Journal of the mechanical behavior of biomedical materials 69 (2017) 412–419

Contents lists available at ScienceDirect

Journal of the Mechanical Behavior of Biomedical Materials

journal homepage: www.elsevier.com/locate/jmbbm

Systematic mechanical evaluation of electrospun gelatin meshes

Annabel L. Butcher[a], Ching Theng Koh[a,b], Michelle L. Oyen[a,*]

[a] Department of Engineering, University of Cambridge. The Nanoscience Centre, 11 JJ Thomson Avenue, Cambridge CB3 0FF, UK
[b] Faculty of Mechanical and Manufacturing Engineering, Universiti Tun Hussein Onn Malaysia, 81310 Parit Raja, Johor, Malaysia

ARTICLE INFO

Keywords:
Electrospinning
Gelatin
Mechanical properties
Fibre diameter
Pore size

ABSTRACT

Electrospinning is a simple and efficient process for producing sub-micron fibres. However, the process has many variables, and their effects on the non-woven mesh of fibres is complex. In particular, the effects on the mechanical properties of the fibre meshes are poorly understood. This paper conducts a parametric study, where the concentration and bloom strength of the gelatin solutions are varied, while all electrospinning process parameters are held constant. The effects on the fibrous meshes are monitored using scanning electron microscopy and mechanical testing under uniaxial tension. Mesh mechanical properties are relatively consistent, despite changes to the solutions, demonstrating the robustness of electrospinning. The gel strength of the solution is shown to have a statistically significant effect on the morphology, stiffness and strength of the meshes, while the fibre diameter has surprisingly little influence on the stiffness of the meshes. This experimental finding is supported by finite element analysis, demonstrating that the stiffness of the meshes is controlled by the volume fraction, rather than fibre diameter. Our results demonstrate the importance of understanding how electrospinning parameters influence the pore size of the meshes, as controlling fibre diameter alone is insufficient for consistent mechanical properties.

1. Introduction

Electrospinning is a cost-effective and convenient method for manufacturing sub-micron fibres (Garg and Bowlin, 2011). The process requires a viscous polymer solution to be ejected through a narrow orifice connected to a high voltage supply. The resulting polymer jet undergoes bending instabilities as it is attracted to an earthed collector, causing the jet to stretch and evaporating the solvent sufficiently to form a mesh of nano- or micro-scale fibres on the surface of the collector (Fang et al., 2011). The versatility of electrospinning has caused a rapid increase in its popularity and it is currently being investigated for use in many biomedical and environmental applications, as well as in energy collection and storage (Fang et al., 2011). Electrospinning has become increasingly favoured in tissue engineering, in particular for soft tissues (Sill and von Recum, 2008). This is due to electrospun nanofibres having a structure very similar to the nanofibrous collagen found in the extracellular matrix (ECM) of many tissues (Mow and Huiskes, 2005). These collagen structures are often responsible for the mechanical properties of the tissues. Mimicking these mechanical properties is key to creating a functional artificial tissue, although it is often overlooked in favour of creating the right biological environment.

Although the electrospinning process is highly versatile, it is not well understood how the process can be manipulated to affect the mechanical properties of electrospun fibres. This is partly due to the wide range of variables within the process, such as the applied voltage, solution flow rate, and the distance between the polymer source and collector (Ko and Wan, 2014). The effect of some of these process variables on the morphology of the electrospun fibres has been studied through experiments (Pillay et al., 2013; Deitzel et al., 2001; Samatham and Kim, 2006; Pelipenko et al., 2013; Nezarati et al., 2013; Harrison et al., 2012) and computer models (Thompson et al., 2007). However, understanding of their effects is limited. The solution properties, such as polymer concentration, solution viscosity and electrical conductivity can also affect the electrospun fibres, including their mechanical properties. Changing the polymer concentration, polymer proportions within a composite solution, or adding ions have all been shown to have a significant impact on mechanical properties (Nugeet al., 2013; Ji et al., 2014; Yin et al., 2013; Machado et al., 2013; Kimura et al., 2014).

Gelatin is commonly electrospun as it is chemically similar to collagen yet much less expensive, more readily available and presents a reduced immunological risk (Elzoghby, 2013). It is also electrospinnable in non-toxic solvents, such as acetic acid (Mindru et al., 2007; Song et al., 2008; Gu et al., 2009; Linh et al., 2010; Tonsomboon and Oyen, 2013; Koh et al., 2013), although little attention has been paid to the variability of molecular weight within gelatin. The polymer is

* Corresponding author.
E-mail address: mlo29@cam.ac.uk (M.L. Oyen).

http://dx.doi.org/10.1016/j.jmbbm.2017.02.007
Received 26 August 2016; Received in revised form 17 November 2016; Accepted 6 February 2017
Available online 07 February 2017

formed from the hydrolytic conversion of collagen, producing a distribution of molecular weights from below 10,000 g mol^{-1} and to over 400,000 g mol^{-1} (Schrieber and Gareis, 2007). Therefore gelatin products are not differentiated by their molecular weight as many natural polymers are, but by their bloom strength. This "strength" or resistance to deformation is dependent upon the structure and molecular mass of the gelatin, and commonly ranges from 50 to 300 g, measured by an arbitrary procedure defined in the food industry (Schrieber and Gareis, 2007).

As bloom strength and solution concentration are both considered to have an effect on the strength of gelatin, the two parameters are combined into a single property known as gel strength. Two gelatins with equal gel strength are considered interchangeable within the food industry, defined as in Eq. (1), where GS is gel strength, C is concentration and B is bloom strength (Schrieber and Gareis, 2007). Gel strength has been shown to have a linear correlation with gelatin's compressive modulus (Chua and Oyen, 2009), but its effect on electrospinning is unknown.

$$GS = C^2B \quad (1)$$

This work reports a methodical study undertaken to examine the relationship between the solution properties, the morphology of the meshes produced and their mechanical properties. The concentration and bloom strength of the gelatin solutions are varied, and the conductivity and viscosity of the solutions measured before electrospinning. The morphology and mechanical properties of the meshes are assessed using scanning electron microscopy and uniaxial tensile testing, respectively. Clear relationships are found between solution properties and fibre diameter, while the mechanical properties of the resulting meshes appear to be independent of the fibre diameter.

2. Materials and methods

2.1. Gelatin preparation

2.1.1. Gelatin solutions

Gelatin solutions were prepared using a solvent system of 90% aqueous acetic acid (Sigma Aldrich, UK). Four different bloom strengths of porcine gelatin were used: 100 g, 180 g, 250 g, and 300 g (Sigma Aldrich, UK). In order to differentiate between the effect of concentration and that of bloom strength, each strength of gelatin was made into a 10 wt% and a 12 wt% solution; eight different solutions were electrospun in total. The solutions were then stirred using a magnetic stirrer for 2–3 h at room temperature. All electrospinning was carried out within 7 days of the solution being prepared.

