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

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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 polyethylene 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/v) 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 with 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.

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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 H2O) 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 was measured using a conductivity meter (Ysi 3200). Subsequently, the aged solutions were electrospun with the aid of an electrospriner (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) that has outside and inner diameters of 1.3 and 0.7 mm respectively was loaded with the different solutions and was attached to the nozzle (60 ml/min) up to 700 °C, at a heating rate of 10 °C/min.

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, \( \lambda = 0.154 \text{ nm} \) 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 electrosprun 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 (90 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:

\[
\text{Swelling degree} \% = \left( \frac{M - M_i}{M_i} \right) \times 100
\]

Where \( M_i \) is the initial dry weight of the nanofibrous mats, and \( M \) is the swollen weight of the nanofibrous mats after surface bloting 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 for 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 (Di) was determined based on the mass loss according to the following equation:

\[
D_i = \left( \frac{W_0 - W_i}{W_0} \right) \times 100
\]

where \( W_0 \) is the initial weight of the electrosprun nanofibers, and \( W_i \) 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 × 108 CFU/ml). A 10 μl of that suspension was diluted in 9 ml Muller Hinton broth to prepare (1 × 107 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/
Chitosan solutions resulted in a small variation in the conductivity of the solutions from 1510 uS with the PCS solutions containing 30% H to 1480 and 1400 uS with the 20%H and 10% H, respectively. The conductivity of a solution is related to its amount of ions. Thus, the decrease in the honey concentration results in a decrease in the amount of ions present in the solution and consequently led to a reduction in its conductivity. Subsequently, the aged HPCS solutions with increasing honey concentrations were electrospun into nanofibers at a feed rate of 10 μl/min and distance of 15 cm between the nozzle and the collector. Different voltages ranging from 10 kV to 29 kV were applied to obtain the optimum value which corresponds the lowest voltage that allowed collection of uniform nanofibers that were free of any beads, sticking or clusters. For each applied voltage, a sample of nanofibers was collected after five minutes of electrospinning followed by examining its morphology under the SEM (data not shown). It was observed that 24 kV is the minimum voltage that allowed for a uniform nanofiber deposition. As apparent from Fig. 1, it was noted that increasing the honey concentration led to increasing the diameter of the nanofibers. For instance, the HPCS nanofibers with 10% honey exhibited a mean fiber diameter of 284 ± 97 nm (Fig. 1a & b) which increased to 371 ± 110 nm, and 464 ± 185 nm upon increasing the honey concentration to 20% (Fig. 1c & d), and 30% (Fig. 1e & f), respectively. Although the increase in conductivity of a solution results in a reduction in the diameter size of electrospun nanofibers, the main parameter affecting the diameter of the nanofibers is the solution concentration [32]. And because of the high concentrations of the honey loaded within the developed HPCS nanofibers, the increase in the fiber diameter is a direct consequence for increasing the amount of honey loaded within the nanofibers as can be observed from Fig. 2a & b, where it is apparent that honey is embedded within the chitosan/polyvinyl alcohol nanofibers. It was also observed that the amount of honey loaded within the nanofibers influences the fiber diameter distribution. As seen in Fig. 1d, addition of 20% honey to the chitosan/polyvinyl alcohol nanofibers allowed for the most focused fiber diameter distribution, as most of the nanofibers exhibited diameters between 300 nm and 450 nm. Whereas, the addition of 10% and 30% honey to the chitosan/polyvinyl alcohol nanofibers resulted in broad distribution of the diameters of the fibers (Fig. 1b & f).

Fig. 1. SEM images of the electrospun honey/polyvinyl alcohol/chitosan (HPCS) nanofibrous mats with increasing concentrations of honey (a, c, e) and their diameter distribution (b, d, f); (a, b) HPCS (10%:7%:3.5%), (c, d) HPCS (20%:7%:3.5%), and (e, f) HPCS (30%:7%:3.5%).
Fig. 2. TEM (a) & SEM (b) images of honey/polyvinyl alcohol/chitosan (HPCS) nanofibers (30:7:3.5 w/w) illustrating the inclusion of honey within the nanofibers.

