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Electrospinning chitosan/poly(ethylene oxide) solutions with essential oils: Correlating solution rheology to nanofiber formation

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ABSTRACT

Electrospinning hydrophilic nanofiber mats that deliver hydrophobic agents would enable the development of new therapeutic wound dressings. However, the correlation between precursor solution properties and nanofiber morphology for polymer solutions electrospun with or without hydrophobic oils has not yet been demonstrated. Here, cinnamaldehyde (CIN) and hydrocinnamic alcohol (H-CIN) were electrospun in chitosan (CS)/poly(ethylene oxide) (PEO) nanofiber mats as a function of CS molecular weight and degree of acetylation (DA). Viscosity stress sweeps determined how the oils affected solution viscosity and chain entanglement (C_e) concentration. Experimentally, the maximum polymer:oil mass ratio electrospun was 1:3 and 1:6 for CS/PEO:CIN and:H-CIN, respectively; a higher chitosan DA increased the incorporation of H-CIN only. The correlations determined for electrospinning plant-derived oils could potentially be applied to other hydrophobic molecules, thus broadening the delivery of therapeutics from electrospun nanofiber mats.

1. Introduction

The electrospinning process fabricates non-woven mats composed of continuous nano- to micro- meter scale diameter fibers (Engel, Schiffman, Goddard, & Rotello, 2012; Reneker, Yarin, Fong, & Koombhongse, 2000). This well-established, scalable (Persano, Camposeo, Tekmen, & Pisignano, 2013) technique has been utilized to form fibers from over 100 different polymers including, polyelectrolytes (Ohkawa, Cha, Kim, Nishida, & Yamamoto, 2004; Pakravan, Heuzey, & Ajji, 2011), biopolymers (Chang, Lee, Wu, Yang, & Chien, 2012; Kong & Ziegler, 2012; Saquing et al., 2013), and synthetic polymers (Kai, Jin, Prabhakaran, & Ramakrishna, 2013; Luo et al., 2015). Additionally, researchers have further tailored the functionality of fibers by loading solid agents into the polymer precursor solutions, including TiO_2/graphene for increased electrical performance (Zhang et al., 2012), quantum dots for fluorescent detection (He et al., 2012), and single-walled carbon nanotubes for antibacterial activity (Schiffman, Wang, Giannelis, & Elimelech, 2011). In these cases, the solid agent was suspended in a concentrated polymer solution, which provided the chain entanglement necessary to "carry" the solid agent along the electrospinning process (Saquing et al., 2013). Alternatively, researchers have synthesized nanoparticles within a fiber mat post-electrospinning, thus avoiding the need to optimize precursor rheology (Persano et al., 2012). However, much less research has been conducted on electrospinning nanofibers from polymer solutions that contain immiscible phase liquids (Angeles, Cheng, & Velankar, 2008; Briggs & Arinzeh, 2014; Díaz, Barrero, Márquez, & Loscertales, 2006; Li, Ko, & Hamad, 2013; Qi, Hu, Xu, Wang, 2006; Rieger & Schiffman, 2014; Sanders, Kloefkorn, Bowlin, Simpson, & Wnek, 2003; Xu et al., 2005, 2006). A handful of reports used harsh organic solvents to emulsion electrospin specific polymers, by relying on a surfactant to carry the immiscible phase biological cargo — proteins (Briggs & Arinzeh, 2014; Yang et al., 2008), DNA (Yang et al., 2011), and water-soluble drugs (Xu et al., 2005; Xu, Chen, Ma, Wang, & Jing, 2008) — and protect them against coalescence (Angeles et al., 2008; Briggs & Arinzeh, 2014; Li et al., 2013; Qi et al., 2006; Sanders et al., 2003; Xu et al., 2005, 2006).

