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Research paper

The viscoelastic response of electrospun poly(vinyl alcohol) mats

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ABSTRACT

Native biological tissues are viscoelastic materials that undergo time-dependent loading in vivo. It is therefore crucial to ensure that biomedical materials have a suitable viscoelastic response for a given application. In this study, the viscoelastic properties of electrospun poly(vinyl alcohol) are investigated using tensile load relaxation testing. A five-parameter generalised Maxwell constitutive model is found to characterise the experimental response. The effect of polymer concentration and electrospinning voltage on model parameters is investigated in detail. The stiffness coefficients for the relaxation process appear to be dependent on the electrospinning conditions used whereas the time constants remain relatively unchanged. It is also observed that the stiffness parameters are linearly correlated with the equilibrium modulus, indicating that a single underlying material property dictates the relaxation moduli. Lastly, it is found that the viscoelastic model parameters are not predicted by the fibre diameter. These results provide an important understanding in designing electrospun mats with desired time-dependent properties.

1. Introduction

Ensuring the mechanical integrity of biomedical materials is critical for their success in applications such as tissue engineering (Mauck et al., 2009), wound healing (Zhong et al., 2010) and drug delivery (Sill and von Recum, 2008). Often, biomedical materials are required to mimic the mechanical properties of native biological tissues e.g. for use as tissue engineering scaffolds (Mauck et al., 2009; Sill and von Recum, 2008). Biological tissues are inherently viscoelastic materials (Fung, 1993) that undergo time-dependent loading such as pulsatile loading in blood vessels. Moreover, at the cellular level, the viscoelastic properties of the substrate material have been shown to influence cell growth and differentiation (Cameron et al., 2011; Chaudhuri et al., 2016). Therefore, it is necessary to characterise and match the viscoelastic response of biomedical materials for particular applications.

In the past two decades, a significant amount of attention has been focused on electrospinning for the fabrication of biomedical materials (Huang and Zhang, 2003; Schiffman and Schauer, 2008). Electrospinning provides a relatively simple, cost effective and versatile technique to create non-woven fibrous materials with fibre diameters in the nano- and micrometer range, making it suitable to mimic the structure of native extracellular matrix (Sill and von Recum, 2008). The properties of electrospun mats depend on a number of processing variables such as the solvent used, humidity, solution concentration and applied voltage (Huang and Zhang, 2003). While there has been some focus on the effect of these processing parameters on the resulting Young's modulus and strength of the samples (Huang et al., 2004; Lee and Deng, 2011; Butcher et al., 2017), their influence on the viscoelastic response remains to be investigated thoroughly.

Poly(vinyl alcohol) (PVA) is commonly used to fabricate electrospun mats due its easy electrospinnability, use of water as solvent and cost effectiveness (Koski et al., 2004; Zhang et al., 2005). Electrospun PVA and its derivatives have been investigated for use as tissue engineering scaffolds (Kim et al., 2006; Shalumon et al., 2009), wound healing and drug delivery patches (Taepaiboon et al., 2006; Zhou et al., 2008), antibacterial coatings (Ignatova et al., 2006) and biosensing membranes (Ren et al., 2006; Wang et al., 2010). For any of these applications undergoing time-dependent loading, the viscoelastic properties of the material become important. In this study, PVA is used as a model material and the viscoelastic response of electrospun PVA characterised using tensile load relaxation testing. In particular, the effect of solution concentration and electrospinning voltage on the time-dependent response is investigated to determine whether processing variables can be used to tune the material viscoelastic response and, if so, in what way. The sample microstructure is observed using scanning electron microscopy and compared to the corresponding mechanical response. It is found that while the electrospinning conditions do influence the viscoelastic properties of the electrospun PVA, this effect does not correlate with the fibre morphology.

