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Regression analysis of manufacturing electrospun nonwoven nanotextiles

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Abstract Electrospinning is a simple and relatively inexpensive method of producing nanofibres by solidification of a polymer solution, stretched by an electric field. In the present work, the results of a systematic investigation of the effects of varying manufacturing parameters on the electrospinning of nanotextiles are reported. The physical and mathematical descriptions of the electrospinning process still remain challenging despite several reported parametric studies performed under various experimental configurations. Since the quality of the nanofibres produced using electrospinning is defined by their fineness and variations of diameter, the relationship between fibre diameter and production parameters has been studied here using multiple regression analysis (MRA) to facilitate quality control of the produced nanofibres. The governing parameters investigated are the concentration and feed rate of polymer solution, applied voltage and the relative humidity of the enclosed area. The results show that polymer concentration and feed rate have significant and controlled impacts on producing fibres with diameters in the nano-range. Voltage and humidity also have considerable effects although their contributions to fibre stretching cannot be well-controlled. It is evident that the relationship derived from the two major factors, polymer concentration and feed rate, can predict the produced fibre diameter more accurately compared to that derived from all factors.

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Introduction

Nanofibres are of industrial and scientific interests due to their large aspect (length-to-diameter) ratios and high surface areas per unit volume. Production of synthetic filaments using electrostatic forces (electrospinning) has been known for more than 100 years. This technique has been used to convert a large variety of polymers into nanofibres and may be the only process that has the potential for mass production of nanofibrous mat or nanotextile [1–3]. The potential applications of these nanotextiles include the areas of tissue engineering (scaffolds), drug delivery media, filtration media, clothes protection. The porosity and pore size of the resulting nanofibrous mat or fabric are crucial for filter media to determine the efficiency as well as pressure drop and permeability. This filtering efficiency increases with a reduction in fibre diameter [4]. Researchers around the globe have used various biocompatible and biodegradable synthetic polymers including poly(lactic) and glycolic acids (PLA and PGA, respectively) and their copolymers (PLAGA), polycaprolactone (PCL), polydioxanone (PDO), etc. for scaffold fabrication [1, 3, 5]. Electrospun nanotextiles meet the essential design criteria of ideal engineered tissue scaffolds based upon their abilities to support and guide cell growth. Most publications indicate that an electrospun nanofibrous structure is capable of supporting cell attachment and proliferation. Cells seeded on this structure tend to maintain phenotypic shape and have their growths guided according to nanofibre orientation. For tissue-engineering applications, Li et al. [6] have developed an electrospun structure, composed of

poly(D,L-lactide-*co*-glycolide) PLGA fibres ranging from 500 to 800 nm in diameter. The structure features a morphologic similarity to the extracellular matrix of natural tissue, which is characterised by a wide range of pore diameter distribution, high porosity and effective mechanical properties. Electrospinning can also be used to create biocompatible thin films with useful coating design and surface structure. These films can be deposited on implantable devices in order to facilitate the integration of these devices with the recipient body. Silk-like polymer with fibronectine functionality (extracellular matrix proteins) has been electrospun to make biocompatible films used on prosthetic devices, aimed to be implanted in the central nervous system [7]. Recently, biodegradable PLLA based conducting nanofibrous textile has been manufactured, which may be of special interest in tissue engineering [8]. There has also been a report on encapsulation of particles into electrospun polymeric nanofibres [9, 10]. This was achieved by adding insoluble particles, such as gold, pollen spore, algimate, etc., to the polymer solution. Substances, e.g. soluble drugs, antibacterial agents, were also added for wound healing or functionalising the fibres. A skin mask was produced by directly electrospinning fibres onto the skin surface in order to protect and heal wounds eventually [10]. Nanotextiles made of fibres from biodegradable polymers may be helpful in adjusting the degradation rate of a specified biomaterial under in vivo environment [2]. Although no systematic research on the influence of nanofibre diameter on the degradation behaviour of polymers has been reported, there is evidence that diameters do affect the degradation features and related mechanical properties of the materials used. Furthermore, it has been theorised that the cells attach and organise well around fibres with diameters smaller than the diameters of the cells [2, 11].

