ELECTROSPINNING OF ALIGNED NANOFIBERS FOR BIOMEDICAL APPLICATION USING A ROTATING DRUM COLLECTOR

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Abstract. Both random and aligned collagen fibers exist in extracellular matrix. The alignment of fiber in tissue engineering scaffolds can affect the cell morphology. Therefore, knowledge in the control of microstructure alignment in electrospun scaffolds is crucial. In this study, a rotating collector drum was designed and fabricated to collect aligned gelatin fibers using an electrospinning technique. The speed of the fabricated rotating drum collector was adjustable and controlled at 1000 rpm, 2000 rpm, and 3000 rpm. Electrospun scaffolds having aligned fibers were successfully produced using the rotating drum at 3000 rpm and compared with random nanofibers collected with the static plate. The speed of the rotating drum collector increased the alignment of the fibers and had little influence on fiber diameter. The results provide process parameters for the fabrication of aligned gelatin fibers.

Keywords: Rotating Drum, Electrospinning, Aligned Fibers

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Introduction

Fiber alignment can vary from randomly oriented fibers that overlap in all directions to fibers that are closely aligned in parallel direction. The alignment of fiber has some influence on cell response in tissue engineering. For example, certain lineages benefit from fiber alignment because it creates an inductive environment such as human tendon stem/progenitor cells on aligned fibers compared to random fibers [1]. When compared to random fibers, aligned fibers drive human mesenchymal stem cells toward cardiomyogenesis and promote myoblast differentiation [2,3].

Furthermore, bone, skeletal muscle, neuron and vascular tissue engineering, as well as fuel cells and gene delivery are all possible applications for aligned nanofibers. [4-7]. Aligned nanofibers have been shown to assist in the regeneration of highly organised structures such as nerve cells, tendons, and ligaments. They can also provide topographic guidance to cells, allowing for cell adhesion, proliferation, and migration.

Electrospinning, drawing, phase separation, template synthesis, and self-assembly are some of the processes used to produce nanofibers [8]. The electrospinning technique is a straightforward way collecting polymer nanofibers [9]. High voltage, a syringe needle, and a collector are the three main parts of electrospinning. There have two electrodes used to create an electric field, one clipping the needle of syringe and the other connecting to the collection plate where the polymer fibers are deposited. High electric potential creates an electrically charged jet of polymer solution or melt, which solidifies or dries to form a polymer fibre. A charged jet stream of polymer solution is discharged from the needle syringe when the electric field overcomes the surface tension of the droplet [10].

Classic electrospinning which produces random fibers using static collector, cannot mimic the needed of practical applications when aligned fibers is required. In this study, a rotating drum was designed and fabricated to produce aligned gelatin nanofiber using an electrospinning technique. It is because well-aligned or highly ordered nanofibers are important and required in the biomedical field to imitate the fibers in the extracellular matrix (ECM) [11,12]. Further, the scaffold's fibers alignment has a direct impact on its mechanical characteristics and functional performances [13]. The rotating drum is an ideal collector for nanofibers deposited align on its surface. The rotating drum is also easy to be conducted as compared to another electrospinning method.

The degree of fibers alignment of PCL was affected by the speed of rotating the drum [14]. The velocity of rotating collector can be classified into three conditions. First, the rotating velocity is too low to launch fibers orientation. Second, the fibers alignment increase with the increment of rotating speeds to an optimum level. Third, overloading rotating collectors induces fibers fracture and turbulent airflow around the collectors' circumference. Fiber alignment decreases as a result of this condition [15]. Electrospinning process is sensitivity to the environment condition including humidity, and therefore the investigation of new process parameters is needed [16].

In this study, the fibers was produced by using the cold water fish skin gelatin as a polymer solution. The gelatin polymer solution was used to produce fibers on a static plate in electrospinning process in previous study. The morphology of gelatin collected from the static plate was in random orientation [17]. This study is aimed to produce gelatin fibers with degree of alignment using a rotating drum.

Materials and Methods

Rotating Drum. A rotating drum collector was fabricated and used in the electrospinning machine as shown in Figure 1. The diameter of the middle shaft of the collector drum is 8cm. The surface area of the shaft was larger than the static plate and able to collect a large surface area of nanofibers meshes. The collector drum was designed to rotate with an adjustable speed up to 3000 rpm. Oldham coupling was used to connect the motor which drives the rotating drum collector in mechanical power transmission assemblies. The misalignment in parallel between the motor shaft and the collector drum shaft was resolved using the Oldham coupling design. When the misaligned problem had been solved, the stability of the rotating drum setup was increased.

The bases and motor holder were made up of stainless steel and the rotating drum holder is made up of nylon. The electrical insulating properties of nylon help to prevent any unwanted flow of current to the earth coming from its supporting points. It also has excellent abrasion and wears resistance and low coefficient of friction.

