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Short communication

Process optimization for fabrication of gellan based electrospun nanofibers

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ABSTRACT

In this investigation, the nanofiber formation ability of gellan, a FDA approved low cost natural polysaccharide, has been achieved for the first time using electrospinning technique. The gellan based ultrafine nanofibers were fabricated by using a blend mixture of gellan with another biodegradable polymer polyvinyl alcohol (PVA). The morphology of resulting gellan–PVA nanofibers was analyzed using field emission scanning electron microscopy (FESEM). The mass ratio of 50:50 for gellan:PVA was recorded as an optimum solution ratio to obtain uniform bead free nanofibers with an average diameter of 40 ± 15.8 nm. Data depicted that among different parameters evaluated, viscosity and the mass ratio of gellan:PVA were the key parameters that influence the nanofiber morphology and diameter.

1. Introduction

Electrospinning is a versatile, progressive and widely used technique for making the highly porous nano-scale fibers. The high surface area-to-volume ratio of these electrospun nanofibers make them a potential candidate for a broad range of biomedical applications such as tissue engineering, wound healing and drug delivery (Homayoni, Ravandi, & Valizadeh, 2009; Vashisth et al., 2013). Recently carbohydrate polymers (Bhattarai & Zhang, 2007; Toskas et al., 2013) have taken an edge over their synthetic counterpart due to their abundant nature and unique structural characteristics. Gellan is a natural exopolysaccharide obtained from *Sphingomonas elodea* (Gong et al., 2009). Its chemical structure consists of repeating tetrasaccharide units of two D-glucose, D-glucuronic acid and L-rhamnose in a linear fashion. The advantageous properties of gellan as biomaterial include the cytocompatibility, bio-adhesiveness and presence of free carboxylic group (Silva-Correia et al., 2011). Moreover, gellan hydrogels (Gong et al., 2009), gellan films (Lee, Chen, & Tsao, 2010; Li, Kamath, & Dwivedi, 2001), gellan microcarriers (Agnihotri, Jawalkar, & Aminabhavi, 2006; Wang, Gong, Lin, Shen, & Wang, 2008) have been proved as a prospective material for biomedical engineering. However, despite huge range of applications, it is potential as gellan nanofibers have not been explored till date to best of our knowledge. This could be due to the limited solubility and the very high viscosity of gellan solutions. Like, most natural polysaccharides, gellan also shows a complex sol–gel behavior and a non-typical solvation in water (Liu, Wang, Gao, & Bai, 2013) which make it difficult to electrospun. Earlier, Stijnman, Bodnar, and Tromp (2011) have tried to electrospun 1% aqueous gellan solution but were unsuccessful to produce gellan fibers. They reported that, instead of jet formation, the gellan solution produced only deformed droplets and an unstable Taylor cone at the spinning needle tip. This behavior of gellan solution was found to be due to its anionic nature, low or a high low-shear viscosity and strong shear thinning behavior at low-shear rates. These limitations were circumvented by adding another hydrophilic co-biopolymer PVA as a supporting polymer. In present study, the effects of solution parameters (viscosity, surface tension, specific gravity and mass ratio of polymers) along with process parameters (voltage, flow-rate, tip-to-collector distance) on the gellan–PVA nanofibers formation and on its morphology were evaluated. Here, we highlighted gellan as a novel biopolymer that could be electrospun to fabricate nanofibers useful for tissue engineering, drug delivery and regenerative medicine applications.

2. Experimental

2.1. Materials

Gellan (Gelrite; MW 1000 kg/mol) was purchased from Sigma Aldrich. PVA (Mw = 140,000) was procured from Himedia (India). Deionized water (diH_2O) was used throughout to prepare polymeric solutions.

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Table 1
The composition and properties of gellan–PVA solutions.

Sample code	Solution composition[a] (wt%)			Solution properties		
	Gellan	PVA	Gellan–PVA ratio	Viscosity (mPaS)	Surface tension (mN/m)	Specific gravity
G1	1.5	10	100:0	5339 ± 74.7	52.7 ± 0.07	1.010
G2	1.5	10	90:10	5255 ± 80.8	52.4 ± 0.01	0.992
G3	1.5	10	70:30	4727 ± 142.5	51.8 ± 0.00	0.986
G4	1.5	10	50:50	4067 ± 41.6	49.9 ± 0.05	0.971
G5	1.5	10	30:70	2675 ± 60.5	49.3 ± 0.05	0.897
G6	1.5	10	10:90	2591 ± 41.8	48.4 ± 0.00	1.012
G7	1.5	10	0:100	2292 ± 22.2	48.0 ± 0.00	0.972

[a] The solvent was deionized water.

