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High concentration honey chitosan electrospun nanofibers: Biocompatibility and antibacterial effects

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ABSTRACT

Honey nanofibers represent an attractive formulation with unique medicinal and wound healing advantages. Nanofibers with honey concentrations of <10% were prepared, however, there is a need to prepare nanofibers with higher honey concentrations to increase the antibacterial and wound healing effects. In this work, chitosan and honey (H) were cospun with polyvinyl alcohol (P) allowing the fabrication of nanofibers with high honey concentrations up to 40% and high chitosan concentrations up to 5.5% of the total weight of the fibers using biocompatible solvents (1% acetic acid). The fabricated nanofibers were further chemically crosslinked, by exposure to glutaraldehyde vapor, and physically crosslinked by heating and freezing/thawing. The new HP–chitosan nanofibers showed pronounced antibacterial activity against *Staphylococcus aureus* but weak antibacterial activity against *Escherichia coli*. The developed HP–chitosan nanofibers revealed no cytotoxicity effects on cultured fibroblasts. In conclusion, biocompatible, antimicrobial crosslinked honey/polyvinyl alcohol/chitosan nanofibers were developed which hold potential as effective wound dressing.

1. Introduction

Electrospinning is recognized as an efficient method for producing nanofibers (Li & Xia, 2004). The electrospun fibers show the advantages of high porosity and large surface to volume ratio (Altstädt, Lovera, Schmidt, Schmidt, & Fery, 2008). Moreover, nanofibers resemble the natural extracellular matrix and were reported to promote proliferation and migration of cells (Bhardwaj & Kundu, 2010). Electrospun nanofibers represent an efficient formulation for drugs and natural remedies as they allow loading high concentration of combinations of natural and synthetic materials and controlled/sustained release (Meinel, Germershaus, Luhmann, Merkle, & Meinel, 2012).

Honey has profound medicinal and nutritional properties (Khan, Abadin, & Rauf, 2007). It exhibits antimicrobial activity, debriding and deodorising action as well as anti-inflammatory, antioxidant and wound healing activities (Lusby, Coombes, & Wilkinson, 2002). In 2013, Maleki et al. were able to fabricate honey/polyvinyl alcohol nanofibers. Unfortunately, the maximum concentration that could be incorporated within the electrospun nanofibers was 2.25% honey of the total weight of the nanofibrous mat (Maleki, Gharehaghaji, & Dijkstra, 2013). Recently, Wang and He (2013), worked on fabrication of high honey concentration nanofibers, however, the maximum concentration of included honey was 9% with 10% polyvinyl alcohol of the total weight of the nanofibrous mat (Wang & He, 2013). Thus, there is a need to fabricate nanofibers composed primarily of high honey concentrations. Such concentrations will maximize the therapeutic and nutritional benefits of honey nanofibrous formulations in smaller dosage forms.

Chitosan is a biodegradable, biocompatible polymer with antibacterial, aqueous adsorption and wound healing ability (Schiffman & Schauer, 2007). Also, it can promote tissue regeneration and achieve hemostasis (Busilacchi, Gigante, Mattioli-Belmonte, Manzotti, & Muzzarelli, 2013; Muzzarelli, Greco, Busilacchi, Sollazzo, & Gigante, 2012). Chitosan meets also the demands of several industrial and biomedical activities

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(Muzzarelli, 2010; Muzzarelli, Greco, Busilacchi, Sollazzo, & Gigante, 2012b; Muzzarelli, El Mehtedi, & Mattioli-Belmonte, 2014).

Because of the high viscosity of chitosan in solutions, electrospinning of chitosan was only possible by using toxic or highly concentrated acidic solvents (Geng, Kwon, & Jang, 2005; Homayoni, Ravandi, & Valizadeh, 2009; Su et al., 2011). Residues of such solvents are unfavorable especially in applications requiring biocompatible materials. Aqueous salts of chitosan were prepared, but the concentration of the incorporated chitosan did not exceed 1% (Charernsriwilaiwat, Opanasopit, Rojanarata, Ngawhirunpat, & Supaphol, 2010; Charernsriwilaiwat, Opanasopit, Rojanarata, & Ngawhirunpat, 2011). Another approach for electrospinning chitosan in more biocompatible solvents was via co-spinning with other readily spun polymers. Among them, co-spinning chitosan with polyvinyl alcohol is one of the most common composites (Liao et al., 2011; Yan et al., 2012; Zhou et al., 2007). Still, nanofibers prepared by this method could only incorporate limited chitosan concentrations.

It is the aim of the present work to co-spin high concentrations of chitosan and honey with polyvinyl alcohol using biocompatible solvents. This would maximize the benefit of these two important materials in the smallest dosage form.

