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Controlled release of cefazolin sodium antibiotic drug from electrospun chitosan-polyethylene oxide nanofibrous Mats

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ABSTRACT

Antimicrobial electrospun chitosan–polyethylene oxide (CS-PEO) nanofibrous mats containing cefazolin, fumed silica (F. silica) and cefazolin-loaded fumed silica nanoparticles (NPs) were produced for biomedical applications. The FE-SEM images revealed that the F. silica and F. silica-cefazolin NPs had average diameters of 40 ± 10 and 60 ± 15 nm, respectively. Also, the fibers diameters were approximately 160 ± 30, 90 ± 20 and 70 ± 15 nm for the pure CS-PEO, CS-PEO-1% F. silica and CS-PEO-1% F. silica-0.5% cefazolin nanofibrous mats, respectively indicating addition of F. silica and cefazolin loaded F. silica NPs to the CS-PEO mat led to decreasing the nanofiber diameter. Both of the CS-PEO mats containing 2.5% cefazolin and 1% F. silica-0.50% cefazolin showed 100% bactericidal activities against both *S. aureus* and *E. coli* bacteria. The cefazolin release from mats was sharply increased within first 24 and 6 hours for the CS-PEO mats including 2.5% cefazolin and 1% F. silica-0.50% cefazolin but after that the drug was released very slowly. The improved hydrophilicity, higher tensile strength and sustained drug release for CS-PEO-1% F. silica-0.50% cefazolin suggested that it was the best nanocomposite tissue/device for biomedical applications among the mats CS-PEO-2.5% cefazolin and CS-PEO-1% F. silica. The wound healing ability of the CS-PEO-F. silica-cefazolin mat was evaluated on the wounded skins of the female Wistar rats and it was shown that the wounded skins of the rats were almost entirely healed after ten days using this mat as a wound dressing scaffold.

1. Introduction

One of the significant research areas in the 21st century is nanotechnology and for this reason, numerous researches have been conducted to more understand the potential effects of the nanomaterials on biological systems. The unique physicochemical properties of the nanometer scale materials are mainly related to their small size, high surface area, chemical composition and shape [1] leading to their application in several fields including drug and gene delivery, imaging and diagnostics [2–4].

Nanocomposite nanofibrous matrices fabricated by electrospinning method consisting of nanoscale inorganic fillers and polymer matrix, combine the advantages of both the polymer materials, such as light weight, flexibility, and good moldability, and the inorganic materials, such as high strength, heat stability, and chemical resistance [5,6]. As a result, these composite nanofibers can have enhanced mechanical, electrical, optical, thermal and magnetic properties without losing transparency, and they can be excellent candidates for many multifunctional applications which include membranes, filtration, textile coating, catalysis, energy conversion and storage systems devices [6–8] as well as biomedical fields such as wound healing, drug delivery systems, and tissue engineering scaffolds [9].

Among various composite nanofibers, silica-filled nanofibers have received considerable attention due to silica NPs offer improved properties such as hydrophilicity, toughness, and permeability [10–12]. The silica NPs possess large specific surface areas/pore volumes resulting in high adsorption of drugs into their nanoporous structures leading to controllable release profiles [13–15]. Moreover, it has been shown that silica NPs do not have toxicity to cells [16–18] and they could also increase the dissolution of poorly water-soluble drugs [19,20]. Finally, they could easily be modified to form a functional surface to control the release of drugs in response to different pH environments and temperature [21,22].

The drug release rate can be controlled by the morphology and composition of the electrospun fibers [23,24] and the drug-loaded mats can also be fabricated into various shapes (membrane, tube) for special applications like wound dressing and nerve conduits [25,26]. Therefore, in this study polymer/fumed silica NPs composite mats were prepared by the electrospinning method for the efficient controlled delivery of the cefazolin drug which belongs to the cephalosporins family.

Cephalosporins are antibacterial antibiotics belonging to the group β-lactam antibiotics which are used as therapeutic agents [27]. Since their discovery in 1948 by Brotzu [28], cephalosporins have widely

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been applied because of their substantial activities against Gram-positive and Gram-negative bacteria by inhibiting synthesis of bacterial cell wall in a manner similar to penicillin [29]. Moreover, they can be used in treating allergic patients to penicillin [29] and to treat moderately severe bacterial infections involving the lung, stomach, joint, bone, blood, heart valve and urinary tract. Cefazolin sodium is classified as a first-generation cephalosporin for injectable use in clinical practice due to its efficacy as a therapeutic agent and surgical prophylaxis.

Antibiotics are commonly given systemically to patients before and after various types of surgery. In orthopedic surgery, the administration of antibiotics is especially important because the surgery often involves large external wound lengths and may be accompanied by the implantation of devices [30]. An alternative approach is locally administration of the antibiotics so that the drug is targeted to the surgical site and released in a controlled manner over time in order to release high initial drug concentrations to eliminate any existing bacteria at the surgical site, followed by the extended release of the antibiotic to avoid re-infection. Controlled release necessitates the drug to be encapsulated in a material that postpones its release at the site [31].