Prior to electrospinning the dynamic viscosity, μ, and conductivity, G, of the solutions were measured. An ExStik conductivity meter (Extech Instruments, USA) was used to record the conductivity. Viscosity measurements were made using a size 200 Cannon-Fenske viscometer tube (Sigma Aldrich, USA). Measurements for each solution were repeated and averaged.

2.1.2. Production of electrospun meshes

The electrospinning apparatus consisted of an infusion pump (KR Analytical Ltd., UK), a high voltage power supply (Glassman high voltage INC., UK) and a grounded, stationary collector comprised of a 7 cm-diameter copper plate wrapped with aluminium foil, suspended horizontally opposite the syringe pump. The solutions were loaded into a plastic syringe (BD Plastipak, Spain) with an 18 gauge stainless-steel blunt-ended hypodermic needle (BD plastic, UK), connected to the voltage supply. The processing parameters were kept constant; the feed rate was 0.005 ml/min, the voltage was 18 kV, and the working distance was 10 cm. Each time a solution was electrospun, the conductivity and viscosity were measured. Each solution was electrospun for a period of four hours. Solution preparation and electrospinning were performed in duplicate, so that four electrospun meshes were produced for each of the 8 solutions, giving 32 samples in total. After electrospinning, the aluminium foil was removed carefully from the mesh, and the mesh was stored at room temperature until characterisation.

2.2. Characterisation

2.2.1. Solution degradation

Gelatin has been shown to degrade over time in acidic solutions (Ki et al., 2005). To observe the effect of the acetic acid solvent on gelatin, the viscosity and conductivity of four gelatin solutions were measured over the period of a week. Solutions of 100 g and 250 g bloom strength gelatins were prepared at 10 and 12 wt%. The solutions were stirred using a magnetic stirrer for at least 3 hours at room temperature. Measurements were taken for the viscosity and conductivity at 4, 8, 12, 24, 48, 96 and 168 h, as described in Section 2.1.1.

2.2.2. Morphology

The morphology of the electrospun meshes was characterised using scanning electron microscopy (EVO LS 15, Carl Zeiss, UK) at an accelerating voltage of 10 kV. Small sections of the electrospun meshes, approximately 5 mm diameter, were taken from each sample and coated with a thin layer of gold to produce a conductive surface. The sections were taken from the area adjacent to that used for mechanical characterisation. The fibre diameters, ϕ, of the gelatin meshes were analysed from the SEM images using ImageJ (NIH, USA) (Tonsomboon and Oyen, 2013). For each mesh, a minimum of 20 measurements were taken to give the average fibre diameter for that sample. The directionality function within ImageJ was used to determine fibre orientation, to assess if the fibres in the mesh showed any alignment.

2.2.3. Mechanical characterisation

Mechanical properties of all samples were measured using uniaxial tensile testing. Specimens were prepared by cutting the samples into rectangular strips of 30×5 mm, taken from the centre of the sample to avoid any possible edge anomalies from the electrospinning process. To assist gripping of the thin specimens and to minimise clamping effects, 5 mm on each end of every sample were wrapped in laboratory tape, giving all specimens a dimension of 20×5 mm. The specimen thickness was measured using digital calipers. The uniaxial tensile tests were performed with an Instron testing machine (model 5544, Canton, USA) with a 500 N load cell. The specimens were stretched at a rate of 0.05 mm/s until failure. A minimum of five tests were conducted for each sample, and used to calculate the tensile strength, σ_{TS}, tensile elastic modulus, E, and failure strain, e.

2.2.4. Statistical analysis

All statistical analyses were performed using Origin (Origin 8, OriginLab, Northampton, MA). Briefly, all data in each plot were concatenated and a linear regression was used to assess the statistical significance. The significance value was set at $p < 0.05$.

3. Results

3.1. Solution properties

Viscosity and conductivity are known to have a significant effect on the electrospinning process (Ramakrishna et al., 2005). The viscosity and conductivity of four solutions were recorded over seven days to view the extent of gelatin degradation in the acidic conditions. Fig. 1 shows the viscosity of all solutions decreases over time, although the effect is greater for larger gel strengths. All solutions show an increase in conductivity over a week, the extent of which is independent of gel strength. The majority of the increase is seen in the first 24–48 h, followed by a slower rate of increase.

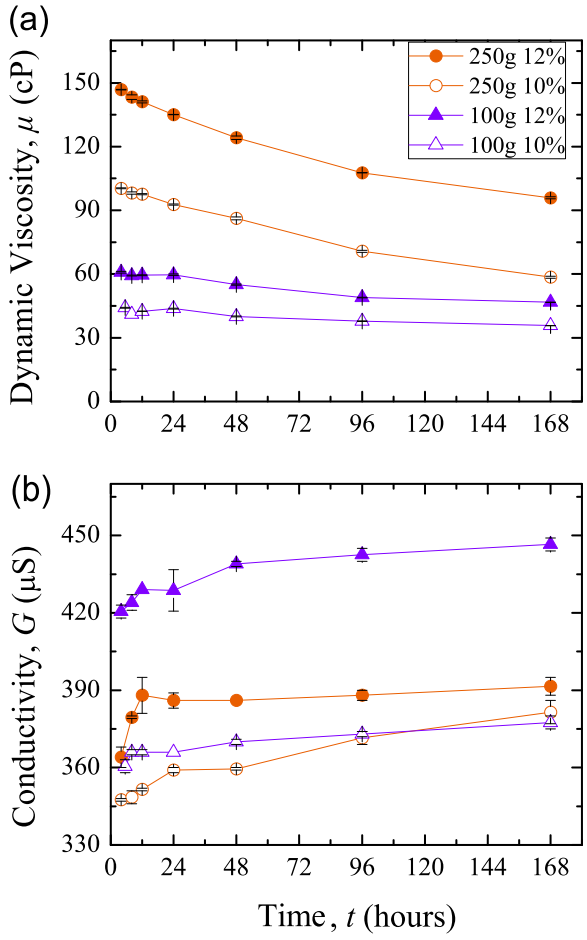

Fig. 1. Degradation of four different gel strength solutions, measured by (a) dynamic viscosity, μ, and (b) conductivity, G, measured over seven days.

