Effect of Glycerol on Solution Properties Governing Morphology, Glass Transition Temperature, and Tensile Properties of Electrospun Zein Film

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ABSTRACT: An attempt was made to improve the tensile strength and elongation of electrospun zein film by addition of a plasticizer, namely, glycerol to the film forming solution. A surfactant viz. Tween®40 and NaCl were also added into the film solution to facilitate the electrospinning process. The effect of glycerol content on selected zein solution properties, i.e., surface tension, electrical conductivity, and viscosity, which in turn affect the mechanical property, morphology, and glass transition temperature (T_g) of the film were investigated. Morphology of the electrospun film was examined via scanning electron microscopy (SEM), whereas the film component was characterized by Fourier transform infrared (FTIR) spectroscopy. It was observed that as the glycerol content increased, the diameter of the electrospun fiber increased as a result of decreasing electrical conductivity with increasing surface tension of the solution. The flow-behavior index of the solution indicated that glycerol had a significant impact on the solution flow property, hence improved fiber diameter uniformity. Besides, the tensile strength and elongation of the film were improved as glycerol content increased, but the reverse was noted at 10 wt %. T_g of the zein film decreased as the glycerol content increased. © 2010 Wiley Periodicals, Inc. J Appl Polym Sci 118: 910–919, 2010

Key words: electrospinning; glycerol; mechanical property; nanofibers; zein

INTRODUCTION

Tissue engineering offers a promising new approach to repair tissue defects and regenerate new tissue. Engineered tissue is required to serve as an adhesive substrate for implanted cells and as a physical support to guide the formation of new organs.[1] In specific cases, the tissue also provides skin therapeutic function as a topical/transdermal drug carrier.[2] Types of natural tissues can be mainly categorized into hard and soft tissues.[3] The examples of the former are bone and teeth, whereas those of the latter may include ligament, skin, and arterial tissues. The elastic modulus of the hard tissues is generally in gigaorder (10^9) down to sub-gigaorder, whereas the soft tissues are much softer with their modulus varying from 400 (e.g., tendon) down to 0.1 (e.g., skin) MPa.[3] For uses in different parts of human body, engineered tissues are fabricated to have a wide range of tensile modulus.[4]

Many synthetic and natural polymeric biomaterials have been utilized in tissue engineering applications. The group of natural biomaterials may include chitosan, collagen, cellulose acetate, etc., whereas their synthetic counterparts can be derived from polylactic acids (PLA) and poly(lactic-*co*-glycolic acid) or PLGA.[5–8] Between the two types, natural biopolymers are in general more biocompatible. Furthermore, some of the synthetic ones are not biocompatible leading to some physiological side effects. For example, PLGA increases the tissue-inflammable response. Owing to their biocompatibility, biodegradability, and biological stability, the natural biomaterials are promising for regeneration of damaged tissue and control release of drug.[9–12]

Zein is a major storage protein of corn. It is a natural biopolymer, usually available in powder form. Zein can be separated from corn by solvent extraction, where ethanol is typically recognized as a good solvent. The major amino acids contained in zein protein are Leu, Glu, Ala, and Pro.[13,14] As the

protein is insoluble in water, it dissolves well in ethanol because of the high degree of nonpolar amino acid. It is nonallergenic and defined as generally recognized as safe (GRAS) by the US-FDA. In addition, zein films are moisture, oxygen, and lipid barriers, as well as have anti-microbial property.[14] Several research groups have successfully produced zein films for tissue engineering.[1,6,15] These films are nontoxic[15] (or biocompatible) to bioenvironment, which cells can adhere and proliferate.[6] However, single component zein films produced are generally very brittle and difficult to handle.[16–19] As tensile strength and elongation at break are strongly relevant to such applications, attempts have been made to improve zein film properties by adding plasticizers. Plasticizers are small molecules that can interfere with the molecular interactions and dynamics (or hydrogen and other bonding) among polymer chains, defining a large part of their physical characteristics. The plasticizers reduce the intermolecular forces within the film matrix, hence mobility of the biopolymeric chains increases.[20,21] Their inclusion gives rise to a reduction in glass transition temperature and an increase in film flexibility.[22–26] For thermally prepared-zein films, glycerol was proved to be an excellent plasticizer as it significantly increased the tensile strength and elongation at break.[21]

Electrospinning is a novel approach to produce nanofibrous films resembling the extracellular matrix, thus suitable for biomimetics applications. By applying a high voltage to a polymer solution, the electrical force will work together with other solution factors such as surface tension, viscosity, results in a formation of nanojet of the well-defined continuous nanofiber.[27–30] The diameter of the electrospun fibers is varied from sub-micrometers down to a few nanometers depending on the solution properties and processing conditions.[29,30] High surface to mass ratio of the films produced together with its simplicity and cost effectiveness makes electrospinning technique to be one of the most interesting and practical methods used for nanofiber fabrication. In this study, the mechanical properties of zein films were expected to be improved by moderating film electrospun solution via adding glycerol as a plasticizer.

MATERIALS AND METHODS

Materials

Zein protein was purchased from Sigma-Aldrich Chemical Company, St. Louis, MO. From the molecular weight determination by SDS-PAGE, α-zein shows major bands around 19–24 kDA.[14,31] Ethanol (90% in aqueous, Commercial Grade, Samsung Fine Chemicals, Korea) was used as a solvent for zein solutions. Glycerol (AR Grade, Carlo Erba, Italy) was exploited as a plasticizer. Tween®40 (AR Grade, Fluka, Germany) was utilized as a surfactant. NaCl (AR Grade, Univar, Seattle, WA) was the ionizability of salt. Deionized water was used to prepare 0.1M NaCl solution.

