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Preparation and characterization of electrospun nanofibers containing glutamine

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ABSTRACT

Oral mucositis is a painful inflammation of mucous membranes commonly after chemotherapy or radiotherapy. The aim of this study was to develop mucoadhesive nanofibers containing glutamine via electrospinning and to characterize them for the treatment of oral mucositis. Different mucoadhesive polymers were tried for preparing nanofibers and sodium alginate nanofibers were chosen after the characterization studies. Glutamine-loaded nanofibers were produced and characterized. Glutamine loaded onto nanofibers was confirmed by differantial scanning calorimetry and fourier transform infrared spectroscopy analyses. As a result, scanning electron microscopy observations showed that the glutamine loaded nanofibers had average diameter of 160 nm. Glutamine amount was found to be 0.452 mg/cm^2. Work of mucoadhesion, tensile strength and elongation at break values of the glutamine loaded nanofibers were found to be 0.165 mJ/cm^2, 2.61 mPa and 6.62% respectively. In vitro dissolution tests showed that more than 85% of the drug was diffused from the nanofibers at the end of 4 h. Stability studies showed that there was no significant changes at 4 and 25 °C/65% relative humidity storage conditions. Therefore, these results demonstrate that glutamine loaded nanofibers could have potential as an oromucosal drug delivery system for the treatment oral mucositis.

1. Introduction

Electrospinning is the most efficient, versatile, handy, low-cost and high speed technique to produce nanofibers with diameter ranging from 50 to 1000 nm or greater. Electrospun nanofibers from natural and synthetic polymers have been widely used in industrial (membranes, filters, textiles and templates for hollow fibers) (Bagheri, Rezvani, & Banihashemi, 2016; Kang, Kim, Singh, Jang, & Kim, 2015; Li, Wu, Yan, & Guan, 2015; Matulevicius, Kliucininkas, Prasauskas, Buivydiene, & Martuzevicius, 2016; Wang, Bai, Xie, Jiang, & Qiu, 2016) and biomedical applications (tissue engineering scaffolds, wound dressing, vascular grafts and drug delivery systems) (Blakney, Ball, Krogstad, & Woodrow, 2013; Catto et al., 2015; Chou, Carson, & Woodrow, 2015; Gong et al., 2016; Jalaja, Naskar, Kundu, & James, 2016; Lee, Jeong, Kang, Lee, & Park, 2009; Potrc et al., 2015; Zhao et al., 2016). Electrospinning is used to produce three-dimensional, ultrafine fibers. The advantages of electrospun fibers for drug delivery are: high surface area to volume ratios; the ability to directly and locally adhere the scaffold onto the site of infection; and the ability to tune the physical properties of the scaffold to control and optimize drug delivery (Aduba et al., 2013; Akduman, Özgüney, & Kumbasar, 2016).

The electrospun nanofibers of natural polymers are more advantageous than synthetic ones for different biomedical applications because of their similarity, often identical to the macromolecular substances inherently present in the human body (Panthi, Park, Kim, & Park, 2014). Natural polymers such as chitosan (Chen, Wang, Wei, Mo, & Cui, 2010; Kim & Lee, 2014), chitin (Min et al., 2004; Noh et al., 2006), alginate (Fang, Liu, Jiang, Nie, & Ma, 2011; Leung et al., 2014), gelatin (Aoki, Miyoshi, & Yamagata, 2015; Okutan, Terzi, & Altay, 2014), cellulose (Christoforou and Doumanidis, 2010; Ohkawa, Hayashi, Nishida, Yamamoto, & Ducreux, 2009), collagen (Dong, Arnoult, Smith, & Wnek, 2009; Fischer, McCoy, & Grant, 2012) and hyaluronic acid (Uppal, Ramaswamy, Arnold, Goodband, & Wang, 2011) have been used to fabricate nanofiber matrices.

Oral mucositis is a painful and serious disease of patients receiving chemotherapy and also head/neck cancer patients receiving radiotherapy. Various drugs (chlorhexidine gluconate, povidone iodine, tobramycin, polimixina E, amphotericin B, bacitracin, gentamicin, benzydamine HCl, sucralfate, glutamine, zinc sulphate) were tried in the management of oral mucositis (Brasil, Serpa, Franca, & Castro, 2012; Rodríguez-Caballero et al., 2012). However, there is no standard protocol for prevent or treatment of

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oral mucositis (Brasil et al., 2012; Sonis, 2011). Glutamine levels decrease in the damaged tissue and in ulcerative oral mucositis disease (Chattopadhyay, Saha, Azam, Mukherjee, & Sur, 2014). Glutamine is a nonessential amino acid, produced in the human body. Glutamine is a source of energy for fibroblasts, epithelial cells, enterocytes, lymphocytes, macrophages and rapidly proliferating cells (Ku, Durak, Bayyurt, & Bilgen, 2014). Glutamine is a precursor of glutathione and glutathione is one of the most important antioxidant molecules. Followed by events such as surgery, sepsis and injury intracellular glutamine reservoirs decrease more than 50% (Amores-Sánchez & Medina, 1999; Stehle & Kuhn, 2015). The degree of normal tissue damage from radiation or chemotherapy may be affected from adequate tissue glutamine stores (Savarese, Savy, Vahdat, Wischmeyer, & Corey, 2003). In these cases, glutamine supplementation therapy in patients accelerates the healing time and in patients receiving glutamine, duration and severity of the disease decreased (Chattopadhyay et al., 2014; Peterson, Jones, & Petit, 2007; Rodríguez-Caballero et al., 2012). There are different applications of glutamine for the therapy of oral mucositis: oral solution for systemic effect (Aquino et al., 2005; Leitao et al., 2008), suspension (swish and expectorate) for local effect (Huang et al., 2000), suspension (swish and swallow) for systemic effect (Anderson et al., 1998; Anderson, Schroeder, & Skubitz, 1998; Peterson et al., 2007). These studies have shown that oral glutamine (local or systemic) have beneficial effects on reducing severity of oral mucositis.