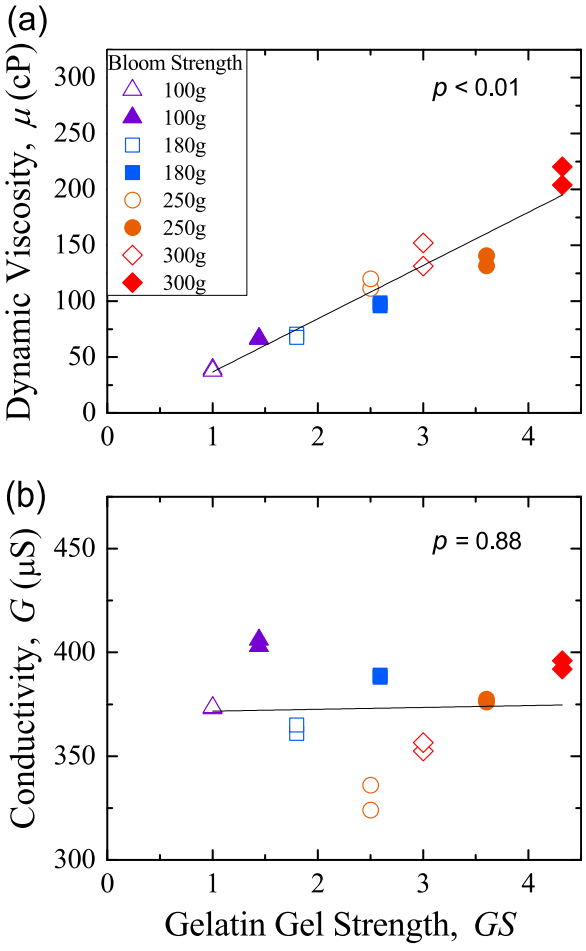

Fig. 2. Effect of gel strength, GS (Eq. (1)) on solution properties, (a) dynamic viscosity, μ, and (b) conductivity, G. Open symbols represent solutions made with 10 wt% gelatin, solid symbols show those made from 12 wt% gelatin.

The dynamic viscosity of the gelatin solutions increased significantly ($p < 0.01$) with the gel strength (see Fig. 2). However, conductivity appears to be largely unaffected by the gel strength of the solutions (p=0.88), although there is a notable difference due to concentration. As the effect of gel strength on conductivity is insignificant, the effect of conductivity on the electrospinning process will not be considered further in this paper.

3.2. Morphology of electrospun fibres

All solutions could be electrospun to produce meshes containing uniform and continuous gelatin fibres. Four hours of electrospinning provided meshes thick enough to perform mechanical testing. Fig. 3 shows representative SEM images for each of the electrospun solutions, together with the directionality of the fibres for each mesh. An increase in concentration or in bloom strength typically leads to an increase in fibre diameter. The variability in the fibre diameter also increases with gel strength. The direction of the fibres within the meshes shows that the fibres are randomly oriented, and therefore the orientation of the meshes does not need to be considered during mechanical testing.

Fig. 4 shows the diameter of the fibres increased significantly with both gel strength and viscosity ($p < 0.01$), unsurprising as the two are related (Fig. 2a). Both also show an increase in the standard deviations for greater gel strength solutions.

3.3. Mechanical properties of electrospun meshes

Tensile testing of the meshes produces an s-shaped stress strain curve, as shown in Fig. 5. Initially the curve is similar to the J-shaped curves seen for many biological materials. The initial increase in stiffness indicates the percentage of loaded fibres is increasing, followed by a linear region, used to approximate an elastic modulus for each sample. Finally a decrease in stiffness is seen as fracture spreads across the sample before failure. The tensile strength was taken at the point of greatest stress, and the strain at this point was also recorded.

Fig. 6 shows that Young's modulus and tensile strength increase as gel strength decreases (p=0.02 and p=0.01 respectively), while the failure strain decreases with decreasing gel strength ($p < 0.01$). While all three properties show a statistically significant relationship with gel strength, there is great variability in the data.

The mechanical properties of the meshes were also plotted against the viscosity of the solutions, shown in Fig. 7, expecting to see similar results as against gel strength. Interestingly, there is no statistically significant dependence of modulus (p=0.61) or strength (p=0.33) on viscosity. However, the failure strain depends significantly on viscosity (p=0.02).

The macro-scale mechanical properties of the meshes are expected to be dependent on their micro-structures. Therefore, the fibre diameter is expected to have a significant effect on the mesh mechanical properties. However, while Fig. 4 shows that fibre diameter is significantly affected by solution viscosity, the modulus and strength of the meshes are not, indicating fibre diameter may not affect the mechanical properties as thought. Fig. 8 shows the relationship between the fibre morphology and the mechanical properties of the mesh. Interestingly, there is a statistically significant relationship for strength (p=0.03) and failure strain ($p < 0.01$) with fibre diameter, but

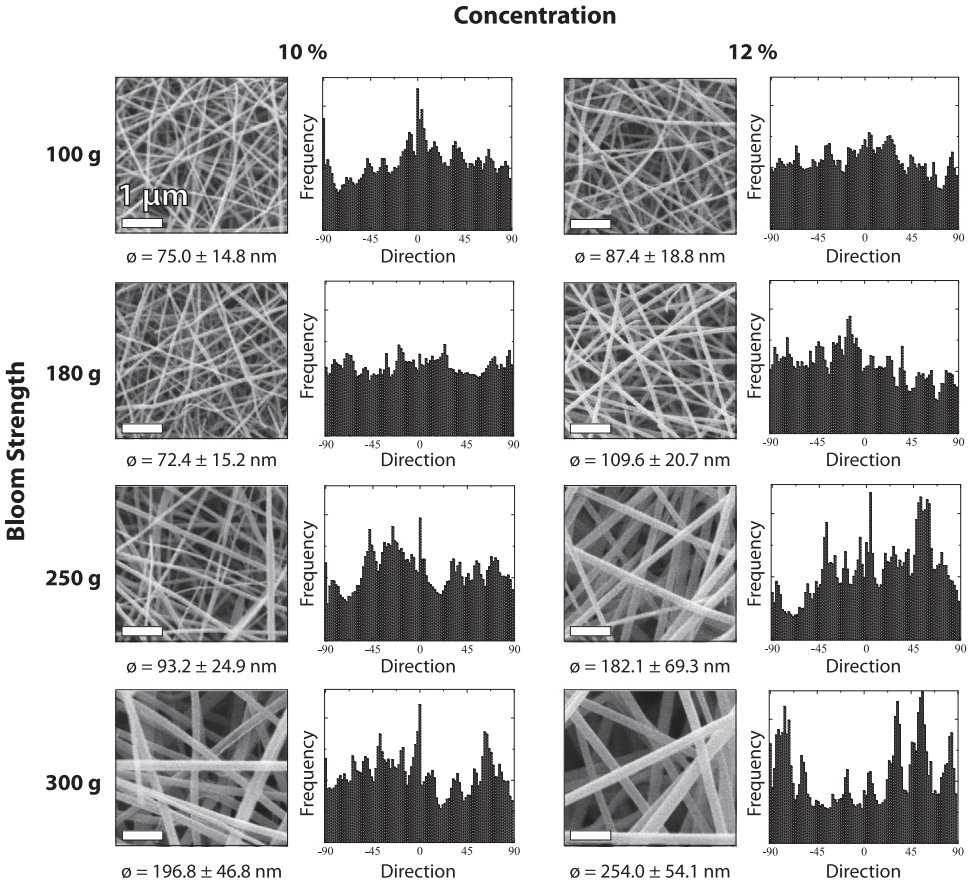

Fig. 3. Representative SEM images and directionality graphs for each of the eight different electrospun solutions. Directionality is plotted against relative frequency. Concentration, *C*, increases from 10 wt% to 12 wt% left to right, the bloom strength, *B*, increases from 100 g to 300 g from top to bottom. All scale bars are 1 µm. Fibre diameter, ϕ, is shown as average ± standard deviation (n=20).

no clear correlation between the modulus and fibre diameter (p=0.09).