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2. Materials and methods

2.1. Materials

Poly(vinyl alcohol) (PVA) with 89–98 kDa molecular weight, 99 + % degree of hydrolysis and 1750 ± 50 degree of polymerisation was obtained from Sigma Aldrich, UK. PVA solutions with concentrations of 8%, 10%, 12%, 14% and 16% w/v were prepared by dissolving the appropriate mass of PVA in deionised water at 80 °C and stirring for 2 h to make transparent solutions. Each solution was allowed to cool at room temperature and used within 2 days.

2.2. Scaffold fabrication by electrospinning

An in-house electrospinning set-up was used in this study. The polymer solution was contained in a 20 ml plastic syringe (BD Plastipak, Spain) with a 21G stainless steel blunt needle (BD Plastic, UK). A high voltage power supply (Glassman High Voltage, UK) was connected to the PVA solution via an alligator clip attached to the syringe needle. The flow rate was set at 0.2 ml/h using a syringe pump (Alaris Medical, UK) and a square 8 × 8 cm collector covered in aluminium foil was used to collect the electrospun fibres with a tip-to-collector distance of 10 cm. Electrospinning was then performed for approximately 8 h for each set of conditions. The average temperature and humidity recorded during the fabrication of all electrospun samples was 21 °C and 43%, respectively. Samples were prepared at applied voltages (V_o) of 10, 12, 14, 16, 18 and 20 kV. No cross-linking was performed. Of the entire parameter space considered, the 16% PVA, 10–12 kV samples could not be prepared due to high solution viscosity leading to blockages in the syringe pump, and the 8% PVA concentration produced flaky samples without a suitable film structure which could not be mechanically tested but were included in the morphology assessment.

2.3. Microstructure characterisation

The morphology of electrospun PVA meshes was observed using a scanning electron microscope (EVO LS 15, Carl Zeiss, UK) at an accelerating voltage of 10 kV after gold coating. The images obtained were analysed using the software ImageJ (Schneider et al., 2012). The mean fibre diameter value, d, was calculated by averaging the diameter of 100 fibres to obtain a statistically representative value.

2.4. Mechanical testing

The viscoelastic response of electrospun PVA was investigated using displacement controlled tensile load relaxation tests. To this extent, at least $n = 3$ mechanical test specimens with grip-to-grip gauge dimensions of 20 mm × 5 mm were prepared from each electrospun mat. Each strip was placed in between two glass slides and the average strip thickness obtained from a set of five readings using a pair of digital vernier callipers (Hangzhou Maxwell Tools, China). Care was taken to avoid compression of the samples. Laboratory tape was applied to the ends of the specimens and the specimens gripped using custom made stainless-steel clamps.

All mechanical testing was performed on a universal testing machine (model 5544, Instron, Canton, MA) with a 500 N load cell (also Instron). A strain-to-failure test was carried out only on the 8%, 18 kV sample with an extension rate of 0.05 mm/s. From the tensile response thus obtained, it was determined that load relaxation tests were to be performed at 1.0% and 1.5% engineering strains ($\triangle l/l$). Two hold strains were used to check whether the material response was linear or non-linear viscoelastic. For the load relaxation tests, specimens were extended at 1.5 mm/s to the peak strain followed by a 150 s hold and return to gauge length. The specimen was allowed to equilibrate for 5 min before the test was repeated for the second hold strain.

2.5. Material model

As will be shown later, the electrospun PVA demonstrated a linear viscoelastic response in the testing regime investigated in this study. A generalised Maxwell model (also called Maxwell-Wiechert model or Prony series), commonly used for linear viscoelastic materials, is used to characterise the relaxation response. The constitutive relationship for the generalised Maxwell model with N chains is:

$$\sigma(t) = E(t)\varepsilon_o \tag{1}$$

$$E(t) = E_e + \sum_{i}^{N-1} E_i \exp\left(\frac{-t}{\tau_i}\right) \tag{2}$$

where $\sigma(t)$ is the observed stress response with time t, ε_o is the hold strain and $E(t)$ is the relaxation response defined as a function of the equilibrium modulus E_e, the time constant τ_i for a relaxation process and the corresponding stiffness modulus E_i for the particular relaxation process.