Fig. 3. Mercury porosimetry results of pore diameter versus delta volume of intruded mercury (a1, c, e), and pore diameter versus delta surface area (b, d, f) of the honey/polyvinyl alcohol/chitosan (HPCS) nanofibrous mats with increasing honey concentrations; (a, b) HPCS (10%:7%:3.5%), (c, d) HPCS (20%:7%:3.5%), (e, f) and HPCS (30%:7%:3.5%).
3.2. Effect of increasing the honey concentration on the porosity of the HPCS nanofibers

In both tissue engineering and wound healing applications, the porosity of the nanofibrous scaffolds will affect the migration and proliferation of cells, growth of the blood vessels and the exchange of the waste products and nutrients between the cells and their surrounding micro-environment [33]. Mercury porosimetry is a well-known method for measuring porosity of materials, and it is based on the fact that mercury does not wet solid surfaces. The sample is completely surrounded with mercury that does not intrude through the pores except after applying pressure. As the pressure increases, mercury intrudes through the large pores first, and by further increase in pressure, the mercury intrudes into the fine pores. After the pressure reaches to the maximum the porosity and pore volume are calculated. It is of note that, mercury porosimetry measures pore sizes between 0.0018 and 400 μm, however pore sizes smaller than 0.0018 μm cannot be measured via mercury porosimetry thus presenting a source of error in the mercury porosimetry results [34,35]. As shown in Fig. 3, mercury porosimetry was used for quantitative assessment of the pore size distribution and porosity of the HPCS nanofibers with increasing honey concentrations (10%, 20% and 30%). Increasing the honey concentration resulted in a slight decrease in the overall porosity. In addition, it was noted that the porosity of all the HPCS nanofibers developed in this study, irrespective the honey concentrations, have demonstrated enhanced porosity compared to previously spun chitosan and chitosan/polyvinyl alcohol nanofibers [36,37]. The developed HPCS nanofibers with 10% and 20%, 30% honey have demonstrated porosity of 97.8% and 95.1% respectively, compared to 79.9% in previously spun non-sonicated chitosan nanofibers [36].

Recently, it was reported that the increase in the overall porosity due to increase in the pore size and fiber diameters enhances the viability of cells. Whereas, increasing the overall porosity due to the simultaneous reduction in fiber diameter and pore diameter could reduce the cell viability [38]. In our study, it was observed that the pore diameter was greatly influenced by the change in the honey concentration within the HPCS nanofibers. The HPCS nanofibers with 10% and 30% honey exhibited wider distribution of pore diameter and most importantly exhibited increased number of pores having large pore diameter reaching to 140 μm (Fig. 3b & f). This was also observed in the volume of mercury intruded through the fibers where most of the volume intruded was through pores having pore diameters between 35 μm and 138 μm (Fig. 3a & b). These results could be attributed to the relatively large fiber diameter as well as the wide distribution of the diameters of the nanofibers of the 10% and 30% honey HPCS nanofibers, respectively. Similar results were observed by Ryu et al., who observed that by increasing the polymer concentration from 15 to 30%, the fiber diameter increased from 90 to 480 nm and subsequently the porosity as determined by mercury porosimetry increased from 25% to 80% [39]. Moreover, Ko et al., have demonstrated that the porosity of SiO2-ZrO2 composite nanofibers ranged from 81.3% to 91.7% by increasing the amount of ZrO2 from 10 to 20% due to an increase in the fiber diameter with an increase in the ZrO2 content. Thus, a direct relation between the increase in the fiber diameter and pore diameter and porosity was verified through different previous studies [40]. Such increased pore diameter achieved in the 10% and 30% honey nanofibers is favorable in cell related applications such as tissue engineering and wound healing [37]. Although the 20% honey HPCS nanofibers showed large fiber diameter of 371 ± 110 nm but they also exhibited a focused diameter distribution (Fig. 1d), this in turn resulted in a more focused pore diameter distribution at less than 20 μm (Fig. 3d). However, a large volume of mercury can still be seen intruded from the pores with pore diameters between 40 μm and 160 μm (Fig. 3c). This could be attributed to the fact that they can allow much greater volume to be intruded through them due to their larger surface area compared to the pores with small diameters as less than 20 μm.