2.2. Solution preparation

Gellan (1.5 wt%) and PVA (10 wt%) solutions were prepared by dissolving them separately in diH_2O. Gellan–PVA blend solutions with different blend ratios (0:100, 10:90, 30:70, 50:50, 70:30, 90:10 and 100:0) were prepared. All the prepared solutions were degassed before use.

2.3. Electrospinning

Nanofibers fabrication from the prepared solution was achieved by applying a high voltage power ranging from 15 to 21 kV to the metallic needle (21 G), attached to 3 ml syringe at the flow-rate from 0.1 ml/h to 0.4 ml/h, controlled by a syringe pump (Harvard apparatus 11 plus syringe pumps). Aluminum foil was used to collect the nanofibers with the horizontal distance ranging from 6 to 18 cm from the needle tip. All the electrospinning processes were carried out under ambient conditions (temperature 25 ± 2 °C, relative humidity 30 ± 1%). The electrospun nanofibers were dried overnight in desiccator to facilitate complete removal of any residual solvent.

2.4. Characterization

The shear viscosities of the gellan–PVA blend solutions were measured at different revolutions (3–120 rpm) using Brookfield viscometer (DV-II pro) with LV-4 spindle. Surface tension and specific gravity of these solutions were determined using du-Novy tensiometer (KRÜSS GmbH, Germany) and specific gravity bottle (25 ml), respectively. The surface morphology of electrospun gellan–PVA nanofibers was examined using FESEM (Ultra Plus 55, ZEISS). For FESEM analysis, the nanofibrous samples (10 mm × 10 mm) were coated with a thin layer of gold using sputter coater (Biotech SC005) for 60 s. The average diameters of the nanofibers were calculated at 50 different points from the FESEM images using Image J software.

3. Results and discussion

3.1. Electrospinning

Electrospinning employs the usage of electrostatic forces to draw fibers from the solution and particularly suited for the production of nano-sized fibers from the large complex molecules. This process is affected by several solutions and process parameters (Vashisth et al., 2013). In the present study, the solution parameters such as concentration, viscosity, surface tension and specific gravity were optimized for fabrication of gellan–PVA nanofibers as listed in Table 1. The viscosity and surface tension of gellan based solutions were observed to be enhanced with increased proportion of gellan in gellan–PVA blend solution, whereas the specific gravity of the solutions changed irrelevantly. Our findings revealed that the aqueous solution of gellan alone at any concentration is not able to produce any fibrous structure using electrospinning