Polymer solution preparation

A standard solution was prepared by dissolving zein in 90% ethanol. The concentration of zein in the solution was 40% w/v of solvent. The mixture was stirred at room temperature for 1 h using a magnetic stirrer. Then the plasticizer, glycerol, was added into the primary zein solution to obtain zein solution samples with glycerol added for 2, 5, 8, and 10 wt %. After that, Tween®40 and 0.1M NaCl solution were added at 1.9 and 0.1 wt % of zein, respectively. The components were then mixed using a homogenizer (IKA labortechnik, T25 Basic, China) at 13,500 rpm for 5 min. The temperature was controlled at 7°C ± 0.5°C during homogenization. The droplet size distribution of glycerol in glycerol-zein emulsion was analyzed using a nanosizer (Malvern, United Kingdom). This was conducted within 4 h after homogenization to evaluate the stability of the emulsions. This period was determined with respect to the production time for 1 batch of the electrospun zein film.

Determination of solution properties

Solution flow property

The apparent viscosity of polymer solutions was investigated using a rheometer (MCR 150, Physica Parr, Germany) with a CC17 concentric cylinder measuring system (Øcup = 18.08 mm, Øbob = 16.66 mm) at a controlled shear rate between 0.01 and 400 s^{-1}. Temperature was controlled at 25°C using a TEZ150P Peltier thermal unit. The reported apparent viscosity was chosen within the linear range of the shear stress-shear rate relationship. Measurement data of shear stress (τ, Pa) versus shear rate ($\dot{\gamma}$, s^{-1}) were fitted to the power law model,[27] the consistency coefficient (K, Pa s^n) and flow behavior index (n) were then evaluated. Consistency coefficient directly indicates fluid consistency, whereas the flow behavior index reflects fluid viscosity property and indicates types of fluid; $n = 1$ if the fluid is Newtonian, $n < 1$ if the fluid flow is psuedoplastic (shear thinning fluid), and $n > 1$ if the fluid is dilatant (shear thickening fluid). The power law constitutive equation is shown in eq. (1).

$$\tau = K\dot{\gamma}^n \qquad (1)$$

Hear τ is shear stress, $\dot{\gamma}$ is shear rate, K is flow consistency coefficient, and n is flow behavior index.

Surface tension

The surface tension of the polymer solutions was measured with a digital tensiometer (Model DCT11, Dataphysics, Germany) using ring method. Sample solution (20 mL) was poured into a 50-mL glass beaker. The surface tension at the maximum pulling force was recorded. The temperature was controlled at 25°C.

Electrical conductivity

The electrical conductivity of the polymer solutions was measured at 25°C using a digital conductivity meter (Model CG855, Schott, Germany).

Electrospinning

Electrospinning apparatus setup is shown in Figure 1. Polymer solution (10 mL) was loaded into a glass syringe equipped with a stainless steel needle (O.D. 0.69 mm). The syringe was fixed on the syringe pump (NE1000, New Era Pump Systems, Farmingdale, NY), which was used to control the solution feed rate at 20 μL/min. The needle was connected to the high-voltage generator (ES30P-5W, Gamma High Voltage, Ormond beach, FL) operating in the positive DC mode from 0 to 30 kV. A precleaned and grounded stainless plate was used to collect the fibers. The distance from the needle tip to the stainless plate was 15 cm. It was noted that clogging from solidified zein at the tip of the spinneret was found during the electrospinning process. To avoid this problem, electrospinning technique with a special apparatus setup so called "solvent coating"[32] was used to effectively overcome the spinneret clogging problem that ordinarily paralyzed the continuity of the nonwoven fibrous film. This was setup by the use of an additional unit, which was fixed onto the main electrospinning unit (Fig. 1). This extra unit is composed of a syringe containing only the solvent: 90% ethanol that was aligned perpendicularly to the main electrospinning tip and was mounted on another syringe pump. The ethanol feed rate was kept at 6 μL/min, which was proved to have no effects on the functional properties of the as-spun zein fibers.[32] All experiments were carried out at 25°C ± 1°C and a relative humidity (RH) of 50% ± 5%.

Film thickness measurement

The film thickness was measured using a caliper micrometer (Model 102–309, Mitutoyo, Tokyo, Japan) with a precision of ±1 μm. Each film sample was measured at 10 random positions close to the testing area. An average film thickness was calculated for uses in the mechanical characterization. Zein films with thickness of 130 ± 30 μm were used for tensile property examination.
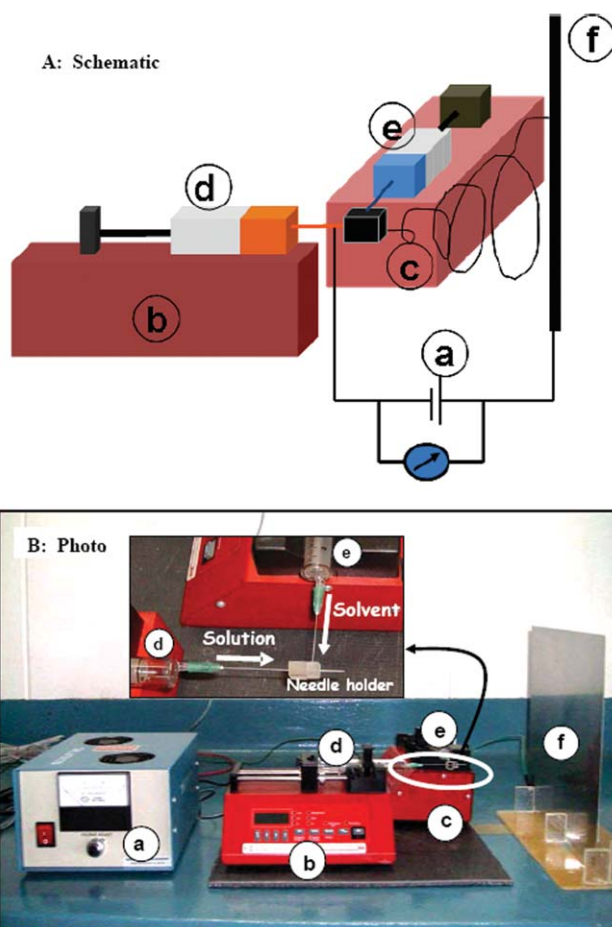

