the first day. It is generally demonstrated that the structural and mechanical properties of membrane are two key factors to direct cell proliferation.[30] With respect to PCL-80k sample, the excellent cellular viability is mainly benefited from the uniform fibrous morphology, highly porous structure and sufficient mechanical behavior.

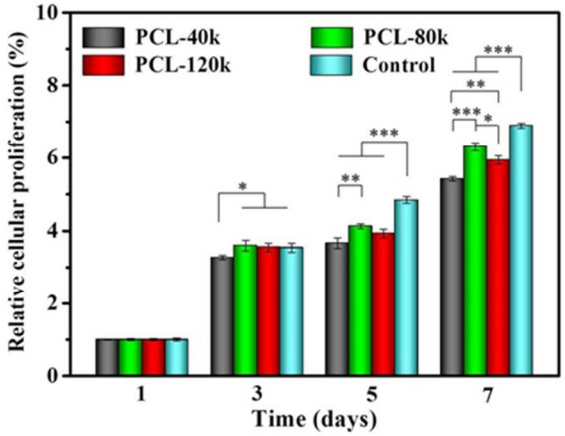

Fig. 7 Relative cellular proliferation of L929 cells on electrospun fabrics and TCP (*i.e.*, control) with different molecular weights after *in vitro* incubation for 1, 3, 5 and 7 days (*$p < 0.05$, **$p < 0.01$ and ***$p < 0.001$). Data were presented as mean ± standard deviation ($n = 3$).

3.3 *In Vivo* Evaluation of Intestinal Adhesion-Prevention

To evaluate the anti-adhesion effects of electrospun PCL membranes, animal study was carried out. All animals stay healthy and no inflammation in the wound is monitored during the whole experiment. Fig. 8 shows the photographs of the surgical site during the operation or at the time when the rats are sacrificed after 14 days post surgery. Different from the control group, the animals treated with PCL samples exhibit an obvious reduce in postoperative adhesion, which could be ascribed to their hydrophobic surfaces and physical barrier effect of non-woven mats. The contact angle measurement is conducted and the corresponding images are illustrated in Fig. S2, ESI†. High value of the contact angle above 125º pointed towards the hydrophobic behavior of non-woven mats, which might be conducive to improve the anti-adhesion ability of membranes.[41]

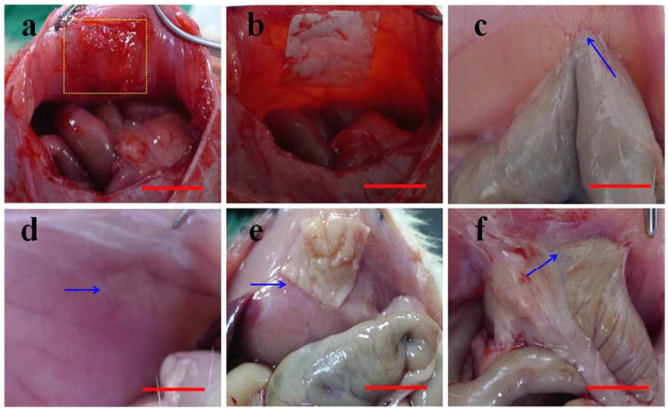

Fig. 8 Photographs of animal experiments of post operative adhesions. Views during the operation (a and b), and gross observation of anti-adhesion effect of electrospun PCL membranes with different molecular weights in rats cecum abrasion model after 14 days: group treated with PCL-40k samples (c), PCL-80k samples (d) and PCL-120k samples (e), no barrier materials were used as untreated (f). Blue arrow indicated remaining membrane adhered to abdominal wall.

In the control group, views between the abdominal wall and cecum surface show vast fibrous tissue layers (Fig. 8f). A relatively slight tissue adhesion is observed in the PCL-40k group, meanwhile,

no membrane residues are observed (Fig. 8c). In the PCL-80k and PCL-120k (Fig. 8d, e) groups, the adhesions seem absent while the electrospun mats display visible remnants. It is observed that the remaining membranes stick on the treated abdominal wall defect site, and the residual area of membranes in PCL-120k group is much larger than that in PCL-80k group, which can be explained by the result of *in vitro* degradation (as shown in Fig. 4).

To determine the degree of adhesion quantitatively, the distribution of adhesion scores (Fig. 9A) and adhesion area (Fig. 9B) of the rats were calculated. Animals without any treatment of abdominal defects all exhibit severe adhesions between the injured cecum and abdominal wall with moderate and high grade adhesion (score 2 and score 3, respectively), and the average adhesion area reaches to the value of 3.27 cm^2. The group performed with electrospun PCL-40k membrane shows slight and moderate grade adhesion (score 1 and score 2), where the percentage of adhesion is 50% and the average adhesion area is 0.28 cm^2. Perfect anti-adhesion effect was presented in terms of PCL-80k and PCL-120k groups, in which only slight grade adhesions are observed. Their percentages of adhesion are 16.67 and 33.33%, respectively, and the corresponding average adhesion area was 0.01 and 0.12 cm^2, separately. To this end, the electrospun PCL membranes with varying molecular weights behave differently in reducing the postoperative adhesion, with regard to the mats with molecular weight above 80,000 g/mol, they all show the ability to completely prevent adhesion.

