Prevention of peritendinous adhesions with electrospun polyethylene glycol/polycaprolactone nanofibrous membranes

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\textbf{A B S T R A C T}

Postoperative adhesion formation is the major complication that could occur after acute tendon surgery. The application of an anti-adhesive membrane at the post-surgical site is deemed as a potential way to solve this problem by preventing adhesive fibrotic tissue development. In this study, we fabricated electrospun composite poly(ethylene glycol) (PEG)/poly(caprolactone) (PCL) nanofibrous membrane (NFM) to prevent peritendinous adhesions, which could act as a barrier between the tendon and surrounding tissues, without interrupting mass transfer and normal tendon gliding. PCL/PEG NFMs of 0% PEG (PCL), 25% PEG (25PECL), 50% PEG (50PECL) and 75% PEG (75PECL) were prepared and characterized for physico-chemical properties. The PCL NFM shows the lowest protein permeability while 25PECL NFM exhibited the largest fiber diameter, smallest pore size and the largest ultimate stress and strain. The 75PECL NFM had the lowest water contact angle and the highest Young’s modulus. In vitro cell adhesion and migration experiments with fibroblasts indicate that all NFMs could prevent cell penetration, with 75PECL NFM having the least cell attachment. In vivo application of 75PECL NFM on the repaired site of rabbit flexor tendon rupture model demonstrated improved efficacy compared with the PCL NFM and a commercial anti-adhesion barrier (Sepraflim\textsuperscript{TM}), from gross observation, histological analysis and functional assays. We concluded that 75PECL NFM could function as an effective anti-adhesion membrane after tendon surgery in a clinical setting.

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1. Introduction

Although there has been improvement in rehabilitative programs, surgical technique and surgical materials, postoperative adhesion still remains one of the most troublesome complications after acute tendon injury, taking place in 4–10\% of patients [1–3]. Adhesions may interfere the normal gliding of tendon, restrict range of motion and cause annoying pain [4]. Also, some patients require re-operation or prolonged rehabilitative programs [5]. The flexor digitorum profundus (FDP) and flexor digitorum superficialis (FDS) tendons in zone II of the hand are especially vulnerable to adhesion formation [6]. Initial trauma or excision to the tendon sheath, tendon immobilization, gap at the injured site and tendon ischemia have all been reported to be related to the formation of peritendinous adhesion [7]. During tendon healing, a physical barrier membrane could block the penetration of fibrotic tissue from the surrounding environments and thus would prevent fibroblasts in-growth and peritendinous adhesion. To this end, a variety of bioresorbable anti-adhesion barriers such as Sepraflim\textsuperscript{TM} and Interceed\textsuperscript{TM} have been used for intra-abdominal and gynecological surgeries and shown great success in reducing severity and occurrence of postoperative adhesion [8, 9]. However, with the fast degradation rate in vivo, those membranes are not feasible to prevent peritendinous adhesion, as the duration of adhesion around repaired or immobilized tendons may be up to 3 weeks [3]. Considering the current scenario, development of a biodegradable and biocompatible anti-adhesion barrier membrane with longer degradation time is demanding and could warrant the prevention of peritendinous adhesion.

The tendon sheath is composed of an outer fibrotic layer and an inner synovial layer and is a unique protective layer around the tendon to provide peritendinous lubrication and gliding functions. The outer fibrotic layer averts extrinsic tendon healing and adhesion...
formation while the inner synovial layer secretes synovial fluid, an important source for tendon nutrition and a lubricant for tendon gliding [10]. Dysfunction of tendon sheath due to injury or surgical trauma would result in adhesion significantly [3]. Hence, the most ideal barrier membrane that could decrease the incidence of post-traumatic adhesion should also maintain the tendon gliding function.

Electrospinning is a versatile method for manufacturing nanofibrous membranes (NFM)s for biomedical applications. This process could generate a non-woven nanofibrous mesh by applying high voltage to a polymer solution [11]. Having microporous structure, an electrospun NFM would be an excellent candidate to develop a peritendinous anti-adhesion barrier, by preventing fibroblast penetration from surroundings, without hindering nutrients and wastes transports at the tendon surgery site. Poly(caprolactone) (PCL) is a biodegradable polyester and has been applied in many medical devices. It offers several advantages such as low costs, stability under ambient conditions, readily availability in large quantities and good mechanical properties [12]. A PCL film demonstrated superior anti-adhesive effect compared to Seprafilm™ in avoiding intra-abdominal adhesion in rats [13]. However, the high hydrophobicity and stiffness of PCL may limit its applicability as anti-adhesion barrier film [14]. Poly(ethylene glycol) (PEG), a hydrophilic and highly biocompatible polymer, has been widely utilized in drug delivery [15], protein modification [16] and many other biological applications. SprayGel®, made from PEG alone, has been devoted in preventing intra-abdominal adhesion, but the short degradation time of ~6 days restricts its efficacy [17]. Previous studies demonstrated that the hydrophobicity of PCL could be reduced by blending with PEG and such blending could also extend the degradation time of PEG to a longer duration [18,19].