The aim of this study was to develop mucoadhesive nanofibers containing glutamine via electrospinning and to characterize them. Application of the mucoadhesive nanofibers could improve the efficiency of the therapy in oral mucositis by prolonging the contact time of the drug with mucosa. Thus, glutamine loaded nanofibers could present advantages of both systemic and local applications. Little research has been carried out with nanofibers containing glutamine. For this purpose, four natural polymers such as sodium alginate, chitosan, k-carrageenan and hydroxypropyl methylcellulose were used. After the properties of nanofibers obtained were compared, the most suitable one was loaded with glutamine.

2. Experimental

2.1. Materials

Sodium alginate (Protanal LF10/60 89 kDa), poly(ethylene oxide) (PEO) (Polyox WSR-205, Mw 600 kDa) and hydroxypropyl methylcellulose (HPMC K4M, HPMC E4M) were kindly donated by FMC BioPolymer (Norway) and Colorcon (UK), respectively and used without further purification. Glutamine (Reagentplus, ≥99%) and k-carrageenan were purchased from Sigma Aldrich. Chitosan (Middle viscous) was purchased from Fluka (UK).

2.2. Preparation of the electrospinning solutions

PEO solutions were prepared in the concentration range 5–7% (w/v) based on the studies in the literature (Keun, Ho, Seung, & Ho, 2004; Uyar & Besenbacher, 2009). Aqueous solution of PEO or polymer/PEO mixtures were prepared by dissolving in distilled water at room temperature. 0.7 ml pure glacial acetic acid was added to the distilled water for the preparation of 100 ml chitosan solutions. Sodium alginate, HPMC and chitosan were used at a constant concentration of 2% (w/v) whereas k-carrageenan was 1% (w/v). PEO and polymer solutions were mixed at 70:30 or 80:20 volume ratio and stirred for 24 h at room temperature to obtain homogeneous solutions and were used for electrospinning (Table 1).

2.3. Characterization of polymer mixtures

2.3.1. Measurement of viscosity value

Rheological experiments were performed with a stress-controlled cone and plate rheometer (Brookfield, DV-III Rheometer with spindle type CPE-41, USA). All the samples were measured at 25 °C. Shear stress and viscosity values were obtained at different shear rates. All of the rheological measurements were repeated on at least three different samples. The viscosity values of the polymer solutions obtained at 20 rpm shear rate were compared.

2.3.2. Conductivity measurements

Ionic conductivity measurements of the solutions (three different samples) were carried out using a conductivity meter (Hanna Instruments, HI 9033, USA). The values were obtained as mS/cm.

2.3.3. Surface tension measeruments

Drops were formed on the tip of the needle equation to measure surface tensions of solutions or mixtures using pendant drop observation (Attension-Theta Lite, Biolin Scientific, Finland) and then surface tensions were calculated using Young Laplace equation.

2.4. Electrospinning

Electrospinning process was performed using NE-100 Laboratory Scale Electrospinning Unit (Inovenso Ltd., Turkey). First, nanofibers (blank nanofibers) without glutamine from polymer solutions were produced. Each polymer solution was placed into a 10 ml plastic syringe capped with an 18-gauge blunt needle. The positive lead from a high voltage supply was connected via an alligator clip to the external surface of the needle. A rectangular (20 × 10 cm) aluminium foil was used as a static collector and connected to the ground. It was not possible to work at constant process parameter for all mixtures due to the fact that different mixtures have different solution characteristics such as viscosity or conductivity. All parameters were tested to ensure the continuous production of nanofibers. The process parameters feed rate, distance of needle tip to the collector and applied voltage were used differently for each formulation (Table 2). The process time was kept for one hour which was an appropriate period to collect nanofiber mats from aluminium foil without damage. Next, after the characterization studies of nanofibers without drug, the appropriate formulation was chosen and drug was loaded to this formulation. Glutamine (35 mg/ml) was added to the polymer solution taking into account that the solubility of glutamine in water (Zhang & Zhong, 2015). After that polymer mixture was mixed over night on magnetic stirrer. Each formulation was produced three times.

2.5. Characterization of the nanofibers

2.5.1. Scanning electron microscopy (SEM) studies

Images of electrospun fibers were obtained with a Quanta 400F (FEI Company, USA) field emission scanning electron microscopy. The nanofibers were coated with gold/palladium. The images were taken in different parts of the nanofibers in 2000, 8000, 60,000 magnification. Average fiber diameters were determined by measuring fibers randomly selected from SEM images. The thickness of nanofibers was measured by cross-sectional SEM images.

2.5.2. Mucoadhesion studies

Texture Analyzer (TA.XT. PlusTexture Analyzer, Stable Micro Systems, UK) with a 5 kg load cell equipped with a mucoadhesive holder was used for the determination of mucoadhesion properties of the nanofibers. Freshly excised sheep cheeck mucosa was used as the model tissue and it was frozen at −20 °C. A 2 mm thick