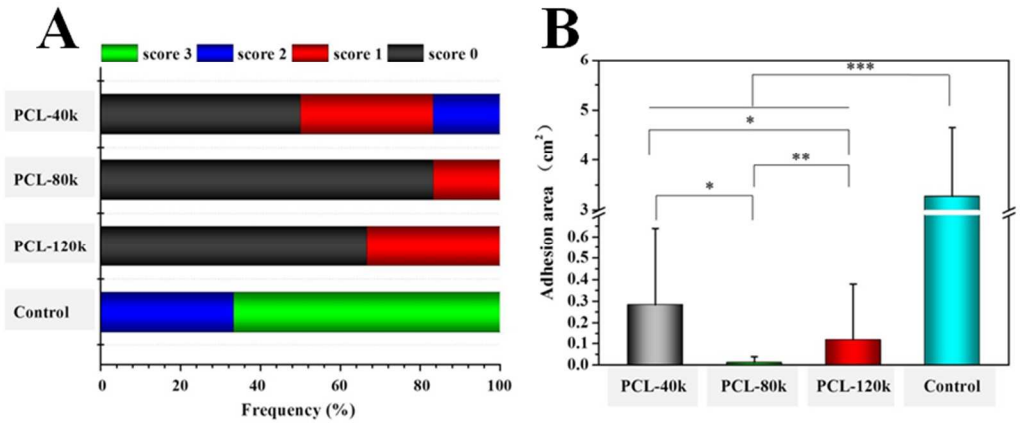

Fig. 9 Distribution of adhesion scores of rats treated with electrospun PCL membranes with different molecular weights or without using any barrier materials as control (A); statistic results of the adhesion area of cecum to the defected abdominal wall (B).

It is accepted that the regenerated collagen can deposit on the wound and result in a mutual adhesion between the peritoneum.[42] With the aim to explore the distribution and density of the regenerated collagen after surgery, the histological observations of the adhesion sites by H&E staining (Fig. 10a − d) and Masson tritchrome staining (Fig. 10e − h) were performed. In the control group, extensive fibrous tissue adhesion (Fig. 10d) appears, meanwhile, vast collagen fibers (Fig. 10h) are scattered between abdominal wall and cecum. With regard to the group treated with PCL-40k samples, lower grade adhesions (Fig. 10a) and less collagen (Fig. 10e) are traced. Moreover, the amount of white cavities with relative loosen structure are presented, resulting from the degradation of membranes. In clear contrast, the groups dealt with physical barrier of PCL-80k and 120k samples have almost no adhesion (Fig. 10b and c) and contain less collagen (Fig. 10f and g), further verifying that the electrospun PCL membranes with different molecular weights have distinct anti-adhesion ability.

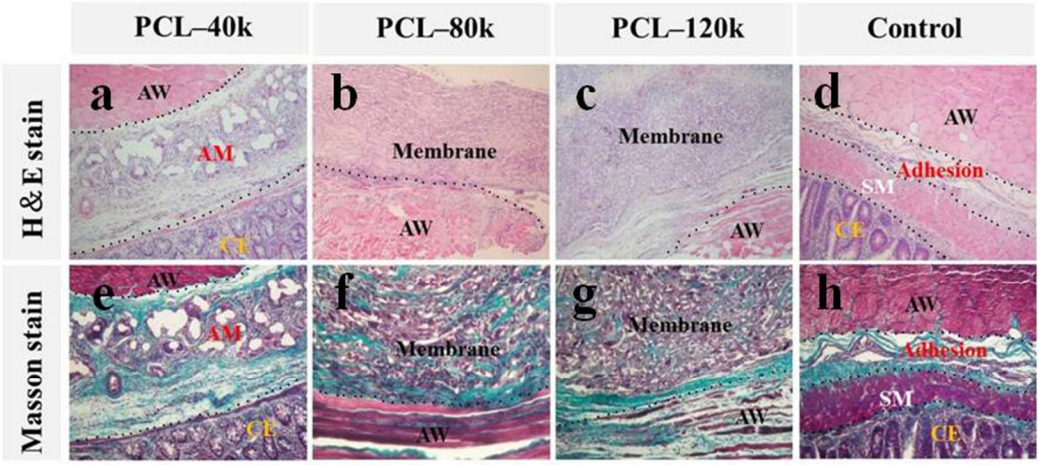

Fig. 10 Representative micrographs of H&E straining (a − d) and Masson's tritchrome staining (e − h) for the defected site at 14 days after surgery: group treated with PCL-40k samples (a, e), group treated with PCL-80k (b, f) and 120k samples (c, g), no barrier materials were used as control (d, h). CE: cecal mucosa, SM: visceral smooth muscle, AW: abdominal wall, AM: adhesion and membrane. Magnification: 100 ×.

Taking into consideration the above analysis, we speculate that the

electrospun PCL-40k samples could only reduce peritoneal tissue adhesion partly, primarily due to its incomplete structure as well as fast degradation rate. On the contrary, the groups performed with electrospun PCL-80k and PCL-120k samples behave excellently in reducing the postoperative adhesions, but it is notable that the degradation rate of membranes (M_n = 120,000 g/mol) is too slow, which might have an negative effect on the normal function of tissues. Thus, the electrospun PCL-80k samples are regarded as the optimal material as intestinal anti-adhesion barrier.

4. Conclusions

Adhesions are unavoidable consequences of surgery and other trauma. Herein, PCL films fabricated by electrospinning are utilized to reduce adhesions. Typical properties of electrospun membranes, such as morphological observation, mechanical properties, degradation kinetics and anti-adhesion effect, were substantially dependent on the molecular weights of PCL. By clear contrast, PCL-80k membranes exhibit a regular fibrous morphology and the best mechanical properties. More importantly, a significant reduction of post-operative peritoneal adhesion is revealed, which can be regarded as the excellent-candidates for the anti-adhesion applications.

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