We hypothesized that PEG/PCL blended NFM will be effective in preventing peritendinous adhesion by obstructing fibroblasts invasion and meeting the demands of a biomimetic sheath to prompt tendon gliding and provide adequate wastes and nutrients exchange (Fig. S1). To test this hypothesis, NFM of various compositions were manufactured and characterized for morphology, porosity, hydrophilicity and protein permeation rate. In vitro cell penetration and adhesion studies were conducted to evaluate the ability of NFM for prevention of migration and adhesion of fibroblasts. Rabbit FDP tendon model was used to validate the efficacy of PECL NFM in preventing peritendinous adhesion in vivo.

2. Materials and methods

2.1. Materials

PCL (molecular weight = 80,000 Da), PEG (molecular weight = 6000 Da), antibiotics and trypsin-EDTA were purchased from Sigma-Aldrich. CellTiter96® AQUeous one solution was purchased from Promega. Dulbecco's Modified Eagle's medium (DMEM, Sigma) and fetal bovine serum (FBS, HyClone) were used for cell culture.

2.2. Preparation of NFM by electrospinning

12% (w/v) PEG/PCL polymer blend solutions with altering PEG compositions (0%, 25%, 50% and 75%) were prepared in a 4:1 methylene chloride and N,N'-dimethylformamide mixed solvent system. Such solutions were employed in electrospinning to fabricate PCL, 25 PECI, 50PECI and 75PECI NFM. A glass syringe containing polymer solution, fitted with a 23-gauge stainless steel needle was mounted on a syringe pump (KD Scientific). A high-voltage power supply (Glassman) provided a 20 kV voltage difference between the needle tip and a grounded collector (aluminum foil). The electrostatic force drew the polymer solution horizontally from the needle to reach a collector placed 15 cm away from the needle tip. The flow rate of the polymer solution was controlled at 2.0 ml/h.

2.3. Characterization of electrospun NFM

The morphology of NFM was observed with a scanning electron microscope (SEM, Hitachi S3000N). The average fiber diameters were calculated by measuring at least 100 random fibers from ten SEM images using the ImageJ software. Capillary flow porometry (PMI CFP-1100-AL, Porous Materials Inc.) with a 15.9 dynes/cm surface tension wetting agent (Galwick®) was used for pore size measurement. The water contact angle was determined with a contact angle analysis system (First Ten Ångstroms) using distilled deionized water. The contact angles at 25 °C were calculated using an automated fitting program (FTA-125), by measuring the angle of droplet after 3 s of water drip. Each value reported was the average of four measurements from four NFM replicates. Chemical analysis was performed by Fourier-transform infrared (FT-IR) spectroscopy using a Horiba FT-730 spectrometer, over a scanning range of 600–2000/cm with a resolution of 2/cm. The uniaxial tensile properties of the NFM was determined by a materials testing machine (Tinius Olsen H1KT) using a 10 N load cell at 5 mm/min elongation rate. The test specimen with dimensions 1 cm × 5 cm × 200 μm was prepared and vertically mounted at two mechanical gripping units at its ends, leaving a 3 cm gauge length for mechanical loading. The ultimate tensile strength, elongation-at-break (ultimate tensile strain) and Young's modulus were obtained from the stress–strain curve [20]. The result values were obtained by taking the average of 6 tests, for each sample.

