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Preparation of novel ultrafine fibers based on DNA and poly(ethylene oxide) by electrospinning from aqueous solutions

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Abstract

Novel DNA/Polyethyleneoxide (PEO) electrospun fibers were obtained from aqueous solution. Key solution properties related to electrospinning: conductivity, surface tension and viscosity were determined. The ionic conductivity of the solution increased significantly with the addition of DNA and only slightly with increasing amounts of PEO; the surface tension decreased with the addition of PEO; the viscosity increased with the addition of either DNA or PEO. It was found that solutions containing both DNA and PEO had ideal properties for electrospinning. The use of these solutions resulted in the formation of ultrafine fibrous mats with fiber diameters of 50–250 nm. It was also found that the average diameter of electrospun fibers decreased with decreased feed rate, increased tip-to-collector distance and increase in the potential employed during electrospinning.
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Keywords: DNA; Ultrafine fibers; Electrospinning; Solution properties; Fiber diameter distribution

1. Introduction

Electrospinning is a simple, rapid, inexpensive and non-mechanical technique for production of ultrafine fibers. First reported by Formhals in 1934 [1], electrospinning has been used with a number of conventional polymers including poly(ethylene oxide) (PEO), polystyrene, polyacrylonitrile and polyvinyl chloride/polyurethane with or without the addition of other, conducting polymers [2–7]. Electrospun products have been employed in areas such as high performance filters [2], high surface area electrodes [6] and fiber templates [7].

In a typical electrospinning process, the polymer feed solution is provided via a needle using a syringe pump. A high electric field is generated between the needle tip and an electrically grounded target such as Aluminium. This results in the formation of a Taylor cone at the needle tip [8], as the voltage reaches a critical value (typically 5–25 kV). When this critical value is reached the electrostatic forces

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overcome the surface tension of the polymer solution at the tip and an electrically charged jet is ejected [9]. As this jet travels towards the target, it begins to elongate by rapidly rotating in a spiral path [10]. The solvent evaporates and deposits a non-woven mat of nanometer to micrometer diameter fibers onto the target [11].

Martin and Cockshott reported the use of electrospinning to produce biomaterials [12] and many such studies have subsequently been reported. For example, Ohkawa et al. prepared cationic polysaccharide (chitosan) fibres from trifluoroacetic acid and analysed the effect of the electrospinning solvent and the chitosan concentration on the morphology of the resulting fibres [13]. Nanostructured electrospun fibres of aliphatic polyesters (such as poly (ε-caprolactone)) have also been obtained from mixtures of methylene chloride and *N*,*N*-dimethylformamide and the influences of solution properties such as dielectric constant, viscosity, surface tension and conductivity on the morphology of the resulting mats have been determined [14]. Recently the electrospinning of polymer nanofibrous scaffolds for tissue engineering has attracted considerable attention. For example, Min et al. have investigated the possibility of fabricating a biodegradable nanostructured composite matrix for tissue engineering through electrospinning poly (lactic-co-glycoclic acid) (PLGA) and chitin from 1,1,1,3,3,3,-hexafluoro-2-propanol and formic acid. The PLGA/chitin matrix obtained showed promise as a tissue engineering scaffold with a three-dimensional structure resembling the collagen–glycosaminoglycans composite structure in the extracellular matrix [15]. Nanofibrous membranes based on dextran have been electrospun from water or water/dimethyl sulfoxide mixtures producing biocompatible or bioresorbable materials [16].

In this work we have developed electrospinning protocols that enable production of novel bio unltrafine fibers containing DNA from aqueous solution. Deoxyribonucleic acid (DNA) is a nucleic acid that contains the genetic instructions specifying the biological development of all cellular forms of life (and many viruses). PEO was previously reported as a host polymer to improve processability and biocompatibility of solutions of natural proteins and conducting polymers without the use of organic solvents [17–19], since it is known to be biocompatible [20] and has previously been electrospun from aqueous solution [17,21]. We have also quantitatively analysed the key factors: solution conductivity, surface tension and viscosity that affect the formation of the jet and determine the success of electrospinning. In addition, the influence of process parameters e.g. tip-to-collector distance, applied potential between the needle and the target and feed rate on fiber morphology and diameter distribution has been determined.

