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A novel chitosan-polyethylene oxide nanofibrous mat designed for controlled co-release of hydrocortisone and imipenem/cilastatin drugs

Yousef Fazli[a], Zahra Shariatinia[a,*], Iraj Kohsari[a,b], Amirreza Azadmehr[c], Seied Mahdi Pourmortazavi[d]

[a] Department of Chemistry, Amirkabir University of Technology (Tehran Polytechnic), P.O. Box: 15875-4413, Tehran, Iran
[b] Department of Physiology and Pharmacology, School of Medicine, Sabzevar University of Medical Sciences, Sabzevar, Iran
[c] Department of Mining & Metallurgical Engineering, Amirkabir University of Technology (Tehran Polytechnic), P.O. Box: 15875-4413, Tehran, Iran
[d] Faculty of Material and Manufacturing Technologies, Malek Ashtar University of Technology, P.O. Box 16765-3454, Tehran, Iran

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ABSTRACT

Antimicrobial chitosan–polyethylene oxide (CS-PEO) nanofibrous mats containing ZnO nanoparticles (NPs) and hydrocortisone-imipenem/cilastatin-loaded ZnO NPs were produced by electrospinning technique. The FE-SEM images displayed that the spherical ZnO NPs were ~70–200 nm in size and the CS-PEO nanofibers were very uniform and free of any beads which had average diameters within the range of ~20–130 nm. For all of the nanofibrous mats, the water uptakes were the highest in acidic medium but they were decreased in the buffer and the least swellings were obtained in the alkaline environment. The drug incorporated mat preserved its bactericidal activity even after it was utilized in the release experiment for 8 days in the PBS buffer. The hydrocortisone release was increased to 82% within first 12 h while the release rate of imipenem/cilastatin was very much slower so that 20% of the drug was released during this period of time suggesting this nanofibrous mat is very suitable to inhibit inflammation (by hydrocortisone) and infection (using imipenem/cilastatin antibiotic and ZnO NPs) principally for the wound dressing purposes.

1. Introduction

Nowadays, electrospinning has become the most commonly used technique for the fabrication of nanofibers due to its simplicity, adaptability, cost-efficiency and versatility (Tamm et al., 2016). Electrospun nanofibers have outstanding properties that make them appropriate as wound dressings, i.e. high oxygen permeability, variable pore size and high surface area to volume ratio. Additionally, nanofibrous mats are morphologically similar to the extracellular matrix (ECM) enhancing the cell migration and proliferation. Therefore, the electrospun mats are especially suitable for different biomedical applications such as wound dressing (Unnithan et al., 2016), drug delivery systems (Murase et al., 2015), tissue engineering (Dippold et al., 2016), vascular grafts (Elsayed et al., 2016) and supports for the human body (Lin and Fu, 2016). It is known that in order to accelerate the healing process of wounds, the wounded area must be protected from environmental factors by its covering with a wound dressing but it should be kept moist enough to accelerate healing (Roshmi et al., 2015). However, there should be no accumulation of fluid between the wound and the dressing to avoid infection. The treatment of severe burns necessitates the suppression of bacterial growth, particularly when eschar and damaged tissues are present. Consequently, an antimicrobial treatment of infected wound is required for the inhibition of bacteria proliferation (Tan et al., 2015).

Chitosan is a biopolymer which is well known as being able to accelerate the wound healing in human (Tian et al., 2016) and this is due to its substantial antibacterial activity against a broad spectrum of bacteria (Jiang et al., 2015; Li et al., 2016; Pan et al., 2015). It is also a biocompatible, biodegradable, abundant and cheap polymer that has haemostatic property (Gu et al., 2016). CS has frequently been electrospun (Kohsari et al., 2016a, 2016b; Samprasit et al., 2015) and it was shown that the compatibility of the CS and PEO polymeric materials with surgical wound sites has led to their employment as controlled release carriers for local antibiotic delivery (Mahmoud and Salama, 2016; Zeng et al., 2016). Moreover, the excellent spinnability of PEO leads to its addition to the poor spinnable CS solution in order to fabricate CS-PEO electrospun nanofibers (Jiang et al., 2014, 2016).

* Corresponding author.
E-mail addresses: shariati@aut.ac.ir, shariatiz@yahoo.com (Z. Shariatinia).

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To enhance the antibacterial properties of electrospun CS-based mats, compounds such as antibiotics or antiseptics are employed (Wang et al., 2010). These antibacterial agents endow several promising properties to the nanofibrous mats that will have several biomedical applications such as wound dressing (Abrigo et al., 2014; Karami et al., 2013; Lin et al., 2012; Merrell et al., 2009; Nitanan et al., 2013; Unnithana et al., 2012). Indeed, nanofibers have a high capacity for loading of biological substances and active materials such as ZnO NPs (Rath et al., 2016; Shalumon et al., 2011) and antibiotics (El-Shanshory et al., 2015; Kataria et al., 2014; Moura et al., 2014; Namazi et al., 2016; Unnithana et al., 2012).

Imipenem (Primaxin) is an intravenous β-lactam antibiotic which is the first member of the carbapenem class of antibiotics which are extremely resistant to the β-lactamase enzymes created by numerous multiple drug-resistant Gram-negative bacteria (Vardakas et al., 2012). Accordingly, they have a vital role in the treatment of infections not easily treated with other antibiotics (Pavez et al., 2016). Imipenem exhibits a broad spectrum of antibacterial activity against aerobic and anaerobic Gram-positive and Gram-negative microorganisms (Sun et al., 2016). It is mainly significant for its activity against *Pseudomonas aeruginosa* and the *Enterococcus* species. Imipenem acts as an antimicrobial compound by inhibiting cell wall synthesis of different Gram-positive and Gram-negative bacteria (Lai et al., 2015). It is quickly degraded by the renal enzyme dehydropeptidase 1 when administered alone, thus it is always co-administered with cilastatin to avoid its inactivation. Cilastatin is a chemical compound inhibiting the human enzyme dehydropeptidase (Nakane et al., 2015) which exists in the kidney and is responsible for degradation of the antibiotic imipenem. Hence, cilastatin is intravenously combined with imipenem in order to protect it from dehydropeptidase and prolong its antibacterial potency. Cilastatin itself does not have antibiotic activity though it is active against a zinc-dependent beta-lactamase that usually provides antibiotic resistance to certain bacteria. Therefore, cilastatin is not an antibiotic by itself but it is considered as a beta-lactamase inhibitor (Nakane et al., 2015).