2. Experimental

2.1. Materials

Chitosan (Mwt: 240 kDa, DDA: 84%; Chitoclear, cg110, TM 3728; Primex; Siglufjordur, Iceland). Polyvinyl alcohol (Mwt: 85,000; Sigma Aldrich, St. Louis, USA), acetic acid (glacial, 99–100%; Merck, Wadeville, South Africa), glutaraldehyde (25% in H_2O; Sigma Aldrich, St. Louis, USA). Nutrient broth & nutrient agar (Becton Dickinson and Company, USA). Trypsin (85450C-25G; Sigma Aldrich), RPMI_1640 with L-Glutamine (R8758; Life Science), fetal bovine serum (10270-106; Gibco), thiazolyl blue tetrazolium bromide–MTT (M2128-1G; Sigma Aldrich), PBS, trypan blue and triton X (Sigma Aldrich, St. Louis, USA). Clover honey was obtained from the faculty of Agriculture, Cairo University. The viscosity of the honey was 15,300 mpas and its total soluble solid content was 81%.

2.2. Preparation of the polyvinyl alcohol/chitosan (P-chitosan), honey/polyvinyl alcohol (HP) and honey/polyvinyl alcohol/chitosan (HP–chitosan) solutions

Different solutions composed of different weight ratios of P-chitosan and HP as well as HP–chitosan were prepared as follows: P-chitosan (7%:1.5%, 7%:2.5% and 7%:3.5%); HP (20%:10% and 30%:10%), and HP–chitosan (30%:7%:1.5%, 30%:7%:3.5%, 30%:5%:5.5%, 30%:5%:4.5%, 20%:7%:3.5% and 40%:7%:3.5%). Solutions were prepared in 1% acetic acid. Each of the as prepared solutions of HP–chitosan was aged at room temperature for different time intervals.

2.3. Viscosity measurements

The viscosity of the polyvinyl alcohol (7%), P-chitosan (7%:3.5%), HP (30%:7%), and HP–chitosan (30%:7%:3.5% and 10%:7%:3.5%) samples were determined using a viscometer (Myr; VR-3000, Viscotech Hispania, Tarragona, Spain). The solutions were aged at room temperature for a week. The viscosity of all samples was tested at different time intervals (0, 24, 48 h and 1 week). The average value of three measurements was reported as mean ± SD.

2.4. Electrospinning of polyvinyl alcohol/chitosan (P-chitosan), honey/polyvinyl alcohol (HP) and honey/polyvinyl alcohol/chitosan (HP–chitosan) nanofibers

Each of the as-prepared solutions of P-chitosan, HP and HP–chitosan with different weight blending ratios was electrospun into nanofibers via the electrospinner (E-spin, NanoTech, Kalyanpur, India). The solutions were loaded in a 5 ml plastic syringe that was attached to a stainless steel needle (22 gauge) as a nozzle. The electrospun polymer solutions were subjected to different voltages (Gamma High Voltage Power Supply, USA) for adjustment of the optimum voltage for each of the spun solutions. The flow rate of the solution was maintained at 10 μl/min and the distance between the nozzle and the collector was maintained at 15 cm. Collection of the samples was done on a ground collector wrapped with an aluminum sheet.

2.5. Cross-linking of fiber mats

Physical and chemical methods were used to crosslink the fiber mats of HP–chitosan. Glutaraldehyde (GA) was used for chemical crosslinking. The fiber mats were placed in a closed desiccator that was saturated with GA vapors (40 ml). Exposure of the nanofiber mats to the GA vapors was done for different time intervals (30, 60, 120 and 180 min as well as 48 h and 72 h). Subsequently, enhancement of the crosslinking reaction and removal of unreacted (GA) were done via heating the nanofiber mats in an oven under vacuum at 70 °C for 24 h as well as at 40 °C for 24 h. Physical crosslinking was performed by freezing/thawing and heating techniques. Freezing and thawing was performed via freezing the fiber mats for 15 min in liquid nitrogen followed by thawing at room temperature for 15 min for three successive cycles. Heating was carried out under vacuum in an oven (Jeiotech, OV-11, South Korea) at both 110 °C, 100 °C for 15 min and 80 °C for 25 min as well as at 70 °C for 24 h.

2.6. Characterization and measurements of the electrospun nanofibers

The morphologies of the electrospun nanofibers were observed using scanning electron microscopy (FESEM, Leo Supra 55, Zeiss Inc., Oberkochen, Germany). Fourier transform infrared spectroscopy (FTIR) was performed for the raw polyvinyl alcohol and chitosan and the HP–chitosan nanofibrous mats using FTIR (Thermo scientific, Nicolet 380, USA). The transmission mode with KBr pellets was used for bulk chitosan and polyvinyl alcohol as well as and HP–chitosan nanofibrous mats.

2.7. Degree of swelling and weight loss

The HP–chitosan nanofibrous mats were tested for the degree of swelling and weight loss that were calculated according to Eqs. (1) and (2), respectively. Both tests were carried out in phosphate buffered saline [PBS], pH (7.4) at 37 °C for 1, 4 and 24 h.

$$\text{Degree of swelling}\,(\%) = \left[\frac{M - Mi}{Mi}\right] \times 100 \quad (1)$$

Sharma, Dinda, & Mishra (2013)

$$\text{Weight loss}\,(\%) = \left[\frac{Mi - Md}{Mi}\right] \times 100 \quad (2)$$

where M is the swollen weight of the nanofibrous sample which was dried using a filter paper, Md is the dried mass of the nanofibrous sample after being immersed in buffer medium, measured by drying the swollen mats at 40 °C until constant weight was reached, and Mi is the initial dry mass of sample.