2.4. Permeability of serum albumin

The permeability coefficient of NFM using bovine serum albumin (molecular weight = 68,000 Da) was measured in a side-by-side permeation chamber at 37 °C [21]. The NFM was placed between two half-cells of the chamber, in which each side acting as the donor and the receptor cell. A permeating solution of bovine serum albumin prepared in phosphate buffered saline (PBS) was added to the donor cell and the receptor cell was filled with PBS. The entire content of the receptor cell was removed and replaced with fresh PBS at intervals. The protein concentration in the receptor cell was determined by a colorimetric method at 595 nm using a protein assay kit from Bio-Rad. Permeability coefficients P(cm/s) were calculated by

\[ \ln \left( 1 - \frac{C_t}{C_0} \right) = -\frac{A}{V} P t \]  

where \( C_t \) is the solute concentration in the receptor cell at time t, C0 is the initial solute concentration in the donor cell, V is the volume of each half-cell and A is the effective area of the membrane available for solute permeation. All measurements were repeated six times.

2.5. In vitro cell culture

Human foreskin fibroblasts (Hs68) cells (ATCC CRL-11372) were purchased from the American Type Culture Collection (Arlington, VA). Cells with passage numbers 4–6 were used. A disk-shaped NFM (1.4 cm in diameter) was sterilized under UV light for 4 h and placed in a 24-well culture plate (Nunc). An aliquot of 0.1 ml cell suspension (1 x 10^5 cells/ml) was seeded onto the surface of each pre-wetted membrane and incubated at 37 °C for 4 h to allow cells attachment. The membrane was transferred to a new well and 1 ml cell culture medium (DMEM containing 10% (v/v) FBS and 1% (v/v) antibiotic–antimycotic) was added to each well. Samples were placed in a 37 °C humidified 5% CO2 incubator for 24 h and the attached cell number was determined by DNA assay (Hoechst...
33258) [22]. The morphology of cells adhered on the NFM surface was evaluated through SEM observation. Samples were rinsed with PBS, fixed in 4% glutaraldehyde at room temperature for 2 h, washed with PBS three times for 20 min, post-fixed in 1% osmium tetroxide (in 0.1 M phosphate buffer) at room temperature for 2 h and finally washing with distilled water for three times (20 min each). Alcohol gradient drying from 50 to 100% was performed to remove water, followed by critical point drying and sputter coating. Samples were coated with gold, at 20 mA for 60 s, followed by examination in a SEM (Hitachi S-3000N).

To investigate the mechanism underlying the anti-adhesion effect, we evaluated the effects of NFM on cell migration and viability using Hs68 cells. The cells were cultured in DMEM containing 2% or 10% FBS in a double chamber dish divided by a porous membrane (Transwell cell culture inserts, Corning). The cells were inoculated at a density of 2.5 × 10^5 cells/well in the upper chamber (containing 2% FBS) with a NFM (200 μm thickness) placed at the bottom of the cell insert. Blank experiments were performed as controls without placing a NFM at the bottom of the cell culture insert. Cell migration through the membrane after 24 h, caused by the gradient of FBS concentration in the upper (2%) and lower (10%) chamber, was determined by measuring the number of viable cells in the lower chamber with MTS assay using the CellTiter96® AQueous one solution kit. The kit contains a novel tetrazolium salt which interacts with metabolically active cells to produce a soluble formazan dye. Colorimetric measurements were done at 492 nm using an ELISA plate reader (BioTek Synergy HT) and reported as the optical density (OD) value. Direct observation of the cells in the lower chamber was also carried out with an inverted microscope (Olympus IX-71) [23].

2.6. In vivo animal study

The animal study was approved by Institutional Animal Care and Use Committee of Chang Gung University. Sixty-four 3-month-old New Zealand white rabbits, purchased from National Laboratory Animal Breeding and Research Center, Taiwan, were used in this study. The skin of hindpaw was shaved and sterilized after the induction of general anesthesia via intramuscular injections of xylazine (6.7 mg/kg body weight) and ketamine (33.3 mg/kg body weight). The zone-II flexor tendons of the second and third digits from both hindpaws of rabbits were released from the tendon sheath. The FDS tendons were first removed and the FDP tendons divided and completely cut with a scalpel, just distal to the chiasm and proximal to the vincula. In further, the FDP tendons were repaired with modified Kessler full suture. The animals were randomly divided into four groups (untreated control, Seprafilm®, PCL NFM and 75PECL NFM) with sixteen animals in each group. All the NFMs were sterilized by ethylene oxide. For each experimental group, an 8 mm × 10 mm piece of Seprafilm®, PCL NFM or 75PECL NFM was wrapped around the tendon repair site while PBS solution was poured to the tendon repair surface in the control group. The skin was closed with 5-0 Nylon sutures. The operated leg was immobilized in a cast to limit the interphalangeal joint movements. The animals were allowed unrestricted activity and received food and water ad libitum. After 4 and 8 weeks, eight animals from each group were euthanized using lethal doses of pentobarbital (0.5 g/kg bodyweight) and the toes were transected at the metatarsophalangeal joints. The skin incisions were re-opened through the original suture line [24] and the digits were randomly assigned for evaluation of peritendinous adhesions. Such assessments were performed through gross evaluation, histological analysis, range of motion (flexion angle) of the distal interphalangeal (DIP) and proximal interphalangeal (PIP) joints, tendon gliding excursion (distance) and tendon pull-out force. To assess the state of tendon healing, the breaking strength of the healed tendon was measured at week 2.