4. Discussion

Gelatin was electrospun under consistent conditions with eight different gel strength solutions. The morphology and mechanical properties of the resulting fibrous meshes were analysed to determine interactions between solution properties, mesh morphology and mesh mechanical properties. Electrospinning with identical parameters produced uniform non-beaded fibres for all eight solutions, so that the effect of gel strength could be studied in isolation. Gel strength was shown to affect the solution viscosity, the fibre diameter, and the modulus, strength and failure strain of the meshes. However, solution viscosity showed no relationship with mesh modulus or strength, and fibre diameter had no statistically significant effect on modulus either.

The effects of solution conductivity on the mechanical properties and morphology of electrospun meshes were not considered in this study. Others have shown increasing solution conductivity causes a decrease in the diameter of electrospun fibres, due to dramatic bending instabilities in the jet (Bhardwaj and Kundu, 2010). Gelatin is a

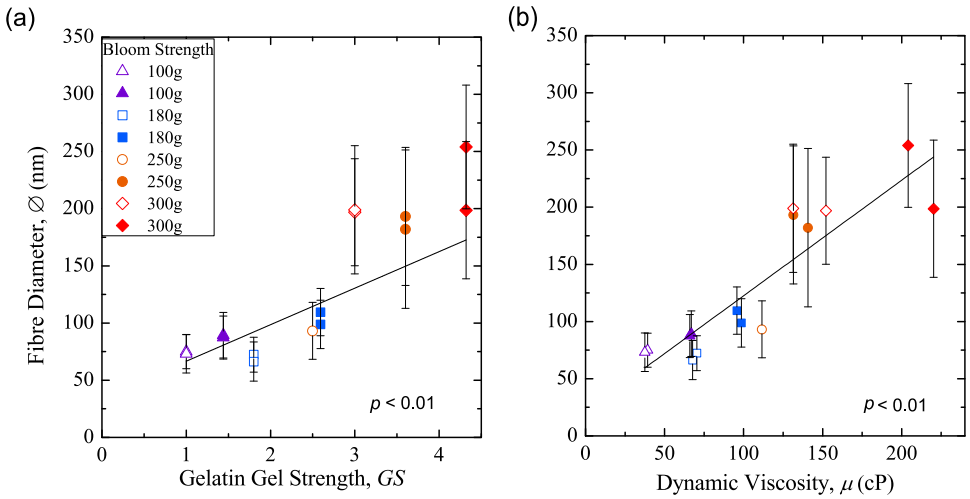

Fig. 4. Fibre diameter, ϕ, against (a) gelatin gel strength, *GS* (Eq. (1)) and (b) dynamic viscosity, μ.

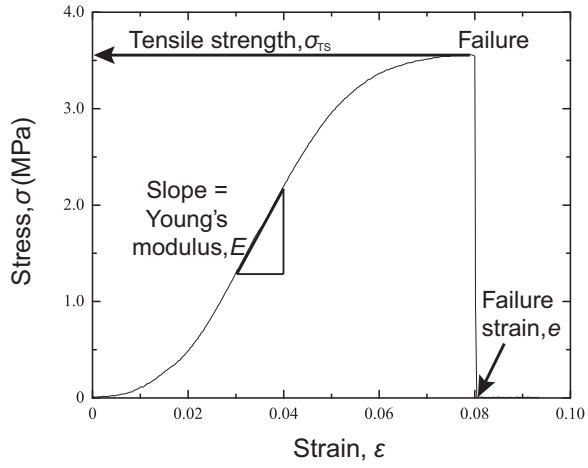

Fig. 5. An example of the stress strain data recorded from tensile testing the meshes. The Young's modulus, E, is measured across the linear portion of the curve, the strength , σ_{TS}, is taken at the peak stress, and the failure strain, e, is recorded at point of failure.

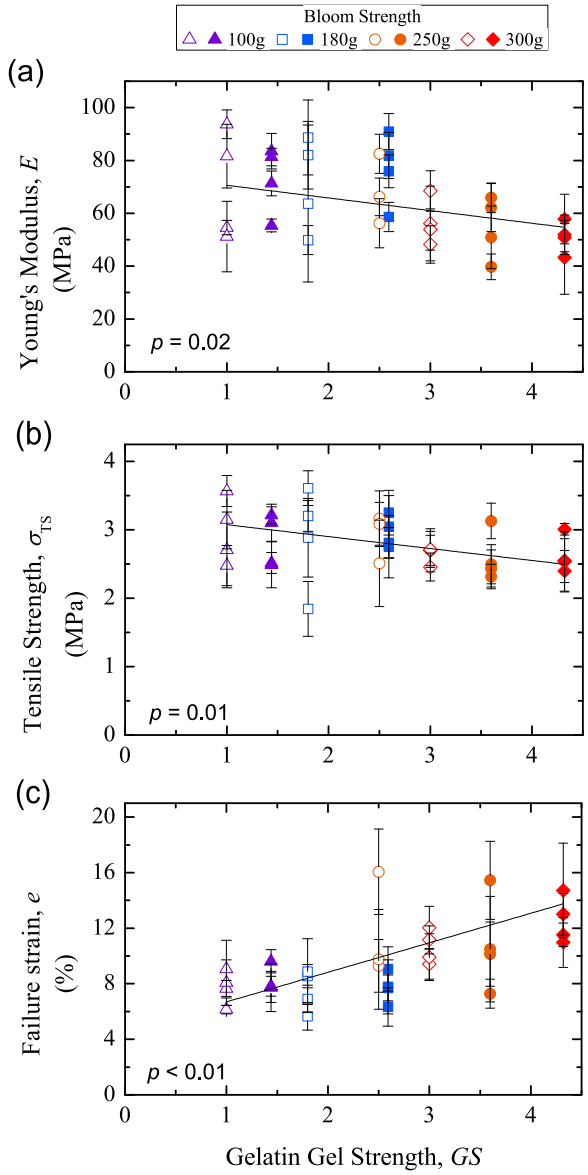

Fig. 6. Effect of gel strength, GS (Eq. (1)) on mechanical properties (a) Young's modulus, E, (b) tensile strength, σ_{TS}, and (c) failure strain, e.