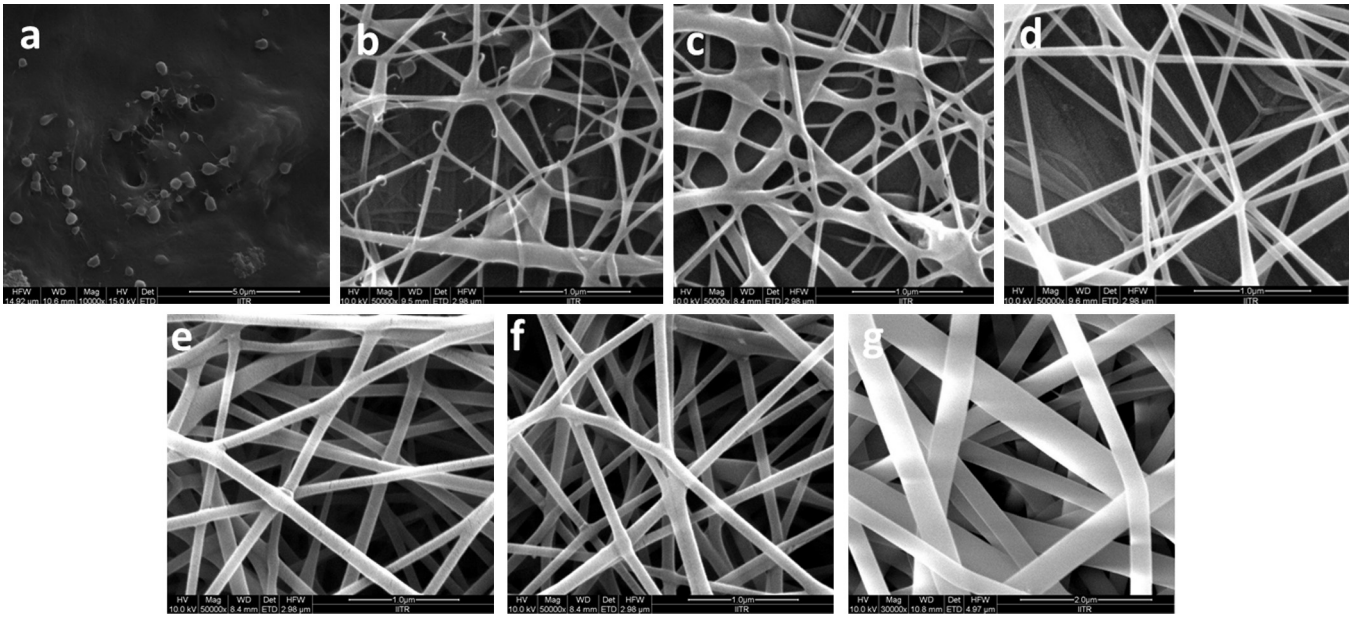

Fig. 1. Morphology of the gellan–PVA nanofibers fabricated at varying gellan:PVA ratio in the blend solutions (a) 100:0, (b) 90:10, (c) 70:30, (d) 50:50, (e) 30:70, (f) 10:90 and (g) 0:100.

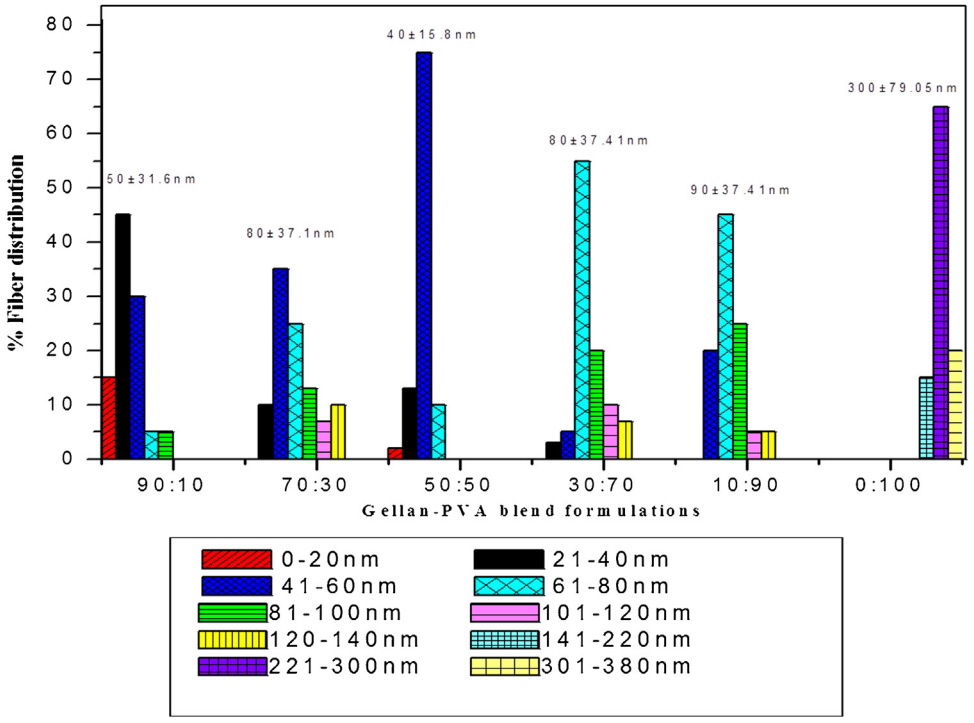

Fig. 2. Diameter distribution histograms for the gellan–PVA nanofibers fabricated at varying ratio of gellan-to-PVA. Data are given as mean ± standard deviation for $n = 50$ in each group.

approach. At lower concentration, the gellan solution leaked out from the syringe tip whereas higher concentration of gellan solution caused the coagulation of the spinning needle due to high viscosity which subsequently obstructs the flow of solution. To combat the above problem and to make gellan aqueous solution spinnable, we used PVA as the subordinate polymer. PVA is a known hydrophilic polymer and spinnable at a wide range of concentrations. The optimum spinnable concentrations of gellan and PVA in the blend were recorded to be 1.5 wt% and 10 wt%, respectively. We noticed that even at lower concentration (~1 wt%), gellan formed gel and was not spinnable. However, on addition of supporting polymer PVA into the gellan solution, the gelling tendency and viscosity of the resultant solution get decreased and now this blend solution was found to have the tendency of electrospinning.

