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The effect of increasing honey concentration on the properties of the honey/polyvinyl alcohol/chitosan nanofibers

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ABSTRACT

The effect of increasing honey concentrations from 10% to 30% within the Honey (H)/polyvinyl alcohol (P)/chitosan (CS) nanofibers was investigated. Changes in the electrospun nanofiber diameters, crystallinity, thermal behavior, porosity and antibacterial activity have been assessed using SEM, XRD, DSC, TGA, mercury porosimeter and viable cell count technique. The HPCS nanofibers were cross-linked and tested for their swelling abilities and degradation behavior. The mean diameter of HPCS nanofibers increased from 284 ± 97 nm to 464 ± 185 nm upon increasing the honey concentration from 10% to 30%. Irrespective the honey concentrations, the nanofibers have demonstrated enhanced porosity. Increasing the honey concentration resulted in a reduction in the swelling of the 1 h cross-linked HPCS nanofibers containing 10% and 30% H from 520% to 100%; respectively. Degradation after 30 days was reduced in the 3 h cross-linked HPCS nanofibers compared to the non-crosslinked HPCS nanofibers. Enhanced antibacterial activity was achieved against both *Staphylococcus aureus* and *Escherichia coli* upon increasing the honey concentration. Changing the honey concentration and the extent of nanofiber crosslinking can be used to adjust different parameters of the HPCS nanofibers to suit their applications in wound healing and tissue engineering.

1. Introduction

Electrospinning is a feasible and simple technique for production of nanofibers [1–4]. The electrospun nanofibers exhibit increased surface to volume ratio with improved and controlled porosity allowing their use in various applications including tissue engineering, [5,6] drug delivery [7,8] wound healing, [9,10] filtration, energy storage, defense, and security [11–13]. Among the different materials spun into nanofibers, chitosan stands as one of the most advantageous biopolymers due to its enhanced properties. Chitosan is well known for its biocompatibility, biodegradability, nontoxicity and non-immunogenicity [14]. Chitosan is also characterized by its cost-effectiveness as it is derived from chitin the second most abundant polymer after cellulose [15]. This has also allowed chitosan to be an important candidate in a large number of applications [16,17]. However, electrospinning of chitosan into nanofibers is not an easy process particularly due to its high charge and viscosity, in addition to the need to use toxic or highly acidic solvents [18,19]. Residues of such solvents are not favorable in biomedical applications. The optimum strategy to avoid this drawback is through co-spinning of chitosan with other easily spun polymers such as polyvinyl alcohol and poly ethylene oxide [20–22]. Such strategy, however allows the electrospinning of only small concentrations of chitosan.

Honey, a carbohydrate rich syrup has been used since ancient times and is now rediscovered for its antibacterial and wound healing activity [23–26]. Honey nanofibers are gaining increasing interest due to the enhanced activity realized upon combining the advantages of the nanofibrous structure especially the increased surface to volume ratio with the advantageous properties of honey. However, due to its low viscosity honey was only electrospun in small concentrations [27,28]. Recently, we have managed to electrospun honey/polyvinyl alcohol/chitosan combinations (HPCS) into nanofibers with high concentrations of chitosan (up to 5.5% *w*/w) and honey (up to 40% w/) via nontoxic solvent (1% acetic acid) and the resulting HPCS nanofibers demonstrated an enhanced nontoxicity and biocompatibility [29].

High concentration HPCS nanofibers represent promising candidates for various biomedical applications due to their biodegradability, biocompatibility and antibacterial effects [30,31]. However, such high concentrations of honey included within the nanofibers is expected to affect the crystallinity, porosity, thermal properties, and degradation behavior of the nanofibers, thus the effect of changing the honey concentration on such properties will be evaluated in the present study along

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with the change in swelling and weight loss extents and antibacterial abilities of the developed HPCS nanofibers.

2. Materials and methods

2.1. Materials

Chitosan (Mw, 240 kDa and DDA of 84%, Chitoclear, cg110, TM 3728) was provided by Primex, Siglufjordur, Iceland. Polyvinyl alcohol of Mw, 85 kDa, and glutaraldehyde (25% in H_2O) were obtained from Sigma Aldrich (St. Louis, USA). Glacial acetic acid of 99–100% purity was purchased from Merck (Wadeville, South Africa). Nutrient broth and Nutrient agar were obtained from Becton Dickinson and Company (USA).

2.2. Electrospinning of honey/polyvinyl alcohol/chitosan (HPCS) mixtures

Various honey/polyvinyl alcohol/chitosan (HPCS) solutions with increasing honey concentrations were prepared using the following weight% ratios; 10:7:3.5, 20:7:3.5, and 30:7:3.5 of honey, polyvinyl alcohol, and chitosan, respectively dissolved in 1% acetic acid. Then, the as-prepared solutions were allowed to age at room temperature for a week. Afterwards, the conductivity of the as-prepared solutions were measured using a conductivity meter (Ysi 3200). Subsequently, the aged solutions were electrospun with the aid of an electrospinner (*E*-spin, NanoTech, Kalyan-pur, India). In brief, a 5 ml plastic syringe was loaded with the different solutions and was attached to the nozzle that has outside and inner diameters of 1.3 and 0.7 mm respectively. Then, different voltages (Gamma High Voltage Power Supply, USA) were applied to the electrospun solutions, and the voltage required for optimum collection of the nanofibers was selected. The distance between the collector and the tip of the nozzle was maintained at 15 cm and the flow rate was maintained at 10 μl/min. All the samples were collected on ground collector covered with an aluminum sheet. The humidity and temperature were maintained at 30–35% and 32 °C, respectively during electrospinning.