2.7. Gross evaluation

A mid-line incision was made at the plantar side of experimental toe to expose the repaired FDP tendon. A macroscopic adhesion grading system was implemented to estimate the severity of adhesion around the repaired tendon by two surgeons who were blinded to the groups. The grading system includes: grade 1, no marked adhesion; grade 2, filmy adhesion that could be readily separated using blunt dissection; grade 3, mild adhesion (>97.5% of the adhesion area could be separated using sharp dissection); grade 4, moderate adhesion (51%–97.5% of the adhesion area could be separated using sharp dissection); grade 5, severe adhesion (<50% of the adhesion area could be separated using sharp dissection) [25,26].

2.8. Histological evaluation

The second and third digits of the rabbits were harvested for histological evaluations. The specimens were fixed in 10% formaldehyde in PBS, sectioned into 4 μm slices and stained with hematoxylin and eosin (H&E) using standard protocols. The severity of adhesion was evaluated by two histopathologists who were blinded to the groups based on four microscopic grades: grade 1, no adhesion; grade 2, mild adhesion (<33% adhesion area on the tendon surface); grade 3, moderate adhesion (34%–66% adhesion area on the tendon surface); grade 4, severe adhesion (>66% adhesion area on the tendon surface) [25].

2.9. Range of motion and gliding excursion

Range of motion analysis of FDP tendon was carried out by transecting the same tendon at the proximal metacarpal level and sutured to a cable, connected to a load transducer of a custom-made range-of-motion device. The metatarsophalangeal joint was fixed by inserting a wire longitudinally through the metatarsal and the proximal phalanx. The proximal, middle, and distal phalanges were fixed to T-shaped pins containing two reflective markers. The prepared digit was further mounted on the range-of-motion device by fixing the proximal wire to a non-slip clamp. A 50 g weight was attached to the extensor tendon to apply an initial tension and ensure a full extension of the digit. The actuator pulled the tendon slowly at a rate of 3 mm/s to cause digital flexion (angular range of motion). The angle measured between the distal phalangeal and the middle phalangeal determined the DIP joint flexion, and the angle between the middle phalangeal and the proximal phalangeal determined the PIP joint flexion [27].

For the functional evaluation based on tendon gliding excursion, we used 2 metal pins through the proximal phalanx to fix digits to a table and applied traction to the tendon so that flexion of the distal 3 joints could be easily performed. The FDP tendon was exposed after removing skin, subcutaneous tissue and the other flexor tendons. The tendon sheath and FDP tendon was marked at the exit from the sheath while a counterweight from the distal phalanx was applied to fully extend the interphalangeal joints. A constant force of 1 N pulled the FDP tendon out of the sheath tunnel and the distance after pulling was measured with a micrometer caliper. This length of tendon gliding was recorded as the gliding excursion of the FDP tendon.

2.10. Biomechanical tests

To evaluate peritendinous adhesions, the breaking strength of the tendon was measured using a materials testing machine (Tinius
Fig. 1. Scanning electron microscope micrographs and pore size distribution histograms of PCL, 25PECL, 50PECL and 75PECL nanofibrous membranes. The insert shows the water contact angle value of the membrane. Bar = 10 μm.

Table 1
The properties of nanofibrous membranes.