3.2. Influence of gellan–PVA ratio on nanofiber morphology

Blend solutions of gellan–PVA were prepared by varying the proportions of gellan:PVA (100:0, 90:10, 70:30, 50:50, 30:70, 10:90 and 0:100). The FESEM image showed that electrospun gellan solution alone produced only droplets at the collector (Fig. 1a). The introduction of PVA at different proportions resulted in fibers with varied morphology and average diameter. It was observed that with increasing content of PVA, the bead defects disappeared and the equal proportion of gellan:PVA resulted in uniform nanofibers with an average diameter of 40 ± 15.8 nm (Fig. 1b–g). The diameter distribution histograms of gellan–PVA nanofibers depicted that increasing PVA proportion in the blend solution leads to enhancement in average fiber diameter (Fig. 2).

To enumerate the effect of solution viscosity on gellan based nanofiber formation, the shear viscosities of the gellan–PVA solutions as a function of shear stress were measured (Fig. 3). Result showed that the viscosity of the gellan–PVA blend solutions decreased with increasing proportions of PVA. The reduction may be attributed to the change/disruption of inter and intra molecular interaction between the gellan chains. From the above observations the solution G4 (Gellan:PVA::50:50) was found to be optimum to produced stable uniform nanofibers hence used for further analysis.

3.3. Effect of applied voltage on gellan–PVA nanofibers

The crucial electrospinning parameter that controls the jet formation onto the needle tip is applied voltage. Jet initiation takes place only when the given applied voltage overcomes the surface tension of solution (Ramakrishna, Fujihara, Teo, Lim, & Ma, 2005). A series of experiments based on varying voltage from 15 kV to 21 kV, were carried out for G4 solution with a constant flow-rate (0.1 ml/h)

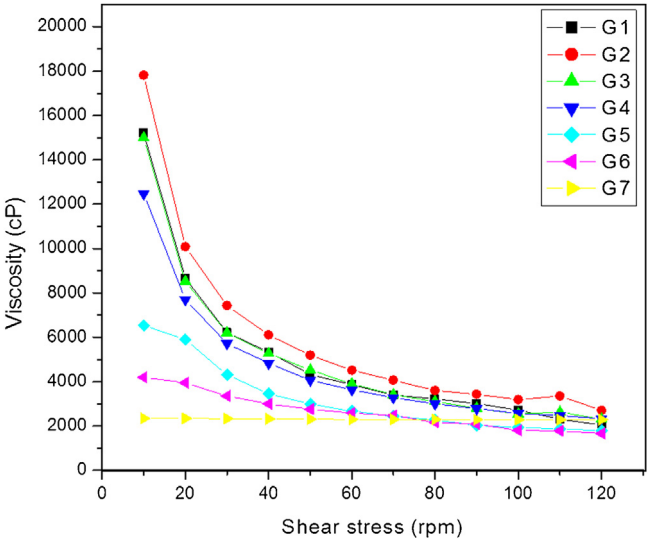

Fig. 3. Shear viscosities of gellan–PVA blend solutions at different proportions of gellan:PVA as a function of shear stress.