The electrospinning process, in its simplest form (Fig. 1), consists of a pipette or a syringe to hold the polymer solution, a collector, a DC voltage supply in the kilovolt range. There is a positive electrode attached to the polymer solution, and a negative electrode attached to the grounded collector. The polymer droplet from the tip of the pipette is drawn into a jet because of the applied high voltage. The charged jet is ejected from the tip at a critical voltage when the repulsive electrostatic force overcomes the surface tension of the polymer fluid. The charge causes the jet to bend in such a way that every time the polymer jet loops, its diameter is reduced due to the evaporation of the solvent in the polymer jet. The jet diameter is eventually reduced to nano-dimensions before it reaches the collector. Once the polymer jet solidifies, the produced nanofibres are collected as a web or mat on the surface of a collector. Although electrospinning appears to be straightforward, it is a rather intricate process that depends on a multitude of

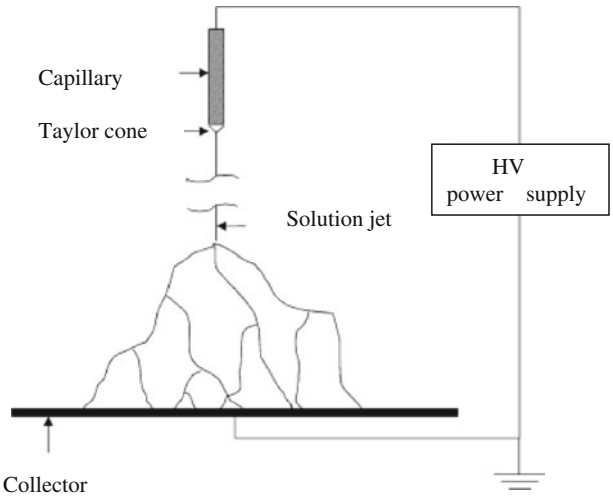

Fig. 1 Basic schematic diagram of electrospinning process [12]

molecular, process related, and technical parameters [3]. It is vital to produce nanotextiles in a controlled manner so that the process gives high quality fibres with precise fibre morphology.

A significant amount of the information regarding electrospinning process comes from empirical observations, but the complexity of the process makes the empirical determination of parametric effects very difficult, if not impractical. It has been well established that both operating parameters and material properties affect the electrospinning process and the resulting fibre morphology [3, 13, 14]. Therefore, an understanding of the process—structure–property relationship—is essential for engineering polymer nanofibres to meet the demands of applications [15]. For nanofibrous textiles to be acceptable in many applications, the fibres should have fine but uniform diameters and a very small (preferably zero) number of beads [13], which are the non-porous polymeric blobs that are created due to many reasons including low polymer concentrations and extreme voltages [13, 16]. A previous study has demonstrated the possibility of making bead-free nanofibres with a good range of diameters through electrospinning and has established suitable parametric combinations using Taguchi methodology [13]. To achieve a better understanding of the relationship between electrospinning parameters and the morphology of the electrospun product, this paper describes a systematic study and the development of predictive models using multiple regression analysis (MRA). The accuracy of a derived model has also been established with the necessary experimental results. Although Thompson et al. [17] have investigated electrospinning process by developing a regression model based on each individual parameter; the present study involves multiple parameters and their combined effects. The analyses have been focused on the experiments producing nanofibres with very few or no beads.

Experimental procedure

Manufacturing of nanotextile

Electrospinning of poly(lactic) acid was accomplished using solutions of PLLA (NatureWorks 3051D) with weight average molar mass about 1.044×10^5 g/mol (measured using gel permeation chromatography). Dichloromethane (DCM) was used as the main solvent and dimethyl formamide (DMF) was used for enhanced conductivity. Experiments were performed on a set-up that included a power supply (capable of generating high voltage) a syringe as capillary tube and a collector as target. A close cabinet, Fig. 2, using transparent poly(methyl methacrylate) thick sheet designed for electrospinning, has been used for dust protection, reduced air turbulence and extra security of the operator. PLLA was dissolved in a mixture of DCM and DMF (Sigma–Aldrich) (60:40 by volume) to prepare different concentrations of 4, 7 and 10% (w/v). The solution was delivered to the tip of a needle through a hypodermic glass syringe (Popper & Sons, Inc) as a capillary tube. The flow rate of the liquid spinnable polymer was controlled using a programmable syringe pump (Cole-Parmer Hz 50/60, cat#. 789100C). The same type of hypodermic needle (20G1TW of 0.9 mm × 25 mm from BD PrecisionGlideTM Needle) has been used throughout the experimental work. The power supply used was from Spellman DEL HVPS INST 230-30R that could make potential difference up to 30 kV. As the electric field increased beyond 7 kV, the hemispherical surface of the solution at the tip of the capillary tube extended to form a cone-like structure, commonly known as Taylor cone [15]. Commercial aluminium foil used for collecting the nanofibres was kept at a distance so that the solvent could evaporate and nanofibres could get enough flight time to dry. Aluminium stubs that had been well cleaned to create a smooth and glossy surface were used to collect the sample for visualisation under scanning electron microscope (SEM). The distance between the collector and the needle tip was maintained at 80–120 mm; white nanofibres adhered smoothly on the aluminium foil with a voltage in the range of 7–12 kV. Saturated salt solutions have been used to maintain the required relative humidity (RH) inside the cabinet. A constant temperature of 20 °C has been maintained throughout the study.

