

Tissue anti-adhesion potential of biodegradable PELA electrospun membranes

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Abstract

The most commonly used anti-adhesion device for separation and isolation of wounded tissues after surgery is the polymeric membrane. In this study, a new anti-adhesion membrane from polylactide–polyethylene glycol tri-block copolymer (PELA) has been synthesized. The synthesized copolymers were characterized by gel permeation chromatography and ¹H nuclear magnetic resonance spectroscopy. PELA membrane was prepared by electrospun. The prepared copolymer membranes were more flexible than the control poly-D-L-lactic acid (PDLLA) membrane, as investigated by the measurements of glass transition temperature. Its biocompatibility and anti-adhesion capabilities were also evaluated. In vitro cell adhesions on the PELA copolymer membrane and PDLLA membrane were compared by the culture of mouse fibroblasts L929 on the surfaces. For in vivo evaluation of tissue anti-adhesion potential, the PDLLA and PELA copolymer membranes were implanted between cecum and peritoneal wall defects of rats and their tissue adhesion extents were compared. It was observed that the PELA copolymer membrane was very effective in preventing cell or tissue adhesion on the membrane surface, probably owing to the effects of hydrophilic polyethylene glycol.

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1. Introduction

Post-surgical tissue adhesion is one of the most urgent problems that need to be overcome as part of improving surgical techniques. Placing a physical barrier between an injured site and the adjacent tissues is a direct approach to prevent adhesions. Various kinds of material, made of animal tissues, biological materials and synthetic polymers, have been reported to be effective in reducing adhesion both in animal models and in clinical practices. Some have been used commercially and clinically [1–4]. Seprafilm™ is a hydrophilic film composed of sodium hyaluronate and

carboxymethylcellulose. It is claimed that this film adheres to a wound surface and mechanically separates the tissue during the post-operative healing phase. However, some surgeons have claimed difficulty in handling because of its brittleness and low mechanical properties. Interceed™ is a knitted fabric composed of oxidized regenerated cellulose and is reported to significantly reduce adhesion when blood contamination is avoided during application [2,5]. As blood evacuation cannot always be done effectively in a clinical situation, surgeons are not always satisfied with Interceed™ for the prevention of adhesion. Polylactide (PLA) is one of the most widely used biomedical polymers owing to its biodegradability and biocompatibility. However, its application has been limited because of its stiffness and hydrophobicity. Introducing polyethylene glycol (PEG)

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can enhance the flexibility and hydrophilicity of PLA [6,7]. In this study, we aimed to develop a novel anti-adhesion membrane poly(lactide–polyethylene glycol) tri-block copolymer (PELA), which has received little attention in the fabrication of non-woven nanofiber membranes.

Electrospinning has been recognized as an efficient method for the fabrication of submicron-sized fibers and various macromolecules have been electrospun into ultra-fine fibers as thin as just a few nanometers [8–10]. From a polymer solution a charged jet is created when the electrical force overcomes surface tension. The jet typically develops a bending instability and then solidifies to form fibers. The important parameters in electrospinning are not only polymer and solution properties such as molecular weight, viscosity, conductivity and surface tension, but also electrospinning conditions, such as applied electric voltage, tip to collector distance and feeding rate [11,12]. Nanofibers have amazing characteristics, such as a very large surface area-to-volume ratio and high porosity with a very small pore size. Therefore, nanofibers are promising materials for many biomedical applications, such as wound dressings [13,14], drug delivery [15,16] and tissue engineering scaffolds [17,18].

An ideal adhesion prevention product should be resorbable, easy to apply and capable of being fixed in position. The goal of this study is to develop a new anti-adhesion membrane for clinical use. The synthesized membrane was characterized and its performance evaluated and compared with poly-D,L-lactic acid (PDLLA) membrane, one of the commonly used anti-adhesion membranes.

2. Materials and methods

2.1. Materials

PEG-diol ($M_n = 20000$) was purchased from Acros (Beijing, China). PDLLA electrospun membrane was a gift from Sichuan Dikang Sci&Tech Pharmaceutical Co. Ltd. (Chengdu, China). *N,N*-Dimethyl formamide (DMF) (Wuhan, China) and acetone (Wuhan, China) were of analytical grade and used without further purification.

2.2. Synthesis of PELA copolymers

Synthesis of PELA copolymers was carried out according to our previous report [19]. Pure D,L-lactide and PEG-diol were placed in a dried polymerization tube and stannous 2-ethylhexanoate, 0.01–0.03 wt.% dissolved in dried ether, was added. The tube was then placed in an oil bath under reduced pressure. After the appropriate time, the tube was sealed under vacuum and the polymerization was carried out at temperatures ranging from 170 to 200 °C. When the polymerization was completed, the reaction product was dissolved in acetone, precipitated in a large amount of water, filtered and washed with hot water in order to remove unreacted PEG homopolymer.

The resulting product was dried in vacuum oven at 60 °C for 3 days to give a colorless, transparent copolymer.

2.3. Electrospinning of nanofibrous membranes

Electrospinning was carried out according to our previous report [20]. In brief, 15–30 wt./vol.% solutions of PELA copolymer in a mixture solvent of DMF and acetone (80:20 vol./vol.) were prepared. The solution was placed in a 20 ml plastic syringe fitted with a needle having a diameter of 0.7 mm. PELA electrospun membranes were fabricated by an electrospinning process at an applied voltage of 20 kV using a high-voltage power supplier (Tianjing, China). A syringe pump was used to feed the polymeric solution into the needle tip and the feeding rate varied from 1 to 2.5 ml h⁻¹. The electrospun membranes were collected on a melt drum rotating at a speed of approximately 200 rpm. The distance between the needle tip and the collector was 15 cm. The resulting fibrous membranes were vacuum dried at room temperature for 7 days prior to investigation.

2.4. Characterization of the electrospinning membranes

2.4.1. Scanning electron microscopy analysis

The surface of the electrospun members were coated with gold and their morphology was evaluated using scanning electron microscope (KYKY-AMRAY-1000B) at an accelerated voltage of 15 kV.

2.4.2. Differential scanning calorimetry (DSC) analysis

The glass transition temperature (T_g) of the membranes was obtained using a DSC thermal analysis apparatus (Perkin-Elmer, DSC-7) in the temperature range from –20 to 100 °C at a heating rate of 10 °C min⁻¹.

2.4.3. ¹H nuclear magnetic resonance spectroscopy (¹H NMR) and gel permeation chromatography (GPC) analysis

The polymer composition was investigated by ¹H NMR spectra recorded on a Bruker Avance 300 spectrometer at room temperature using CDCl₃ as a solvent and trimethylsilane as the internal standard. The M_n and M_w distribution of the copolymers were estimated by GPC.

2.4.4. Determination of water sorption

To determine the equilibrium water content of various polymer membranes, 250 μm thick strips of dimensions 20 × 50 mm² were embedded in 25 ml distilled water at 25 °C. Weights were recorded periodically after blotting with filter paper to remove surface water. Water sorption (%) was calculated as follows:

$$\text{water sorption \%} = (w_t - w_0) \times 100 / w_0 \quad (1)$$

where w_t is the wet weight measured at the time t (h) and w_0 is the weight of the dry sample measured at $t = 0$ h.

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