The experimental load data $P(t)$ is converted to nominal stress $\sigma(t)$ by dividing by the original cross-sectional area A_o (= thickness × width) of each strip to give the engineering stress. Assuming an instantaneous response and a step increase in strain, the initial ramp response was removed from the experimental data. A non-linear curve fitting program (OriginPro 9.1, OriginLab, Northampton, MA) was then used to fit Eq. (2) with an increasing number of chains (N) to the relaxation response.

3. Results and discussion

3.1. Fibre size and morphology

Representative SEM images of samples prepared at an applied voltage of 14 kV and different PVA concentrations are shown in Fig. 1. The 8% PVA concentration samples produced beaded nanofibres at all electrospinning voltages, presumably leading to the flaky sample structure, and were therefore not included in the load relaxation testing. Higher concentration samples produced continuous fibres due to the increased solution viscosity allowing the surface tension to be overcome. For high PVA concentration and high applied voltage, a few thick non-fibrous ribbon-like structures were also observed in the morphology. The average fibre diameter, d, for samples prepared at different electrospinning conditions is shown in Fig. 2. Connecting lines are only shown as a visual aid. The applied voltage does not appear to have a consistent effect on the average fibre diameter whereas increasing solution concentration leads to an overall increase in the fibre size, consistent with previous studies (Supaphol and Chuangchote, 2008).

3.2. Viscoelastic response of electrospun mats

For linear viscoelastic response, the relaxation modulus $E(t) = \sigma(t)/\varepsilon_o$ is independent of hold strain ε_o. Fig. 3(a) shows that the relaxation modulus of a representative specimen for both 1.0% and 1.5% ε_o are nearly identical, indicating that the specimen response is within the linear viscoelastic regime. Generalised Maxwell models with three, five and seven parameters were then fitted to the relaxation response to find the suitable constitutive relationship. From Fig. 3(b), it can be seen that a standard linear solid model (three-parameter fit) does not give a suitable fit to the experimental data whereas a seven-parameter fit is not significantly better than the five-parameter fit. Thus, the five-parameter model was deemed appropriate to characterise the viscoelastic behaviour of electrospun PVA with the constitutive equation as follows:

$$E(t) = E_e + E_1 \exp(-t/\tau_1) + E_2 \exp(-t/\tau_2) \tag{3}$$

Previous studies on electrospun polycaprolactone (Sethuraman

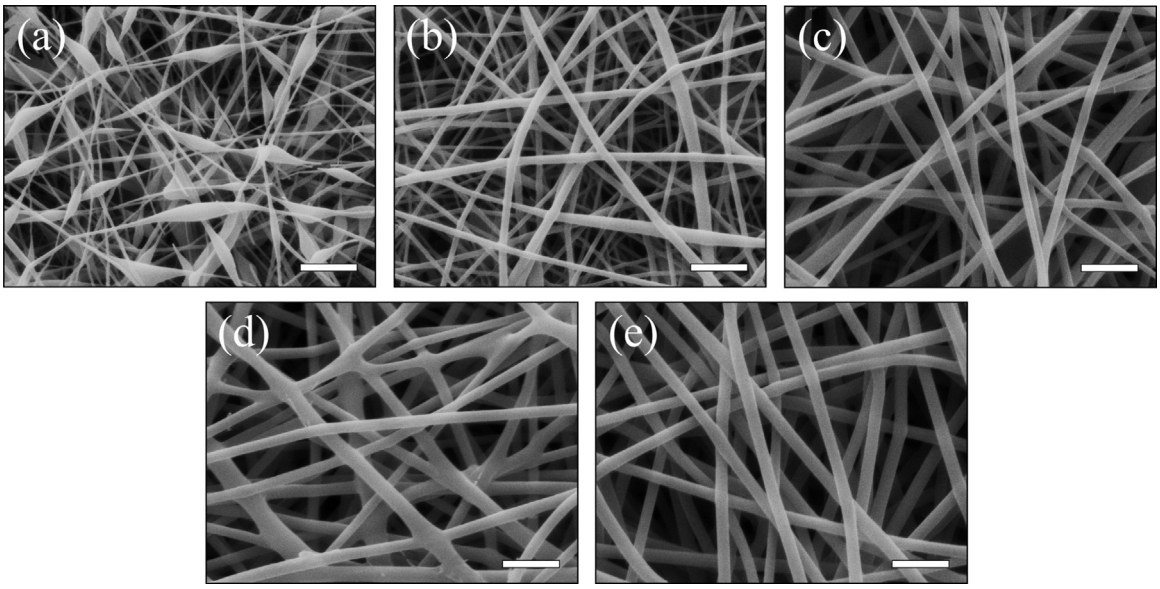

Fig. 1. Representative SEM images of samples electrospun at 14 kV voltage and PVA concentrations of (a) 8, (b) 10, (c) 12, (d) 14 and (e) 16%. The scale bars are 2.0 μm each.

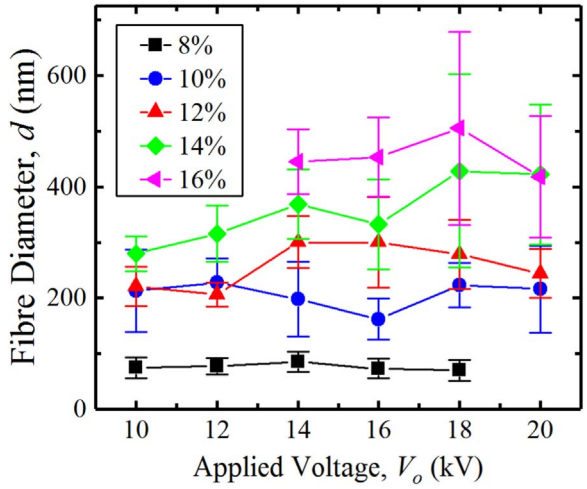

Fig. 2. Average fibre diameter, d, against applied voltage, V_o, for different PVA concentrations. The error bars show one standard deviation.

et al., 2013) and aldehyde based bulk hydrogel films (Tarek et al., 2008) observed that a standard linear solid model was suitable to characterise the time-dependent response of these two materials. Biological tissues, in contrast, are more commonly characterised by five or an even larger number of parameters (Michelle et al., 2004; Machiraju et al., 2006) where the different relaxation time scales have been linked to the different roles of collagen, smooth muscle and elastin (Masayosi and Kimoto, 1966). Thus, it is demonstrated here that electrospun PVA is suitable to mimic complex relaxation processes occurring at two different time scales.

The elastic fraction F of a material is defined as the ratio of the long term equilibrium stiffness to the instantaneous stiffness:

$$F = \frac{E_e}{E_e + E_1 + E_2} \tag{4}$$

Another demonstration of linear viscoelastic behaviour is that the elastic fraction is independent of the instantaneous peak stress σ_o observed after the initial ramp loading (Findley et al., 1976). The elastic fraction for all specimens tested in this study are plotted in Fig. 4 for 1.0% and 1.5% hold strain. The values within three standard deviations of the mean elastic fraction $\overline{F} = 0.43$ are shown as the shaded region