2.3. Characterization and measurements

The surface morphologies of the electrospun nanofibers were observed using scanning electron microscopy (FESEM, Leo Supra 55, Zeiss Inc., Oberkochen, Germany) and transmission electron microscopy (Jeol, Musashino, Akishima, Tokyo, Japan). Image-J software was used for measurement of the diameters of the collected nanofibers. From three different images 100 fibers were measured for each of the developed nanofibrous mats. Subsequently, the average diameter and diameter distribution were determined. The X-ray diffraction patterns of the HPCS nanofibers with increasing honey concentrations were obtained using an XRD diffractometer (Bruker 4040, Karlsruhe, Germany) with a wavelength, $\lambda = 0.154$ nm at 40 kV, 150 mA, and at a scan speed of 4° per minute in the 2θ range of 5°–80°. Porosity measurements and pore size distribution of each of the electrospun HPCS nanofibers were obtained using mercury porosimetry (PoreMaster® mercury intrusion porosimeter, Quantachrome, Florida, USA). Thermal behavior of the nanofibers was investigated using differential scanning calorimetry (DSC 4000, PerkinElmer, Inc., Massachusetts, USA). The nanofibrous mats were weighed and sealed in aluminum pans. Then, the temperature was elevated from room temperature to 200 °C followed by cooling to room temperature and then heating again to 200 °C with a heating rate of 10 °C/min. Moreover, thermogravimetric analysis (TGA) of the nanofibers was performed (TGA Q50, TA Instruments). Samples were heated in a platinum pan under nitrogen atmosphere (60 ml/min) up to 700 °C, at a heating rate of 10 °C/min.

2.4. Assessment of swelling of the cross-linked HPCS nanofibers

The developed HPCS nanofibrous mats with increasing honey concentrations; 10:7:3.5, 20:7:3.5, and 30:7:3.5 (w%) were cross-linked through exposure to glutaraldehyde (GA) vapours for 1 h and 3 h followed by heating at 40 °C to enhance the crosslinking and remove any unreacted GA. The cross-linked nanofibrous mats were then evaluated for their swelling ability. The mats were placed in phosphate buffered saline, PBS of a pH 7.4 at 37 °C, and their swelling ability was determined at 1, 4 and 24 h with the aid of the following relationship:

$$Swellingdegree\ (\%) = [M - M_i/M_i] \times 100$$

Where *Mi* is the initial dry weight of the nanofibrous mats, and *M* is the swollen weight of the nanofibrous mats after surface blotting with a filter paper.

2.5. Degradation rate of the cross-linked HPCS nanofibers

The degradation behavior of the developed HPCS nanofibers with increasing honey concentrations 10:7:3.5, 20:7:3.5, and 30:7:3.5 (w%) was determined in PBS (pH 7.4) at 37 °C and 100 rpm after 30 days. The degradation behavior of both the non-cross-linked (non CL) and cross-linked samples via exposure to glutaraldehyde (GA) vapours for 3 h (3 h CL) followed by heating at 40 °C was determined. The degradation index (D_i) was determined based on the mass loss according to the following equation:

$$D_i = (W_0 - W_t)/W_0 \times 100$$

where W_0 is the initial weight of the electrospun nanofibers, and W_t is the weight of the dried fibers after 30 days.

2.6. Antibacterial activity

The antibacterial activities for the obtained HPCS nanofibrous mats with increasing honey concentrations; 10:7:3.5, 20:7:3.5, and 30:7:3.5 (w%) of honey, polyvinyl alcohol, and chitosan, respectively were determined against *Staphylococcus aureus* and *Escherichia coli*. The nanofibrous mats (0.05 g) after UV sterilization for 20 min were placed in sterile vials containing 3 ml of Muller Hinton broth. A bacterial suspension of each of the bacterial strains was prepared from fresh colonies after overnight incubation at 37 °C and the turbidity was adjusted to 0.5 McFarland standard (1×10^8 CFU/ml). A 10 μl of that suspension was diluted in 9 ml Muller Hinton broth to prepare (1×10^7 CFU/ml) bacterial suspension. A 30 μl aliquot of each organism and from each bacterial dilution was added to each vial containing the nanofibrous mats. Then, the tubes containing the bacterial strains and the nanofibrous mats as well as the controls were incubated at 37 °C with shaking at 100 rpm. After 24 h, samples of 10 μl were taken from the treated bacterial broth and the controls. Serial dilution in nutrient broth was performed for each sample from which 50 μl were spread on nutrient agar plates that were subsequently incubated for 24 h at 37 °C. Following the 24 h incubation, surviving colonies were counted. The dilution that allowed counting 10 to 150 CFU were counted. The experiment was repeated three times and the mean value of CFU was recorded.

3. Results and discussion

3.1. Electrospinning of increasing concentrations of honey within the HPCS nanofibers

Prior to electrospinning, the conductivity of the aged polyvinyl alcohol/chitosan solutions (7:3.5 w%) with increasing honey concentrations (10, 20, and 30 w%) was determined. It was observed that the change in the honey concentration within the polyvinyl alcohol/