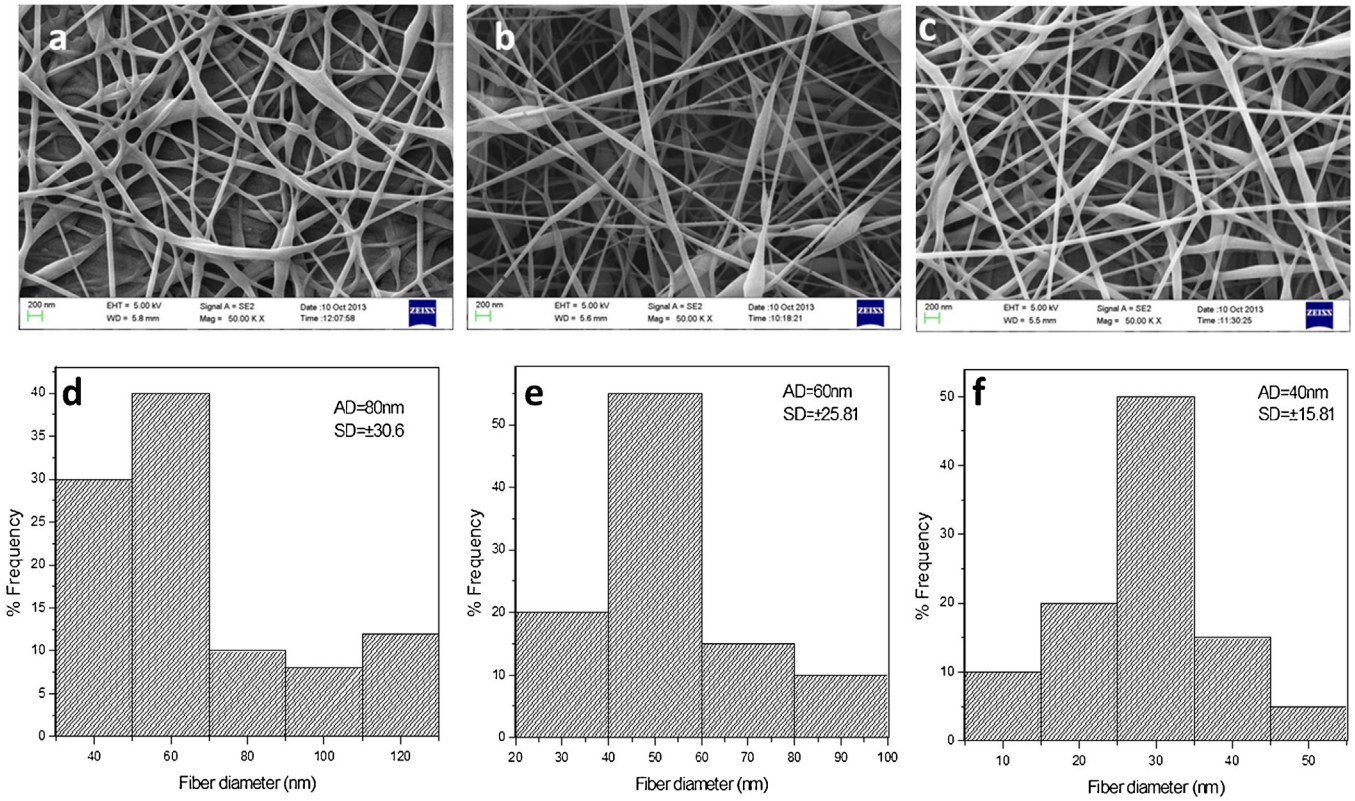

Fig. 4. Morphology and corresponding diameter distribution histograms for the gellan–PVA nanofibers fabricated at varying applied voltage (a and d) 15 kV, (b and e) 18 kV and (c and f) 21 kV. AD = average diameter; SD = standard deviation.