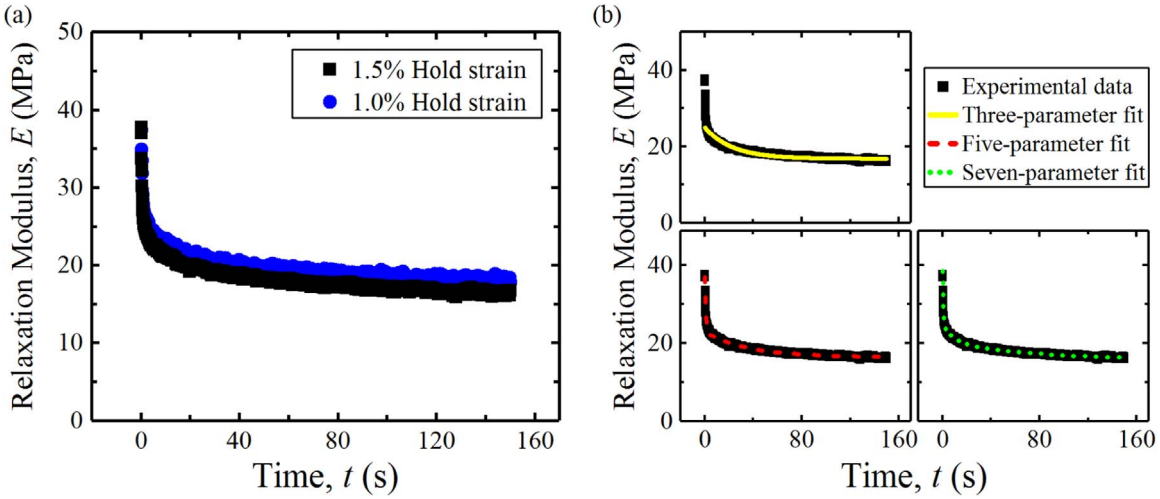

Fig. 3. (a) The relaxation modulus, $E(t)$, for a representative sample showing the linearity of response with respect to hold strain. (b) Results showing generalised Maxwell fit to relaxation modulus, $E(t)$, for a different number of model parameters. Experimental data is for the 1.5% hold strain shown in (a).

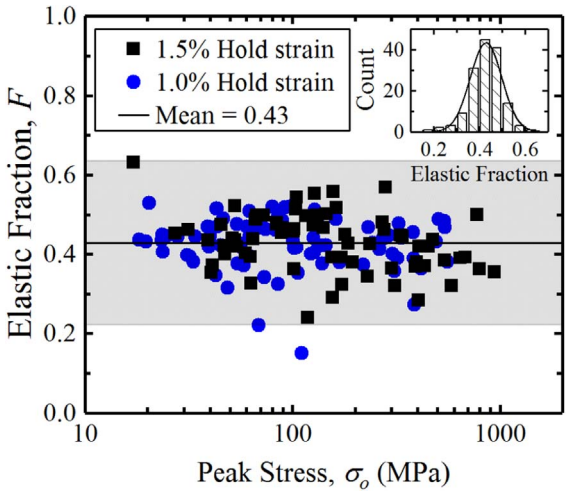

Fig. 4. Plot of elastic fraction, F, against peak stress, σ_o, for all experimental specimens tested in this study. The shaded area represents the mean ± three standard deviations. The inset shows a histogram of the values of elastic fraction obtained from all specimens.

demonstrating that only a few specimens lie outside this range. A nearly normal distribution about the mean value (shown in the inset) is obtained indicating that the global behaviour is indeed linear viscoelastic with the variation in elastic fraction being attributed to experimental factors.

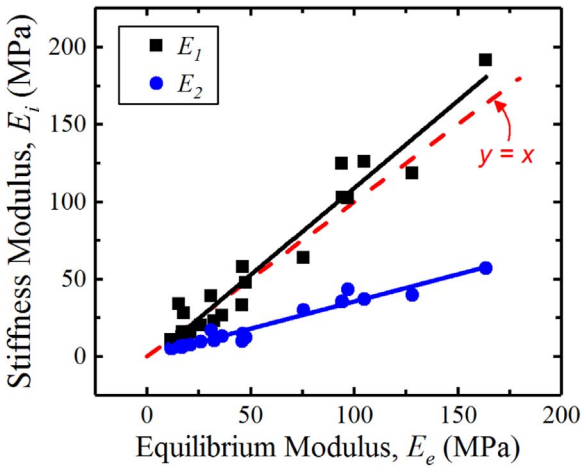

Fig. 6. The stiffness moduli, E_1 and E_2, plotted against equilibrium modulus, E_e, for all electrospun samples. Both linear fits demonstrate a good fit with $p < 0.01$.