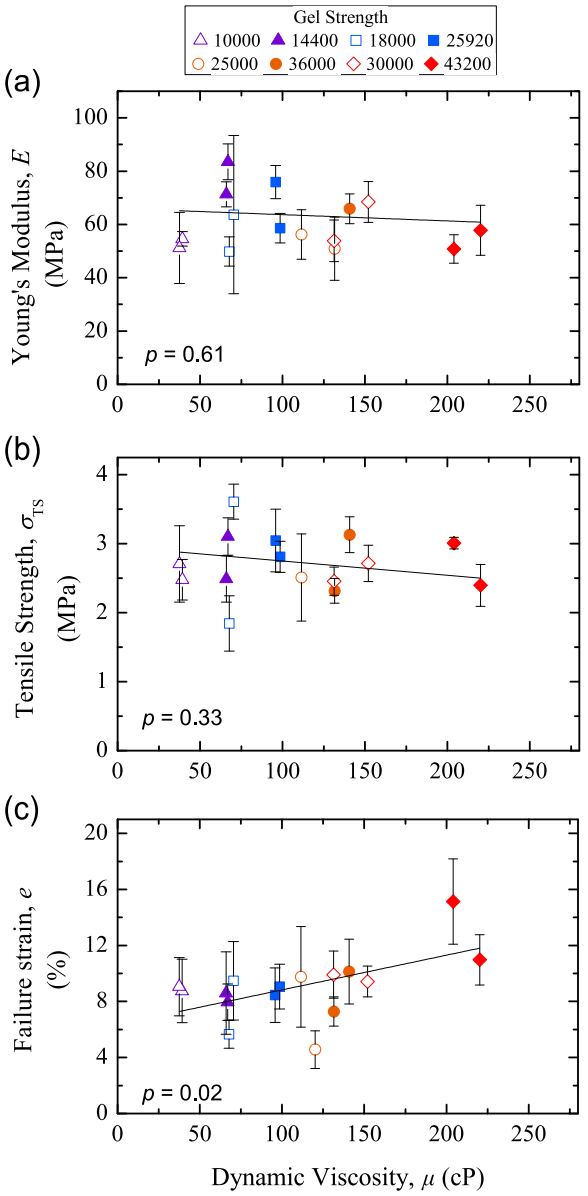

Fig. 7. Effect of solution viscosity, μ, on mechanical properties (a) Young's modulus, E, (b) tensile strength, σ_{TS}, and (c) failure strain, e.

polyelectrolyte, and so increasing the gel strength of a solution should increase the charge density, and therefore the conductivity of the solution. However, this paper showed the effect of gel strength on solution conductivity was insignificant, see Fig. 2, and so the effect of conductivity on mesh morphology and mechanical properties was considered to be beyond the scope of this study.

There was great variability in all the taken measurements, within samples and between samples of the same gel strength. This is most likely due to the large number of variables that affect the electrospinning process, particularly due to the difficulty in controlling environmental variables such as temperature and humidity. Variations in the age of solutions when electrospinning could also account for these differences, suggesting that the age of solution is an additional variable that should be taken into account when electrospinning. Variability within a sample could be explained by the heterogeneous structure of the meshes. Small changes in fibre density across a sample, and the nature of randomly oriented fibres, could account for the large standard deviations.

Electrospun fibre diameter is shown to be dependent on gelatin gel strength and solution viscosity, in accordance with other studies

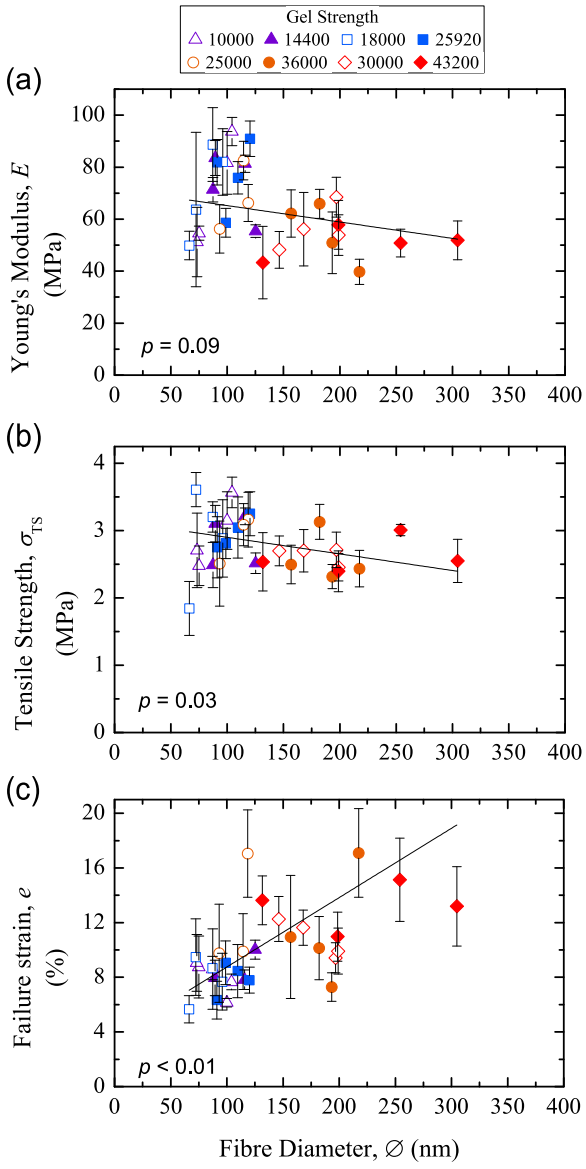

Fig. 8. Fibre diameter effect on mechanical properties (a) Young's modulus, E, (b) tensile strength, σ_{TS}, (c) failure strain, e.

(Nezarati et al., 2013; Harrison et al., 2012; Erencia et al., 2014). The standard deviation of the fibre diameter increases with gel strength, suggesting it would be beneficial to use gelatin solutions of smaller gel strength to create more reproducible samples. As collagen fibrils in soft tissue ECM are closer in size to the smaller diameter fibres seen here, this is convenient for tissue engineering applications.

