Colloids and Surfaces B: Biointerfaces 133 (2015) 221–230

Contents lists available at ScienceDirect

Colloids and Surfaces B: Biointerfaces

journal homepage: www.elsevier.com/locate/colsurfb

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

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ARTICLE INFO

Article history:
Received 17 November 2014
Received in revised form 3 June 2015
Accepted 4 June 2015
Available online 11 June 2015

Keywords:
Anti-adhesion
Nanofibrous membranes
Polycaprolactone
Polyethylene glycol

ABSTRACT

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 physicochemical 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 (Seprafilm™), 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.

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 avoid fibroblasts in-growth and peritendinous adhesion. To this end, a variety of bioresorbable anti-adhesion barriers such as Seprafilm™ and Interceed™ 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

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http://dx.doi.org/10.1016/j.colsurfb.2015.06.012
0927-7765/

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 (NFMs) 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 polyester, 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 NFMs 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, NFMs 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 NFMs for prevention of migration and adhesion of fibroblasts. Rabbit FDP tendon model was used to validate the efficacy of PECL NFMs 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 PECL, 50PECL and 75PECL NFMs. 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-AI, 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 600–2,000/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

$$\ln\left(1 - 2\frac{c_t}{c_0}\right) = -2\frac{A}{V}Pt \quad (1)$$

where C_t is the solute concentration in the receptor cell at time t, C_0 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 (1×10^5 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% CO_2 incubator for 24 h and the attached cell number was determined by DNA assay (Hoechst