The material parameters (E_e, E_1, E_2, τ_1 and τ_2 in Eq. (3)) for each electrospun mat are obtained by averaging across the corresponding specimens, with each specimen's parameters being calculated by averaging over the two hold strains. The results are shown in Fig. 5. The stiffness parameters E_e, E_1 and E_2 all appear to follow a similar trend

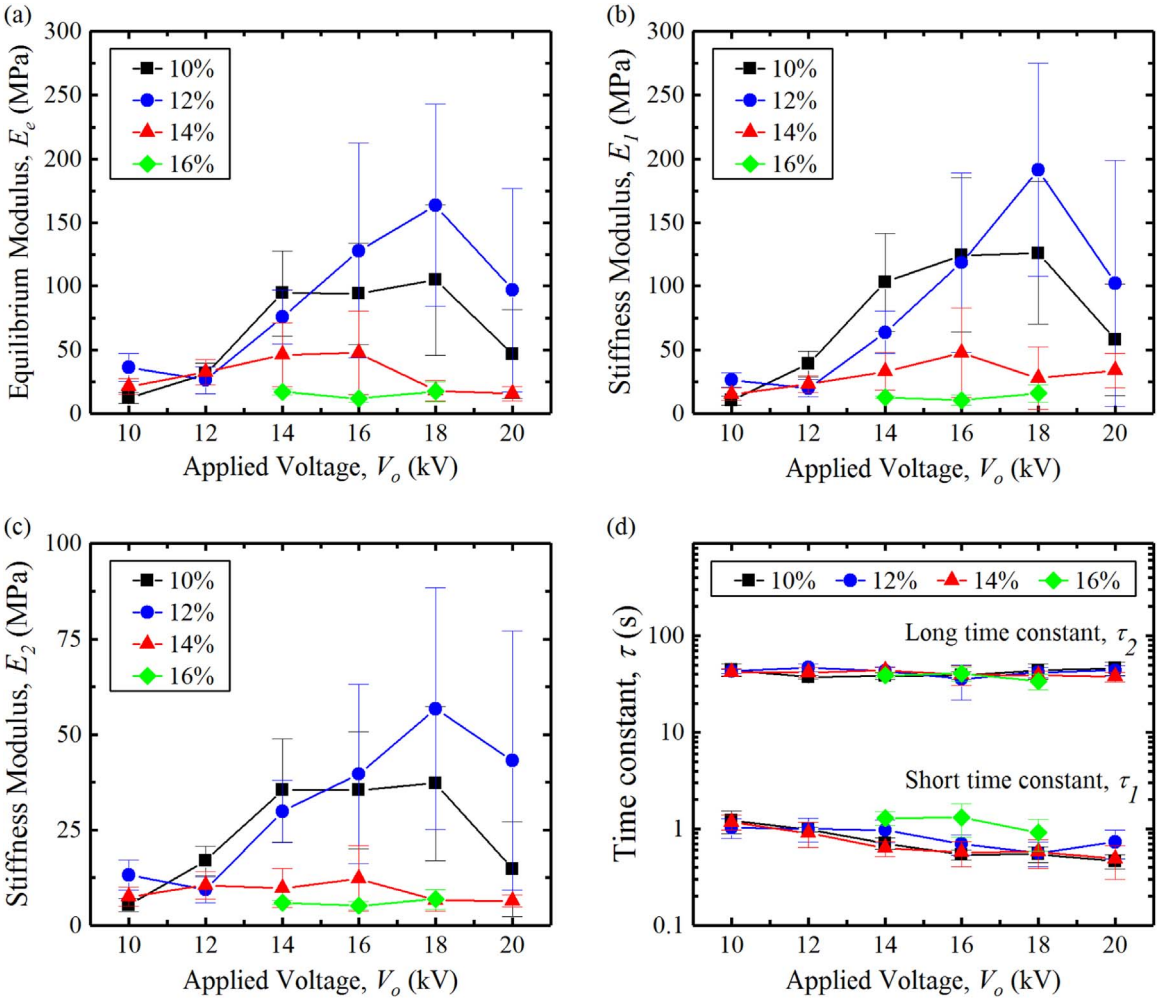

Fig. 5. Plots of model parameters, (a) equilibrium modulus E_e, (b) stiffness modulus E_1, (c) stiffness modulus E_2 and (d) time constants τ_1 and τ_2, against applied voltage, V_o, for different PVA concentrations. The error bars show one standard deviation.

