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Long-term drug release from electrospun fibers for in vivo inflammation prevention in the prevention of peritendinous adhesions

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ABSTRACT

Physical barriers such as electrospun fibrous membranes are potentially useful in preventing peritendinous adhesions after surgery. However, inflammatory responses caused by degradation of barrier materials remain a major challenge. This study aimed to fabricate electrospun composite fibrous membranes based on drug-loaded modified mesoporous silica (MMS) and poly (L-lactic acid) (PLLA). Using a co-solvent-based electrospinning method ibuprofen (IBU)-loaded MMS was successfully and uniformly encapsulated in the PLLA fibers. The electrospun PLLA–MMS–IBU composite fibrous membranes showed significantly lower initial burst release (6% release in the first 12 h) compared with that of electrospun PLLA–IBU fibrous membranes (46% release in the first 12 h) in in vitro release tests. Moreover, the release from PLLA–MMS–IBU was also for significantly longer than that from PLLA–IBU (100 vs. 20 days). In animal studies both PLLA–IBU and PLLA–MMS–IBU showed improved anti-adhesion properties and anti-inflammatory effects compared with PLLA fibrous membrane alone 4 weeks after implantation. Further, animals implanted with PLLA–MMS–IBU for 8 weeks showed the lowest inflammation and best recovery compared with those implanted with PLLA–IBU and PLLA, most likely as a result of its long-term IBU release profile. Therefore, this study provides a platform technique for fabricating fibrous membranes with long-term sustained drug release characteristics which may function as a novel carrier for long-term anti-inflammation and anti-adhesion to prevent peritendinous adhesions.

1. Introduction

Adhesion is a physiologically important part of wound healing. However, undesirable adhesions after surgical treatment often induce serious complications, including pain and functional obstruction of patients, or even re-operative surgery. Peritendinous tissue adhesions frequently occur as a common complication after tendon injury and subsequent surgery. In general, peritendinous adhesion formation is associated with various factors, such as tissue trauma, ischemia, foreign body reaction, infection, and hemorrhage. Current treatments include the use of biomaterial barriers and rehabilitation. Among them, physical barriers are the most widely accepted method for minimizing adhesion formation [1]. Recently electrospun nanofibrous membranes, characterized by fibrous nanostructures, a high specific surface area, and interconnected porous structure with high porosity and variable pore size distribution, have emerged as promising anti-adhesion barriers [2,3]. This is for two reasons: (1) the microporous structure allows the passage of nutrients from outside the tendon sheath to promote intrinsic healing [4]; (2) electrospun fibrous membranes are more flexible and easier to use than commonly used anti-adhesion films (e.g. poly(D,L-lactic acid) and Seprafilm® films) in the prevention of abdominal wall adhesions [5]. In addition, electrospun fibrous membranes are also ideal scaffolds for drug delivery and tissue engineering [6–8].

On the other hand, post-operative inflammation also contributes to surgical adhesion and should be avoided [9]. The degradation products of commonly used biodegradable polymers such as polylactic acid (PLA) often induces severe foreign body reaction, which results in an inflammatory response and consequent tendon adhesion formation [9]. Ibuprofen (IBU) has long been used as a kind of non-steroidal anti-inflammatory drug (NSAID) to prevent adhesion formation by preventing mass migration of inflammatory cells [10]. A previous study has shown that electrospun IBU-loaded poly(L-lactide acid)–polyethylene glycol (PELA) fibrous membranes can prevent peritendinous adhesion formation through anti-adhesion and anti-inflammatory actions in the early stages

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[5]. However, after complete release of IBU 1 month post-implantation and the beginning of PLA degradation at 2 months tendon adhesion recurred. Therefore, long-term drug delivery carriers are desirable to release therapeutic agents continuously at a sustained rate over a period of several weeks or months [11]. Unfortunately, it is difficult to achieve long-term drug release using current electrospun fibrous membranes of biodegradable polymers because of their large surface area to volume ratio. The release of electrospun biodegradable barriers usually last for 2–5 weeks [12–14]. While forming core–shell structures in the micro/nanofibers through emulsion electrospinning or coaxial electrospinning helped slow down the release rate, the total release time could only be increased to no more than 7 weeks [15–19].

