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Antibacterial electrospun chitosan–polyethylene oxide nanocomposite mats containing bioactive silver nanoparticles

Iraj Kohsari[a], Zahra Shariatinia[a,*], Seied Mahdi Pourmortazavi[b]

[a] Department of Chemistry, Amirkabir University of Technology (Tehran Polytechnic), PO Box 15875-4413, Tehran, Iran
[b] Faculty of Material and Manufacturing Technologies, Malek Ashtar University of Technology, PO Box 16765-3454, Tehran, Iran

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ABSTRACT

The antimicrobial chitosan–polyethylene oxide (CS–PEO) nanofibrous mats were developed by electrospinning technique for wound dressing applications. Indeed, a green route was introduced for fabrication of antibacterial mats loaded with 0.25% and 0.50% (w/w) of bioactive silver nanoparticles (Ag NPs, ~70 nm diameter) reduced by *Falcaria vulgaris* herbal extract. The mats were characterized by FE-SEM, EDAX, elemental mapping, FT-IR, contact angle, TGA/DSC as well as tensile strength analysis. All of the nanofibers had an average ~200 nm diameter. Interestingly, both of the CS–PEO mats containing 0.25% and 0.50% bioactive *F. vulgaris*-Ag NPs revealed 100% bactericidal activities against both *Staphylococcus aureus* and *Escherichia coli* bacteria. The silver release from nanofiber mats was sharply increased within first eight hours for both CS–PEO mats including 0.25% and 0.50% *F. vulgaris*-Ag NPs but after that the Ag nanoparticles were released very slowly (almost constant). The improved hydrophilicity, higher tensile strength and much greater silver release for CS–PEO-0.50% *F. vulgaris*-Ag NPs relative to those of the CS–PEO 0.25% *F. vulgaris*-Ag NPs suggested that the former was superior for biomedical applications.

1. Introduction

Nowadays, polymeric nanofibers have gained much increased interest in science and technology due to they are applied in a wide variety of industrial fields and present a bright future (Lee, Kim, Khil, Ra, & Lee, 2003). It is known that the diameters of fibers made by conventional methods like spinning from melt or solution are usually in the range of 5–500 μm. For this reason, producing polymer nanofibers with diameters in the nanoscale range by the electrospinning method has recently attracted much increased interest (Xu et al., 2015; Yao, Pantano, Pugno, Bastiaansen, & Peijs, 2015). The electrospun textiles have inherent small pore size and large specific surface area (Deitzel, Kleinmeyer, Harris, & Beck Tan, 2001; Reneker & Chun, 1996), thus they have found valuable applications in military protective clothing and filters, non-traumatic wound dressing, drug delivery carriers, haemostatic devices, tubular shapes for blood vessels and nerve regeneration, 3D-scaffolds for bone and cartilage regeneration, cosmetics, optics like nano-sensors (thermal, piezoelectric, biochemical and fluorescence optical chemical sensors), and electronics (Burgshoef & Vancso, 1999; Huang, Zhang, Kotaki, & Ramakrishna, 2003; Norris, Shaker, Ko, & MacDiarmid, 2000).

The natural polyaminosaccharide chitosan (CS) has extensively been used in the fibrous architecture for tissue scaffolds (Chen, Chen, & Lai, 2012; Chen, Wang, Wei, Mo, & Cui, 2010; Huang et al., 2011; Mottaghitalab et al., 2011), wound dressing (Gu et al., 2013) and antibacterial films and coatings (Shariatinia & Fazli, 2015; Shariatinia & Nikfar, 2013; Shariatinia, Nikfar, Gholivand, & Abolghasemi Tarei, 2015). A chitosan nanofiber scaffold can diminish infection in *in vivo* implantation due to its antibacterial properties. Furthermore, besides the non-toxicity and the morphological similarity of CS nanofibers to native skin ECM, their oxygen permeability (originating from their porosity characteristics) make them suitable for wound healing applications (Jayakumar, Prabaharan, Kumar, Nair, & Tamura, 2011). Another suitable polymer for devices contacting with living organisms is poly(ethylene oxide) (PEO) because of its low toxicity (Herold, Keil, & Burns, 1989). PEO was electrospun from aqueous solution and fibers with diameters in the range of 500–5000 nm were fabricated (Doshi & Reneker, 1995).

In is well known that pure CS is difficult to electrospin and the nanofiber creation is facilitated by its blending with other polymers (Kriegel, Kit, McClements, & Weiss, 2009; Shalumon et al., 2010; van der Schueren, Steyaert, de Schoenmaker, & de Clerck, 2012). Additionally, the combination of CS with other polymers

* Corresponding author. Tel.: +98 2164542766; fax: +98 2164542762.
E-mail addresses: shariati@aut.ac.ir, shariatiz@yahoo.com (Z. Shariatinia).

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may lead in a higher analogy of the scaffold to natural ECM components and induces superior properties which are essential in tissue regeneration. Combining CS with PEO might lead to novel materials suitable for diverse applications in the biomedical field. For instance, CS–PEO nanofibers can be utilized as a 3-D scaffolds for cartilage tissue repair due to their good adhesion, proliferation and viability for chondrocytes (Subramanian, Vu, Larsen, & Lin, 2005).

