International Journal of Pharmaceutics 529 (2017) 642–653

Contents lists available at ScienceDirect

International Journal of Pharmaceutics

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

Evaluation of three-layered doxycycline-collagen loaded nanofiber wound dressing

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ARTICLE INFO

Article history:
Received 26 May 2017
Received in revised form 29 June 2017
Accepted 8 July 2017
Available online 10 July 2017

Keywords:
Nanofiber
Wound dressing
Coaxial electrospinning
Doxycycline
Collagen

ABSTRACT

Nanofiber wound dressings have great potential for both acute and chronic wound healing. The aim of this study is to develop a wound dressing by the electrospinning method and to determine its in vitro characteristics. The viscosity and the surface tension values of the polymer solutions used in the electrospinning were measured and their suitability for electrospinning was evaluated. Nanofiber wound dressing consists of three layers. The first and the second layers are sodium alginate and chitosan nanofibers, respectively. The core of the coaxial nanofibers that comprises the third layer of the wound dressing contains 1% polycaprolactone and 4.5% collagen, the shell comprises 2.5% doxycycline and 2.5% polyethylene oxide. The developed wound dressing comprises aligned nanofibers, with a contact angle of 38°, a work of bioadhesion value of 0.485 mJ/cm^2 on rat skin, a tensile strength of 2.76 MPa, an elongation at break value of 7.65%, a specific surface area of 9.65 m^2/g and a porosity of 52.3%. The amount of doxycycline content was found to be 260 μg/cm^2 and the complete drug release was achieved in 15 min. No cytotoxic effect was shown in cell culture studies with keratinocyte cell lines. As a result of the stability studies, it was found that the morphological, mechanical, bioadhesion and wettability properties and the amount of doxycycline remained stable for a period of 12 months at 4 °C/ambient humidity condition. The developed three-layered wound dressing could be an alternative for wound healing applications.

1. Introduction

Nanofiber is the term given to fibers whose diameters are less than 1 μm (Lin et al., 2012). Nanofiber-based products obtained from synthetic or natural polymers have unique properties such as high surface area/volume ratio, high porosity and permeability (Aduba et al., 2013; Li et al., 2005; Luong-Van et al., 2006; Natu et al., 2010). Electrospinning is a commonly used method for the production of nanofibers from polymer solutions. Unlike conventional electrospinning, coaxial electrospinning allows to produce core-shell nanofibers (Sun et al., 2003). The main advantage of this method is that incompatible polymers or drugs can be loaded into the core or shell with different solutions.

Nanofiber based wound dressings have some advantages compared to conventional wound dressings due to their large surface area and 3-dimensional microporous structure (Cai et al., 2010; Chen et al., 2010b; Çalamak et al., 2014; Kanani and Bahrami, 2010). Thus, nanofiber wound dressings have the ability to mimic the extracellular matrix (ECM). In addition, nanoporous wound dressings are oxygen-permeable; however, these pores are small enough to prevent the penetration of the exogenous bacteria into the wound, and they can protect the wound from bacterial penetration and dehydration. In addition to protecting the wound, local application of therapeutic agents is ensured.

Wound healing is the regeneration of impaired tissue through different overlapping phases (Martin and Leibovich, 2005; Park and Barbul, 2004). If the wound is healed in a continuous and normal process and occurs in 8–12 weeks, it is called an acute wound, while a wound where the healing is delayed due to a local or systemic factor, like diabetes, and occurs over months to years it is called a chronic wound (Dhivya et al., 2015). In chronic wounds, the inflammatory phase is prolonged and usually wound becomes infected. Infected wounds increase risks of patient morbidity and mortality. Proliferation and remodelling phases are supressed due to the prolonged inflammatory phase. Especially in chronic wounds, the amount of matrix metalloproteinase (MMP) enzyme that breaks down the growth factors with matrix proteins

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http://dx.doi.org/10.1016/j.ijpharm.2017.07.027
0378-5173/

increases, while tissue metalloproteinase inhibitor (TIMP) enzyme levels reduce (Löffek et al., 2011; Lu et al., 2011). The high MMP level and impaired MMP/TIMP ratio cause the wound to become chronic. Healing time can be accelerated using suitable wound dressings in both acute and chronic wounds (Sarabahi, 2012).

Doxycycline (DOX), a member of tetracycline class antibiotics, has activity against aerobic and anaerobic gram-positive and gram-negative bacteria (Anumolu et al., 2010; Kogawa and Nunes, 2012). Except antibacterial effect of DOX, it has a wound healing effect due to the binding of divalent metal ions like the zinc present in the active site of MMP in the wound area and direct inhibition of active MMPs (Stechmiller et al., 2010). For example, in a study, seven diabetic patients with chronic lower extremity ulcers were treated with carboxymethyl cellulose hydrogel containing 1% DOX and compared to placebo (Chin et al., 2003). Four patients were healed in DOX group, while only one wound was healed in placebo group. Granulation tissue and epithelization occurred with topical DOX administration after 30 weeks. Akalin et al. (2004) investigated the effects of oral and topical DOX on 45 patients with chronic periodontitis. Local DOX administration significantly accelerated healing and no adverse effects were seen in local DOX groups. Thus, topical DOX might be an alternative to use oral DOX. DOX-collagen loaded microspheres were administered for the treatment of the infected excisional wound, and it was seen that DOX neutralized or inhibited MMPs and thus stimulating healing (Adhirajan et al., 2009).