Another class of hydrophobic bioactive agents are essential oils. Due to the rise of antibiotic resistance, research into plant-derived agents has surged because they can inactivate microbes non-specifically (Bakkali, Averbeck, Averbeck, & Idaomar, 2008; Kavanaugh & Ribbeck, 2012). Within the past two years, a number of essential oils including *Cinnamomum* (Rieger & Schiffman, 2014), *Thymus vulgaris* (Karami, Rezaeian, Zahedi, & Abdollahi, 2013), *Chamomilla recutita* (Motealleh et al., 2014), *Cymbopogon* (Liakos et al., 2015), *Mentha piperita* (Liakos et al., 2015), *Acidum tannicum* (Xu, Weng, Gilkerson, Materon, & Lozano, 2015), *Eremanthus erythropappus* (de Oliveira Mori et al., 2015), and *Centella asiatica* (Yao, Yeh, Chen, Li, & Huang, 2015) have been electrospun

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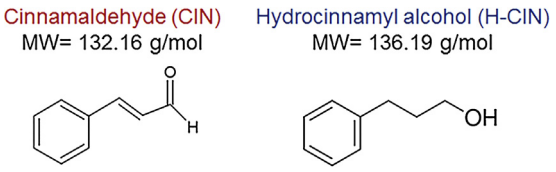

Fig. 1. Cinnamaldehyde (CIN) or hydrocinnamyl alcohol (H-CIN) was incorporated into chitosan (CS)/poly(ethylene oxide) (PEO) solutions at a variety of different total polymer:oil (p:o) mass ratios.

within nanofiber mats as promising drug delivery vehicles. In general, these papers focused on how the essential oil containing nanofiber mats influenced antibacterial activity and/or mammalian cell proliferation. We previously electrospun cinnamaldehyde (CIN), a hydrophobic essential oil, using a chitosan (CS)/poly(ethylene oxide) (PEO) solution and quantified the incorporation, release, and antibacterial activity of CIN (Rieger & Schiffman, 2014). CS, a polycationic derivative of chitin, was chosen because it is a non-toxic, antibacterial, biodegradable, and biocompatible biopolymer (Bhattarai, Edmondson, Veiseh, Matsen, & Zhang, 2005; Vulcani et al., 2015) used for biomedical applications, including, drug delivery, wound dressings, and tissue engineering scaffolds (Croisier & Jérôme, 2013; Jayakumar, Prabaharan, Nair, & Tamura, 2010; Subramanian, Vu, Larsen, & Lin, 2005). Due to the intrinsic antibacterial activity of CS, our CS/PEO nanofiber mats achieved a full inactivation of *Escherichia coli* S20918 and a ~50% inactivation of *Pseudomonas aeruginosa* S20930. The release of CIN from CS/CIN/PEO nanofiber mats increased their cytotoxicity against *P. aeruginosa*. After 180 min, 81 ± 4% of the *P. aeruginosa* was inactivated by the CS/CIN/PEO nanofiber mats (Rieger & Schiffman, 2014). Despite the advancement of demonstrating the ability to deliver hydrophobic agents, the electrospinning parameters that control the quantity of the immiscible phase that can be incorporated into the nanofiber mats has not yet been investigated.

The solution properties needed to electrospin CS/PEO solutions into nanofiber mats, including, total polymer concentration, CS molecular weight, and acid concentration have been experimentally determined (Klossner, Queen, Coughlin, & Krause, 2008; Pakravan et al., 2011). However, the previous work was limited in scope. Here, we determine for the first time the chain entanglement concentration (C_e) of CS/PEO solutions as a function of CS molecular weight and degree of acetylation (DA). Additionally, we examine the C_e of CS/PEO in the presence of immiscible liquid phase agents. There are over 300 essential oils, and of those, nearly all of them rely on one of three chemical structures – phenols, aldehydes and alcohols – to exhibit bioactivity (Bakkali et al., 2008). Thus, further investigation into the electrospinning parameters for adding oils to CS/PEO is needed. Here, two structurally different essential oils, CIN and hydrocinnamic alcohol (H-CIN) (Fig. 1) were investigated. CIN is an aldehyde that can react with CS to form Schiff bases (Cordes & Jencks, 1962; Guinesi, 2006; Marin et al., 2014; Marin, Simionescu, & Barboiu, 2012). H-CIN was chosen as a model alcohol that does not form Schiff base with CS. Understanding the parameters that enable the electrospinning of hydrophobic molecules stabilized by CS-containing solutions provides a platform to broaden the potential biomedical applications of electrospun nanofiber mats.