The resulting DNA/PEO ultrafine fibers exhibit three dimensional, microporous structures indicating potential with applications in areas such as biosensing or as novel platforms for cell culturing.

2. Experimental

2.1. Materials

PEO (M_W 300 000 Ca) was purchased from Aldrich. DNA–Na (M_W 3 000 000, N% 15.5%, P% 7.96%) was supplied by Nippon Chemical Feed Co. Ltd., Japan. All chemicals were used without further purification.

2.2. Solution preparation

2.2.1. Pure DNA aqueous solution

DNA (2 wt%), DNA (4 wt%), DNA (8 wt%), DNA (8 wt%) and DNA (10 wt%) were prepared by dissolving DNA in Milli-Q water respectively.

2.2.2. Pure PEO aqueous solution

PEO (2 wt%), PEO (4 wt%) and PEO (6 wt%) were also dissolved in Milli-Q water.

2.2.3. DNA/PEO blend solution

DNA/PEO solutions (6 wt%, 8 wt% and 10 wt%) with a 1:1 mass ratio of DNA to PEO were prepared by dissolving DNA/PEO in Milli-Q water. In addition, 10 wt% DNA/PEO solutions with different mass ratio (3:7, 4:6, 6:4, and 7:3) of DNA to PEO were prepared in Milli-Q water respectively.

Furthermore, 4 wt% DNA/6 wt% PEO electrospun fibers were obtained at different feed rate (R), distance of tip-to-collector (D) and potential employed (ΔE) to confirm the influence of R, D and ΔE on average diameter of resulting fibers (d).

2.3. Characterization

The conductivity of each solution was measured using a Model 20 pH/Conductivity Meter (Denver Instrument). The surface tension of solutions was

determined using KSV contact angle analyzer (goniometer, KSV instruments Ltd.) and the viscosity of solutions was tested by DV-II viscometer. The resulting mat's morphology was determined using a Leica Cambridge 440 stereoscan scanning electron microscope (SEM). The average diameter of the electrospun fibers was measured from SEM micrographs by using Video Pro 32 (Leading Edge Pty. Ltd.,). Samples were sputter coated with gold prior to SEM imaging.

2.4. Electrospinning

The electrospinning set up consisted of a Gamma High Voltage Power Supply (ES50P10W/DAM), a 5 ml glass syringe and a PrecisionGlide™ 19G needle (1.1 mm diameter). The electrospinning was conducted at various tip-to-collector distances (10–25 cm), applied potentials between the needle and collector (5–20 kV) and feeding rates (50–200 μl/min), respectively.

3. Results and discussion

Solution conductivity, surface tension and viscosity have been shown to be key parameters in determining the suitability of polymer solutions for electrospinning [22].

These properties of pure DNA, pure PEO and DNA/PEO containing solutions were determined (Table 1).

3.1. Pure DNA aqueous solution

No fibers could be successfully collected during electrospinning pure DNA aqueous solutions. For lower concentration of DNA solution (less than 2 wt%), the viscosity was very low and thus the viscoelastic force was not large enough to counter the higher Coulombic force. The charged jet breaks up into droplets as a result of surface tension [23]. With the addition of DNA, the viscosity and conductivity increased significantly. For higher concentration of DNA, the increase in viscosity brought the higher viscoelastic force, which was enough to prevent the break-up of the jet and favor the jet traveling to the grounded target [24]. The sharp changes in conductivity arise from the protonation of the functional group on DNA. The higher solution conductivity results in enough electrical force to overcome the surface tension of the solution. However, if ionic conductivity is too high, the formation of beads favoured [25]. Thus, the difficulty in electrospinning pure DNA solutions is attributed to the high conductivity of the solution.