3.3. Effect of increasing the honey concentration on crystallization and thermal properties of the HPCS nanofibers

The XRD diffraction patterns of pure polyvinyl alcohol and chitosan have been previously reported [41,42]. Moreover, the XRD patterns of polyvinyl alcohol/chitosan (PCS) nanofibers and films were reported by Jia et al., who observed that nanofibers of the PCS exhibited deteriorated crystalline structure compared to the films [43]. Fig. 4, illustrates the XRD diffraction patterns of the prepared HPCS nanofibers with increasing honey concentrations. The HPCS nanofibers exhibited an amorphous microstructure with a single broad peak around 2θ = 20°. Such XRD patterns are in coherence with those observed for the previously prepared polyvinyl alcohol/chitosan nanofibers [43]. Thus the addition of honey did not affect the diffraction model of the polyvinyl alcohol/chitosan nanofibers and consequently the increase in the honey concentration within the HPCS nanofibers had no effect on their diffraction pattern.

Fig. 4. XRD diffraction patterns of the honey/polyvinyl alcohol/chitosan (HPCS) nanofibers with increasing honey concentrations. The weight blending ratios of the electrospun mats were 7% polyvinyl alcohol (P), 3.5% chitosan (CS), and increasing concentrations of honey (H): 10%, 20%, and 30%.

Fig. 5. TGA (a) DSC (b) thermograms of the honey/polyvinyl alcohol/chitosan (HPCS) nanofibers with increasing honey concentrations. The weight blending ratios of the electrospun mats were 7% polyvinyl alcohol (P), 3.5% chitosan (CS), and increasing concentrations of honey (H): 10%, 20%, and 30%.
The deterioration of the crystalline structure of the electrospun nanofibers was previously reported [44,45]. Such deterioration could be attributed to the fast deposition and drying of the elongated electrospun nanofibers thus hindering the crystallization [43].

TGA of the HPCS nanofibers with increasing honey concentrations (10%, 20% and 30%) was performed. As observed in Fig. 5a, the examined samples demonstrated similar thermal degradation process that takes place in several steps. The first step of weight loss is attributed to moisture elimination which resulted in loss of less than 10% of the weight of the examined nanofibers below 120 °C.

It is of note that at 120 °C the HPCS nanofibers having 10% honey exhibited the highest weight loss of ~8% whereas the weight loss decreased by increasing the amount of honey within the HPCS nanofibers to ~6% and ~3% with the 20% and 30% honey, respectively. This indicates that the HPCS nanofibers with higher honey concentrations exhibited higher initial moisture content, which is a result of the hygroscopic nature of honey. The second and major weight loss of approximately 50% of the weight occurred after 120 °C till 400 °C and is mainly attributed to the thermal decomposition of the polymer structure as well as the degradation of the honey components followed by carbonization of the honey contents. In the final step of the thermal decomposition at temperatures above 500 °C, the polymer backbone has been ruptured in addition to the oxidation of the organic matter found in honey. These observations are in agreement with what has been previously reported [46,47]. TGA clearly demonstrates that the fabricated HPCS nanofibers with different honey concentrations exhibit good thermal stability below 120 °C.

The DSC thermograms of the HPCS nanofibers with increasing concentrations of honey (10, 20, and 30 w%) are illustrated in Fig. 5b. The three DSC thermograms showed no peaks. This further proves the deteriorated crystalline structure of the developed HPCS nanofibers. Moreover, it was previously proven that increasing the chitosan content within the PCS nanofibers to ~1% resulted in further deterioration of the crystallinity of the fibers [43,48]. Thus, in the present work the increase in the chitosan concentrations to 3.5% within the developed HPCS nanofibers combined with the nanofibrous structure and the honey loaded within the nanofibers resulted in a significant deterioration in the crystalline structure and development of the nanofibers in amorphous form resulting in absence of any peaks in the DSC thermogram. Obviously, increasing the honey concentration loaded within the nanofibers did not affect the DSC thermograms of the obtained HPCS nanofibers.

3.4. Effect of increasing the honey concentration on the swelling of the HPCS nanofibers

Honey and chitosan nanofibrous mats represent top candidates for wound dressing applications and determining their swelling capabilities would allow prediction of their exudate management ability [49].