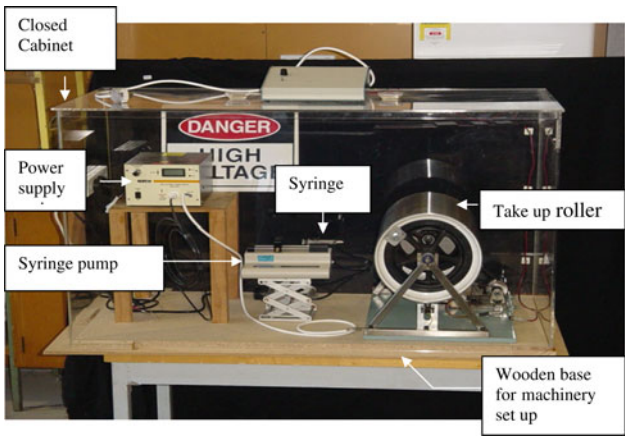

Fig. 2 Electrospinning set-up for this study

Selection of parameters

The four factors, namely (i) concentration of polymer solution, (ii) feed rate, (iii) applied voltage and (iv) RH of the closed area, have been considered at three different levels, as shown in Table 1, to produce nanofibres under various conditions. The properties of a polymer solution have significant influences on the electrospinning process and the resulting fibre morphology. The conductivity and viscosity of the solution determine the extent of elongation of the stretched polymer solution [18, 19]. The viscosity of the polymer solution depends on both the molecular weight of the polymer and the concentration of the polymer solution [13, 19]. In the current study, the molecular weight of the polymer was not varied and neither the conductivity of the polymer solution was considered as a varying parameter due to the constant proportion of the two solvents (60:40 by volume). This solution was capable of making almost bead-free nanofibres, hence there was no need to enhance the solution conductivity by adding any salt or electrolyte. However, the feed rate of the polymer solution is an important parameter because it determines the amount of solution available for stretching to form nanofibres and plays a vital role in determining the fibre diameter and bead formation [20]. As recommended in reported studies [19–21], a concentration of 4–10% w/v and a feed rate of 0.5–2 mL h^{-1} were chosen for this study, Table 1. It is to be noted that although a feed rate of 0.5 mL h^{-1} performed well at times, it had a tendency to clog the needle tip. So precautions had been taken by cleaning the tip frequently.

Higher electrical forces (i.e. larger applied voltage) provide extra stretch to elongate the fibres and thus produce

Table 1 Factors and levels used in the experiments

Factors	Description	Level 1	Level 2	Level 3
A	Concentration of polymer solution (%w/v)	4	7	10
B	Feed rate of polymer (mL h^{-1})	0.5	1	2
C	Voltage (kV)	8	10	12
D	Relative humidity (%)	20	40	75

Level 1, 2 and 3 has been depicted as 0, 1 and 2, respectively, in the experimental layout plan

nanofibres with reduced diameters. The starting voltage in this study was 6 kV and the spinning rate increased with increasing voltage. At voltages higher than 15 kV, the spinning rate became too fast to control and the jet flow got diverged. Consequently, 8–12 kV was selected as an appropriate voltage range. With increased voltage, the acceleration of the fibre also increases [19]. This reduces the flight time of the electrospinning jet, resulting in increased diameter as jet does not have enough time to stretch and elongate before depositing on the collector. However, it has been recommended that independent of the polymer, solvent and the solution concentration, a voltage of 1 kV per centimetre between the nozzle and collector can be used [4].

Ambient parameters such as RH, temperature and gas composition also influence the electrospinning process and the outcome. A change in temperature causes the variation of the solvent evaporation rate and the solution viscosity, which are two opposing factors affecting the mean fibre diameter [22]. However, in the present research, temperature has been found not to be critically important, hence it will not be considered for further analysis. But it was realised that the RH of the environment would determine the rate of solvent evaporation in the solution. It may make the nanofibres thicker or thinner, depending on the chemical nature of the polymer and high RH may even cause the formation of pores on the fibre surface. Generally, finer diameters have been found in lower RH environment during electrospinning of PLLA, and a higher RH has caused rather coarse diameters (average 500 nm) with a possibility of bead forming while keeping other parameters the same as the set of previous research where bead-free fibres were produced. In order to understand the overall effect of changing RH on the resulting fibre diameter, three different RH values, 20, 40 and 75%, have been investigated in this study.