<table>
<thead>
<tr>
<th>Membrane</th>
<th>Fiber diameter (nm)</th>
<th>Pore size (μm)</th>
<th>Ultimate tensile strength (MPa)</th>
<th>Elongation-at-break (%)</th>
<th>Young's modulus (MPa)</th>
<th>Permeability coefficient (× 10⁻⁵ cm/s)</th>
</tr>
</thead>
<tbody>
<tr>
<td>PCL</td>
<td>475.3 ± 128.0</td>
<td>1.53 ± 0.13</td>
<td>1.35 ± 0.07</td>
<td>92.70 ± 6.88</td>
<td>6.00 ± 1.17</td>
<td>1.5 ± 0.5*</td>
</tr>
<tr>
<td>25PECL</td>
<td>621.1 ± 161.1</td>
<td>0.92 ± 0.09*</td>
<td>2.67 ± 0.71*</td>
<td>181.76 ± 74.37*</td>
<td>6.02 ± 0.52</td>
<td>24.9 ± 7.6</td>
</tr>
<tr>
<td>50PECL</td>
<td>540.3 ± 163.5</td>
<td>1.36 ± 0.07</td>
<td>1.36 ± 0.09</td>
<td>97.60 ± 1.39</td>
<td>7.53 ± 0.30</td>
<td>25.6 ± 10.8</td>
</tr>
<tr>
<td>75PECL</td>
<td>446.9 ± 211.5</td>
<td>1.54 ± 0.25</td>
<td>1.27 ± 0.11</td>
<td>76.18 ± 7.80</td>
<td>10.30 ± 4.48*</td>
<td>17.1 ± 5.7</td>
</tr>
</tbody>
</table>

*p < 0.05 compared with other groups. Data are expressed as mean ± SD (n = 6).
Olsen H1KT) with a 50 N load cell. The digit was amputated through the metatarsophalangeal joint, leaving a 2 cm longer attached FDP tendon, firmly fixed at the bottom by a custom-made static clamp. The distal digit was attached to the hook via a hole drilled through the distal phalange and connected through a steel hook to the top crosshead. The FDP tendon was pulled at 5 mm/min and the movement of the crosshead ultimately pulled the tendon out of the tendon sheath. The pull-out force (N) was calculated by measuring the maximum force necessary to pull the tendon out of the tendon sheath.

To evaluate tendon healing, the proximal and distal ends of a repaired rabbit FDP tendon were fixed on the nonslip clamps (HT-51) of a materials testing machine (Tinius Olsen H1KT) with a 50 N load cell. The tendon was pulled uniaxially at a rate of 5 mm/min until terminal rupture occurred. The breaking force of the repaired tendon was recorded as the maximum tension force.

2.11. Statistics

Results are expressed as mean ± standard deviation. Statistical software SPSS 10.0 (Chicago, USA) was used to analyze the data by one-way ANOVA analysis of variance; \( p < 0.05 \) was considered significant.

3. Results and discussion

3.1. Preparation and characterization of electrospun NFM

Pristine PCL NFMs are highly hydrophobic and stiff in nature, causes difficulties in handling during surgical procedures. Therefore, various combinations of blended PEG/PCL NFMs were suggested to improve the hydrophilicity and flexibility. To determine the best PEG/PCL ratio for application in vivo, electrospun NFMs with a PEG mass ratio ranging from 0% to 75% were prepared and characterized for its physico-chemical properties. The 100% PEG NFM was eliminated due to its fast dissolution in PBS. SEM observations confirmed the smooth and bead-free nanofiber morphology for all NFMs (Fig. 1). The water contact angles were 115.9, 66.9, 56.3, and 34.4°, respectively, for PCL, 25PECL, 50PECL and 75PECL NFM (Fig. 1 inserts). As expected, the incorporation of hydrophilic PEG into PCL resulted in reduction of hydrophobicity of pristine PCL membrane. Increasing PEG concentration also

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Fig. 2. Fourier transform infrared (FTIR) spectroscopy and X-ray diffraction (XRD) analysis of PCL, 25PECL, 50PECL and 75PECL nanofibrous membranes.

Fig. 3. Migration of fibroblasts through a PCL, 25PECL, 50PECL or 75PECL nanofibrous membranes after 24 h, driven by serum albumin concentration gradient in a double chamber dish divided by a cell culture insert. The number of migrated cells in the lower chamber was determined by MTS assays and direct microscopic observations. The run without the nanofibrous membrane was used as the control. Bar = 500 μm. \(^* p < 0.05\) compared with other groups. Data are expressed as mean ± SD (n = 5).
progressively yielded more hydrophilic surfaces, as observed from reduced water contact angles. When PEG concentration was increased to 75%, the water contact angle decreased to those values reported for self-assembled monolayers of PEG [28]. This suggests that PEG has a significant wetting coverage over the PCL polymer chain, by masking the latter from its hydrophobic properties.

