Structure development in electrospun fibres of gelatin

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Abstract. Gelatin fibres have been successfully electrospun from water by heating a gelatin solution above the sol-gel transition temperature, and allowing cooling in a controlled environment as the fibres are produced. The development of structure in these fibres is monitored using wide angle x-ray scattering, in this way the presence of the triple helix structure, which provides the physical cross-linkages in the gel, could be probed. There is clear evidence that these structures are obtained in gelatin electrospun from aqueous solutions. In contrast fibres electrospun from a solution of gelatin in glacial acetic acid, showed no evidence of the triple helix structure.

1. Introduction
Electrospinning is a technique for producing nanoscale fibres from a solution with the aid of an electric field [1]. This study looks into the gelling process which can be found in biopolymers such as gelatin in aqueous solutions; and its effect on the electrospinning process. The production of nanofibres is an essential step in the optimisation of a range of biomedical and pharmaceutical applications. These include tissue engineering, drug release, and wound dressings [2]. Gelatin, with its inherent biocompatibility, is an attractive material choice for such applications. Unfortunately, until now gelatin has only been electrospun when dissolved in selected non-aqueous solvents; these include 2,2,2-trifluoroethanol, glacial acetic acid and acetic acid / dimethyl sulfoxide [3], [4]. The advantage of making a nanofibre from water is that the product would be non-toxic, an attractive option for biomedical applications.

Gelatin is a complex biopolymer system containing principally glycine, proline and 4-hydroxyproline [5]. These amino acids are bonded together forming a polymer chain, the state of which is dependent on temperature. In aqueous solution it exhibits a gel at temperatures below ~ 30 °C. The gelation is driven by hydrogen bonding, and in particular, the formation of short sequences of triple helix structures similar to that observed in collagen. Above this temperature the triple helix structure partially unravels reducing the number of hydrogen bonds, and resulting in a change of state from gel to solution [5]. The generation of nanoscale gelatin fibres using electrospinning requires the destabilisation of the triple helix structures. This can be achieved through the use of an acidic solvent or through the hitherto unexplored approach of electrospinning a solution of gelatin and water at an elevated temperature, which is described here for the first time.

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2. Experimental Procedure

2.1 Materials
In this work the gelatin used was bovine gelatin sourced from Gelita with a Bloom value of 210. All chemicals and samples were obtained commercially and used without further purification, except for water which was distilled prior to use. Solutions were made by shaking the required quantity of solid with the hot solvent until all the solid was observed to have dissolved.

2.2 Electrospinning
Electrospinning was performed as shown in Figure 1. A glass syringe mounted in a syringe pump was fitted with a 22 gauge needle of length 2.5 cm (and with an internal diameter of 0.71 mm). A flat aluminium electrode was placed normal to the needle at distances varying from 10 to 40 cm. A Glassman High Voltage power supply was used which allowed defined voltages over the range 7.5-20 kV. In these experiments (unless otherwise stated) a potential of 13kV was used with a needle to collector distance of 14 cm. The temperature of the solution was maintained at the desired temperature (Ts) using a heating stage, while an air conditioning unit provided some control of the ambient temperature (Ta) within an environmental chamber.

2.3 Wide angle x-ray scattering (WAXS)
WAXS data on fibre samples at 5 °C over an extended |Q| range were obtained using a symmetrical transmission diffractometer equipped with a graphite monochromator and pinhole collimation and a Cu K source (with a wavelength of 1.54 Å). The intensity values from samples were obtained as a function of |Q| over the range 0.2 to 6 Å⁻¹ in steps of 0.02 Å⁻¹ where |Q| = 4πsinθ/λ, 2θ is the scattering angle and λ is the incident x-ray wavelength. The fibres were held on a temperature controlled plate at 5 °C to ensure they did not melt (and hence retained their molecular structures).