The electrospun nanofibres were studied using a SEM (Philips XL30S). With an image processing software (Uthansca), measurements of nanofibre diameter were made from the micrographs. Scanning micrographs were taken from five different places of a sample, produced in a pre-set condition and from each micrograph, eight individual fibre strands were examined resulting in a total of 40 fibres being considered from the same set of experimental condition to determine the average fibre diameter produced from each experiment.

Design of experiment

For this study, four factors with three different levels of each have been considered, as shown in Table 1. However, in order to facilitate the analytical process, instead of normal factorial design (3^4), a 9-run orthogonal array [23], Table 2, based on Taguchi's Design of Experiment technique, was chosen due to its capability to check the interactions among various factors [23, 24]. The sequence in which these trials were carried out has been randomised and the three levels of each factor have been represented by '0', '1' and '2' in the matrix. Each of the nine conditions was repeated once and the other 18 combinations of conditions, yielding uniform nanofibres were used to produce the electrospun samples. Therefore, a total of 36 experiments have been carried out in an effort to ensure the accuracy of the MRA.

Table 2 Experimental layout plan using nine runs (L_9)

Experiment number	Electrospinning parameter level			
	A Polymer concentration	B Feed rate	C Voltage	D Humidity
1	A_0	B_0	C_0	D_0
2	A_0	B_1	C_1	D_1
3	A_0	B_2	C_2	D_2
4	A_1	B_0	C_1	D_2
5	A_1	B_1	C_2	D_0
6	A_1	B_2	C_0	D_1
7	A_2	B_0	C_2	D_1
8	A_2	B_1	C_0	D_2
9	A_2	B_2	C_1	D_0

Results and discussion

Model development

In order to establish the relationship between the fibre diameter and each of the two primary factors of electrospinning, polymer concentration and feed rate, a linear regression analysis was carried out considering individually the effects of varying polymer concentration and feed rate on the resulting fibre diameter. The results are shown in Fig. 3a, b with two forms of data fit, a simple linear form and a power law. From these individual linear regression results, it appears that despite scattering of the fibre diameter formed from each polymer concentration or feed rate, the polymer concentration–fibre diameter correlations show better agreements in predicting the experimental behaviour compared to those from feed rate–fibre diameter equations. Both the power law and the linear relationships describe the experimental trend with very similar accuracy, judging from the close R^2 values [25]. Therefore, either form of relationship can be used to predict the diameter of the

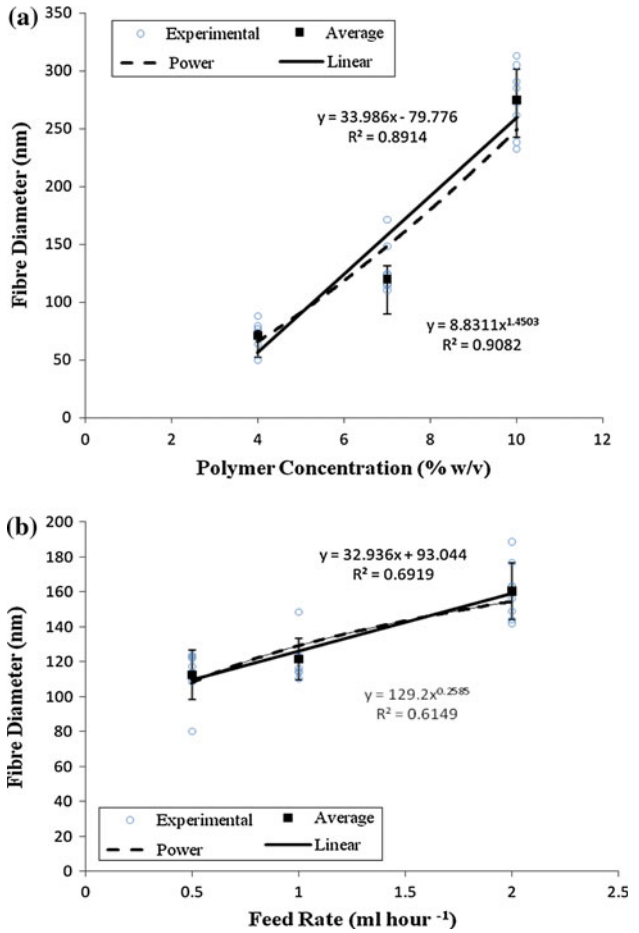

Fig. 3 Typical regression results of fibre diameter against **a** polymer concentration and **b** feed rate (Error bars indicating ±1 standard deviation)

nanofibres when considering only one particular operational parameter. The polymer concentration is shown to have a stronger effect on the final diameter with its resulting trend lines displaying generally higher slopes, Fig. 3a, b.