The tensile modulus, strength and failure strain all show a statistically significant dependence on the solution gel strength. The modulus and strength decrease at higher gel strengths while failure strain increases. Electrospun gelatin mats will therefore have to compromise between strength and ductility. Despite the strong dependence of viscosity on gel strength, there is no such correlation between the material properties and the viscosity of the solution. There is also no statistically significant relationship between the tensile modulus and the fibre diameter of the meshes, and the relationship between tensile strength and fibre diameter is weak. This statistically significant dependence of mechanical properties on gel strength but not on fibre diameter suggests the changing gel strength is affecting another aspect of the mesh morphology.

These results are in contrast to other studies that have demonstrated a relationship between fibre diameter and strength, often

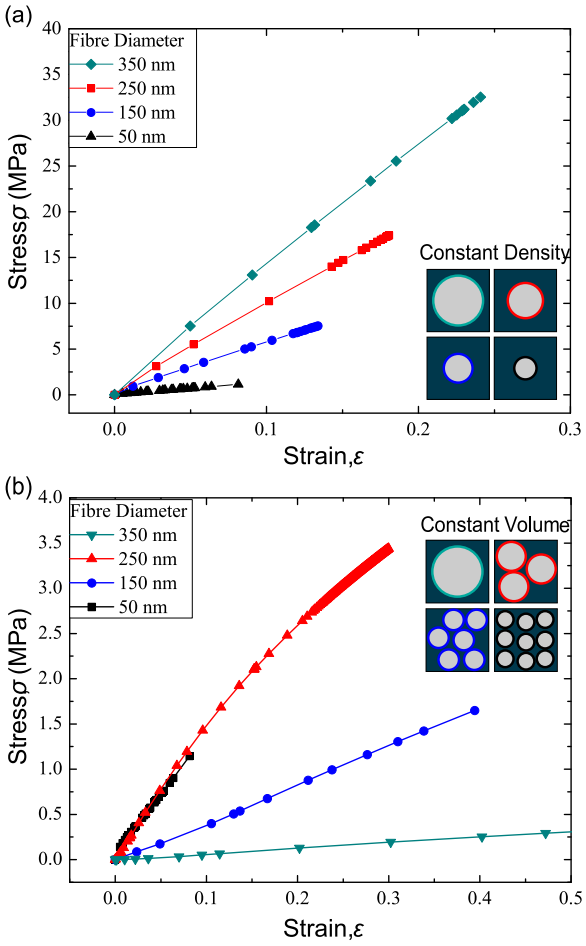

Fig. 9. Stress strain response from finite element analysis models under uniaxial tension. Different fibre sizes for (a) models of constant density and (b) of constant volume.

Table 1
Model specification. Volume, $V = \pi(d/2)^2 \rho A$, where A is the area of the simulation, 200 × 50 μm.

	Diameter, d (nm)	Fibre Density, ρ (mm^{-1})	Volume, V (mm^3)
Constant Density	50	7768	1.52E-07
	150	7768	1.37E-06
	250	7768	3.81E-06
	350	7768	7.47E-06
Constant Volume	50	7768	1.52E-07
	150	820.76	1.45E-07
	250	307.23	1.51E-07
	350	157.29	1.51E-07

showing tensile mechanical properties to improve as fibre diameter decreases (Linh et al., 2010; Wong et al., 2008; Huang et al., 2004). However, McManus et al.showed contradictory results, with values for tensile modulus and peak stress increasing as fibre diameter increased (McManus et al., 2006). The findings of this study suggest that there are other aspects of the electrospun mesh morphology that have greater significance to the mechanical properties than fibre diameter, such as pore size or the interaction between fibres. Further analysis of the mesh morphology is restricted due to the limited resolutions of many 3D imaging techniques, for example micro-computed tomography (micro-CT) has a resolution ranging from 1 to 50 micrometers (Ho and Hutmacher, 2006). These electrospun fibres have diameters ranging from 50 to 350 nm, and the pore sizes can be estimated to vary from 0.5 to 5 μm, so these imaging techniques will not provide a sufficient

level of detail to indicate which aspect of the mesh morphology is having the greatest impact upon the mechanical properties.

Computational modeling has been used to interpret these experimental results. A 2D model of randomly oriented sub-micron fibres was created using finite element (FE) analysis as described elsewhere (Koh et al., 2015). The model was put under uniaxial tension by fixing the nodes at one end and assigning a displacement to nodes at the other. All other nodes, including edges, were free to move horizontally and vertically, and the stress strain response was recorded. The fibre diameters were varied between the minimum and maximum diameters of gelatin fibres produced in this study. The mass of gelatin in each of the meshes produced here was approximately equal as all the electrospun meshes were produced using the same flow rate for a fixed length of time. The area of the meshes remained equal, and the thicknesses were similar, suggesting the volume fraction of gelatin fibres within the meshes remained approximately equal in all samples, despite variances in fibre diameter. The volume fraction can be kept constant in simulations by varying the number of fibres, or number density, see inset Fig. 9b. For comparison, models with constant fibre density and varying volume fraction were also analysed (inset Fig. 9a), in which there is greater space between each fibre. The models are described in Table 1.

Fig. 9 shows the stress strain data for the models of constant density and constant volume; the 50 nm data points in both plots are from the same model. Models with a constant density of fibres show an increase in stiffness as fibre diameter increases, as expected from simple “rule of mixture” models. The stiffness for the constant volume models, however, shows no systematic dependence on fibre diameter, supporting the experimental findings of this study. Nevertheless, the variation in modulus indicates that other properties of the meshes are important. This will be investigated in future studies.

It is likely the change in stiffness seen in the constant density models is caused by decreasing pore size rather than increasing fibre diameter. This demonstrates that controlling the fibre diameter of electrospun meshes is not sufficient to produce materials with consistent mechanical properties. Other aspects of the mesh morphology must be measured to ensure consistency between samples, such as the pore size.

While fibre diameter has shown to not be predictive of mesh mechanical properties, this study has also shown that changing the solution concentration or molecular weight in the electrospinning process has little effect on the mechanics of the mesh. This is indicative that electrospinning can produce meshes with similar mechanical properties despite large variations in solution properties. Electrospinning is a highly adaptable manufacturing method due to the large number of process variables, however, this study has also shown the method to be robust, producing similar materials despite changes in solution parameters. This consistency of the mechanical properties is necessary if electrospinning is to become a commercial tool for tissue engineering applications.

5. Conclusions

Electrospun gelatin fibres have been produced with diameters approaching those of the collagen found within cartilage and other soft tissues. Mechanical properties were found to vary unexpectedly between meshes made from the same solutions, and between meshes with similar fibre diameters. The stiffness was shown to be largely unaffected by fibre diameter, a result that was supported by FE models, as long as the volume fraction of fibres was constant. It is therefore clear that pore size has a great influence on electrospun mesh mechanical properties, and more studies are needed to assess the impact of electrospinning variables on pore size, and the impact of pore size on mesh mechanical properties. The sub-micron fibrous meshes showed similar mechanical properties despite changes in mesh morphology, suggesting electrospinning is a robust process suitable for commercialisation.