Figure 1 Setup of the electrospinning apparatus for the production of electrospun zein film: (a) high-voltage generator, (b) first syringe pump controlling feed rate of electrospun solution, (c) second syringe pump controlling feed rate of solvent, (d) spinneret of electrospun solution, (e) spinneret of solvent, and (f) grounded collector plate. [Color figure can be viewed in the online issue, which is available at www.interscience.wiley.com.]

Film preconditioning

Referring to the method of Ghanbarzadeh and Oromiehi,[22] the electrospun zein films were dried at 50°C for 24 h under vacuum to remove the residual solvent. The solvent-removed zein films were then cut into 25 mm wide × 100 mm long strips using extra sharp blade. The film strips were conditioned for 24 h at 50% ± 2% RH with saturated salt solution of magnesium nitrate hexahydrate (AR Grade, Qrëc, New Zealand), according to ASTM standard method D618.[33]

Mechanical property measurement (tensile test)

Tensile property characterization of the preconditioned zein film strips was carried out using a texture analyzer (TA.XT.Plus, Stable Micro System, Surrey, UK) with a 50-kg load cell. The grip surfaces were lined with thin silicone to prevent slippage as

TABLE I
Apparent Viscosity at a Shear Rate of 305 s^{-1}, Power Law Indices: K and n, Surface Tension, Electrical Conductivity
of Zein Solutions without Glycerol and with Glycerol Added for 2–10 wt %

Samples (with/without glycerol added; wt %)	Apparent viscosity $\eta_{a,305} \times 10^3$ (Pa s)	Power law consistency coefficient (K)	Power law flow-behavior index (n)	Surface tension (mN/m)	Electrical conductivity (μS/cm)	Average fiber-diameter (nm)
Without glycerol	225 ± 2[a]	0.005 ± 0.003[a]	1.69 ± 0.11[d]	24.46 ± 0.03[a]	531 ± 1[d]	483 ± 119[a]
With 2% glycerol	229 ± 5[a]	0.009 ± 0.003[a,b]	1.58 ± 0.05[b,c]	24.53 ± 0.01[b]	529 ± 2[c,d]	474 ± 108[a]
With 5% glycerol	226 ± 4[a]	0.009 ± 0.004[a,b]	1.60 ± 0.09[b,c]	24.55 ± 0.01[b,c]	527 ± 1[c]	452 ± 110[a]
With 8% glycerol	223 ± 5[a]	0.013 ± 0.005[b]	1.52 ± 0.07[a,b]	24.6 ± 0.00[c,d]	520 ± 2[b]	617 ± 133[b]
With 10% glycerol	221 ± 2[a]	0.026 ± 0.003[c]	1.39 ± 0.02[a]	24.63 ± 0.05[d]	510 ± 1[a]	928 ± 187[c]

The last column shows the average fiber-diameter of the film obtained from each solution sample. The different super-scripts (a–d) in the same column indicate the significant differences ($P < 0.05$) of the values. The results of solution property are averaged from triplicate determinations. The fibers diameters are obtained from measuring 200 fibers/sample.

well as breakage of the gripped film strips. The initial grip separation and crosshead speed were set at 50 mm and 50 mm/min, respectively. Tensile strength was calculated by dividing the maximum load before the breaking point by typical cross sectional area of the tested film. Tensile elongation was determined by dividing the film maximum elongation before rupture point by the initial gage length. Tensile modulus was defined as the slope of the stress–strain curve in the elastic (linear) range. For each film sample, the tensile property examination was performed on fifteen replicates and the average values were reported.

Scanning electron microscopy

The morphology of the electrospun zein film was evaluated using a scanning electron microscope (SEM) (JSM 5800, JEOL, MA). Square pieces of the electrospun zein films was attached on SEM aluminum stub and sputtering coated with gold-palladium mixture in Polaron E5100 coater at a condition of 2 kV, 5 mA for 2 min with an argon backfill at 8 Pa. The average diameter for each sample of the electrospun fibers was determined by image analysis (NIH, MD) from at least 200 fibers.

Differential scanning calorimetry

The glass transition temperature of the electrospun film was characterized by using a differential scanning calorimetry (DSC) (DSC 822^e, Mettler toledo, Switzerland) in nitrogen atmosphere. Calibration was performed using indium as a reference material. The sample weighing 3.5 ± 0.2 mg was hermetically sealed in DSC pan. An empty pan was used as a reference. The temperature was ramped from 25°C to 150°C, 150°C to 25°C, and 25°C to 200°C according to the temperature of the first heating, subsequent cooling, and the second heating, respectively. The scanning rate was 20°C/min. The T_g was indicated by the

DSC software; STARe thermal analysis version 6.1 (Mettler Toledo AG, Switzerland). The reported T_g was averaged from at least three separate scans.

