Biomedicine & Pharmacotherapy 83 (2016) 33–40

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Preparation of asiaticoside-loaded coaxially electrospinning nanofibers and their effect on deep partial-thickness burn injury

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ARTICLE INFO

Article history:
Received 15 April 2016
Received in revised form 25 May 2016
Accepted 9 June 2016

Keywords:
Electrospinning
Coaxial nanofiber
Burn wound
Wound healing
Asiaticoside

ABSTRACT

Sodium alginate and chitosan were in favor of wound healing. However, the two polymers were not compatible in one formulation due to the electrostatic interaction. Coaxially electrospinning technology could make two or more noneletrospun polymers to be electrospun in independent core and shell layer. Asiaticoside-loaded coaxially electrospinning nanofibers of alginate, polyvinyl alcohol (PVA) and chitosan (alginate/PVA/chitosan) were prepared and evaluated. Morphologies and microstructure of nanofibers were observed with scanning electron microscopy (SEM) and transmission electron microscopy (TEM). Drug release *in vitro* of coaxial nanofibers was also evaluated. Deep partial-thickness burn injury were established and used to evaluate the improved healing effect of asiaticoside-loaded coaxial nanofibers. Drug-loaded coaxial nanofibers prepared with the optimized formulations and technologies had the obvious core-shell structure. Coaxial nanofibers showed faster drug release profiles *in vitro* and this facilitated wound healing. Its healing effect on rats with deep partial-thickness burn injury was also significant based on morphology, wound healing ratio, and pathological sections. Positive expression of vascular endothelial growth factor (VEGF), cluster of differentiation 31 (CD31), and proliferating cell nuclear antigen (PCNA), and down regulation of tumor necrosis factor (TNF) and interleukin-6 (IL-6) also validated the improved effect of wound healing. In general, the asiaticoside-loaded coaxial nanofibers had obvious core-shell structure with smooth surface and uniform diameter. Its healing effect on deep partial-thickness burn injury of rats was obvious. Asiaticoside-loaded coaxial nanofibers provide a novel promising option for treatment of deep partial-thickness burn injury.

1. Introduction

Deep partial-thickness burn injury is one of the most common burns. It involves destruction of the entire epidermis and a substantial part of the dermis. Although superficial burns can re-epithelialize fairly rapid with minimal scarring, deep partial-thickness burn injury may take a few weeks to heal and tend to form more severe scars [1]. The treatment challenges of deep partial-thickness burn injury are how to protect wound from bacterial infection, promote wound healing, and hinder scar formation.

Asiaticoside, a major triterpenoid component derived from *Centella asiatica* (L.), has been widely used in wound healing [2], antioxidant [3], immunomodulatory [4] and anti-inflammatory activities [5]. Previous studies had also shown that the asiaticoside had antidepressant-like effect, which was entirely inhibited by pharmacological inhibition of brain-derived neurotrophic factor (BDNF) signaling in behavioral experiments [6]. In addition, some studies demonstrated that asiaticoside had restore synaptic function to improve the learning and memory abilities in diabetic rats, which may be attributed to its anti-diabetic and modulating PI3K/Akt/GSK-3 pathway [3]. However, the most important pharmacodynamics action of asiaticoside is its improved effect on burn healing.

Electrospinning is a convenient method to obtain nanofibers with desired diameters. Commonly, an electrospinning process employs a high voltage source to add electrostatic force of polymer solution or melt. When the electric field strength exceeds the

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http://dx.doi.org/10.1016/j.biopha.2016.06.016
0753-3322/

surface tension of the solution, leading edge of the solution changes from a rounded meniscus to a cone and ejects fibers jet from the cone [7]. Fiber produced using this process has diameters on the order of a few micrometers down to the tens of nanometers. The capacity to easily produce materials at this biological size scale has created a renewed interest in electrospinning for applications in drug delivery [8–10].

Chitosan is biocompatible and biodegradable, and show positive effects on wound healing. Previous studies have shown that chitosan-based dressings can accelerate repair of different tissues, facilitate contraction of wounds, and regulate secretion of the inflammatory mediators, such as interleukin 8, prostaglandin E, interleukin 1β, etc [11]. Its antimicrobial effect was also obvious [12,13].

Alginates are natural polysaccharide polymers from brown algae [14] and sodium alginate is a salt of alginic acid. However, the repulsion force between polyanions of alginate may prevent alginate forming fibers in static electric field [15]. Intermolecular twisting by high molecular polymers, such as polyethylene oxide and polyvinyl alcohol (PVA), may decrease the repulsion force between alginate polyanions and facilitate electrospinning [16].

The aim of the study was to prepare asiaticoside-loaded coaxially electrospinning nanofibes with sodium alginate, chitosan, and PVA. It could inhibit microorganism growth based on chitosan, promote wound healing based on chitosan and alginate, and prevent scar formation based on asiaticoside. Preparation, morphology, drug release *in vitro*, and improved healing effect of coaxial nanofibers on deep partial-thickness burn injury were evaluated in the paper.