To determine the effects of multiple parameters on the resulting fibre diameters, a MRA has been carried out. The general equations considering polymer concentration and feed rate together for determination of diameter is shown in Eq. 1.

$$D = 10^{0.920} \times \mathrm{PC}^{1.463} \times \mathrm{FR}^{0.266} \quad R^2 = 0.9556 \qquad (1)$$

If all of the essential parameters of electrospinning, including applied voltage and RH, are taken into account, then a more general equation can be derived, as given in Eq. 2

$$D = 10^{-0.676} \times \mathrm{PC}^{1.410} \times \mathrm{FR}^{0.208} \times \mathrm{VOLT}^{0.515} \times \mathrm{RH}^{0.719}$$
$$R^2 = 0.8567 \qquad (2)$$

where D is fibre diameter (nm), PC is polymer concentration (%w/v), FR is feed rate of polymer solution (mL h^{-1}), VOLT is applied electrical voltage (kV) and RH is relative humidity (%) inside the cabinet. Compared to the experimental data obtained for specific elctrospinning conditions, the values calculated by the general equation can predict the trend with sufficient accuracy. However, even with a reasonably high modelling accuracy, predictions made from the derived equations sometimes may not be able to match the experimental data for a particular set of electrospinning conditions.

Using Eq. 1, the effects of PC and FR on the fibre diameter have been displayed in a similar manner as in Fig. 4a, b. These show clearly that most of the experimental data lies within a ±15% zone of the predicted values. Hence, it can be stated confidently that the derived general equations can be used as reasonably reliable guides

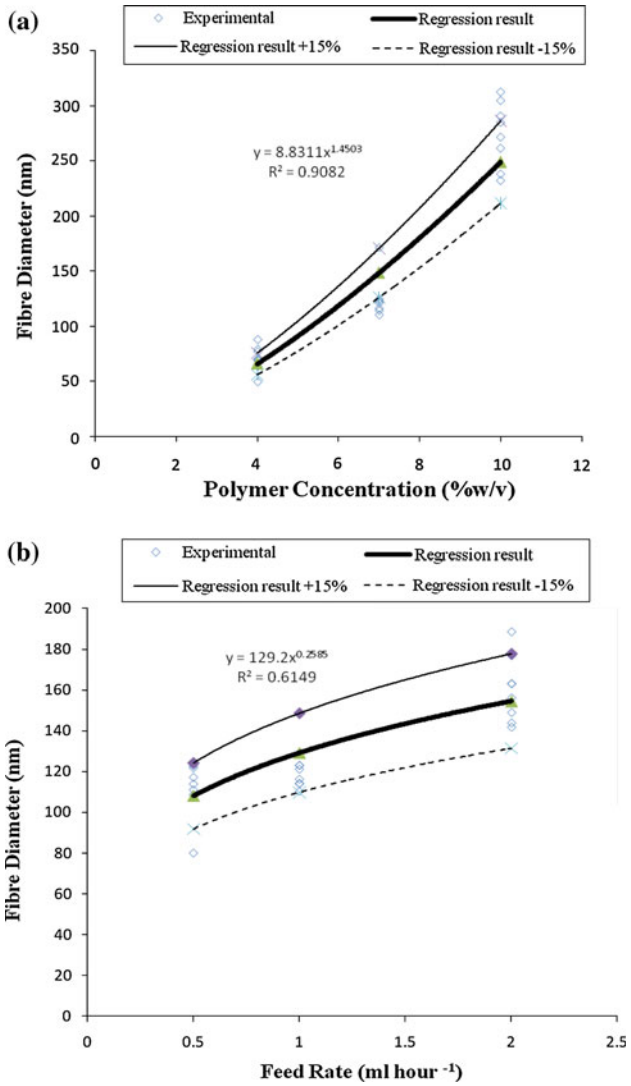

Fig. 4 Typical general regression results of fibre diameter with 15% tolerance against **a** polymer concentration and **b** feed rate

for estimating the fibre diameter of nanofibres produced from electrospinning poly(lactic acid).