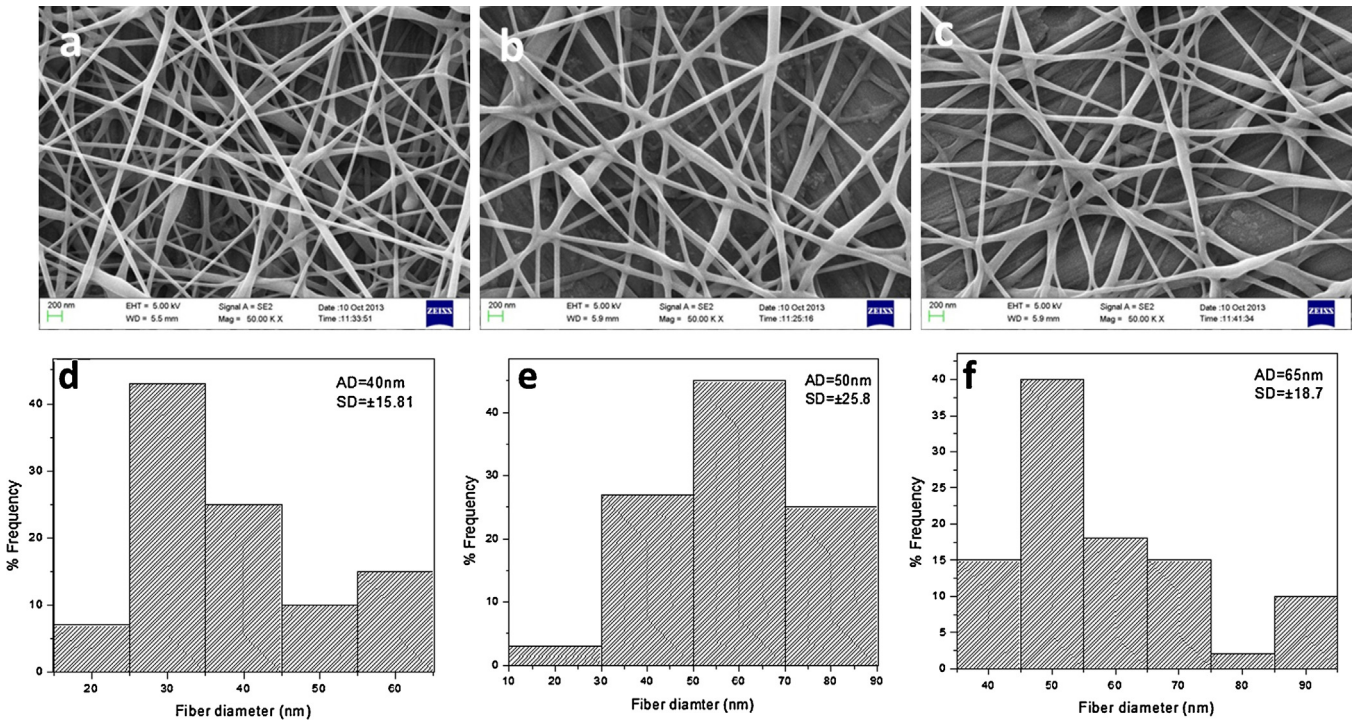

Fig. 5. Morphology and corresponding diameter distribution histograms for the gellan–PVA nanofibers fabricated at varying flow-rate (a and d) 0.1 ml/h, (b and e) 0.2 ml/h and (c and f) 0.4 ml/h. AD = average diameter; SD = standard deviation.