An ideal drug delivery system should offer: (i) a sufficient antimicrobial concentration at the target site, (ii) a slow and constant release of antimicrobial over a prolonged period and (iii) be biodegradable so that a second operation is not necessary [32,33]. Evidently, the compatibility of the chitosan (CS) and polyethylene oxide (PEO) polymeric materials with surgical wound sites [34,35] makes them appropriate candidates for employment as controlled release carriers for local antibiotic delivery.

Herein, antimicrobial chitosan-polyethylene oxide (CS-PEO) nanofibrous mats containing fumed silica and cefazolin loaded-fumed silica particles were produced for wound dressing applications. The antibacterial activities of the mats were evaluated against two widespread wound burn infectious microorganisms including *Staphylococcus aureus* and *Escherichia coli* bacteria. Also, the release profiles of cefazolin loaded mats were evaluated during 15 days.

2. Experimental

2.1. Materials

The high molecular weight chitosan (>75% deacetylation degree, viscosity = 200–800 cps) and poly(ethylene oxide) (average M_v = 400,000) polymeric materials as well as hydrophilic fumed silica (SiO_2, Cab-O-Sil™, S5505) were purchased from Sigma–Aldrich Company and other compounds including acetic acid (CH_3COOH), NaCl, KCl, HCl, Na_2HPO_4, KH_2PO_4, $AgNO_3$ and Muller Hinton agar were received from Merck Company. Also, the cefazolin sodium drug was obtained from Afachemi pharmaceutical Company (Tehran, Iran) and stored in ambient conditions protected from light and moisture.

2.2. Drug loading and encapsulation efficiency

Before performing the drug loading experiment, the fumed silica NPs were calcined at 500 °C for 24 h in order to prepare dehydrated NPs and also to introduce some porosity into the particles which is appropriate for drug loading. The drug loading was done by dispersing the calcined F. silica NPs in an aqueous solution containing exact amount of the cefazolin 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 drug remained was determined using a UV–Vis spectrophotometer at a wavelength of 270 nm [36]. The encapsulation efficiency (EE) percentage was calculated equal to ~50% using the equation $EE = (C_1/C_0) \times 100$, where C_1 is the drug concentration in the F. silica NPs and C_0 is the drug concentration in the initial solution.

2.3. 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 1% F. silica (or cefazolin-loaded F. silica) 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.

2.4. Electrospinning process

Electrospinning was performed in a laboratory spinning unit (ANSTCO-N/VI, Tehran, Iran), which was designed in terms of a vertical working principle. Each solution was placed in a 10 mL syringe and sent to the drum collector (covered with aluminum foil) through a 20 gauge nozzle. The power supply (AC) was set up for a positive voltage of 21 kV. The flow rate of the solution was also determined by setting up the syringe pump at 1 mL/h. The rotational speed of the drum collector was 2500 rpm and its distance was set to 10 cm (optimum distance based on preliminary tests) away from the nozzle. It is noteworthy that in order to produce nanofibrous mats with high qualities, the electrospinning of each sample was prolonged to 10 h. At the time of the experiments, relative humidity and temperature values ranged from 35% to 42% RH and 25 to 35 °C, respectively.

2.5. Characterization techniques

In order to perform the tensile strength analysis, the mats ($n = 3$ for each group) were cut into 5 mm × 25 mm rectangular stripes and their mechanical properties were tested on an electron tensile machine (LLY-006, China) according to the ASTM D638 for plastics. The water contact angle measurements were performed using Tensiometer with water as a probe liquid (Dataphysics Instruments, TC/TPC 150). The swelling behaviors of the pure CS-PEO and CS-PEO mats containing cefazolin, F. silica and cefazolin-loaded F. silica were carried out by immersing them in different media including acidic pH = 4, neutral pH = 7.4 (0.1 M phosphate buffer saline (PBS) solution) and alkaline pH = 13 at 25 °C for three days. The water uptake or swelling percent was calculated according to the following equation, where W_0 is initial weight of dry mat and W_s is swollen weight of mat at equilibrium.

$$\text{Swelling percent} = [(W_s - W_0)/W_0] \times 100$$

The field–emission scanning electron microscopy (FE–SEM) micrographs were taken from Philips instrument (XL30), under vacuum, accelerated at 20 kV in order to study the morphology and size of the F. silica/drug-loaded F. silica NPs and nanofibrous mats. The EDAX analysis was performed using the EDAX accessory on the FE-SEM instrument. The Fourier–transform infrared (FT–IR) spectra were recorded on a Bruker spectrometer in the 500–4000 cm^{-1} wavelength range so that the Attenuated Total Reflectance (ATR) mode was selected due to our samples were not transparent. The thermogravimetric analysis (TGA) and differential scanning calorimetry (DSC) were obtained using a Perkin Elmer TPI-PE (Temperature Programmer Interface for Perkin Elmer) instrument under oxygen atmosphere with a heating rate of 10 °C/min up to 600 °C. The XRD patterns were taken with an INEL EQUINOX 3000 X–ray diffractometer using Cu K_α radiation (λ = 1.5406 Å) to get insight about the crystalline nature of the samples. The amounts of cefazolin drug released from the fibrous mats were examined by UV–visible spectrophotometer (Perkin Elmer, USA) at the wavelength of 270 nm [36].