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Strategy on the Production of Bead-Free Electrospun Gelatin Scaffolds

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ABSTRACT

Electrospun scaffolds consist of micro-scale or nano-scale porous fibrous networks. These electrospun scaffolds had become increasingly popular in tissue engineering filed as it could provide nano-environment for cell culture and produced by using biodegrable polymer. One of the important key to provide such environment for cell culture is the porosity of the electrospun scaffolds as it is highly related with the cell-cell interaction. The porosity of the electrospun scaffolds could be affected by bead formation which is one of the common problems faced in electrospinning process. However, the formation of beads are difficult to be controlled as it depends on environmental factors such as humidity and operating temperature. Controlling these two environmental factors normally requires an expensive control system. This paper aims to solve the problem of bead formation by adjusting material concentration and process parameters without controlling the environmental factors. The parameters studied in this paper include polymer concentration, flow rate, distance between the syringe needle tip and collector and applied voltage. The microstructure of the electrospun scaffolds produced were visualised using scanning electron microscopy (SEM) and were analysed in terms of bead formation and fiber diameter. This study shows that polymer concentration is the best strategy to prevent bead formation in gelatin scaffolds while other process parameters such as applied voltage, distance between the syringe needle tip and the collector as well as flow rate can be used to control the fiber diameter. An understanding of the effects of each parameter provides a guideline to control microstructure morphology by producing bead-free electrospun gelatin scaffolds.

Keywords: *electrospun, gelatin, bead, fiber diameter*

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Introduction

The electrospinning method is a technique commonly used to produce nanofibers and fibrous scaffolds. The fibrous scaffolds are able to mimic the microstructure of an extracellular matrix. Hence, these fibrous scaffolds are crucial in tissue engineering as they are able to provide a suitable micro-environment for cells to grow and repopulate [1]. Furthermore, electrospinning is also able to produce fibrous scaffolds by using biodegradable polymer such as gelatin which is crucial in tissue applications [2, 3].

The electrospinning technique is a simple technique that requires a syringe needle to eject the polymer solution and a collector to collect the polymer solution so that fibrous scaffolds can be formed [4]. The process of electrospinning involves of applying electric potential on the polymer liquid and collector. When electric potential applied to the polymer solution, the electrical forces and the surface tension of the polymer solution would create a protrusion. The charges would accumulate at the protrusion and pulls the solution outwards to form conical shape, which is called as Taylor’s Cone. Continuous supply of electric potential at that stage would initiate the jet process and the polymer would deposit on the collector [5, 6]. Although electrospinning is a simple technique, it is hard to control the microstructure morphology of the fibrous scaffolds. This is because the electrospinning technique is sensitive to both environmental and process parameters [7]–[9]. Environmental factors such as humidity and operating temperature can cause the formation of beads on fibrous scaffolds [8]. Therefore, the protocol used for producing fibrous scaffolds in seasonal countries is cannot be applied in tropical countries. However, process parameters can be used to accommodate these problems so that bead-free electrospun scaffolds could be produced. Process parameters such as flow rate, applied voltage, distance between the syringe needle tip and the collector, solvent system, type of collector, weight ratio and polymer concentration of polymer solutions have an impact on bead formation and the morphological properties of fibrous scaffolds [9]–[18]. Solving bead formation and controlling morphological properties such as fiber diameter are crucial as failure in doing so could affect their interaction with cells and the mechanical properties of fibrous scaffolds [17]–[26].

The aim of this study is to effectively prevent bead formation without the need for a design system to control humidity and temperature. The findings of this study would lead to the production of bead-free gelatin electrospun scaffolds. Furthermore, this study may provide a guideline for controlling the morphology properties of gelatin electrospun scaffolds by modifying process parameters.

Methodology

Sample Preparation

The gelatin fibrous scaffolds were produced by replicating the recipe of Song et al. (Table 1: A) [27]. A 10 wt% of gelatin was dissolved in a solvent system of acetic acid and co-solvent water with a ratio of 9:1 and was stirred at room temperature overnight. The solution was loaded into a syringe (5 ml) with a 23g needle in the electrospinning machine. The polymer solution was dispensed at a flow rate of 0.06 ml/hr. The distance between the syringe needle tip and the collector was set to 8 cm and an applied voltage was applied at 12 kV.