Fourier transform infrared spectroscopy

Infrared spectra of electrospun zein films were recorded using a Fourier transform infrared (FTIR) spectrometer (Spectrum GX, Perkin-Elmer, Waltham, MA) in a range of 4000–600 cm^{-1} to investigate the functional groups and structure conformation of biopolymer chains in the electrospun zein films. Each measurement was an average of 16 scans at 4 cm^{-1} resolution. Result analysis (deconvolution and second derivative) of the obtained spectra was conducted by using Peakfit^{TM} v.4.12 software (Seasolve Software, MA). The spectra regions of 1800–1400 and 3700–2400 cm^{-1} of the original spectra were fitted with Gaussian peak fit. The number of deconvoluted peaks was determined automatically with adjusting the parameter values of baseline, bandwidth, and amplitude as linear-D2, 30, and 1.0, respectively. All the deconvolutions were carried out with R^2 of 0.999.

Statistical analysis

One-way ANOVA was used to analyze the variance. Differences between the results were tested using the LSD option and Duncan means comparison test with a significant level of 0.05 ($P < 0.05$). Software of SPSS (Statistical Product and Service Solutions) version 15.0 was used.

RESULTS AND DISCUSSION

Properties of zein solution

Viscosity, surface tension, and electrical conductivity of the electrospun zein solution with and without glycerol added were measured a well as are shown in Table I. The apparent viscosity of the pure zein solution at a shear rate of 305 s^{-1} ($\eta_{a,305}$) was 225 ±

2 MPa s, whereas those of zein solution with glycerol added for 2, 5, 8, and 10 wt % were 229 $\pm$ 5, 226 $\pm$ 4, 223 $\pm$ 5, and 221 $\pm$ 2 MPa s, respectively. The apparent viscosity was not significantly influenced by the glycerol added ($P \geq 0.05$). As the added glycerol ratio was increased from 0 (no glycerol) to 10 wt %, however, the trend of power law flow-behavior index decreased evidently ($P < 0.05$) from 1.69 $\pm$ 0.11 to 1.39 $\pm$ 0.02, the zein solution flow property shifted toward the direction of shear thinning. The change of flow behavior index reflected influence of glycerol in increasing molecular chain mobility, as a more facilitating electrospinning process was observed (data shown in terms of increase in fibers diameter uniformity). Besides, as the glycerol content increased, surface tension increased but electrical conductivity of the zein solution decreased. The decrease in electrical conductivity could be attributed to the charge shielding effect of the added glycerol molecules on protein zein molecules,[34,35] thereby yielding an increase in contact surface area among the molecule at the surface leading to the increase in surface tension of the zein solution upon enhancing glycerol content.

Morphology of the electrospun zein fibers

As shown in Figure 2, the morphologies of the zein electrospun fibers with and without the plasticizer are all flat or ribbon-like with the average diameters varying with the plasticizer contents. The average diameter of pure zein fibers was 483 $\pm$ 119 nm, whereas those of zein fibers containing 2, 5, 8, and 10 wt % glycerol were 474 $\pm$ 108, 452 $\pm$ 110, 617 $\pm$ 133, and 928 $\pm$ 187 nm, respectively. The ribbon-like structure of the fibers was due to rapid evaporation of solvent from the jet surface during traveling from the spinneret to the grounded collector, thereby resulting in a thin layer of solid skin with a liquid core. As a result of the atmospheric pressure, the solid surface collapsed in tandem with further solvent slowly evaporating from the core. Then the initial circular cross section developed an elliptical shape and, thereafter, the fibers transformed into a ribbon-like structure.[27] On the investigation of the effects of the plasticizer on fiber diameters, the results from Table I revealed that glycerol significantly reduced electrical conductivity of the polymer solution ($P < 0.05$) leading to larger fiber diameters. Therefore, the solution's low-electrical conductivity and the resulting low-Coulombic repulsive force within the stretching jet might be the cause of larger fiber diameter.[26]

It was also found that power law index of flow behavior of the solution reduced as the amount of glycerol increased up to 5 wt % and higher. Normally, a polymer solution with a low value of flow behavior index reflects its high-shear thinning
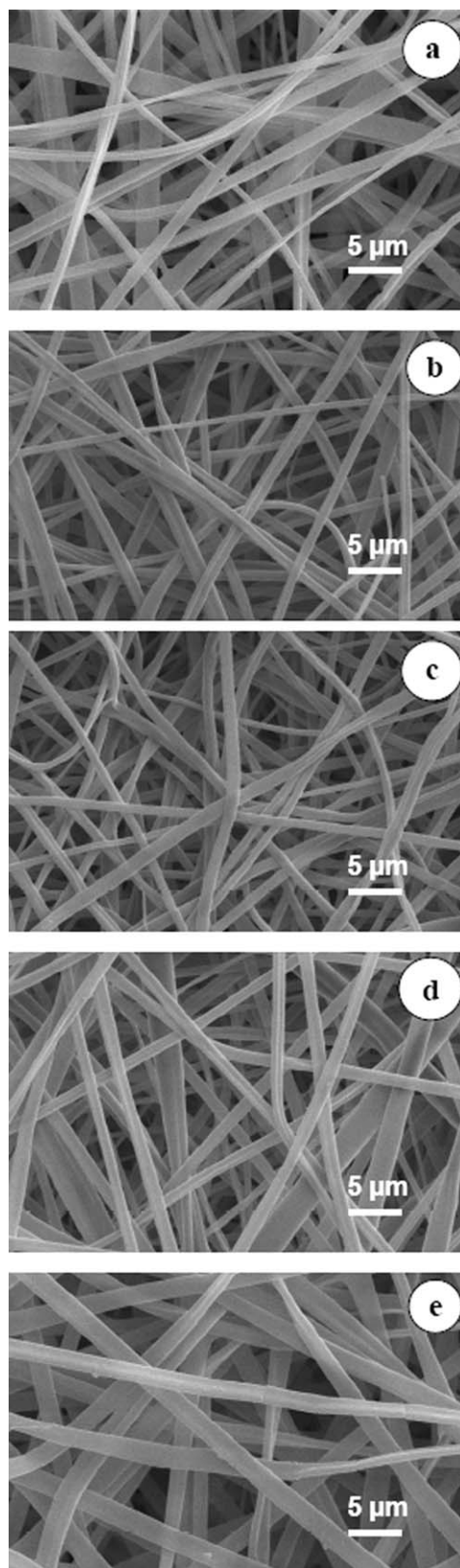