Recently we have illustrated that the swelling capabilities of the noncrosslinked HPCS nanofibrous mats with varying degrees of chitosan and honey ranged from 46% to 197% [29]. Such values illustrate the low swelling capability of the noncrosslinked HPCS nanofibrous mats. In the present study the swelling capability of the crosslinked fibers with increasing honey concentrations was investigated.

Crosslinking allows maintaining the nanofibrous structure of the developed HPCS nanofibrous mats in aqueous media, thus, allowing for improved porosity. However, increasing the crosslinking degree will decrease the swelling ability of the nanofibers due to the increased rigidity of the network as a result of increasing both the inter- and intra-molecular interactions [50]. Consequently, the effect of changing the honey concentration was studied at two mild crosslinking degrees. These include exposing the nanofibers to GA vapours for 1 h and 3 h with subsequent heating at 40 °C to enhance crosslinking and remove any residual GA. Such crosslinking treatments were selected as they represent the shortest possible exposure to GA vapours that allowed crosslinking, thus avoiding deteriorating the nontoxicity and biocompatibility of the developed nanofibers upon excessive exposure to the GA vapours. Moreover, the HPCS nanofibers that were crosslinked via exposure to GA vapours for 3 h with subsequent heating were proved nontoxic and biocompatible via MTT cytotoxicity evaluation [29].

Increasing the honey concentration within the nanofibers decreased its swelling ability at both the tested crosslinking degrees (Fig. 6a & b). It could be observed from the figures that the HPCS nanofibers with 10% honey and 1 h of crosslinking with the GA vapours exhibited superior swelling capabilities reaching to 520% at 1 h and 300% after 24 h (Fig. 6a). On the other hand, the effect of the crosslinking time on the swelling capabilities of the HPCS nanofibers varied according to their incorporated honey concentration. For the HPCS nanofibers with 10% honey, increasing the crosslinking time from 1 h to 3 h decreased their swelling capabilities noticeably from 520% to 273%. Whereas, HPCS nanofibers with 20% honey exhibited increased swelling ability with the increase in the crosslinking time from 1 h to 3 h. Noticeably, the HPCS nanofibers with 30% honey demonstrated the lowest swelling ability at both crosslinking times.

Although honey is known for its high water uptake ability [51], it also has high water solubility. Such high water solubility results in increasing the degradation rates of the nanofibers and consequently losing their compact porous structure that can hold in water [52]. Thus, this eventually results in massive decrease in swelling ability. This was observed by the very low swelling abilities of the HPCS nanofibers with 30% honey.
Interestingly, the increase in the crosslinking efficiency by increasing the exposure time to the GA vapours allows the nanofibers to maintain a more compact nanofibrous structure [53, 54] thus, the percent of released and solubilized honey decreases. This allows the honey to be maintained within the nanofibers for longer periods of time, and thus its water uptake capabilities could be realized. On the other hand, the increase in the crosslinking degree decreases the swelling ability as it hinders the intermolecular motion and chain disentanglements within the nanofibrous scaffold. These two opposite effects on the swelling abilities of the nanofibrous scaffolds could be observed in the results presented in Fig. 6a & b. In the HPCS nanofibers with 10% honey, the amount of honey within the nanofibers is small thus the effect of the swelling hindering due to crosslinking was more pronounced than the water uptake ability of the maintained honey. Whereas, when the concentration of honey increased to 20%, the water uptake ability of the maintained honey in this case exceeded the hindering effect of crosslinking on swelling which allowed the HPCS nanofibers with 20% honey to exhibit a noticeable increase in swelling ability even at 24 h by increasing the crosslinking time to 3 h. The HPCS nanofibers with 30% honey however showed slight decrease in the swelling ability at 24 h with increasing the crosslinking time. This is because at such high concentration of honey such crosslinking treatments could not overcome the increased solubility of the HPCS nanofibers with 30% honey which affects the compact structure of the nanofibrous scaffold. These results reveal the importance of optimization of the crosslinking degree as well as the honey concentrations within the developed HPCS nanofibers to adjust the water uptake ability according to the desired application.