2. Experimental

2.1. Materials and chemicals

Low molecular weight chitosan (LMW CS, poly(D-glucosamine), M_w = 460,000 Da), medium molecular weight chitosan (MMW CS, poly(D-glucosamine), M_w = 1,000,000 Da), poly(ethylene oxide) (PEO, M_w = 6,00,000 Da), ReagentPlus® grade acetic acid (AA, ≥99.0%), cinnamaldehyde (CIN, ≥93%, FG, M_w = 132.16 g/mol), hydrocinnamic alcohol (H-CIN, ≥98%, FCC, M_w = 136.19 g/mol), deuterium oxide, and acetic acid-d4 (AA-d4) were obtained from Sigma-Aldrich (St. Louis, MO). Sodium hydroxide (NaOH) was obtained from Fisher Scientific (Fair Lawn, NJ). Deionized (DI) water was obtained from a Barnstead Nanopure Infinity water purification system (Thermo Fisher Scientific, Waltham, MA).

2.2. Modification and characterization of CS

A modified medium molecular weight chitosan (MOD-MMW CS, M_w = 1000,000 Da) was synthesized to provide a direct comparison of molecular weight and DA. MOD-MMW CS was produced through the deacetylation of the MMW CS by suspending 5.0 g of MMW CS in 100 mL of 45 w/w% NaOH. The solution was heated at 70 °C for 45 min. The MMW CS was then filtered and washed with DI water until a neutral pH was achieved (Yuan, Chesnutt, Haggard, & Bumgardner, 2011). The resultant powder was then dried for 12 h in a vacuum oven at 25 °C.

Proton nuclear magnetic resonance (^{1}H NMR, Bruker Avance 400) along with SpinWorks3, an NMR analysis software, were employed to quantitatively determine the DA of the LMW, MMW, and MOD-MMW CS. Solutions for ^{1}H NMR containing 1.0 w/v% LMW, MMW, or MOD-MMW CS were dissolved in 0.5 M AA-d4 (500 μL). To analyze the interactions between CS and CIN or H-CIN, new ^{1}H NMR solutions at the previously mentioned solution parameters and polymer:oil (p:o) mass ratios of 1:0.2 and 1:0.4 were prepared.

2.3. Preparation of CS/PEO and oil loaded CS/PEO solutions

A 1:1 weight ratio of LMW, MMW, or MOD-MMW CS/PEO (0.5 g/0.5 g) in 0.5 M AA (20 mL) corresponding to total polymer concentrations ranging from 0.25 to 5.0 w/v% solutions were mixed for 24 h at 20 rpm using an Arma-Rotator A-1 (Bethesda, MA). CIN or H-CIN (Fig. 1), was added to a LMW, MMW, or MOD-MMW CS/PEO solution to form an oil loaded solution ranging from 1:0.2 to 1:12 p:o mass ratio. These solutions were mixed for an additional 24 h, at which point, the solution changed from transparent to opaque. Throughout the mixing process, the solution had a pH value of 4. Within this manuscript, all solutions were prepared in a similar manner using a 1/1 weight ratio of CS/PEO.

2.4. Characterization of CS/PEO and CS/PEO(CIN or H-CIN) solutions

Oil-loaded solutions with and without PEO were imaged using a Zeiss Optical Microscope (Axio Imager A2) to qualitatively examine (i) the polydisperisty of the oil droplets and (ii) the effect of PEO addition on oil droplet size for 1:0.5, 1:1, 1:2, and 1:5 p:o mass ratios. The contact angle of the CS/PEO and oil-loaded CS/PEO solutions were determined using a home-built digital Olympus camera imaging setup to capture solution droplets. Solutions for contact angle analysis had a total polymer concentration of 2.5 w/v% for MMW and MOD-MMW CS/PEO, and a total polymer concentration of 5.0 w/v% for LMW CS/PEO. All oil loaded solutions were mixed with a 1:1 p:o mass ratio. *Image J* 1.45 software (National Institutes of Health, Bethesda, MD) was used to measure the contact angle. The average contact angle along with the standard deviation for each solution was obtained by measuring three droplets.

LMW, MMW, and MOD-MMW CS/PEO solutions with total polymer concentrations ranging from 0.25 to 5.0 w/v% were used for