Figure 2 Scanning electron microscopic images (magnification = ×5000) of electrospun zein fibers produced from zein solution containing glycerol at a percentage weight ratios of: (a) 0%, pure zein fibers, (b) 2%, (c) 5%, (d) 8%, and (e) 10%. The bar scale is 5 μm.
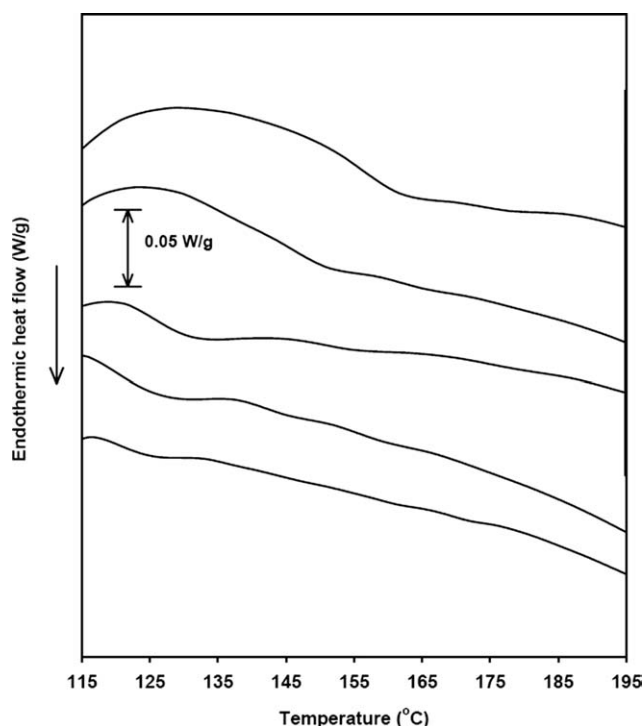
Figure 3 DSC thermograms of the electrospun zein fibers with/without glycerol added. Each line, from top to bottom, represents the thermogram of the elctrospun zein fiber without glycerol, with added glycerol of 2, 5, 8, and 10 wt %, respectively.

property. Here, it was hence believed that the transformation from shear thickening into the direction of shear thinning (reduction in values of n) might be the important factor behind the increase in fiber diameters uniformity (considered in terms of $SD/\overline{X}$), as the glycerol content increased. Besides, in the experiment, it was also observed that the electrospinning process was more facilitated when the glycerol added content increased, as observed from deposition rate of the as-spun fibers on the grounded collector.

Thermal property of electrospun zein film

Thermal characteristics of zein films with and without glycerol by DSC are shown in Figure 3. Illustrations in Table II are glass transition temperatures (T_g) determined from the resulting thermogram as the midpoint between onset and endset temperatures of a step change in the heat flow curve. The T_g of electrospun pure zein film was 154.1°C $\pm$ 0.4°C. The T_g decreased with the increase in glycerol ratio. They were 138.9°C $\pm$ 2.0°C, 127.1°C $\pm$ 1.4°C, 126.4°C $\pm$ 0.3°C, and 125.5 $\pm$ 0.4°C for the films containing 2, 5, 8, and 10 wt % of glycerol, respectively. As stated in many reports[35–37] about the T_g of protein-based film that its T_g was mainly attributed to inter- and intra-molecular attractive force of the molecules,

the T_g of the film containing higher ratio of glycerol, hence decreased as a result of decrease in average molecular weight of protein molecules. As further illustrated in the following section on FTIR analyses, the plasticizing effect of glycerol on reducing T_g of the electrospun films was milder than that of the cast films which were reported in other studies.[22,38,39] This might be due to the higher molecular orientation within electrospun fibers in comparison with that of the cast films.

Composition analysis of electrospun zein film by Fourier transform infrared spectroscopy

The infrared spectra of the elctrospun zein films with and without glycerol are shown in Figure 4. The characteristic peak of —OH group of the glycerol-zein co-spun fibers was observed at about 1045 cm^{-1}.[40] Although this peak was not detected in the pure zein spun fibers, its intensity increased as the glycerol content increased. This finding confirmed the presence of glycerol in the as-spun zein fibers. The characteristics peak at about 3550–3200 cm^{-1} was attributed to the intermolecular bonded hydroxyl group, whereas those ranging between 1700–1600 and 1600–1500 cm^{-1} indicated the presence of amide I and amide II, respectively.[21,35,40–43] The two vibrational stretching bands of amide I and II are the two most important bands of polymer chain with protein backbone.[40–42] The characteristics peak of amide I is attributed mainly to the C=O stretching, whereas that of amide II resulted primarily from N—H bending and C—N stretching.[42,43] From an analysis of the second derivative of spectra ranging between 1800 and 1400 cm^{-1}, all the as-spun fibers showed the characteristics peak of amide I band at 1655 cm^{-1}, whereas that of amide II of the pure zein fibers appeared at 1542 cm^{-1} with a shift to around 1545 cm^{-1} for the fibers containing glycerol. These results implied that glycerol had a greater effect on the conformation change of amide