Comparing the values of R^2 from two equations, it is also evident that the general equation derived using two parameters, polymer concentration and feed rate, gives a better estimate of the resulting fibre sizes and further inclusion of voltage and humidity renders results worse. Nevertheless, the R^2 value of 0.857 for Eq. 2 has shown that it can be used to model the multi-factor experimental behaviour with sufficient accuracy. Reflecting on the results mentioned earlier from the linear regression, it is also clear from both equations that the effect of polymer concentration on the final fibre size is much stronger than that of feed rate. On the other hand, although the exponents for VOLT and RH show higher values than that of FR, as found in a previous research [13], both VOLT and RH are not deemed as critical as the PC and FR (the solution properties) for deciding the final fibre diameters in a controlled environment due to the following facts. First, FR plays an important role in controlling the amount of polymer to be electrospun and second, RH is not an easily controllable operational factor. In addition, the stretching of nanofibres through electrical forces depends not only on the magnitude of the voltage applied but also on the distance between the needle tip and the collector (referring to the voltage factor of 1 kV cm^{-1}). The present research has included most of the major parameters with three levels; therefore, the model developed has performed efficiently to find out the contributions of production parameters and has become more reliable.

Microscopic study and validations of MRA

The effects of PC and FR modelled using Eq. 1 can be firmly supported by the findings from a previous research that suggested the polymer concentration is the most significant factor of electrospinning; analysis of variance has shown that polymer concentration contributes 76% in deciding fibre diameter [13]. PC has played the only role to maintain the solution viscosity when the molar mass remains unchanged. Fibre diameter has become larger, Fig. 5a–c, with the increase of PC. With 10% PC, coarse fibres, Fig. 5c, of great uniformity and bead-free structures are found. This is due to the increase of the polymer concentration resulting in greater polymer chain entanglements within the solution, which is necessary to maintain the continuity of the jet during electrospinning. For the effect of FR, it has also been suggested [13, 19–21] that for a given voltage, when feed rate is increased, there is a greater volume of solution that can be drawn away from the needle tip. As a result, the solvent may not get sufficient time to dry and may require longer distance to evaporate the larger quantity of solvent necessary for producing a considerable quantity of nanofibres, thus rendering those nanofibres with increased diameter as shown in Fig. 5d–f. The accuracy of predicting the fibre size using the regression result, Eq. 1, has been well supported by the microscopic strictures of Fig. 5a–f. As shown from the experimental data, the scattering of the diameter values is more severe with increased polymer concentration, Fig. 4a, and there is a similar pattern for feed rate with a lesser extent of variation, Fig. 4b. This is attributed to the fact that both parameters estimate the amount of spinnable material. In addition to its more significant effect on deciding the final fibre size, PC also displays a considerable capability of controlling the size variation in the electrospun fibres compared to FR. Higher PC produces coarse fibres, but it makes visibly more uniform diameter, Fig. 6a, compared to the produced fibres with less concentration.

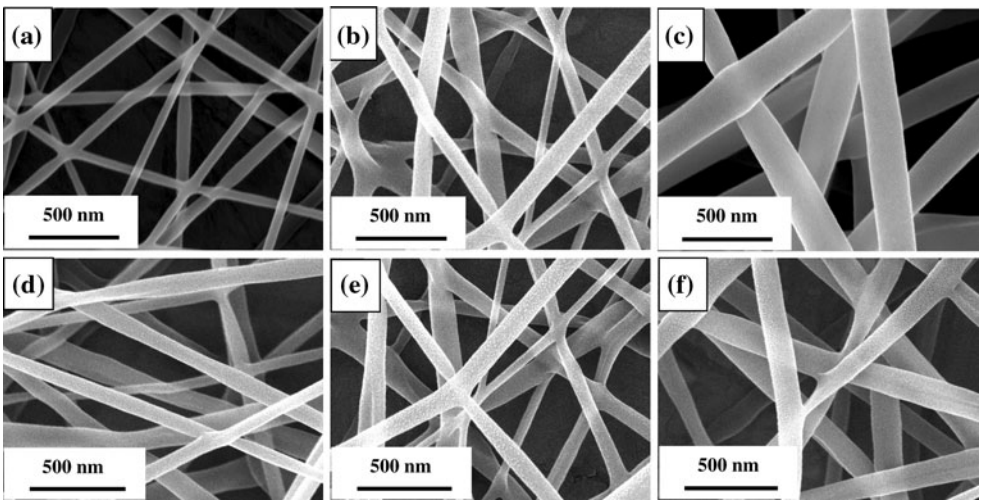

Fig. 5 Scanning micrographs of nanofibres with suitable parametric conditions $B_1C_1D_1$ using different polymer concentrations. **a** 4% **b** 7% and **c** 10% w/v, and nanofibres with conditions $A_1C_1D_1$ using different feed rate **d** 0.5 **e** 1 and **f** 2 mL h^{-1}