2. Materials and methods

2.1. Materials

Alginate (viscosity, 200 ± 20 mPa S) and chitosan (degree of deacetylation, 0.89, MW 60 kDa) were purchased from Sinopharm Chemical Reagent Co., Ltd, (Beijing, China). PVA (mean MW 77,000) was purchased from Beijing Yili Fine Chemicals Co., Ltd, China. Asiaticoside and centella triterpenes cream (a wound healing commercial product, contained asiaticoside 2.5%) were obtained from Shanghai Shyndec Pharmaceutical Co., Ltd., China. Organic solvents were of analytical grade. The other chemicals were of reagent grade. ELISA kits for determination of interleukin-6 (IL-6) and tumor necrosis factor-α (TNF-α) were purchased from the NeoBioscience Technology Co., Shenzhen, China.

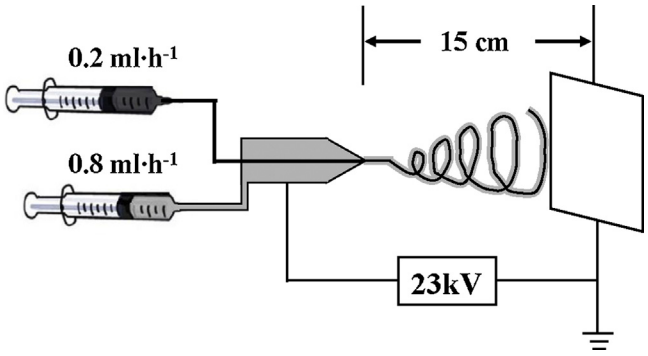

Fig. 1. Graph of coaxially electrospinning instrument to prepare nanofibers.

2.2. Animals

Healthy male Sprague-Dawley rats (weight, 180–200 g) were obtained from the Vital River Laboratory Animal Technology Co., Ltd. (Beijing, China). All of the animals were free access to standard diet and water. The rats were kept in the polypropylene cages coated with sawdust submitted to controlled temperature (22–28 °C) and lighting (12 h light/dark cycle). Principles in good laboratory animal care were followed and animals' experiment was in compliance with the Guidelines for the Care and Use of Laboratory Animals in the institute. The animals were sacrificed to obtain the tissues.

2.3. Electrospinning formulation and technology

A mixture of 0.8% (w/v) alginate and 7% (w/v) PVA was added into the 10% (v/v) acetic acid solution followed by stirring for 3 h at 90 °C to obtain the shell-forming solution. The core-forming solution was prepared after 3% (w/v) chitosan was dissolved in the 2% (v/v) acetic acid solution. The asiaticoside was added into the chitosan solution as the drug-loaded core-forming solution. The coaxial nanofibers were prepared with the electrospinning equipment (SS-2535H, Beijing Ucalery Technology Development Co., Ltd, China) installed with two coaxial injectors. The needle was connected to the emitting electrode of positive polarity and a rotating collector with aluminum foil was connected to the negative polarity. A high voltage of 23 kV was applied to the droplet of injected solution. The needle tip was placed 15 cm away from the collector (Fig. 1). The flow rate of shell-forming and core-forming solutions were 0.8 and 0.2 ml/h, respectively. The asiaticoside-loaded coaxially electrospinning nanofibers were obtained when simultaneously spraying under a high-pressure electrostatic field.

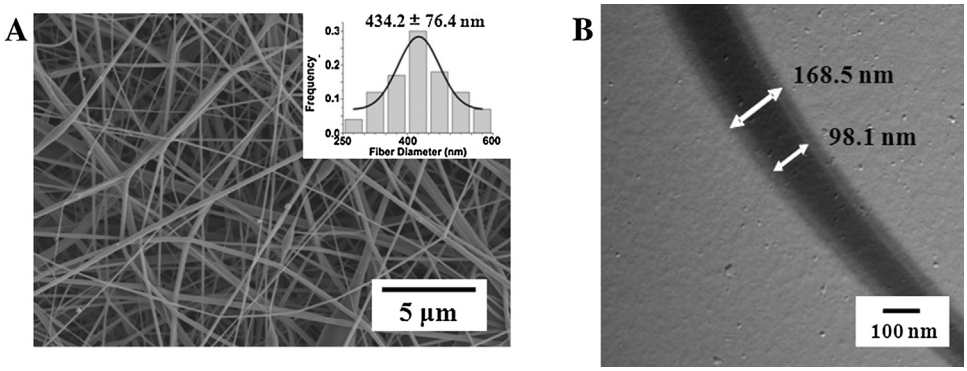

Fig. 2. SEM images and diameter distribution of asiaticoside-loaded coaxially electrospinning nanofibers (A) with obvious core-shell structure (B).