TABLE II
Effects of Glycerol on the Glass Transition Temperatures of Electrospun Zein Films without Glycerol and with Glycerol Added for 2–10 wt %

Samples (without/with glycerol added; wt %)	Glass transition temperature, T_g (°C)
Without glycerol	154.1 $\pm$ 0.4[c]
With 2% glycerol	138.9 $\pm$ 2.0[b,c]
With 5% glycerol	127.1 $\pm$ 1.4[a,b]
With 8% glycerol	126.4 $\pm$ 0.3[a]
With 10% glycerol	125.5 $\pm$ 0.4[a]

The different superscripts (a–c) in the same column indicate the significant differences ($P < 0.05$) of the values. The results are reported in terms of mean $\pm$ SD from triplicate determinations.
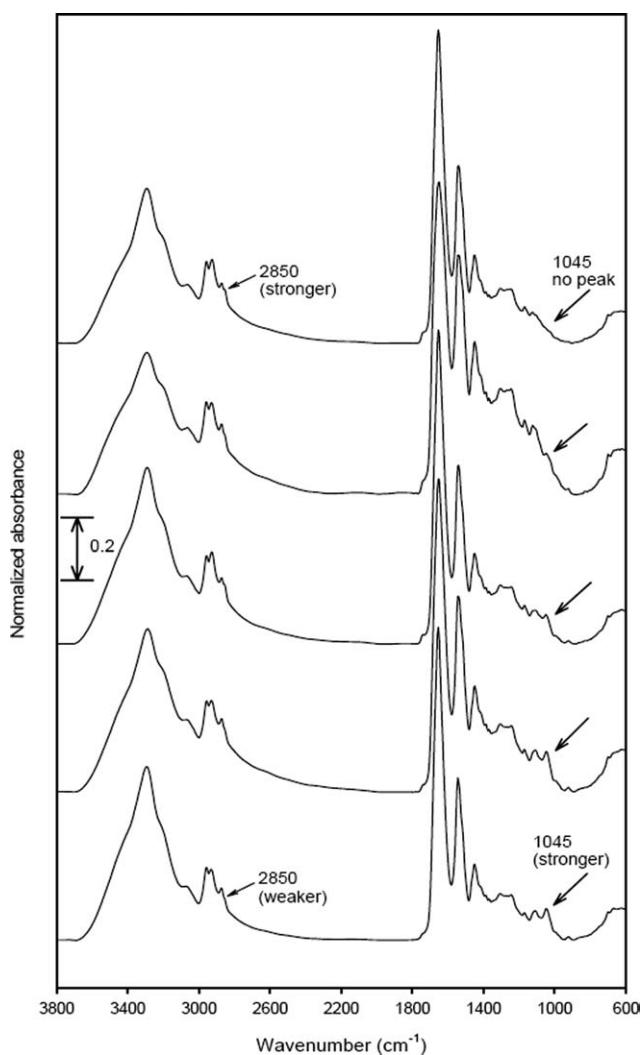
Figure 4 Infrared spectra of the electrospun zein film with/without glycerol added at wavenumber ranging from 4000 to 600 cm^{-1}. Each line, from top to bottom, represents the infrared spectra of the elctrospun zein fiber without glycerol, with added glycerol of 2, 5, 8, and 10 wt %, respectively.

II than that of amide I structure. Interestingly, upon addition of glycerol, the amide I region (1700–1600 cm^{-1}) exhibited a more eminent change in intensity at around 1695–1685 cm^{-1} (intermolecular β-sheets) and 1684–1670 cm^{-1} (intramolecular β-sheet) in comparison with that in 1659–1648 cm^{-1} region (α-helical primary). This suggested that glycerol had a stronger influence on the β-sheet structures than on the α-helical primary structures.