With PEO containing solutions, the conductivity remains low (1.67–1.79 uS/cm) and increased slightly with increasing PEO concentration (Table 1 nos. 6–8). A significant difference was observed in the conductivity values between the pure PEO and DNA solutions. The surface tension of pure PEO solutions decreased slowly with the addition of PEO due to the adsorption of the PEO

Table 1
Properties of aqueous solutions (100 mL) containing DNA/PEO

Solution no.	DNA (g)	PEO (g)	Amount of water (g)	Conductivity (S/cm)[a]	Surface tension (mN/m)[b]	Viscosity (mPa s)[c]
1	2	0	98	32.2	64.21	28.2
2	4	0	96	66.7	70.15	49.4
3	6	0	94	74.1	74.51	369.6
4	8	0	92	79.1	77.79	1068.8
5	10	0	90	104.0	74.26	63766.5
6	0	2	98	1.67	66.68	91.82
7	0	4	96	1.75	65.39	233.07
8	0	6	94	1.79	62.12	369.62
9	3	3	94	38.8	64.84	233.07
10	4	4	92	41.4	63.74	1104.14
11	5	5	90	53.6	61.58	2589.68
12	3	7	90	28.8	62.62	2337.77
13	4	6	90	41.9	64.11	2537.88
14	6	4	90	59.2	63.38	1998.76
15	7	3	90	80.4	61.14	8899.07

[a] Determined by a model 20 pH/conductivity meter (Denver Instrument).
[b] Determined by a KSV contact angle analyzer (goniometer, KSV instruments Ltd).
[c] Determined by a DV-II viscometer, shear rate using 20 s^{-1} for viscosity testing.

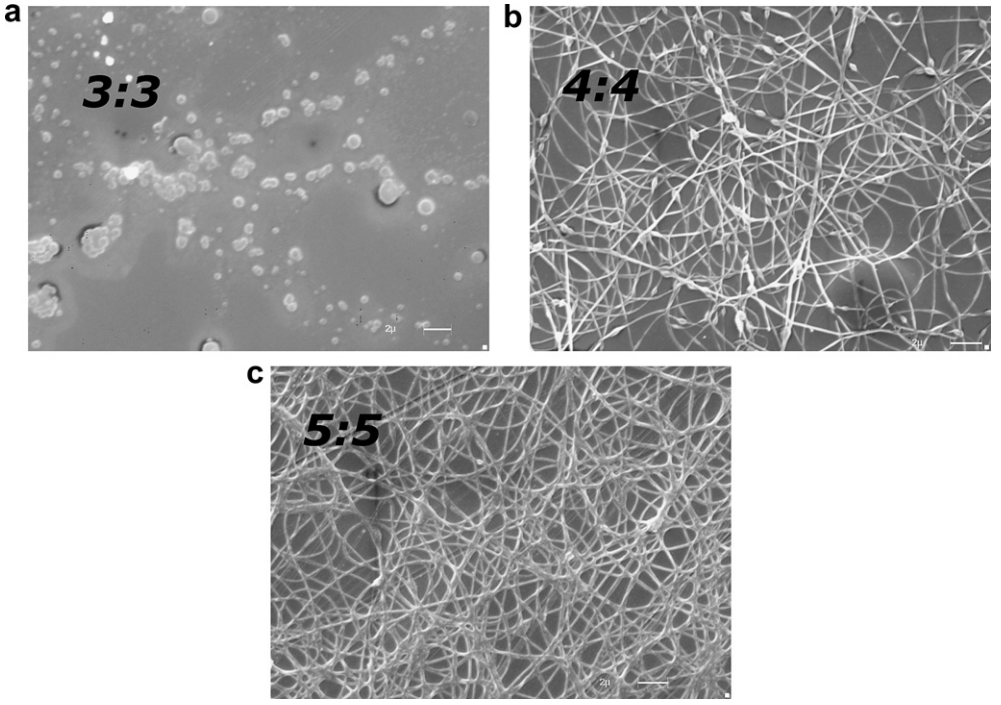

Fig. 1. SEM micrographs of the electrospun fibers from: (a) 3 wt% DNA/3 wt% PEO, (b) 4 wt% DNA/4 wt% PEO and (c) 5 wt% DNA/5 wt% PEO in Milli-Q water (Scale = 2 μm).