3. Results

3.1 Electrospinning fibres from a solution of gelatin and acetic acid.
Gelatin was dissolved in acetic acid to form a 30% w/v concentration solution. This was then electrospun to produce smooth fibres, without beading, as shown in the SEM image in figure 2a. The fibres produced had an average fibre diameter of approximately 1000 nm, which is of a similar order of magnitude to the fibres produced by Choktaweesap et al [4]. In order to establish the presence of any triple helices, similar fibres were produced for a WAXS experiment, the results of which are shown in figure 2b. These show a disordered polymer with no peaks at Q = 2.2 Å⁻¹, which are characteristic of a triple helix when gelatin is dissolved in water and in the gel state as shown by Bigi et al [6].
3.3 Electrospinning fibres from a solution of gelatin and water.

We have developed a methodology which allows gelatin fibres to be spun directly from aqueous solutions by holding the solution at an elevated temperature. The air temperature is adjusted to optimise the process, controlling the rate of gelation to facilitate electrospinning. In this way fibres can be directly electrospun from an aqueous gelatin solution as shown in figure 3a. The fibres were free from beading and essentially cylindrical; with variable diameters depending on the conditions chosen. A typical distribution of fibre diameters is shown in figure 3b.

It was found that electrospinning aqueous gelatin fibres is not possible for Ts < 36 °C; at these temperatures presumably the solutions become too viscous. A general trend is observed for the fibres prepared at different temperatures, in that an increasing diameter is coupled with increasing values of Ts as shown in Table 1. This can be correlated with the change in viscosity, and the greater delay in any gelation. As a comparison, gelatin fibres electrospun from a 30% w/v acetic acid solution using similar experimental conditions, other than the temperature, Ts = 25 °C, generated fibres with a diameter of ~ 1000 nm.

3.2 Structure and Gelation

Triple helices found within the collagen molecular structure have been identified by x-ray diffraction by their characteristic diffraction rings. The rings correspond to two distances, 11 Å and 2.9 Å. The first refers to the diameter of the triple helix and the second relates to the distance between the amino acid residues along the helix [6]; thus we analysed the fibres with a view to monitoring the x-ray scattering at values for |Q| of 0.571Å⁻¹ and 2.17 Å⁻¹. As an initial probe of the way in which such helices form, the WAXS curves (Figure 3) were obtained at room temperature from gelatin and water samples cast at the temperatures shown and the water removed by reduced pressure. The sharper peaks at ~ 0.5 Å⁻¹ and 2.2 Å⁻¹ are a direct signature of the formation of the triple helix structures; materials prepared at temperatures above the sol-gel transition temperature do not exhibit this feature.

Table 1. Fibre diameter as a function of Ts at which the fibres were electrospun.

| Ts (°C) | Average Fibre Diameter (nm) |
|--------|-----------------------------|
| 25     | no fibres                   |
| 37     | 295                         |
| 43     | 269                         |
| 49     | 306                         |
| 52     | 376                         |
Figure 4. Wide-angle x-ray scattering data recorded for gelatin samples cast from aqueous solutions at the temperatures indicated and dried at that temperature.

3.4 Structure in aqueous gelatin fibres
Unlike those fibres spun from acetic acid, (Figure 2b) the wide-angle scattering of the aqueous gelatin fibres shows that there is significant triple helix content. For example, Figure 5 shows the WAXS of fibres spun with $T_s = 45 \degree C$ and $53 \degree C$ although the triple helix content is significant, it is rather less than that exhibited by a room temperature gel. Interestingly the features arising from the triple helix appeared stronger both at a higher spinning temperature, and when the fibres were left to dry.

Figure 5. Wide-angle x-ray scattering data for gelatin fibres prepared by electrospinning from aqueous solution with $T_s = 45 \degree C$, $53 \degree C$ and $T_a = 36 \degree C$.

4. Conclusions
Nanoscale fibres of gelatin can be directly prepared from aqueous solution using thermal electrospinning techniques for $T_s > 36 \degree C$. Fibres prepared from aqueous solution exhibit a size distribution which is dependent on the temperature of the electrospinning solution. Wide-angle x-ray scattering shows that these fibres prepared by this thermal electrospinning process show some triple helix content, but less than that observed in the equivalent room temperature gelatin gels. Gelatin fibres prepared from acetic acid solution at $25 \degree C$ show no triple helix content. The successful production of non-toxic gelatin nanofibres provides an additional route to biomedical applications.

5. References
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