For the amide II peak, distinct changes in spectral intensity [Fig. 5(a–e)] were observed in two regions of deconvoluted peaks i.e., ~ 1545–1509 (trend line) and 1498–1482 cm^{-1} (reduced intensities). In general, amide II intensities in glycerol-plasticized protein samples are attributed to the hydrogen bonding environments.[41] Thus, the results suggested that the changes in structural conformation involved mainly
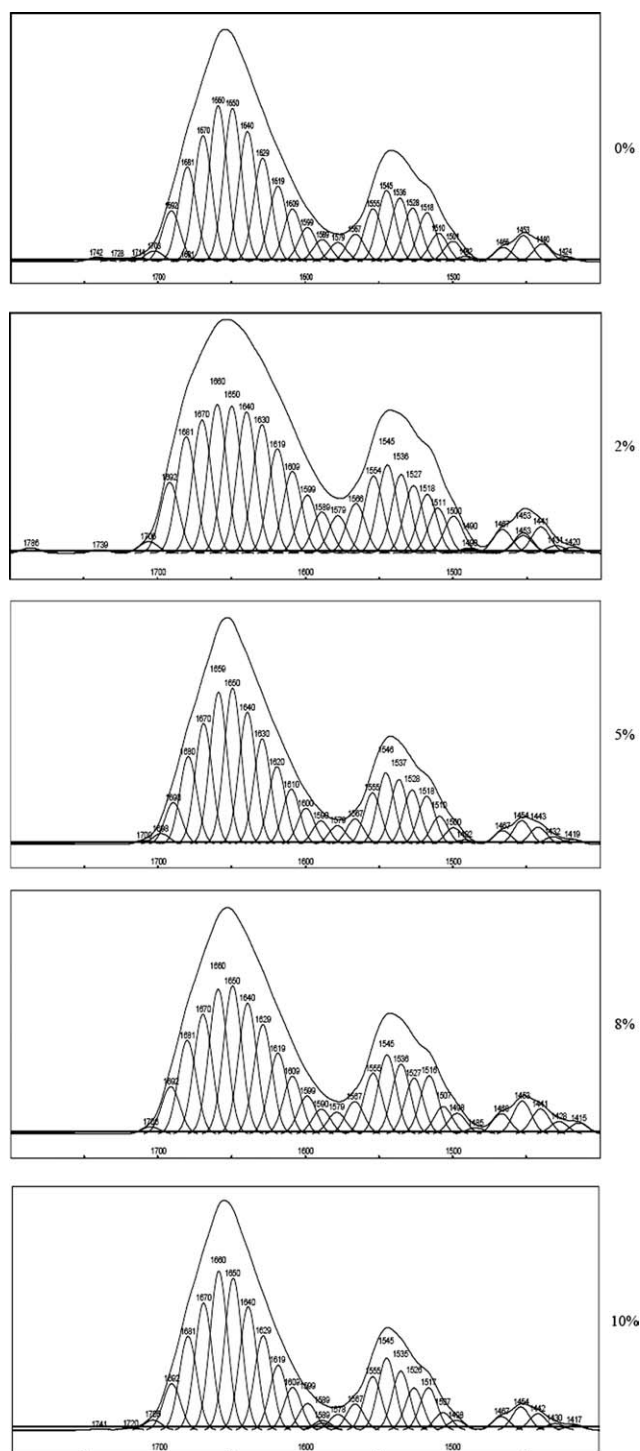

Figure 5 Deconvoluted peaks of the FTIR spectra ranging between 1800 and 1400 for the electrospun zein fibers containing glycerol without/with glycerol added for 2–10 wt %. The deconvolution was carried out using Peakfit^{TM} software. Band shapes were conducted based on Gaussian calculation and with a linear baseline. The top curve shows the integrated result of the deconvoluted peaks. The integrated curve obtained upon the deconvolution was carried out with R^2 of 0.999 fitting to the original spectrum.
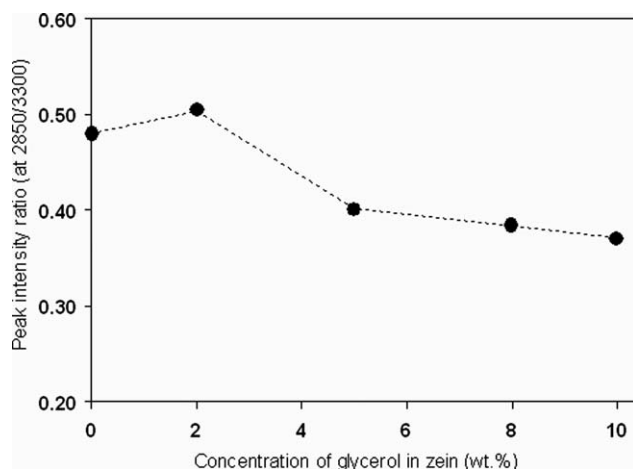
Figure 6 Ratios of the relative intensities of deconvoluted peak of amide I at the wavenumber of 2850 cm^{-1} (thiol group) to 3300 cm^{-1} (hydroxyl group) against concentration of glycerol.

with H-bonds. The effect of glycerol on the change in polymer structure of electrospun zein fibers was also indicated by the absorbance peak between 2800 and 2700 cm^{-1} (primary and secondary amine molecules). After deconvoluting the peak around 2850–2600 cm^{-1} (figure not shown), it was revealed that the resulting peaks (2864, 2808, 2757, and 2705 cm^{-1}) shifted to higher wave numbers (2866, 2811, 2762, and 2711 cm^{-1}, respectively) upon addition of glycerol. This suggested that glycerol–protein hydrogen bonding reduced the degree of freedom of protein subunits.[44,45] Moreover, the infrared spectra of pure zein film showed an absorbance peak at $\sim$ 2850 cm^{-1} which corresponded to thiol end functional groups found in cysteine and methionine,[43] the two sulfur-containing amino acids of zein protein.[14,46] In Figure 6, the glycerol concentration was plotted against the intensity ratio of peak at $\sim$ 2850 (thiol groups) to $\sim$ 3300 cm^{-1} (hydroxyl groups). It was shown that the ratio decreased with increasing glycerol content. This indicated the correlation between the presence of glycerol and the change in zein molecular conformation.

As indicated by FTIR and DSC that glycerol played an important role in the zein molecular chain conformation which normally in turn influenced the mechanical property of the as-spun film, tensile properties of the fabricated films with and without glycerol were thus investigated. Then, the results obtained from DSC, FTIR, and tensile tests were concomitantly deliberated to get an insight into the influence of plasticizing with glycerol on the modification of films' tensile property.