One of the main threats in the health care is the emergence of microbial organisms which are resistant to antibiotics. As a result, researchers have tried to develop new effective antimicrobial agents such as nanoparticles which may show far lower tendency to induce microbial resistance than antibiotics (Shahmohammadi Jebel and Almasi, 2016). Inorganic nanocrystalline metal oxides like ZnO are especially interesting and suitable for biological applications. The inorganic antibacterial materials reveal advantages over organic antibacterial materials because of their superior durability, less toxicity and greater selectivity and heat resistance (Sarwar et al., 2016). Also, some metal oxides NPs, such as ZnO NPs, have selective toxicity to bacteria and human cancer cells (Condello et al., 2016). The unique properties of ZnO NPs have led to their multifunctional applications including drug delivery, imaging, antibacterial agents and sensing (Lee et al., 2014; Pandurangan et al., 2016).

The novelty of this research is the development of CS-PEO nanofibrous mats as wound dressings for controlled co-release of hydrocortisone and imipenem/cilastatin drugs so that most of the anti-inflammatory hydrocortisone will be released during first 12 h but the imipenem/cilastatin antibiotics will be released very much slower within a long time period to have a dressing with continuous efficiency against bacterial infection. For this purpose, the hydrocortisone was mixed with the CS-PEO polymeric solution but the imipenem/cilastatin mixed drugs were loaded onto the ZnO nano-pores and the drug containing ZnO NPs were incorporated within the polymeric matrix. In such a mat architecture, the ZnO NPs can also be released into the medium to increase the mat bactericidal activity. Interestingly, the mat preserved its antibacterial potency even after it was used in the release experiment for 8 days in the PBS buffer confirming it can be employed as a very appropriate wound dressing to inhibit inflammation (by hydrocortisone) and infection (using imipenem/cilastatin antibiotic and ZnO NPs).

2. Experimental

2.1. Materials

The high molecular mass CS (>75% deacetylation degree, viscosity = 200–800 cps) and PEO (average M_v = 400,000) polymeric materials were purchased from Sigma–Aldrich Company and other compounds including acetic acid (CH_3COOH, (≥99%), NaCl (≥99.5%), KCl (≥99.5%), HCl (37%), Na_2HPO_4 (≥99%), KH_2PO_4 (≥99.5%), $Zn(NO_3)_2{\cdot}4H_2O$ (≥98.5%) and Muller Hinton agar were received from Merck Company. Also, the hydrocortisone and imipenem/cilastatin drugs were obtained from Aburaihan and Afachemi pharmaceutical Companies, respectively (Tehran, Iran), stored at ambient conditions and protected from light and moisture.

2.2. Synthesis of ZnO NPs

ZnO NPs were synthesized by a green method using glycerin as a fuel (Shariatinia and Bagherpour Sardsahra, 2016) and thermal decomposition of the $Zn(NO_3)_2{\cdot}4H_2O$ precursor. For this purpose, an aqueous solution containing $Zn(NO_3)_2{\cdot}4H_2O$ (1 g) and glycerin (5.6 mL) in 1:20 molar ratio was prepared in 20 mL of distilled water and the solution was stirred for 1 h at 90 °C to obtain a homogenueous concentrated solution. Then, it was placed in a furnace and heated up to 700 °C with a heating rate of 5 °C/min for 4 h. After calcination, the white powder of ZnO NPs were washed with acetone and dried at 100 °C.

2.3. Drug loading and encapsulation efficiency

Before performing the drug loading experiment, the ZnO NPs were dried at 100 °C for 10 h in order to prepare dehydrated NPs which are appropriate for the drug loading. The drug loading was done by dispersing the ZnO NPs in an aqueous solution containing exact amount of the imipenem/cilastatin sodium drug in distilled water. The suspension was stirred for 1 h at room temperature. After that, the mixture was centrifuged and the powder was washed with distilled water and dried at 50 °C for 5 h. The amount of imipenem/cilastatin drug remained was determined using a UV–vis spectrophotometer at wavelength of 300 nm (Van Herendael et al., 2012). The encapsulation efficiency (EE) percentage for the imipenem/cilastatin drug was calculated equal to 82% using the equation $EE = (C_1/C_0) \times 100$, where C_1 is the drug concentration in the ZnO NPs and C_0 is the drug concentration in the initial solution.

2.4. Solution preparation for electrospinning

An aqueous solution of 50% (v/v) concentrated acetic acid (CH_3COOH:H_2O) was used as the solvent for CS and PEO and total concentration of the two polymers in the solution was 4% (w/w) with the CS to PEO weight ratio was 75:25. The 3% ZnO NPs (or 2% hydrocortisone-3% imipenem/cilastatin-loaded ZnO NPs) powder was added into the polymer solution and the mixture was stirred overnight in a dark brown bottle. Then, it was sonicated for 20 min to obtain the electrospinning mixture.

2.5. Electrospinning process

Electrospinning was performed in a laboratory spinning unit (ANSTCO-N/VI, Tehran, Iran), which was designed in terms of a