Song et al.'s recipe was then modified to study the effect of parameters on bead formation and fiber diameter. Two levels of gelatin, 15 wt% (Table 1: B-E) and 25 wt% (Table 1: F-G) were dissolved in an acetic acid and water solvent system with a ratio of 9:1. The solutions were stirred overnight at room temperature. The polymer solution was then loaded in a syringe (5 ml) with a 23g needle for the electrospinning set-up. The polymer solution was dispensed at a flow rate of 0.06 ml/hr (Table 1: B and D-G) or 0.12 ml/hr (Table 1: C). Applied voltage was examined by varying voltage between the needle tip and the collector at 12 kV (Table 1: B-D and F) and 15 kV (Table 1: E and G) respectively. The effect of the distance between the syringe needle tip and the collector was studied by varying the distance between 8 cm (Table 1: B and C) and 12 cm (Table 1: D-G). The collector used in this study was a metal plate wrapped with aluminum foil. The fibrous scaffolds collected were dried in a desiccator for at least 24 hours to remove residual solvent. The parameters used to produce electrospun scaffolds are shown in Table 1.

Table 1: The parameters used to produce electrospun scaffolds for each case study

Case Study (CS)	Gelatin Concentration (wt%)	Distance, d (cm)	Flow Rate, *f* (ml/hr)	Voltage, v (kV)
A	10	8	0.06	12
B	15	8	0.06	12
C	15	8	0.12	12
D	15	12	0.06	12
E	15	12	0.06	15
F	25	12	0.06	12
G	25	12	0.06	15

Microstructure Visualization and Quantitative Analysis

The microstructure of the electrospun scaffolds was investigated using a scanning electron microscope (SEM, Hitachi SU1510, Japan). Each sample was gold coated for 30 seconds. The percentage of beads was evaluated from the SEM images with a magnification of 1,000×. Every SEM image was separated into 100 grids. The grid that contained beads was counted and labelled as a bead grid. The percentage of beads is determined by the number of bead grids divided by the total number of grids. The fiber diameter was measured from the SEM images with a magnification of 10,000×. A technique was used to select the fibers to be measured without human judgement. Nine grids were drawn on every SEM image and the diameter of the fiber located at the centre of the grids was measured using Image-J software. The mean and standard deviation of these diameters were calculated.

Result and Discussion

The Formation of Beads

The electrospun scaffolds were reproduced by following the recipe by Song et al. (Figure 1 (a)). The electrospun scaffolds produced came with droplet formation and the formation of droplets was prevented by increasing gelatin concentration from 10 wt% to 15 wt% (Figure 1(a,b)). Based on our observation, 10 wt% of gelatin polymer solution was not sufficiently viscous to form a nice Taylor’s Cone shape on the needle tip and the jet was not formed continuously throughout the electrospinning process. Deitzel et al. [9] had a similar outcome and stated that the polymer fiber may still be wet when it hits the surface of the collector which can cause droplet formation.

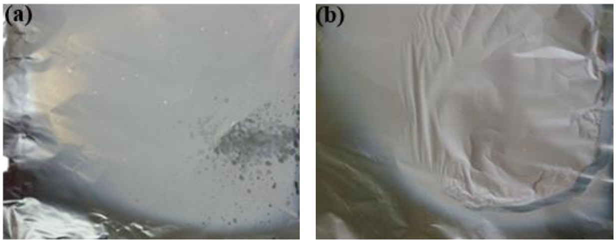

Figure 1: The images of fibrous scaffolds (a) with droplets and (b) without droplets. These fibrous scaffolds were produced by following the process parameters of Table 1: A and B respectively.

Although the 15 wt% of gelatin concentration solution was able to produce electrospun scaffolds without droplet formation, beads were found on the electrospun scaffolds (Table 2: B) (Figure 2(a)). The gelatin concentration was then increased from 15 wt% to 25 wt% and the percentage of beads was greatly reduced from 53% to 1% (Table 2: D and F) (Figure 2(c,e)). Furthermore, a bead-free electrospun scaffold was produced when 15 kV of voltage was applied on 25 wt% gelatin concentration solution (Table 2: G) (Figure 2(f)). The 25 wt% gelatin concentration was more concentrated and had a higher viscosity to form a nice conical shape known as Taylor's Cone. Moreover, a continuous jet was formed throughout the electrospinning process. The increase in gelatin concentration reduced the bead formation drastically and the same outcome was observed in Choktaweesap et al.'s study [28].

The flow rate was increased from 0.06 ml/hr to 0.12 ml/hr and the percentage of beads increased from 83% to 96% (Table 2: B and C) (Figure 2(a,b)). Okutan et al. [13] managed to reduce bead formation by increasing the flow rate, however, it did not worked out in this case study. A nice Taylor's Cone shape was unable to form consistently throughout the electrospinning process when the flow rate was increased. Furthermore, the jet was broken frequently and caused even more bead formation on the fibrous scaffolds [14, 29]. As a result of that, a bead-free fibrous scaffold was unable to be produced.