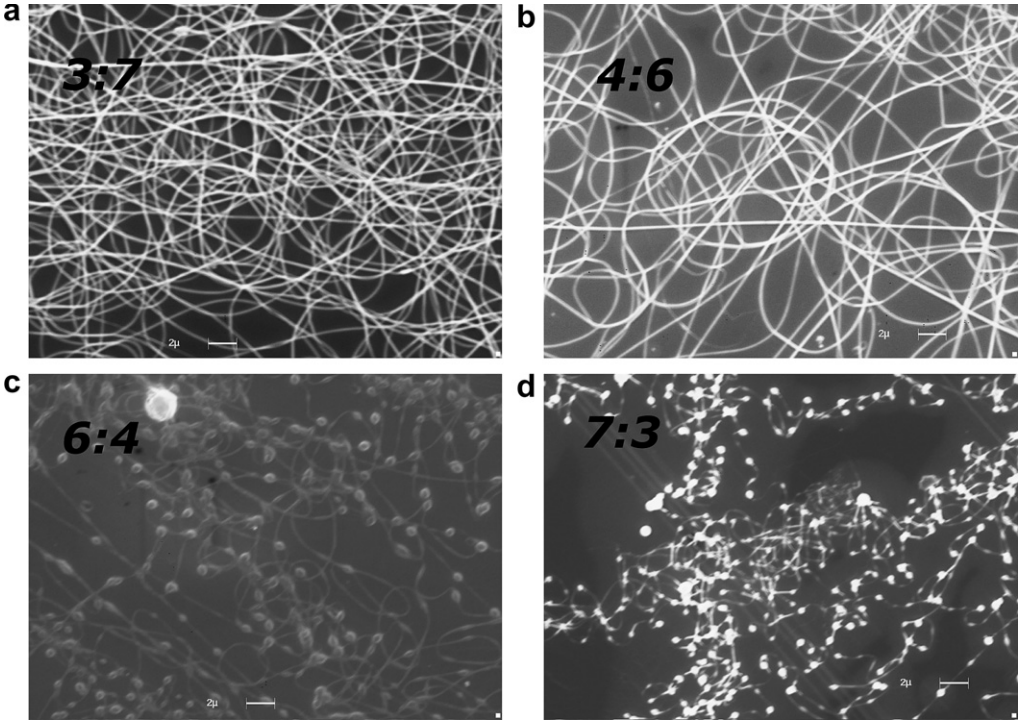

Fig. 2. SEM micrographs of the electrospun fibers from: (a) 3 wt% DNA/7 wt% PEO, (b) 4 wt% DNA/6 wt% PEO, (c) 6 wt% DNA/4 wt% PEO and (d) 7 wt% DNA/3 wt% PEO in Milli-Q water (Scale = 2 μm).

molecules at the solution surface [26]. Surface tension affects the formation of beads and beaded fibers during electrospinning [27]. Surface tension changes the jets into spheres in order to decrease the surface area while electrical force tries to increase the surface area by maintaining a thinner jet. The decrease in surface tension was thus used to produce fibers without beads. Sharply increased viscosity was obtained with increasing concentration of PEO. As with DNA, these solutions only produced beads on the target when electrospinning was attempted. Similar results have also been observed by Son et al. [17]. They found that no fibers could be obtained from pure PEO in water when the concentration of PEO was less than 7 wt%. The failure to successfully electrospin pure PEO at these lower concentrations may be attributed to the low ionic conductivity of the solutions.

Table 1 nos. 9–11 summarises the properties of 6 wt%, 8 wt% and 10 wt% DNA/PEO containing aqueous solutions. Comparing the pure DNA solution with DNA/PEO solutions, the latter had lower conductivity and surface tension which favor the formation of fibers without beads [25]. These mixtures result in solutions with the ionic conductivity, surface tension and viscosity in the range required for successful electrospinning. The formation of the Taylor cone was observed during electrospinning and a nanofibrous mat was obtained on the collector electrode (Fig. 1).

Excellent nanofibrous mats were obtained when using the feed solution containing 5% DNA and 5% PEO (Fig. 1c). The increased conductivity and decreased surface tension combined with appropriate viscosity results in the formation of beautiful nanofibers with the beaded products that were observed at lower polymer concentrations (Fig. 1a and b) eliminated.