The field of nanoparticle research has indicated remarkable growth because of the unique chemical and physical properties from the bulk. Silver nanoparticles (Ag NPs) have gained substantial attention due to their potential applications in medical field especially in the production of biodegradable surgical sutures. Recently, a number of Ag NPs embedded electrospun nanofibers with antimicrobial potential have been prepared (Liu et al., 2015; Wen, Zhang, Hu, Zhang, & Liu, 2015). Although there are several routes available for the synthesis of Ag NPs including chemical reduction, thermal decomposition, electrochemical and microwave assisted processes, biological methods for nanoparticles synthesis using microorganisms, enzymes, and plants or plant extracts offer numerous benefits over chemical and physical methods due to their cost effectiveness, environmentally friendly nature and the ability to be easily scaled up for large scale synthesis (Mittal et al., 2015; Mohapatra, Kuriakose, & Mohapatra, 2015; Saravanakumar, Ganesh, Jayaprakash, & Jang, 2015).

Falcaria vulgaris (locally named ghazzyaghi/poghazeh) from Apiaceae family is a medicinal plant that is consumed as a vegetable in Iran, also for healing skin ulcer, stomach disorders including peptic ulcer, liver diseases and stones of kidney and bladder (Abaghian, Shafaghat, Zarea, Kasimov, & Salimi, 2011; Jaberian, Piri, & Nazari, 2012; Shafaghat, 2010, 2011). The antimicrobial and antioxidant properties of *F. vulgaris* were examined and it was found that it had high content of carvacrol (29.8%) as a main component suggesting it would be able to be a source of natural product with potential antibacterial and antioxidant activity (Jaberian, Piri, & Nazari, 2013). Also, it was reported that hydroalcoholic extract of *F. vulgaris* decreased the gastric ulcer in Rat (Khazaei & Salehi, 2006).

The electrospun CS–PEO nanofibrous mats containing Ag NPs synthesized using UV irradiation (Wang, Cheng, Gao, & Wang, 2015) or $NaBH_4$ (An, Zhang, Zhang, Zhao, & Yuan, 2009) were developed. The nanofibrous CS–PEO mats crosslinked using glutaraldehyde and loaded by ciprofluxacin hydrochloride and moxifloxacin hydrochloride drugs were also prepared (Cheng, Gao, Wang, & Hu, 2015). However, to the best of our knowledge, there is not any report in literature on the preparation of CS–PEO nanofibrous mats containing antibacterial herbal extracts.

The aim of the present work is preparation of chitosan–polyethylene oxide (CS–PEO) nanofiber mats containing 0.25% and 0.50% (w/w) of biosynthesized Ag NPs to produce antimicrobial nanofibrous mats for wound dressing applications. For this purpose, a green method was used consisting of two steps: (1) preparation of Ag NPs using *F. vulgaris* herbal extract as a green reducing agent and (2) electrospinning of the CS–PEO polymeric mixture containing bioactive Ag NPs. The antibacterial activities of the fiber mats were tested against two bacteria including *Staphylococcus aureus* and *Escherichia coli* which are considered as commonly widespread wound burn infectious microorganisms.

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 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.

2.2. Extraction of plant material

The *F. vulgaris* were collected from East Azerbaijan province in the north-west of Iran. The Plant leaves were dried in the shade at room temperature and grounded in a mortar. Fifty grams of the plant were extracted using distilled water by maceration. The extracts were filtered using Whatman filter paper (No. 1) and then were centrifuged at 8000 rpm for 10 min. The supernatant was again filtered using Whatman filter paper (No. 1) under strict aseptic conditions. The collected filtrates were concentrated in vacuum at a temperature below 40 °C using a rotary evaporator (Buchi, Switzerland). The residue obtained was stored in freezer at 20 °C until further use.

2.3. Synthesis of silver nanoparticles

Ag NPs were synthesized by means of simple chemical reduction of silver nitrate using *F. vulgaris* herbal extract. The aqueous extract solution of the plant leaves was added to the $AgNO_3$ aqueous solution and the reaction flask was covered with aluminum foil and subjected to a constant mixing on a magnetic stirrer. The addition of the plant extract (dark green) to $AgNO_3$ aqueous solution (colorless) led to turning the solution color to dark grey indicating formation of silver nanoparticles. In another method to produce Ag NPs, sonication was applied to make uniform particles. The flask containing the identical amounts of aqueous plant extract and $AgNO_3$ aqueous solution was placed in the ultrasonic bath for 15 min. The flask was sealed during sonication to prevent loss of solution. The progress of Ag NPs formation during the biosynthesis was monitored at regular intervals based on surface plasmon resonance of Ag NPs around 450 nm using UV–visible spectrophotometer.

2.4. Solution preparation

In a typical procedure, 50% (v/v) of concentrated aqueous acetic acid solution (CH_3COOH:H_2O) was used as the solvent. Total concentration of the CS and PEO polymers in the solution was 4% (w/w) with the CS to PEO weight ratio was 75/25. The solutions containing CS, PEO and biosynthesized Ag NPs using *F. vulgaris* extract were prepared by addition of 0.25% and 0.50% (w/w) of nanobio silver aqueous solutions into the 50% acetic acid solution including CS and PEO. The CS–PEO-herbal extract-Ag NPs solution was stirred overnight in a dark brown bottle and then it was sonicated for 15 min to get Ag NPs incorporated electrospinning solution.

2.5. Electrospinning process

Electrospinning was performed in the laboratory spinning unit (ANSTCO-N/VI), which was designed in terms of a vertical working principle. A digital photograph of the electrospinning instrument used in this work is presented in Fig. S1. 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. At the time of the experiments, relative humidity and temperature values ranged from 35% to 42% RH and 25 to 35 °C, respectively.