The bead formation was found to reduce from 83% to 53% when the distance between the syringe needle tip and the collector was increased from 8 cm to 12 cm (Table 2: B and D) (Figure 2(a,c)). The polymer jet had insufficient drying time upon reaching the collector because of the shorter distance between syringe needle tip and the collector. As a result of that, the bead formation had increased [14, 29]. In addition, there is an optimum range of distance for the electrospinning process [12, 14].

An applied voltage of 15 kV on 15 wt% of gelatin polymer solution was unable to produce fiber and particles were formed instead of fibers (Figure 2(d)). The poor electrospinability was because of the aggressive pulling of the polymer solution from the needle tip to the collector by a strong electric field. The Taylor's Cone was not sufficiently charged to form a stable jet and this results in the inability to form fiber. However, the same trend was not seen in 25 wt% of gelatin solution. Figure 2(f) shows that 15 kV voltage applied on the 25 wt% gelatin solution produced bead-free electrospun scaffolds. The Taylor's Cone was now able to contain more charges to form a stable jet due to the high viscosity of the gelatin solution. Few studies have shown that there is an optimum range of voltage to be applied [9, 12, 29]. Since the concentration of gelatin solution differs, the optimum range of voltage would differ as well. Thus, two different trend outcomes were obtained in this study.

The results show that the dominant factor to solve bead formation is the polymer concentration. When the gelatin concentration increased from 15 wt% to 25 wt%, bead formation was nearly non-existent for most cases. The change in process parameters for a gelatin solution of 15 wt% reduced the percentage of beads but failed to eliminate bead formation.

Table 2: The percentage of beads of each electrospun scaffold

Case Study (CS)	Percentage of beads (%)
A	Droplets
B	83
C	96
D	53
E	No fiber formed
F	1
G	0

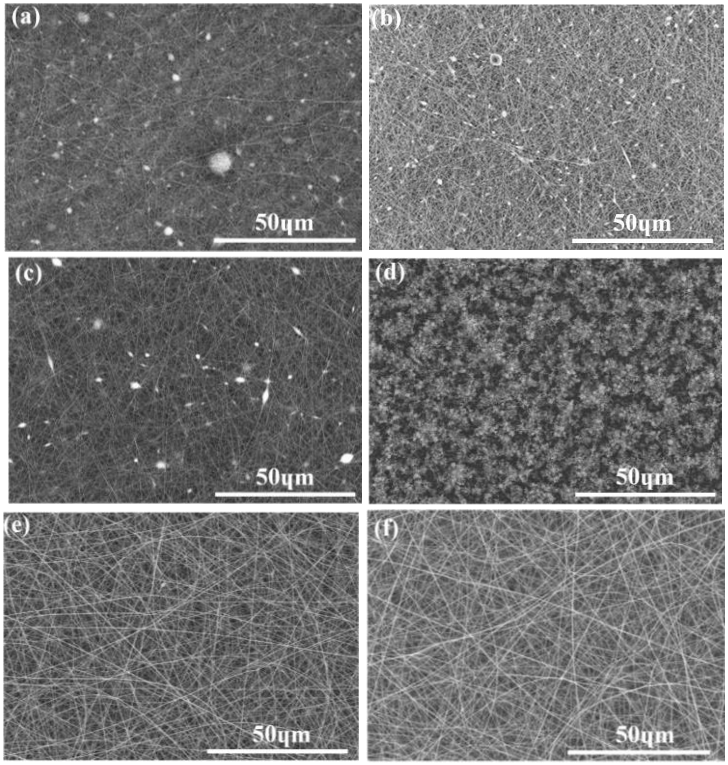

Figure 2: The SEM images of the fibrous scaffolds used for the analysis of bead formation. The SEM images show (a) with a huge amount of beads, Table 1: B (b) with a huge amount of beads, Table 1: C, (c) with a lesser amount of beads, Table 1: D, (d) with no fiber formed, Table 1: E, (e) with

the least amount of beads, Table 1: F and (f) without bead formation, Table 1: G

The Control of Fiber Diameter

In addition to the effect of bead formation, the concentration of a gelatin solution also has great impact in controlling fiber diameter. The fiber diameter was found to increase from 104±20 nm and 233±49 nm for gelatin solutions with concentrations of 15 wt% and 25 wt% respectively (Table 3: D and F) (Figure 3(c,e)). The fiber diameter was larger when the gelatin concentration increased. A similar trend was reported in studies by Huang et al. and Choktaweesap et al. [17, 28]. Although the impact of gelatin concentration on fiber diameter is great, it is not recommended to reduce the concentration of gelatin solutions to produce finer fibers. The bead formation problem may arise when gelatin concentration is reduced.