The ratio of DNA and PEO was then varied while keeping the overall concentration of solids at 10% (w/w). The properties of these solutions are outlined in Table 1 nos. 12–15.

The suitable conductivity, viscosity and surface tension suggest that the solutions labelled 12 and 13 should be amenable to electrospinning. With both solutions, well defined Taylor cones were observed during electrospinning and a continuous nanofibrous mat was obtained. The deposits were

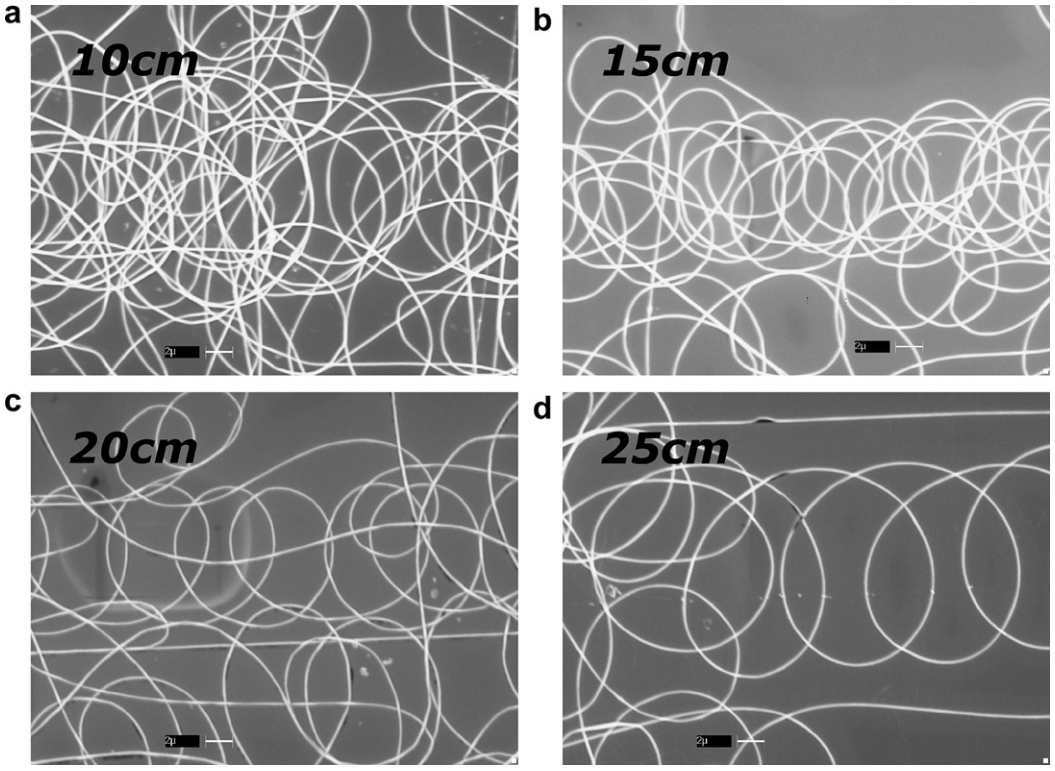

Fig. 3. SEM micrographs of the electrospun fibers from 4 wt% DNA/6 wt% PEO obtained using a range of tip-to-collector distance: (a) 10 cm, (b) 15 cm, (c) 20 cm and (d) 25 cm (Scale = 2 μm).

examined using SEM (Fig. 2) and the ability to form long ultrafine fibers was confirmed (see Fig. 3).

With samples (nos. 14 and 15) containing higher concentrations of DNA more beads were obtained (Fig. 2c and d). This may be attributed to the rapid increase in conductivity of the solution which helps to form beads [25] with increasing DNA percentage in the blends.

3.2. Effect of electrospinning parameters on fiber diameter

The effect of tip to collector distance, applied potential and the solution feed rate on the morphology and diameter of the fibers obtained was examined using SEM.

The solution composition was held constant at 4 wt% DNA/6 wt% PEO.

3.3. Influence of tip-to-collector distance (D)

Electrospun fibers were obtained using different tip-to-collector distances, while the feed rate (R) and applied potential between the needle and collector (ΔE) constant remained at 100 μL/min and 15 kV, respectively. The average diameter of electrospun fibers decreased significantly with increasing tip-to-collector distance (Fig. 4). This is similar to behaviour observed previously within electrospinning of PEO [28].