3.5. Effect of increasing the honey concentration on the degradation rates of the HPCS nanofibers

The effect of increasing the honey concentration within the HPCS nanofibers on the degradation behavior of both the noncrosslinked and crosslinked nanofibers via exposure to GA vapours for 3 h followed by heating at 40 °C for 24 h was studied.

As illustrated in Fig. 7, increasing the honey concentration within the HPCS nanofibers increased the degradation ability of the nanofibers after 30 days of incubation in PBS at 37 °C and 100 rpm. This could be attributed to the high water solubility of honey. Thus, in the HPCS nanofibers with higher honey concentrations such effect could be realized with an increase in the degradation behavior of the nanofibers.

Meanwhile, the crosslinked HPCS nanofibers at all honey concentrations showed decreased degradation ability compared to the noncrosslinked fibers. This is mainly because crosslinking the HPCS nanofibers via exposure to GA for 3 h allows maintaining the nanofibrous structure as was previously proven [29]. Exposure of the HPCS nanofibers to GA allows chemical crosslinking of chitosan either through formation of Michael-type adducts or Schiff’s base structures [55] and thus decreases their degradation rate.

3.6. Effect of increasing the honey concentration on the antibacterial activity of the developed HPCS nanofibers

Both honey and chitosan exhibit antibacterial activity. Honey exerts its antibacterial activity via its acidity, high sugar content as well as its ability for hydrogen peroxide production [56]. Whereas, the polycationic nature of chitosan allows it to interact with the negatively charged membranes of bacteria leading to loss in the permeability of the membrane with subsequent cell leakage and death [57].

Electrospun nanofibers allow enhancement of the antibacterial activity of both chitosan and honey within the developed HPCS nanofibers, mainly due to the increase in the surface to volume ratio [29].

It was previously shown that the antibacterial effect of HPCS nanofibers with 30% honey are significantly affected by the change in the chitosan concentrations within the nanofibers [29]. In the present work the effect of changing the honey concentration within the HPCS nanofibers was investigated as shown in Fig. 8. The increase in the honey concentration within the HPCS nanofibers enhanced their antibacterial activities against both S. aureus and E. coli at 1 × 10^7 CFU/ml (Fig. 8a & b). However, upon increasing the bacterial count to 1 × 10^8 CFU/ml the increase in the honey concentration resulted in an increase in the antibacterial activity against S. aureus, whereas nearly no antibacterial effect was realized against E. coli. These results agree with the previously reported results of the HPCS nanofibers that demonstrated enhanced antibacterial activity against gram positive S. aureus over the gram negative E. coli [29].

4. Conclusions

Different honey concentrations (10%, 20% and 30%) have been electrospun within chitosan (3.5%)/polyvinyl alcohol (7%) nanofibers.
The effect of increasing the honey concentration on the different properties of the electrospun nanofibers has been investigated. Increasing the honey concentration resulted in an increase in the fiber diameter from 284 ± 97 nm with 10% honey to 464 ± 185 nm with 30% honey, whereas the porosity was slightly decreased from 97.8% with 10% honey to 95.1% in the case of the nanofibers incorporating 20% and 30% honey. Interestingly, it was observed that both the HPCS combinations with 10% honey and 30% honey exhibited an increased number of pores with large pore diameter reaching to 140 μm which is advantageous for tissue engineering and wound healing applications. The swelling of the nanofibers was greatly influenced by the concentration of incorporated honey and the degree of crosslinking. Highest swelling extent was observed with HPCS nanofibers having 10% honey, and the least swelling was noted in the HPCS nanofibers having 30% honey. However, the swelling ability of the nanofibers containing 20% honey was noticeably enhanced with increasing the crosslinking degree. The degradation ability of the nanofibers increased with increasing the honey concentration within the HPCS nanofibers and decreased with crosslinking of the fiber mats. The crystallization and thermal behavior of the nanofibers on the other hand were not affected by changing the honey concentration within the developed HPCS nanofibers, increasing the honey concentration within the HPCS nanofibers enhanced their antibacterial activity against both S. aureus and E. coli at 7 × 10^8 CFU/ml. Whereas, at 7 × 10^8 CFU/ml nearly no antibacterial effect was realized against E. coli at all honey concentrations included within the HPCS nanofibers.

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