Figure 3(a,b) and Table 3(B and C) show that the fiber diameter was larger when the flow rate was increased. The flow rate was increased from 0.06 ml/hr to 0.12 ml/hr and the fiber diameter was increased from 113±23 nm to 119±30 nm respectively. This situation was expected as it was similar to Okutan et al.'s study [13].

Figure 3(a,c) and Table 3(B and D) show that the fiber diameter was reduced from 113±23 nm to 104±20 nm when the distance between the syringe needle tip and the collector was increased from 8 cm to 12 cm. Figure 3(e,f) and Table 3(F and G) show that finer and more consistent fibrous scaffolds were produced when applied voltage was reduced. The fiber diameters for an applied voltage of 12 kV and 15 kV on the gelatin solution (25 wt%) were 233±49 nm and 279±109 nm respectively. The same outcome was observed in a study by Ki et al. [12].

Table 3: The fiber diameter of each electrospun scaffold

Case Study (CS)	Fiber Diameter,Ø (nm)
A	Droplets
B	113±23
C	119±30
D	104±20
E	No fiber formed
F	233±49
G	279±109

The results show that the dominant factor needed to produce a finer fiber diameter is gelatin concentration. 15 wt% of gelatin concentration was able produce a fiber diameter of around 100 nm while 25 wt% of gelatin concentration produced a fiber diameter of around 200 nm. However, it is not recommended to control fiber diameter by altering gelatin concentration as it may lead to bead formation. The other processing parameters studied in this paper should be used to produce a finer fiber dimeter as it would be less likely to cause bead formation.

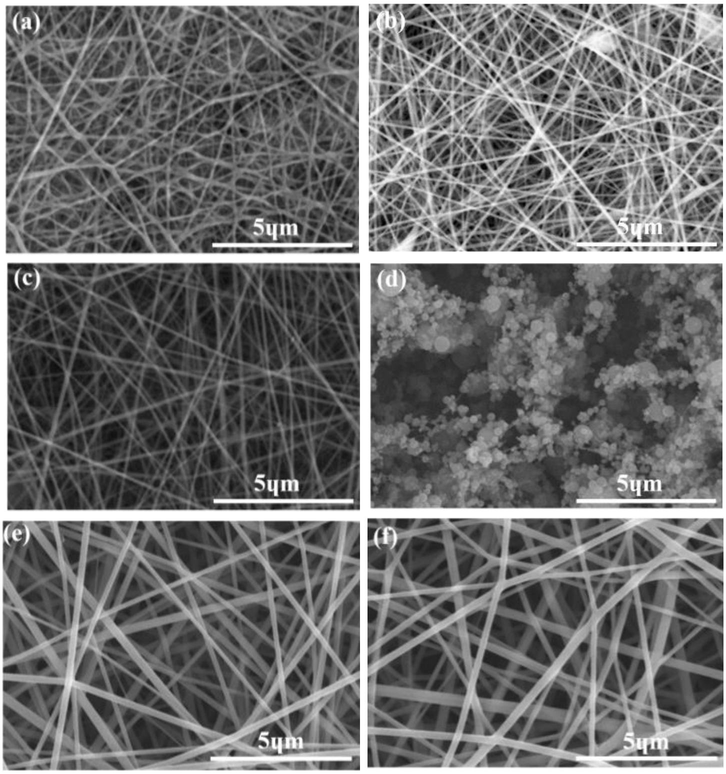

Figure 3: The SEM images of the fibrous scaffolds used for the analysis of fiber diameter. The SEM images showing (a) with a fine fiber diameter, Table 1: B (b) with a fine fiber diameter, Table 1: C, (c) with the finest fiber diameter, Table 1: D, (d) with no fiber formed, Table 1: E, (e) with a large fiber diameter, Table 1: F and (f) with the largest fiber diameter, Table 1: G

Conclusion

Various parameters were studied in terms of their effect on bead formation and fiber diameter. The results show that gelatin concentration had the largest impact on both bead formation and the production of a finer diameter. However, gelatin concentration has to be increased in order to eliminate bead formation while the production of finer fiber requires a reduction in gelatin concentration. Thus, it is highly recommended to increase gelatin concentration to prevent bead formation while other processing parameters like flow rate, applied voltage and distance between the syringe needle tip and the collector can be adjusted to control the fiber diameter so that bead formation would not arise.

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