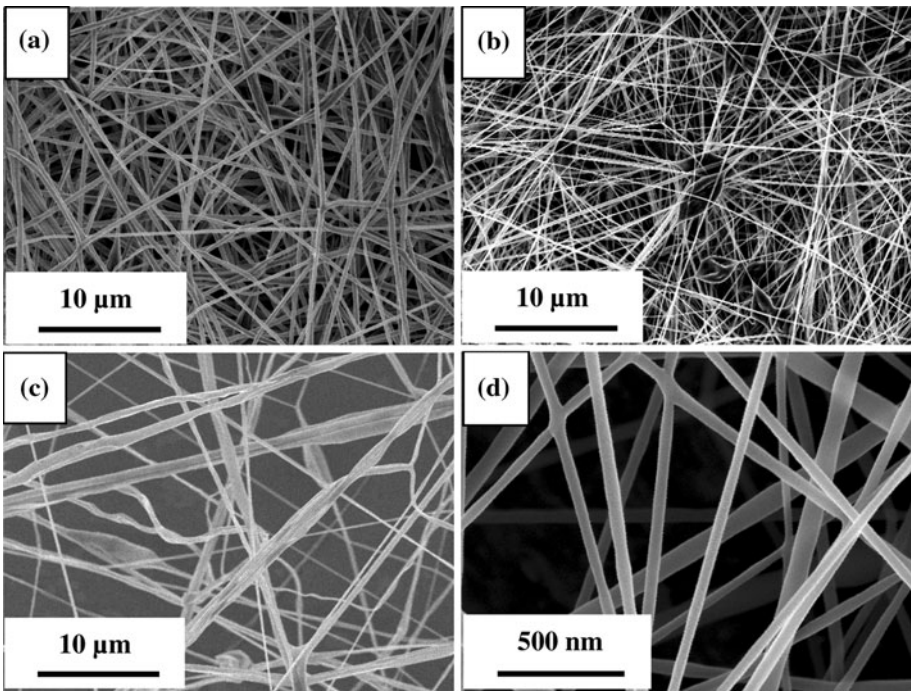

Fig. 6 Scanning micrographs of nanofibres with suitable parametric conditions using different polymer concentrations. **a** Coarse and uniform fibres with 10%, **b** fine fibres with variation in diameter with 4% w/v; and using different relative humidity, **c** sticky and coarse nanofibres with 75%, **d** superfine fibres with 20%

Lower PC makes fine fibres, but with beads and diameter variation, Fig. 6b, which is supported by previous research as well [13]. Electrospinning of bead-free and uniform nanofibres, particularly for fibre diameters less than 100 nm, still remains great challenge [13, 14].

Inclusion of the parameter RH has completed this parametric analysis compared to the previous research carried out to find the suitable parameter combinations with RH assumed to remain constant [13]. To observe the impact of RH on diameter, temperature has been kept unchanged (20 °C) inside the electrospinning cabinet using ice and/or hot air. The effect of humidity on fibres is attributed to the development of porous and sticky fibres. At a higher humidity, polymer solutions solidify slower and thus the fibre formation process cannot be completed within the flight time of the jet, resulting in coarse, irregular and sticky fibres, Fig. 6c. A low RH (20%) has ensured quick and efficient drying and provided extra stretch to make superfine fibres, Fig. 6d. The power supplied should be adequate to overcome the viscosity and surface tension of the polymer solution to form and sustain the jet from the pipette. An increase in electric potential tends to stretch the polymer more resulting in decreased fibre diameter up to a certain level. A maximum voltage of 12 kV has been found suitable for effective stretching, further increase in voltage generates too much acceleration on the polymer solution resulting in nanofibres with irregular sizes due to insufficient flying time for solvent evaporation. Voltage and humidity are not easily controllable parameters for producing the final diameter efficiently; however, these do have strong impacts on shaping the nanofibres.