Collagen is a 3-dimensional network structure composed of multi-fibrils at the nanometer scale in ECM. Type-I collagen found in the tissues is about 50–500 nm in diameter and the diameter distribution is quite uniform. The non-woven matrix structure composed of collagen nanofibers can easily be produced by electrospinning, and this structure is perfectly similar to the collagen-containing ECM structure (Matthews et al., 2002).

Chitosan and alginate are natural polymers with wound healing effects. Chitosan accelerates the migration of cells into the wound area at the inflammatory phase (Min et al., 2004). This allows macrophages to destroy microorganisms, remove dead cells, and stimulate other immune-mediating cells (Kiliç et al., 2013; Min et al., 2004 Min et al., 2004). These stimulated cells help tissue organization and new blood vessel formation. Alginate creates a moist environment in the wound area, absorb the exudate, help hemostasis and absorb proteinases. The effect of chitosan and alginate on wound healing has been shown in several studies (hydrogel, film, sponge, nanofibers) (Catanzano et al., 2015; Chen et al., 2010b; Leung et al., 2014).

Although the effect of topical DOX on wound healing (reduction in MMP, TNF-α, serine protease levels) has been shown in studies, there are limited studies with nanofibers containing DOX for wound healing (Anumolu et al., 2010; Solorzano et al., 1997). Collagen, alginate or chitosan based wound healing products are commercially in use as foam, hydrocolloid, hydrogel or hydrofiber wound dressings. Collagen, alginate or chitosan affect wound healing in different phases (Adhirajan et al., 2009; Dai et al., 2011; Wang et al., 2002). Therefore, the combination of DOX, collagen, chitosan and alginate nanofibers has been, for the first time, prepared in the present study, to be used a complete wound dressing for all phases of wound healing. As a result, the aim of the present study was to develop and characterize a three-layered coaxial nanofiber wound dressing prototype using electrospinning method for both acute and chronic wounds. In addition, cell culture and stability studies were carried out. Nanofiber wound dressing was designed as three layers. The first and the second layers consisted of alginate and chitosan, respectively, for these hydrophilic layers would rapidly contact with wound for shortening the inflammatory phase. The third layer contained collagen-DOX coaxial nanofibers, directly affecting wound healing. DOX was loaded to the shell, whereas collagen was loaded to the core of the coaxial nanofibers. Thus, DOX could be released rapidly from outer shell to show its intended MMP-2 inhibitory effect. The core part of the coaxial nanofiber was thought to create a suitable environment for cell proliferation by forming an ECM-like structure in the wound.

2. Materials and methods

2.1. Materials

Doxycycline hyclate (>98%), type-I collagen (from calf skin), polycaprolactone (PCL, Mn 70–90 kDa), glacial acetic acid and hexafluoroisopropanol (HFIP) were purchased from Sigma-Aldrich (UK). Sodium alginate (Protanal LF10/60, Mn 89 kDa), chitosan (91% deacetylation degree), polyethylene oxide (PEO, Polyox WSR-205, Mn 600 kDa) were gift from FMC BioPolymer (Norway), Golden Shell Pharmaceutical (China) and Colorcon (UK), respectively. Human keratinocyte cell lines (HaCaT) were obtained from CLS Cell Lines (Eppelheim, Germany).

2.2. Preparation of wound dressing

Nanofiber wound dressing was produced in three steps (Scheme 1). In the first step, core-shell polymer mixtures were electrospun and then aligned coaxial nanofibers were obtained. In the second step, the chitosan solution was electrospun over coaxial nanofibers. In the final step, alginate solution was electrospun over chitosan nanofibers. As a result, three-layered wound dressing was carefully collected from aluminium foil and dried at room temperature for 24 h.

2.2.1. Coaxial nanofibers

2.2.1.1. Preparation of electrospinning solutions. Coaxial nanofibers with different core-shell compositions were fabricated using coaxial electrospinning unit (NE300, Inovenso, Turkey). For core mixtures, collagen and PCL were dissolved in HFIP and glacial acetic acid (w/v), respectively (Table 1). The use of HFIP as solvent is considered to be an advantage for dry collection of the collagen on aluminium foil due to low-boiling point (61 °C). For shell mixtures, PEO (2.5%) and DOX (2.5%) were dissolved in aqueous 90% acetic acid solution (Table 1). The acetic acid concentration must be so high, if not it caused blockage at the tip of the coaxial nozzle due to contacting of water and PCL in the Taylor cone.

2.2.1.2. Characterization of electrospinning solutions. Before the electrospinning process, viscosity and surface tension of core and shell mixtures were measured. Additionally, the effect of these parameters on fiber diameter was investigated. A stress-controlled cone and plate rheometer (Brookfield, DV-III Rheometer with spindle type CPE-41, UK) was used for viscosity measurements. Shear stress and viscosity values were obtained at different shear rates. All of the rheological measurements were repeated on at least three different samples.

For surface tension measurements, drops were formed on the tip of the needle to measure surface tensions of polymer mixtures using pendant drop observation (Attension, Theta Lite, Finland) and then surface tensions were calculated using Young Laplace calculations (Zarska et al., 2017).

2.2.1.3. Electrospinning process. The core and shell mixtures were placed into 10 ml syringes each and the feed rate was fixed at 0.25 and 0.35 ml/h, respectively. Voltage values between 10 and 25 kV were tried for the production of coaxial nanofibers and voltage value of 12 kV was selected due to continuous jet formation.