Mechanical properties of electrospun zein film

The applications of zein-based films have been limited by the material brittleness.[20–22] Accordingly, glycerol was deliberately introduced into the film forming solution to improve the electrospun films' elongation as shown in Table III. The corresponding deformation curve is shown in Figure 7. The results showed that the tensile strength of electrospun zein films was increased as the percent content of glycerol increased. As the glycerol content reached 10 wt %, the elongation at break of the zein film was improved for 1.6 times. This could be explained by drawing attention to the fact that glycerol enhanced the solutions' flow consistency (Table I) as indicated by the power law consistency coefficient (K). With a reduction in electrical conductivity, the increase in flow consistency led to an increase in fiber diameters. In a mechanical sense, the fibrous film with larger fibers' diameter possessed greater capability to resist tensile force. Moreover, the hydrogen bonding between glycerol and zein molecules as revealed by the FTIR analysis was also expected to enhance the mechanical performances. Because glycerol acted as a plasticizer for the electrospun zein fibers, the rigidity of as-spun fibers was decreased. Although the average molecular weight of the zein fiber decreased upon plasticization with glycerol, the chain mobility increased. This enhanced the elongation property of the blend films. However, the reverse of the tensile strength was found at 10 wt % glycerol. This might

TABLE III
Tensile Strength (MPa), Elongation at Break (%) and Tensile Modulus (MPa) of the Electrospun Zein Film without Glycerol and with Glycerol Added for 2–10 wt %

Samples (with/without glycerol added; wt %)	Tensile properties		
	Tensile strength (MPa)	Elongation at break (%)	Tensile modulus (MPa)
Without glycerol	0.332 ± 0.100[a]	1.642 ± 0.287[a]	0.192 ± 0.038[a]
With 2% Glycerol	0.472 ± 0.271[a,b]	2.171 ± 0.561[a,b]	0.197 ± 0.061[a]
With 5% Glycerol	0.488 ± 0.200[a,b]	2.359 ± 0.825[a,b]	0.218 ± 0.062[a]
With 8% Glycerol	0.657 ± 0.220[b]	2.460 ± 0.659[a,b]	0.270 ± 0.070[a]
With 10% Glycerol	0.381 ± 0.059[a,b]	2.555 ± 0.090[b]	0.193 ± 0.014[a]

The different superscripts (a and b) in the same column indicate the significant differences ($P < 0.05$) of the values. The results are reported in terms of mean ± SD from 15-replication determinations.
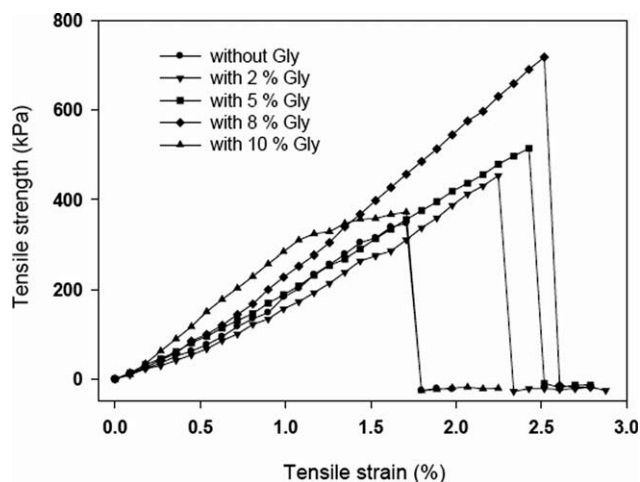
Figure 7 Typical forced-deformation curves of the electrospun zein film without glycerol and with glycerol added for 2–10 wt %.

be due to the anti-plasticizing effect at high-glycerol content. In general, for a composite matrix, when the additive or filler is over loaded, the continuity of polymer network is disrupted leading to a reduction in the tensile strength. Note also that, only at 10 wt % glycerol containing, zein film deformation showed a yield point (at 320 kPa) before a large deformation (at 380 kPa). This suggested that the fibers became more ductile after high loading with glycerol.

The shift of the deconvoluted peaks of amide II region to lower wavenumbers upon addition of glycerol indicated that the protein structure became more flexible. Once the rigidity of the structure decreased, the T_g reduced as a result of increased polymer chain mobility. This finally led to higher elongation of glycerol-zein co-spun fibrous film. However, due to the randomly arrangement direction of the fibers of the spun film, the fibrous film samples exhibited a large distribution of tensile property. It was interesting to note here that substantial increases in the tensile strength and tensile modulus were obtained upon immersing spun films in water. From this, it was also hypothesized that water became a source for hydrogen bonding formation within the polymer network. Besides, our first observation by the light microscope showed that unity in fiber's arrangement direction increased. Nonetheless, in case of using the zein film for tissue engineering application, some additional treatments such as crosslinking is needed to further improve the tensile strength of the spun films.

CONCLUSIONS

This work studied the effects of glycerol as a plasticizer on the zein solution properties, which influentially governed the morphologies of the corresponding electrospun zein fibers. It was found that the additive had a very little effect on the apparent viscosity of the solutions. However, as the amount of glycerol increased, the electrical conductivity of the solution significantly decreased, whereas the power law index shifted from shear thickening into the direction of shear thinning. The SEM micrographs revealed that zein nanofibers were ribbon-liked. The sizes of the fibers expanded upon addition of plasticizer, whereas the size distribution increased with the increase in the electrical conductivity and the reduction in the flow power law constant. Moreover, the glass transition temperatures of the electrospun zein films containing glycerol were lower than that of the films without the additive, giving rise to better elongation to break. This study demonstrated the advantages of using glycerol as a facile and cost-effective approach to improve the tensile property of electrospun zein films. For tissue engineering application, however, this study clearly indicated that the tensile strength of electrospun fibrous zein film was not high enough, with only slight improvement by co-spinning with glycerol (up to 10 wt %). Nonetheless, the elastic properties of the glycerol plasticized-zein fibrous film may be suitable for active compound control release/carrier that is attached on specific engineered tissues. The potential use of the electrospun zein films in such application is being investigated and will be reported in the future publication.

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