When comparing particular experimental data with calculated results using Eq. 2, an average of 10–15% variation can be expected depending on the experimental conditions. A less than 5% of variation in fibre diameter is found with operational conditions of $A_2B_1C_1D_1$ (PC of 10%, FR of 1 mL h^{-1},VOLT of 10 kV and RH of 40%) and $A_2B_1C_2D_1$ (PC of 10%, FR of 1 mL h^{-1}, VOLT of 12 kV and RH of 40%), Fig. 7a, b; however, with experiments $A_0B_2C_2D_2$ (PC of 4%, FR of 2 mL h^{-1}, VOLT of 12 kV and RH of 75%) and $A_2B_2C_2D_2$ (PC of 10%, FR of 2 mL h^{-1}, VOLT of 12 kV and RH of 75%), Fig. 7c, d, more than 15% of variation has taken place. It is expected that the MRA modelling is working well with all the operational parameters kept within the experimental range of this study, such as the results from experiments $A_2B_1C_1D_1$ and $A_2B_1C_2D_1$ where the differences between the estimated and experimental values are small (only 0.7% for condition $A_2B_1C_1D_1$). However, when using the derived model to predict the resulting fibre diameter under the extreme conditions of this study, particularly for VOLT and RH, the predictive capability of the MRA model is compromised, e.g. experiments $A_0B_2C_2D_2$ and $A_2B_2C_2D_2$, where all the conditions are mostly on the high end of the experimental spectrum. With the hard-to-control parameters, VOLT and RH, at their highest levels, the experimental results have become inconsistent to cause the large discrepancy in the estimated outcome.

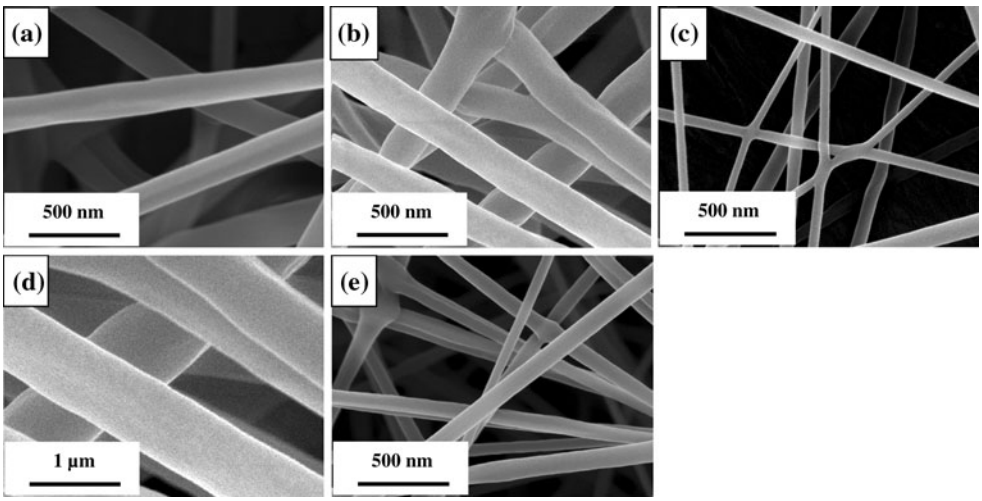

Fig. 7 Scanning micrographs of nanofibres with parametric conditions. **a** $A_2B_1C_1D_1$, **b** $A_2B_1C_2D_1$, **c** $A_0B_2C_2D_2$, **d** $A_2B_2C_2D_2$, **e** confirmatory test

In order to assure the validity of MRA, an electrospinning experiment under randomly selected conditions was performed using PC of 8% (w/v), FR of 1.5 mL h^{-1}, VOLT of 9 kV and RH of 35%. The average fibre diameter from this experiment has been found to be 161.4 nm, Fig. 7e, whereas Eq. 2 gives an estimated fibre diameter of 173.2 nm, which is very encouraging.

Finally, it is worth mentioning that due to the interpolative nature of MRA technique, using the derived results for modelling electrospinning with extreme conditions or beyond covered range (particularly for VOLT and RH) makes the prediction inaccurate and chaotic, and therefore may not be reliable. However, this problem could be overcome by selecting the experimental conditions carefully. The MRA model developed in this study has been proved to reliably predict the resultant fibre diameter within the commonly used operational parameter range for electrospinning [13, 19–21]. The technique can be easily expanded to build the capability for estimating the electrospun fibre diameters by selecting the necessary manufacturing parameters and their range.

Conclusions

A general equation involving major production parameters to predict the resultant fibre diameter for electrospinning of PLLA nanofibres has been derived using MRA. Polymer concentration has been confirmed from the linear regression analysis as the most major parameter in electrospinning of PLLA nanofibres with its controllable effect. The polymer concentration–fibre diameter equation can describe the experimental behaviour more accurately than the feed rate–fibre diameter relationship despite feed rate also having a significant effect on deciding the fibre diameter by controlling the amount of polymer to be electrospun. A general equation using two parameters, polymer concentration and feed rate, gives a better indication when other parameters are suitably maintained. While combining humidity and applied voltage, the prediction of produced fibre diameters becomes less satisfactory but is still acceptable. This study has shown that prior to the mass production of nanotextiles, the experimental time could be significantly reduced by identifying the most suitable production conditions that would yield nanofibres of specific required qualities.

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