The observed average fiber diameter of all NFMs ranged from 447 to 621 nm, with 25PECL showing the largest value and 75PECL the smallest (Table 1). In the composite PECL NFM, the diameter is inversely proportional to PEG content, i.e. more PEG content in the blend leads to smaller fiber diameter, as observed in previous studies [18]. There was also a correlation between the PEG composition in the blend and the average pore size measured by capillary flow porometry. The average pore size is proportional to the PEG content in the composite PECL NFM (Table 1). The pore size distributions were from 0.3 to 3.2 μm and the mode pore sizes ranged from 0.9 to 1.8 μm (Fig. 1). Another correlation can be drawn between the pore size and average fiber diameter, in which smaller average fiber diameter results in larger pore size (Table 1). Indeed, a larger fiber size in the NFM may occupy more space and lead to a smaller pore size. This could be cross-confirmed from the similar pore size and fiber diameter values of PCL and 75PECL NFM. In such a way, micrometer-scaled pores (0.92–1.54 μm) of NFMs exert an important underlying mechanism for anti-adhesion effect, by blocking the passage of extrinsic fibroblastic cells (>8 μm in diameter) that causes peritendinous adhesion, without hampering the diffusion of growth factors and cytokines required for tendon healing process.

FT-IR demonstrated the distribution of functional groups among different NFMs (Fig. 2). The C=O stretching intensity of PCL at 1725/cm decreased with increasing PEG composition [29], whereas C-O-C stretching intensity of PEG at 1107/cm increased with increasing PEG composition [30,31]. This confirmed the homogeneous blending of PEG and PCL in the electrospun NFMs. Other than chemical compositions, the crystal structures of NFMs were analyzed by XRD (Fig. 2) and two major peaks at 21.5 and 23.9° were observed for PCL NFM [32]. Blending of PEG with PCL resulted in an additional diffraction peak at 19.2°. Similar to FTIR results, the peak intensity corresponds to the crystallinity of PEG increased as more PEG is blended with PCL [33].

Mechanical properties like tensile strength and ultimate strain are crucial for an anti-adhesion NFM, considering the possible large strain and stress it would experience during application to

Fig. 4. The attachment of fibroblasts to PCL, 25PECL, 50PECL or 75PECL nanofibrous membranes. The attached cell number was determined after 24 h by DNA assays and scanning electron microscope images. Bar = 50 μm. *p < 0.05 compared with other groups. Data are expressed as mean ± SD (n = 5).
a repaired tendon. The ultimate stress, ultimate strain and Young’s modulus calculated from the stress–strain curves are listed in Table 1. All NFMs show similar typical stress–strain curves with an initial elastic region and an ultimate failure. The PCL and 50PCL NFMs have similar mechanical properties. Among the composite NFMs, increasing PEG content decreased ultimate tensile strength and elongation-at-break but elevated the Young’s modulus. Indeed, the 25PCL showed the largest ultimate tensile strength (2.67 MPa) and elongation-at-break (181.76%), which might result from its largest fiber diameter. The Young’s modulus, also known as elastic modulus, is the ratio of the tensile stress along an axis to the tensile strain and a measure of the stiffness of an elastic material. The 75PCL NFM shows the highest Young’s modulus (10.30 MPa) among all NFMs. Indeed, it may be difficult to choose the optimum anti-adhesion NFM considering strength, modulus and flexibility.

Intrinsic healing is an important concept of tendon healing, as it allows healing without peritendinous adhesion by blocking the fibroblast-ingrowth, migration and collagen synthesis of epitendon [34]. This makes a barrier membrane a rational modality. However, permeation of nutrients and growth factors are critical to a barrier membrane since it might influence the subsequent healing process. To ensure adequate nutrient exchange, the permeability of the serum protein in each NFM was measured. There was no significant difference in the permeability coefficient among composite PCL NFMs, however, significant reduction in permeability was found for PCL NFM (Table 1). This repulse of nutritional exchange is deemed arising from the hydrophobicity of PCL NFM, not related to the pore size, as there was no significant difference in pore size between PCL (1.53 μm) and 75PCL (1.54 μm) NFMs (Table 1). The increase in hydrophilicity of PCL NFM after blending with PEG justifies the changes of contact angles in Fig. 1, which also raises the permeation rates of proteins among other nutrients through the composite NFM and benefits exchanges of nutrients and waste during the tendon intrinsic healing process.