Acknowledgments

The author would like to acknowledge Khaow Tonsomboon for his assistance with electrospinning. This work was supported by the UK Engineering and Physical Sciences Research Council (EPSRC) via the Doctoral Training Award, Department of Engineering, University of Cambridge, grant number 1354760. Supporting research data as required by EPSRC research policy may be accessed at http://dx.doi.org/10.17863/CAM.6258.

References

Bhardwaj, N., Kundu, S.C., 2010. Electrospinning: a fascinating fiber fabrication technique. Biotechnol. Adv. 28 (3), 325–347. http://dx.doi.org/10.1016/j.biotechadv.2010.01.004, [URL ⟨http://www.sciencedirect.com/science/article/pii/S0734975010000066⟩].

Chua, W.K., Oyen, M.L., 2009. Viscoelastic properties of membranes measured by spherical indentation. Cell. Mol. Bioeng. 2 (1), 49–56. http://dx.doi.org/10.1007/s12195-009-0049-7, [URL ⟨http://link.springer.com/10.1007/s12195-009-0049-7⟩].

Deitzel, J., Kleinmeyer, J., Harris, D., Beck Tan, N., 2001. The effect of processing variables on the morphology of electrospun nanofibers and textiles. Polymer 42 (1), 261–272. http://dx.doi.org/10.1016/S0032-3861(00)00250-0, [URL ⟨http://www.sciencedirect.com/science/article/pii/S0032386100002500⟩].

Elzoghby, A.O., 2013. Gelatin-based nanoparticles as drug and gene delivery systems: reviewing three decades of research. J. Control. Release : Off. J. Control. Release Soc. 172 (3), 1075–1091. http://dx.doi.org/10.1016/j.jconrel.2013.09.019, [URL ⟨http://www.sciencedirect.com/science/article/pii/S0168365913008079⟩].

Erencia, M., Cano, F., Tornero, J.A., Macanás, J., Carrillo, F., 2014. Resolving the electrospinnability zones and diameter prediction for the electrospinning of the gelatin/water/acetic acid system. Langmuir : ACS J. Surfaces Colloids 30 (24), 7198–7205. http://dx.doi.org/10.1021/la501183f, [URL ⟨http://dx.doi.org/10.1021/la501183f⟩].

Fang, J., Wang, X., Lin, T., 2011. Functional Applications of Electrospun Nanofibers. In: Lin, T., (Ed.), Nanofibers - Production, Properties and Functional Applications, InTech, Ch. 14. http://dx.doi.org/10.5772/916. URL ⟨http://www.intechopen.com/books/nanofibers-production-properties-and-functional-applications/functional-applications-of-electrospun-nanofibers⟩.

Garg, K., Bowlin, G.L., 2011. Electrospinning jets and nanofibrous structures. Biomicrofluidics 5 (1), 13403. http://dx.doi.org/10.1063/1.3567097, [URL ⟨http://www.pubmedcentral.nih.gov/articlerender.fcgi?artid=3082340 & tool=pmcentrez & rendertype=abstract⟩].

Gu, S.-Y., Wang, Z.-M., Ren, J., Zhang, C.-Y., 2009. Electrospinning of gelatin and gelatin/poly(l-lactide) blend and its characteristics for wound dressing. Mater. Sci. Eng.: C 29 (6), 1822–1828. http://dx.doi.org/10.1016/j.msec.2009.02.010, [URL ⟨http://www.sciencedirect.com/science/article/pii/S0928493109000538⟩].

Harrison, B.S., Atala, A., Tiwari, S.K., Venkatraman, S.S., 2012. Importance of viscosity parameters in electrospinning: of monolithic and core-shell fibers. Mater. Sci. Eng.: C 32 (5), 1037–1042. http://dx.doi.org/10.1016/j.msec.2012.02.019, [URL ⟨http://www.sciencedirect.com/science/article/pii/S0928493112000719⟩].

Ho, S.T., Hutmacher, D.W., 2006. A comparison of micro CT with other techniques used in the characterization of scaffolds. Biomaterials 27 (8), 1362–1376. http://dx.doi.org/10.1016/j.biomaterials.2005.08.035, [URL ⟨http://www.sciencedirect.com/science/article/pii/S0142961205007957⟩].

Huang, Z.-M., Zhang, Y., Ramakrishna, S., Lim, C., 2004. Electrospinning and mechanical characterization of gelatin nanofibers. Polymer 45 (15), 5361–5368. http://dx.doi.org/10.1016/j.polymer.2004.04.005, [URL ⟨http://www.sciencedirect.com/science/article/pii/S0032386104003684⟩].

Ji, Y., Liang, K., Shen, X., Bowlin, G.L., 2014. Electrospinning and characterization of chitin nanofibril/polycaprolactone nanocomposite fiber mats. Carbohydr. Polym. 101, 68–74. http://dx.doi.org/10.1016/j.carbpol.2013.09.012, [URL ⟨http://www.sciencedirect.com/science/article/pii/S0144861713009028⟩].

Ki, C.S., Baek, D.H., Gang, K.D., Lee, K.H., Um, I.C., Park, Y.H., 2005. Characterization of gelatin nanofiber prepared from gelatin-formic acid solution. Polymer 46 (14), 5094–5102. http://dx.doi.org/10.1016/j.polymer.2005.04.040, [URL ⟨http://www.sciencedirect.com/science/article/pii/S0032386105004763⟩].

Kimura, N., Kim, B.-S., Kim, I.-S., 2014. Effects of Fe^{2+} ions on morphologies, microstructures and mechanical properties of electrospun nylon-6 nanofibers. Polym. Int. 63 (2), 266–272. http://dx.doi.org/10.1002/pi.4500, [URL ⟨http://doi.wiley.com/10.1002/pi.4500⟩].

Ko, F.K., Wan, Y., 2014. Introduction to Nanofiber Materials. Cambridge University Press. http://dx.doi.org/10.1017/CBO9781139021333.

Koh, C., Strange, D.G., Tonsomboon, K., Oyen, M.L., 2013. Failure mechanisms in fibrous scaffolds. Acta Biomater. 9 (7), 7326–7334. http://dx.doi.org/10.1016/j.actbio.2013.02.046, [URL ⟨http://www.sciencedirect.com/science/article/pii/S1742706113001165⟩].

Koh, C., Low, C.Y., bin Yusof, Y., 2015. Structure-property relationship of bio-inspired fibrous materials. Procedia Comput. Sci. 76, 411–416. http://dx.doi.org/10.1016/j.procs.2015.12.278, [URL ⟨http://www.sciencedirect.com/science/article/pii/S1877050915037795⟩].