3.4. Influence of applied potential (ΔE)

The influence of the applied potential between the needle and collector (ΔE) on the average diameter of electrospun fibers was investigated (Fig. 5). Fibers were collected at different ΔE while the feed rate and tip to collector distance were kept at 100 μL/min and 15 cm, respectively. The average diameter of the fibers obtained decreased as the potential was increased (Fig. 5). The electrostatic force increased with increasing ΔE, which helps to further dissipate the polymer containing drop during spinning [29] with a concomitant decrease in fiber diameter. This is similar to results observed in previous work on electrospinning chitosan [30].

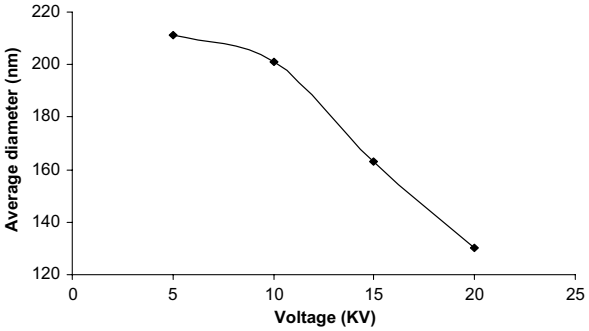

Fig. 5. Relationship between average diameter of fibers and applied potential during electrospinning.

3.5. Solution feed rate (R)

The feed rate of the polymer solution was controlled by the syringe pump. This was varied from 50 μL/min to 200 μL/min while the tip to collector distance and the applied potential were maintained at 15 cm and 15 kV, respectively. The average diameter of the fibers increased rapidly with increasing feed rate (Fig. 6). An increase in the feed rate results in increased bead area and fibre diameter as observed previously by others' electrospinning polystyrene [31].

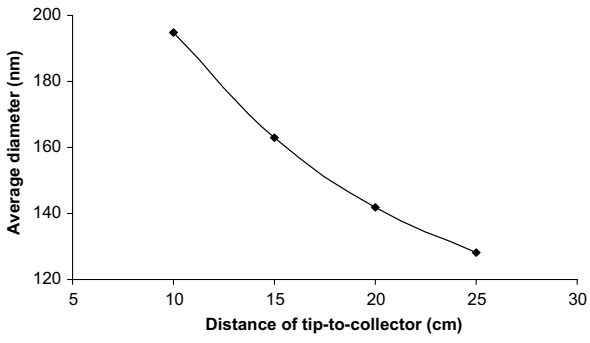

Fig. 4. Relationship between average diameter of fibers and tip-to-collector distance during electrospinning.

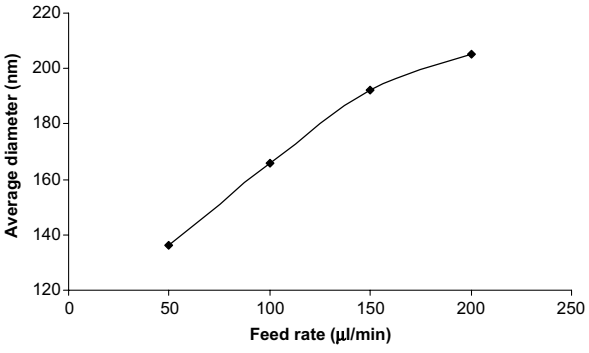

Fig. 6. Relationship between average diameter of fibers and feed rate during electrospinning.

4. Conclusions

DNA/PEO ultrafine fibers with diameters between 50 and 250 nm were successfully prepared from aqueous solution using an electrospinning technique. Three key solution properties: conductivity, surface tension and viscosity were determined and it was found that unltrafine fibrous mats were obtained from DNA/PEO solutions with suitable combination of conductivity, surface tension and viscosity. It was found that the average diameter of electrospun fibers decreased with increased potential applied between the positive and negative electrodes, decreased feed rate or increased tip-to-collector distance employed during electrospinning.

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