3.2. In vitro cell culture

Fibroblasts migration and penetration is the major cause responsible for adhesion formation. By creating a serum concentration gradient across the cell insert during in vitro cell culture, the number of viable cells migrated to the lower chamber through the cell insert was determined by the MTS assay. As shown from Fig. 3, the NFM could significantly reduce the number of penetrated cells. The OD value that is proportional to the cell number dropped to below one-third of the value shown by the control, which did not have a NFM in the cell insert. Blocking cell penetration by the NFM was also confirmed from microscopic observation of the lower chamber. Although limited cells could be observed microscopically in the presence of NFM, the concern of membrane toxicity could be eradicated considering the presence of viable cells detectable through the MTS assay.

To examine the cell attachment on different NFMs, Hs68 cells was inoculated on the NFMs for 24 h. Although there is a trend that PEG concentration monotonically suppressed cell attachment, the attachment remained statistically invariant when the PEG concentration was increased from 0 to 50% in the composite membrane (Fig. 4). However, at the highest level of PEG (75PCL), there is a significant reduction in attached cell number compared with other groups (p < 0.05). Generally, the hydrophilic/hydrophobic characteristic of a membrane is important to influence initial cell adhesion [35]. Many studies have demonstrated that cells adhere, spread and grow more easily on moderately hydrophilic substrates than on hydrophobic or super hydrophilic ones [36]. Although PCL NFM is considered as a hydrophobic substrate from contact angle

![Fig. 5](image-url)
measurements, PCL has good affinity toward fibronectin, which has been shown to play a major role in many types of cell adhesion, including fibroblasts [37]. Anterior cruciate ligament cells cultured on PCL membranes displayed well spread phenotypes with a developed cytoskeleton, attributed to the higher fibronectin adsorption of PCL [38]. On the other hand, PEG is reported to actively regulate the biological responsiveness of protein adsorbed biomaterials. As the PEG concentration in PEG-variant biomaterials increased, the amount of adsorbed fibronectin decreased linearly [39]. The degree of mouse fibroblasts attachment to PEG-variant biomaterials also varied with PEG concentration in a manner similar to the dependence of fibronectin bioactivity on PEG [39]. Taken together, the significant reduction of fibroblasts adhesion to 75PECL NFM is justified by considering the reduced affinity toward fibronectin from the serum protein during in vitro cell culture. Such behavior is extremely relevant during in vivo application.

SEM observation indicated rounded cell morphology for the attached fibroblasts on 50PECL and 75PECL NMFs in comparison with a well spread morphology on PCL NFM (Fig. 4). Specifically, the fibroblasts spread less and occupied less area on the 75PECL NFM compared with other membranes, coinciding with the significantly lower attached cell number determined quantitatively. The 75PECL NFM was thus selected for further in vivo study due to its high modulus, reasonable permeability, effective cell blockage ability, and reduced cell attachment.

3.3. In vivo animal study

To examine the anti-adhesion efficacy in vivo, the rabbit FDP tendon model was used and divided into four groups, implementing 75PECL NFM, PCL NFM, SeprafilmTM or untreated control. In gross evaluation, peritendinous adhesions were found at the repaired site in control, SeprafilmTM and PCL NFM group at both time points (Fig. 5a). A time-dependent increase of adhesion was also observed in these groups. The composite 75PECL NFM showed massive improvement in anti-adhesion effect over the PCL NFM. Throughout the 8 weeks period, 75PECL revealed no evidence of peritendinous adhesion. The grading scale of tissue adhesion based on gross evaluation of the four test groups are summarized in Fig. 5b. The macroscopic adhesion grading scales measured for PCL and 75PECL NMF groups were significantly lower than those measured for the control and SeprafilmTM groups, and the differences between PCL and 75PECL NFM groups were also statistically significant at both 4 and 8 weeks (Fig. 5b). Histologically, severe peritendinous adhesions were observed in control, SeprafilmTM and PCL groups at week 4 and 8 in comparison with the 75PECL group where no adhesion was found (Fig. 6a). As shown in Fig. 6b, the repaired tendon wrapped with PCL and 75PECL NMFs and SeprafilmTM revealed a lower histopathologic adhesion grading scale at week 4 and 8. Thus, adhesion was prevented to a greater extent in these groups than in the tendons of the control group. Furthermore, the grading scale of 75PECL NFM group was...
significantly lower than those of the other groups, indicating the superior prevention effect on peritendinous adhesion. Residual membrane matrix was still identifiable from the histological section for the groups treated with NFM, where no residues were found for the group treated with Seprafilm due to rapid degradation. Indeed, as tendon healing takes longer than 4 weeks, Seprafilm was unable to provide the barrier effect during the extended healing phase of a tendon to prevent peritendinous adhesion. The NFM thus prevail from this perspective. In addition, the physicochemical properties and cellular response of 75PECL NFM in vitro may endow it with the improved anti-adhesion efficacy over PCL NFM in vivo.