Linh, N.T.B., Min, Y.K., Song, H.-Y., Lee, B.-T., 2010. Fabrication of polyvinyl alcohol/gelatin nanofiber composites and evaluation of their material properties. J. Biomed.

Mater. Res. B Appl. Biomater. 95 (1), 184–191. http://dx.doi.org/10.1002/jbm.b.31701, [URL ⟨http://www.ncbi.nlm.nih.gov/pubmed/20737434⟩].

Machado, R., da Costa, A., Sencadas, V., Garcia-Arévalo, C., Costa, C.M., Padrão, J., Gomes, A., Lanceros-Méndez, S., Rodríguez-Cabello, J.C., Casal, M., 2013. Electrospun silk-elastin-like fibre mats for tissue engineering applications. Biomed. Mater. (Bristol, Engl.) 8 (6), 065009. http://dx.doi.org/10.1088/1748-6041/8/6/065009, [URL ⟨http://iopscience.iop.org/1748-605X/8/6/065009/article/⟩].

McManus, M.C., Boland, E.D., Koo, H.P., Barnes, C.P., Pawlowski, K.J., Wnek, G.E., Simpson, D.G., Bowlin, G.L., 2006. Mechanical properties of electrospun fibrinogen structures. Acta Biomater. 2 (1), 19–28. http://dx.doi.org/10.1016/j.actbio.2005.09.008, [URL ⟨http://www.sciencedirect.com/science/article/pii/S1742706105001492⟩].

Mindru, T.B., Mindru, I.B., Malutan, T., Tura, V., 2007. Electrospinning of high concentration gelatin solutions. J. Optoelectron. Adv. Mater. 9 (11), 3633–3638.

Mow, V.C., Huiskes, R., 2005. Basic Orthopaedic Biomechanics & Mechano-biology 3rd Edition. Lippincott Williams & Wilkins, Philadelphia, USA, [URL ⟨http://books.google.com/books?hl=en&lr=&id=_NA-qHOeYLQC&pgis=1⟩].

Nezarati, R.M., Eifert, M.B., Cosgriff-Hernandez, E., 2013. Effects of humidity and solution viscosity on electrospun fiber morphology. Tissue Eng. C Methods 19 (10), 810–819. http://dx.doi.org/10.1089/ten.TEC.2012.0671, [URL ⟨http://online.liebertpub.com/doi/abs/10.1089/ten.tec.2012.0671⟩].

Nuge, T., Hoque, M.E., Yeow, T.K., Nordin, N., Chowdhury, M., 2013. Electrospun gelatin composite nanofibres: a review on structural and mechanical characterizations. Regen. Res. 2 (2), 39–42, [URL ⟨http://www.regres.tesma.org.my/pdf/RR-271113-009~R1~(7)~final.pdf⟩).

Pelipenko, J., Kristl, J., Janković, B., Baumgartner, S., Kocbek, P., 2013. The impact of relative humidity during electrospinning on the morphology and mechanical properties of nanofibers. Int. J. Pharm. 456 (1), 125–134. http://dx.doi.org/10.1016/j.ijpharm.2013.07.078, [URL ⟨http://www.sciencedirect.com/science/article/pii/S0378517313007254⟩].

Pillay, V., Dott, C., Choonara, Y.E., Tyagi, C., Tomar, L., Kumar, P., Du Toit, L.C., Ndesendo, V.M.K., 2013. A review of the effect of processing variables on the fabrication of electrospun nanofibers for drug delivery applications. J. Nanomater. 2013, 789289. http://dx.doi.org/10.1155/2013/789289.

Ramakrishna, S., Fujihara, K., Teo, W.-E., Lim, T.-C., Ma, Z., 2005. An Introduction to Electrospinning and Nanofibers. World Scientific Publishing, [URL ⟨http://www.worldscientific.com/worldscibooks/10.1142/5894⟩].

Samatham, R., Kim, K.J., 2006. Electric current as a control variable in the electrospinning process. Polym. Eng. Sci. 46 (7), 954–959. http://dx.doi.org/10.1002/pen.20565, [URL ⟨http://doi.wiley.com/10.1002/pen.20565⟩].

Schrieber, R., Gareis, H., 2007. Gelatine Handbook: Theory and Industrial Practice. Wiley-VCH, Weinheim, Germany.

Sill, T.J., von Recum, H.A., 2008. Electrospinning: applications in drug delivery and tissue engineering. Biomaterials 29 (13), 1989–2006. http://dx.doi.org/10.1016/j.biomaterials.2008.01.011, [URL ⟨http://www.sciencedirect.com/science/article/pii/S0142961208000203⟩].

Song, J.-H., Kim, H.-E., Kim, H.-W., 2008. Production of electrospun gelatin nanofiber by water-based co-solvent approach. J. Mater. Sci. Mater. Med. 19 (1), 95–102. http://dx.doi.org/10.1007/s10856-007-3169-4, [URL ⟨http://www.ncbi.nlm.nih.gov/pubmed/17577633⟩].

Thompson, C., Chase, G., Yarin, A., Reneker, D., 2007. Effects of parameters on nanofiber diameter determined from electrospinning model. Polymer 48 (23), 6913–6922. http://dx.doi.org/10.1016/j.polymer.2007.09.017, [URL ⟨http://www.sciencedirect.com/science/article/pii/S0032386107009275⟩].

Tonsomboon, K., Oyen, M.L., 2013. Composite electrospun gelatin fiber-alginate gel scaffolds for mechanically robust tissue engineered cornea. J. Mech. Behav. Biomed. Mater. 21, 185–194. http://dx.doi.org/10.1016/j.jmbbm.2013.03.001, [URL ⟨http://www.sciencedirect.com/science/article/pii/S1751616113000799⟩].

Wong, S.-C., Baji, A., Leng, S., 2008. Effect of fiber diameter on tensile properties of electrospun poly(l-caprolactone). Polymer 49 (21), 4713–4722. http://dx.doi.org/10.1016/j.polymer.2008.08.022, [URL ⟨http://www.sciencedirect.com/science/article/pii/S0032386108007003⟩].

Yin, A., Zhang, K., McClure, M.J., Huang, C., Wu, J., Fang, J., Mo, X., Bowlin, G.L., Al-Deyab, S.S., El-Newehy, M., 2013. Electrospinning collagen/chitosan/poly(L-lactic acid-co-ϵ-caprolactone) to form a vascular graf: mechanical and biological characterization. J. Biomed. Mater. Res. A 101 (5), 1292–1301. http://dx.doi.org/10.1002/jbm.a.34434, [URL ⟨http://www.ncbi.nlm.nih.gov/pubmed/23065755⟩].