Mesoporous silica nanoparticles (MSNs) have been suggested as controlled drug delivery carriers because of their unique properties, including a uniform and tunable particle size and pore size, a high surface area and a large pore volume, two functional surfaces, stable physico-chemical properties and a rigid framework, porous structure and superior biocompatibility (biodegradation, interaction with cells, cytotoxicity, blood compatibility and tissue compatibility) [20–23]. Song et al. fabricated a dual drug-loaded poly(lactic acid-co-glycolic acid) (PLGA)–MSN electrospun composite mat. They found that most of the drug on the surface of the MSN was rapidly released over the 324 h of the trial, however, drug within the MSN showed sustained release with only 8–37% being released after 324 h [24]. Therefore, drug-loaded MSN in electrospun composite fibrous membranes were expected to show sustained and long-term release [11]. In this study we aimed to encapsulate drug-loaded, modified MMSs in electrospun poly(L-lactic acid) (PLLA) fibers by a co-solvent method. Since the drug-loaded MMSs are encapsulated within the electrospun fibers the drug entrapped within the MMSs must first be released at a few solution state, and then be released from the polymeric fibers to enter the surrounding medium. Using such a composite release system we were interested to test whether long-term drug release (i.e. more than 100 days) could be achieved and whether it could function to minimize peritendinous adhesion formation, and whether inflammation caused by surgery and barrier material degradation could be minimized through a synergistic anti-adhesion and anti-inflammatory effect, especially in the later stages of healing (Fig. 1). The electrospun PLLA–MMS–IBU fibrous membranes were characterized and evaluated in vitro and in vivo for their ability to prevent tissue adhesion.

2. Materials and methods

2.1. Materials

PLLA (M_w 50 kDa, M_w/M_n = 1.6) was prepared by bulk ring-opening polymerization of L-lactic acid using stannous chloride as initiator [25]. Tetraethylorthosilicate (TEOS) (98% pure), 3-aminopropyltriethoxysilane (APTES) (99% pure), ammonium hydroxide, ethanol, dichloromethane (DCM), hexafluoroisopropanol (HFIP) and other reagents were purchased from Sinopharm Chemical Reagent Co. Ltd. MSNs were synthesized using the ammonia catalyzed hydrolysis of TEOS in aqueous solution [26], and were amine-functionalized by treating with APTES [23], termed modified mesoporous silica nanoparticles (MMSs). Ultrapure water from a Milli-Q biocel purification system (UPI-IV-20, Shanghai UP Scientific Instrument Co., Shanghai, People's Republic of China) was used.

2.2. Preparation and characterization of the fibrous membranes

The drug-loaded MMSs were prepared as follows. IBU was completely dissolved in n-hexane at a concentration of 20 mg ml^{-1}, and MMSs were added to the IBU solution (40 mg ml^{-1}). The mixture was incubated at 37 °C for 24 h. The products, termed MMS–IBU were centrifuged and washed with ethanol and mixing solution (ethanol, HFIP, and DCM) until there was no IBU in the supernatant. The supernatant was collected and UV–vis spectrometry (Unicam UV300) was used to evaluate the amount of IBU loaded in the MMSs [23].

MMS–IBU20 was dispersed in a solution of ethanol and HFIP, and PLLA was dissolved in DCM. The two solutions were mixed (PLLA/mixed solvent 22 wt.%, MMSs/PLLA 10 wt.%, IBU/PLLA 5 wt.%) and electrospun at 11 kV under a steady flow rate of 0.04 ml min^{-1}. The electrospun composite fibrous membrane (PLLA–MMS–IBU) was collected on a grounded plate covered with aluminum foil. The distance between the spinneret and the collector was 15 cm.

Electrospun PLLA and PLLA–IBU (IBU/PLLA 9 wt.%) fibrous membranes were similarly prepared, except that the electrospining solution was prepared by dissolving PLLA and IBU in a solution of ethanol, HFIP and DCM.

The morphology of the fibrous membranes was observed with an environment scanning electron microscope (FEI Quanta 250, The Netherlands). The structure and encapsulation of MMS–IBU in PLLA were observed by transmission electron microscopy (TEM) (Hitachi JEM-2100F, Japan). The samples were prepared and imaged according to the manufacturer's instructions.

2.3. Ibuprofen release study

The loading and efficiency of encapsulation of IBU in the fibers were determined after extraction from the electrospun fibers. Briefly, a 5 mg PLLA–IBU electrospun fiber was dissolved in 1 ml of chloroform. Then 10 ml of phosphate-buffered saline, pH 7.4 (PBS) was added and the mixture agitated for 2 min. A nitrogen

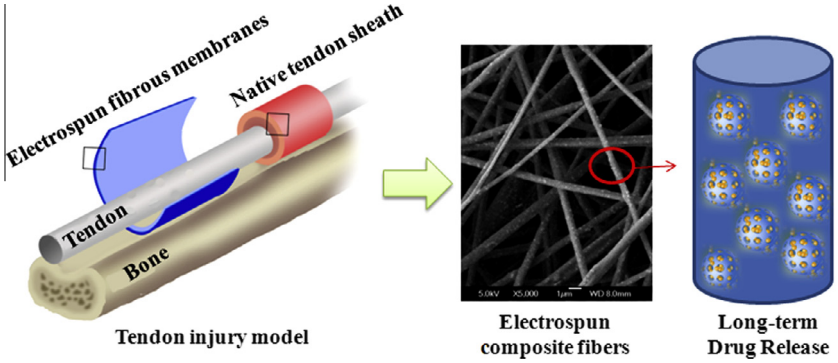

Fig. 1. Schematic illustration of a novel electrospun composite fibrous membrane as both drug carrier and physical barrier for long-term anti-inflammation and anti-adhesion after tendon injury.