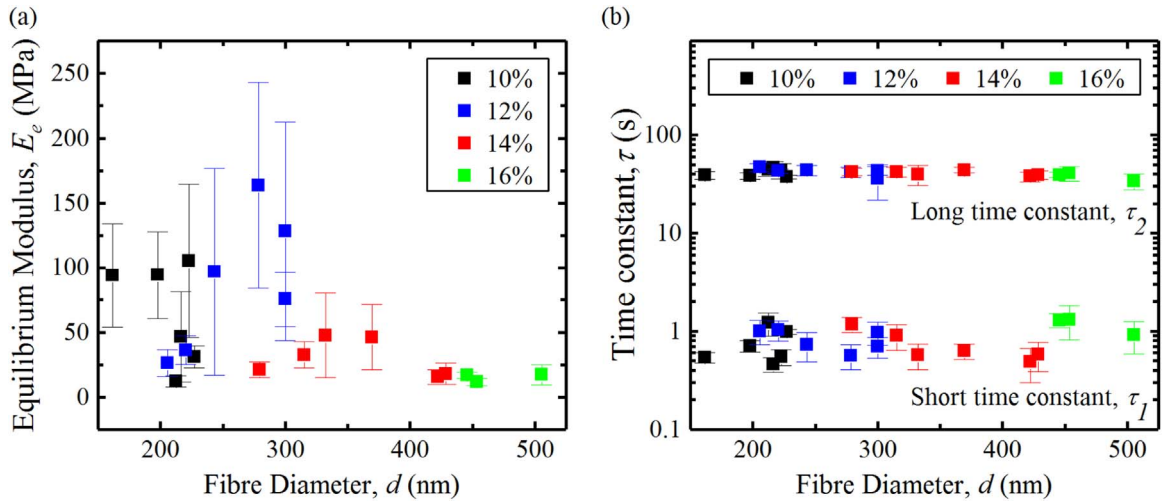

Fig. 7. The values of (a) equilibrium modulus E_e and (b) the time constants τ_1 and τ_2 against the sample average fibre diameter (d). The samples are also distinguished by their PVA concentration. The error bars show one standard deviation.

against electrospinning voltage and concentration, with an increase in the modulus as the electrospinning voltage is increased from 10 kV, reaching a maximum at 16–18 kV and then decreasing with a further increase in voltage. This effect is significantly more pronounced for lower PVA concentrations (see the curves for 10% and 12% PVA) to the extent that the 16% PVA samples have nearly constant, small stiffness parameters for all voltages studied. The standard deviations are also larger for increasing values of stiffness parameters. Thus, overall the stiffness parameters E_e and E_1 are found to be in the range $20 - 200$ MPa and E_2 in the range $5 - 60$ MPa depending on the electrospinning voltage and PVA concentration.

The relaxation time constants are plotted together in Fig. 5(d). The short time constant τ_1 is of the order of one second and the long time constant τ_2 of the order of tens of seconds with average values of 0.8 and 40 s respectively. There appears to be only a small decrease in the short time constant τ_1 with increasing voltage for all PVA concentrations. The 16% PVA sample has a larger τ_1 compared to the other concentrations, each of which have similar values for the short time constant at each voltage. The long time constant τ_2 on the other hand seems to be completely unaffected by solution concentration or applied voltage. Sethuraman et al. Sethuraman et al. (2013) also observed using a standard linear solid model that the relaxation time constant of polycaprolactone (PCL) scaffolds prepared using electrospinning, salt leaching and air drying remains unchanged from one scaffold fabrication technique to another but the stiffness parameters vary. Together with our results, this suggests that perhaps the relaxation time scales are determined by some material-dependent molecular level processes which remain unaffected by the fabrication conditions.