To evaluate the in vivo anti-adhesion performance quantitatively, post-operative tendon functional assay was performed, including range of motion, tendon gliding excursion and biomechanical evaluation. Compared to the control group, there was significant difference in the range of motion of DIP in the three experimental groups at week 4, but only 75PECL showed significant difference at week 8 (Fig. 7). Similarly for PIP range of motion, the significant difference among groups gradually diminished and was only seen for 75PECL compared with other groups at week 4 and 8 (Fig. 7). The progressive decline in the range of motion might be due to adhesion formation after week 4. These findings are in agreement with the gross and histologic results where an adhesion-free peritendinous area was only revealed in the group treated with 75PECL NFM at week 4 and 8. The gliding excursion test examines relative movements between tendons and surrounding tissues. The value of gliding distance showed a similar trend as observed in the range of motion test. The 75PECL demonstrated a significant increase in gliding distance compared with other groups at week 4 (Fig. 7). Although the displaced distance was not significantly different between 75PECL and PCL at week 8, the gliding distance was longer in 75PECL (10.4 ± 1.3 mm) than PCL (7.8 ± 1.0 mm). The degradation times of PCL and 75PECL NFMs in vivo is more than 8 weeks from histology (Fig. 6a). This could render both NFMs not only as an anti-adhesion barrier but also a tendon sheath mimic. Nevertheless, the 75PECL NFM is expected to exhibit superior lubrication power over the PCL NFM. Tendon healing capability should
be due to the enhanced hydrophilicity, which may result in better displacement distance during the gliding excursion test. In due course, this might facilitate early rehabilitation and contribute to less adhesion and better recovery.

The pull-out force envisages the severity of adhesion by determining the maximum force required to pull the tendon away from surrounding tissues. A significantly lower level of pull-out force was shown by the 75PECL NFM compared with other groups at week 8 (Fig. 7). Judging from above results, the 75PECL NFM consistently showed superior anti-adhesion performance in vivo. To address the concern of inappropriate tendon healing by intervention of the NFM, breaking strength test of the healed tendon was conducted. This maximum force required to break a healed tendon at week 2 could provide the healing quality. All healed tendons showed no significant difference in the breaking strength, including the naturally healed tendon in the control group (Fig. 7) [40]. Overall, no dehiscence or decrease in strength of healed tendon was revealed, implying that the 75PECL NFM is a functional barrier membrane to be used after acute tendon injury. Previously, we have successfully conjugated chitosan [41] or hyaluronic acid [42] to a PCL NFM by surface grafting and improve the membrane’s efficacy as an anti-adhesion barrier. This study employs a similar approach by using a biodegradable polymer to augment the anti-adhesion function offered by pristine PCL NFMs. Nonetheless, a fast and facile method for preparation of anti-adhesion composite NFMs was achieved through electrospinning of PEG/PCL polymer blend in contrast to post-modification of the PCL NFM. Further investigation into the molecular mechanism of anti-adhesion, long-duration implantation and large animal studies are necessary for future clinical implications of the 75PECL NFM.

### 4. Conclusion

Electrospun PECL NFMs from PEG/PCL blend were successfully prepared to prevent peritendinous adhesion and also to mimic tendon sheaths. The NFM demonstrated good mechanical properties and could allow the exchange of nutrients and wastes through its microporous structure to promote normal tendon healing. Meanwhile, it provides a secured barrier to prevent post-operative penetration and attachment of fibrotic cells responsible for peritendinous adhesions. In animal model, the 75PECL NFM demonstrated suitable biodegradability and biocompatibility and showed improved anti-adhesion efficacy over Seprafilm™ and PCL NFM from gross evaluation, microscopic examination and functional assays. All evidences suggest that 75PECL NFM is a highly clinical relevant anti-adhesion membrane to prevent peritendinous adhesion and could also replace injured tendon sheath to facilitate normal tendon healing.

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