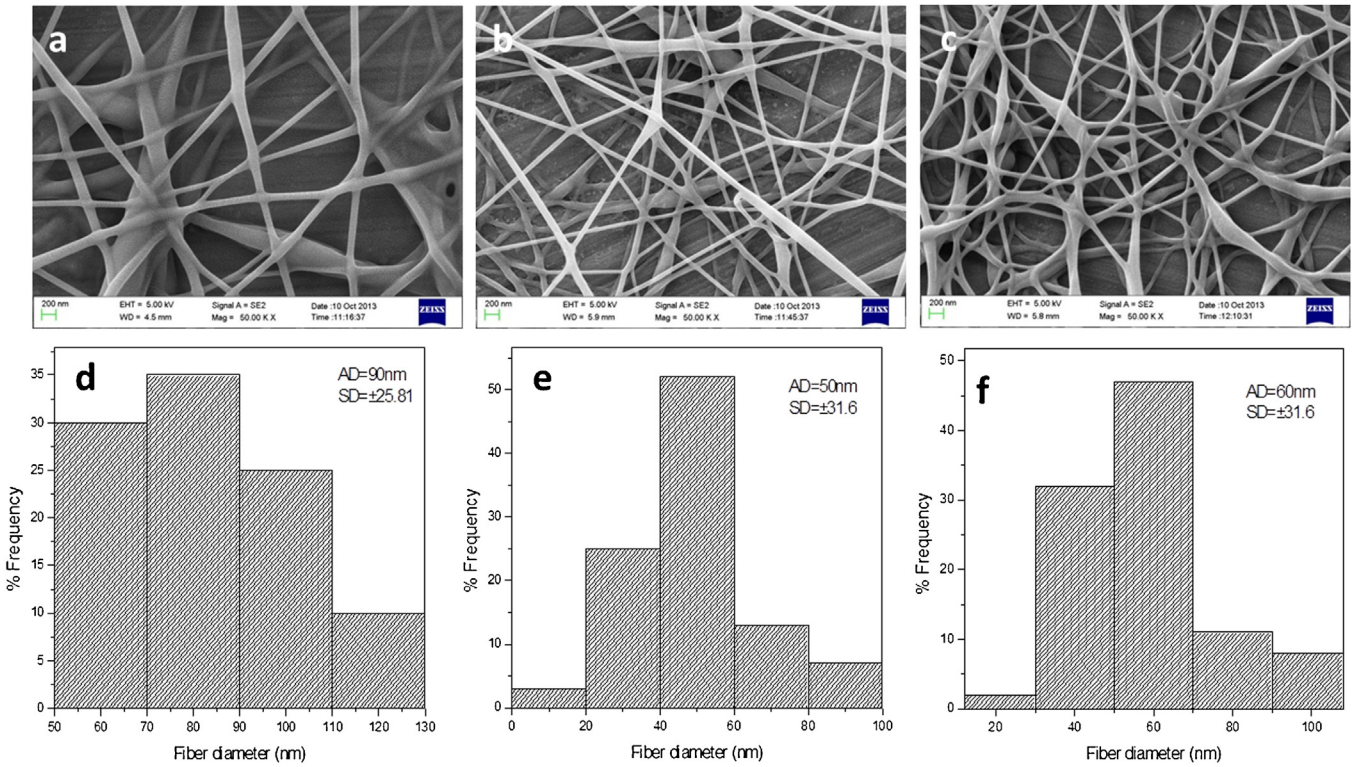

Fig. 6. Morphology and corresponding diameter distribution histograms for the gellan–PVA nanofibers fabricated at varying tip-to-collector distance (a and d) 6 cm, (b and e) 12 cm and (c and f) 18 cm. AD = average diameter; SD = standard deviation.

and constant tip-to-collector distance (12 cm) to investigate the effects of voltage on fiber morphology (Fig. 4). The high applied voltage causes the change in shape of a droplet into a fibrous structure and also lowers droplet dripping rate at the needle tip. The bead defects disappeared and the diameter of the nanofibers decreased with increasing applied voltage. It has been previously reported that higher voltage applied on the needle tip promotes the formation of nanofibers with lesser diameters; this can be attributed to the increase in columbic forces on the accelerated jet due to higher voltage which also causes rapid solvent evaporation to obtain dry fibers on collector (Sencadas et al., 2012; Ramakrishna et al., 2005).

3.4. Effect of flow-rate on gellan–PVA nanofibers

A minimum volume of solution that comes out from the needle tip should be maintained to form a stable Taylor cone in order to produce continuous beads free nanofibers (Teo & Ramakrishna, 2006). Thus to investigate the effect of flow-rate, the experiments were carried out on varying flow-rate from 0.1 ml/h to 0.4 ml/h, for G4 solution with optimized constant voltage (21 kV) and constant tip-to-collector distance (12 cm). It was observed that at flow-rate 0.1 ml/h, a droplet of solution remained suspended at the needle tip all the time and the average fiber diameter of about 45% nanofiber were recorded to be 40 ± 15.81 nm. Whereas, at higher flow-rates (0.2 ml/h and 0.4 ml/h), the nanofibers possessed the average diameters of about 50 nm and 65 nm, respectively (Fig. 5). These observations are in agreement with the previous study which reported that the lower flow-rate yield fibers of smaller diameter (Chang, Lee, Wu, Yang, & Chien, 2012). We also noted that at higher flow-rates, some of the polymer solution (as small droplets) directly shot the grounded collector as a consequence of withdrawal of high volume of solution at the needle tip. Similar observations were also reported by Sencadas et al. (2012).

3.5. Effect of tip-to-collector distance on gellan–PVA nanofibers

The influence of distance between tip-to-collector onto the nanofiber morphology and diameter distribution is revealed in Fig. 6. At shorter tip-to-collector distance (6 cm), the nanofibers did not get enough time to dry and consequently some of the nanofibers stuck together at junctions and resulted into nanofibrous bundle on the collector. At longer tip-to-collector distances (12 cm and 18 cm) the nanofibers exhibited long, straight and cylindrical morphology without any shucking at junctions. The increasing tip-to-collector distance displayed similar results as observed in case of lower voltage. These observations are in line with the previous studies done by Ramakrishna et al. (2005).

4. Conclusion

Gellan based nanofibers without bead defects were fabricated for the first time using aqueous solution of gellan and PVA by electrospinning technique. PVA was used as a subordinate polymer in this study which reduced the repulsive forces within the charged polymeric solution and allowed the electrospinning to generate uniform gellan–PVA nanofiber of 40 ± 15.8 nm. Data showed that viscosity of gellan–PVA solution to be one of the major determinant parameters that controlled the gellan–PVA nanofiber morphology and diameter. The higher content of PVA in the gellan–PVA solution leads to increase in the fibers diameter. On the other hand low applied voltage and longer tip-to-collector distance resulted in origin of almost similar type of nanofiber while high flow-rate did not exhibit much influence on the gellan–PVA electrospun nanofibers.

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