From Fig. 5(a)-(c), it appears that the stiffness parameters follow a similar trend against voltage and solution concentration. To illustrate this effect more clearly, each of the stiffness moduli E_1 and E_2 are plotted against the equilibrium modulus E_e in Fig. 6. A linear correlation (with $p < 0.01$) can be observed between both moduli and E_e. Thus, it can be noted that when comparing a stiff sample to a less stiff one, even though the stress-time response of each sample will be markedly different, each of the stiffness parameters is larger in the same proportion between the two samples. Thus, the relative contributions of the individual relaxation processes remain unchanged from a stiff to a more compliant sample. Moreover, for each sample, it can be observed that E_e and E_1 have similar values as each other, whereas $E_2 \sim E_e/3$, implying that the relaxation processes corresponding to E_e and E_1 are more dominant in the material response. From this correlation, the elastic fraction is also obtained as $F \approx 1/(1 + 1 + 0.33) = 0.43$, the same as the mean value observed experimentally. Using these aforementioned relationships, it is then possible to find all of the stiffness parameters from either the instantaneous or the equilibrium mechanical response.

Lastly, the correlation between model parameters and the average fibre diameter of the electrospun mats is investigated. Since the stiffness moduli have been shown to be correlated to each other, only the equilibrium modulus E_e and the time constants are considered against fibre diameter in Fig. 7. For the equilibrium modulus, there is a greater variation in E_e at small fibre diameters but a consistently small E_e for large fibre diameters. It is clear from the legend that the large diameter values occur at the higher PVA concentrations. The time constants appear to be unaffected for all fibre diameters. Thus, it is observed that the fibre diameter does not affect the viscoelastic properties of the samples. Butcher et al. (2017) observed similar results for the tensile modulus and strength of electrospun gelatin mats finding these properties to be independent of the fibre diameter; it was suggested that some other microstructural property such as pore size might be influencing the mechanical properties. As the tensile modulus corresponds to the stiffness parameters for viscoelastic behaviour, a similar mechanism might be at play here for these stiffness parameters, whereas the time constants appear to be fixed material properties determined by molecular level processes rather than microstructural features.

Overall, these results show that electrospun PVA has a relaxation response spanning short and long time scales of the order of a few seconds and tens of seconds respectively, which is comparable to biological tissues with complicated relaxation responses (Michelle et al., 2004). The stiffness parameters of the viscoelastic response are shown to be correlated to each other, such that any one of them can be used to characterise the other parameters if the correlation expressions are known. Importantly, it is found that the stiffness parameters can be tuned by varying the applied voltage and polymer concentration used during electrospinning, whereas the time constants are a fixed material property. This therefore suggests a constraint on the choice of electrospun materials suitable for required relaxation time-scales, since the time constants cannot be altered by the processing variables. Finally, it is also shown that fibre diameter does not predict the model stiffness parameters, indicating that some other microstructural features need to be considered.

4. Conclusions

Electrospun PVA mats were fabricated at different applied voltages and PVA concentrations and the sample morphology observed using scanning electron microscopy. Tensile load relaxation tests were then

performed to characterise the viscoelastic behaviour. It was shown that a five parameter generalised Maxwell model describes the experimental results well and that the load relaxation testing was done within the linear viscoelastic regime with an elastic fraction that was independent of the applied strain or instantaneous peak stress. The stiffness parameters were found to be correlated to each other with $E_1 \sim E_e$ and $E_2 \sim E_e/3$. Larger stiffness parameters were obtained for PVA concentrations of 10–12% and electrospinning voltages of 16–18 kV. The stiffness decreased with increasing solution concentration and for low or very high voltages. The time constants, on the other hand, were found to be independent of the electrospinning conditions with a short relaxation constant $\tau_1 \sim 0.8$ s and long relaxation constant $\tau_2 \sim 40$ s, indicating that these are a fixed material-dependent property. Lastly, the average fibre diameter was found not to be predictive of the viscoelastic properties of the electrospun mats.

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