Solution Preparations. The gelatin polymer solution was dissolved according to the method of Khoo et al. (2019). The mixture of 90 wt% glacial acetic acids (Merck, Germany) and 10 wt% water was mix with 25 wt% solution of cold water fish skin gelatin with a molecular weight of 60 kDa (Sigma Aldrich, USA) [18]. Before loading the gelatin solution into a 50 mL plastic syringe (Terumo, UK), the gelatin solution was stirred overnight at room temperature.

Electrospinning Process. For electrospinning setup, the gelatin polymer solution was put into a 5ml syringe with a 23g needle. The applied voltage was controlled at 15kV and the distance between syringe tip and collector was controlled at 15cm. The feed rate of the solution was maintained at 0.45ml/hr. Two types of collectors used in this study were a rotating drum with 80mm diameter and a static plate. The speeds of the rotating drum used were 1000rpm, 2000rpm and 3000rpm. After that, the fibrous scaffolds collected were stored in a desiccator with silica gel.

Visualization and Quantitative Analysis. Scanning Electron Microscopy (Hitachi SU1510, Japan) was used to analyse the microstructure morphology of the electrospun scaffolds. Before analyzing, every electrospun scaffold was gold-coated by gold sputter (Fisons, UK) for 60 seconds to make sure the electron conductivity on the non-metal electrospun scaffolds. Then, ImageJ (NIH, USA) was used to process the SEM images and evaluate the fiber diameters. Three SEM images were captured from three random regions for each sample and 3 measurements of fiber diameters were taken for each SEM image to get an average fibers diameter. The fiber orientation was determined by the directionality function within ImageJ to show the alignment of the fibers in the mesh.

Results and Discussion

Comparison of Different Types of Collector. Figure 1 shows the electrospun scaffold deposited on the surface of the static plate and the rotating drum. The electrospun scaffold was deposited fully and distributed equally on the surface area of the rotating drum. The static plate only collected the fibrous scaffold on a particular area. After tearing off the electrospun scaffold, the surface area of the fibrous scaffold collected by a rotating drum was larger than the electrospun scaffold collected by static plate.

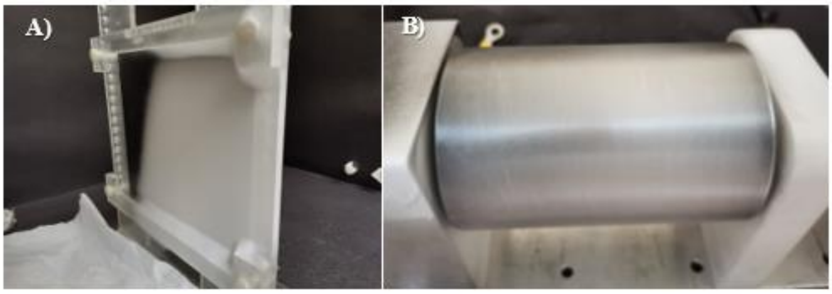

Figure 1. Electrospun scaffold deposited on the surface of A) static plate and B) rotating drum.

Morphology of Electrospun Fibers. Figure 2 shows the SEM images of gelatin fibrous scaffolds collected by static plate and rotating drum with the speed of 1000rpm, 2000rpm, and 3000rpm and direction of fiber for each mesh. The nanofibers collected by static plate and the rotating drum were conducted in the same process parameters and environmental conditions. All scaffolds produced by both collectors show the microstructure of the fibrous network without beads formation. According to Huang et al. (2004), the formation of beads affects the cohesive force between each nanofibers and thus decrease the strength of the fibrous scaffold [19].

The orientation of nanofibers collected by the static plate was random (Figure 2). The nanofibers collected by the rotating drum were organized in aligned orientation. The rotation speeds of the rotating drum affected the alignment degree of nanofibers. The direction of fibers collected at 1000rpm has the highest frequency in the range of -10^{o} and -20^{o}, but the frequency of fibers direction still present in every angle. This shows that the degree of alignment of fibrous scaffold collected at 1000rpm was low and quite random. When the rotation speed of the rotating drum increased to 2000rpm, the alignment degree of nanofibers increased as the direction of fibers more focus in the range of 0^{o} and -60^{o}. The orientation of nanofibers collected at 3000rpm shows that the most aligned nanofibers were within the direction range of -20^{o} and 20^{o}. When the speed of the rotating drum increased, the pulling force acting toward fibers was increased too. With the aid of pulling force of rotating drum, the fibers were pulled in a certain direction parallel which increased the alignment degree of fibers. Therefore, the rotation speed of the rotating drum affected the alignment degree of nanofibers. It was reported that neurite outgrowth on highly aligned nanofiber substrates can be up to 20% longer than on randomly aligned fibers. [20].

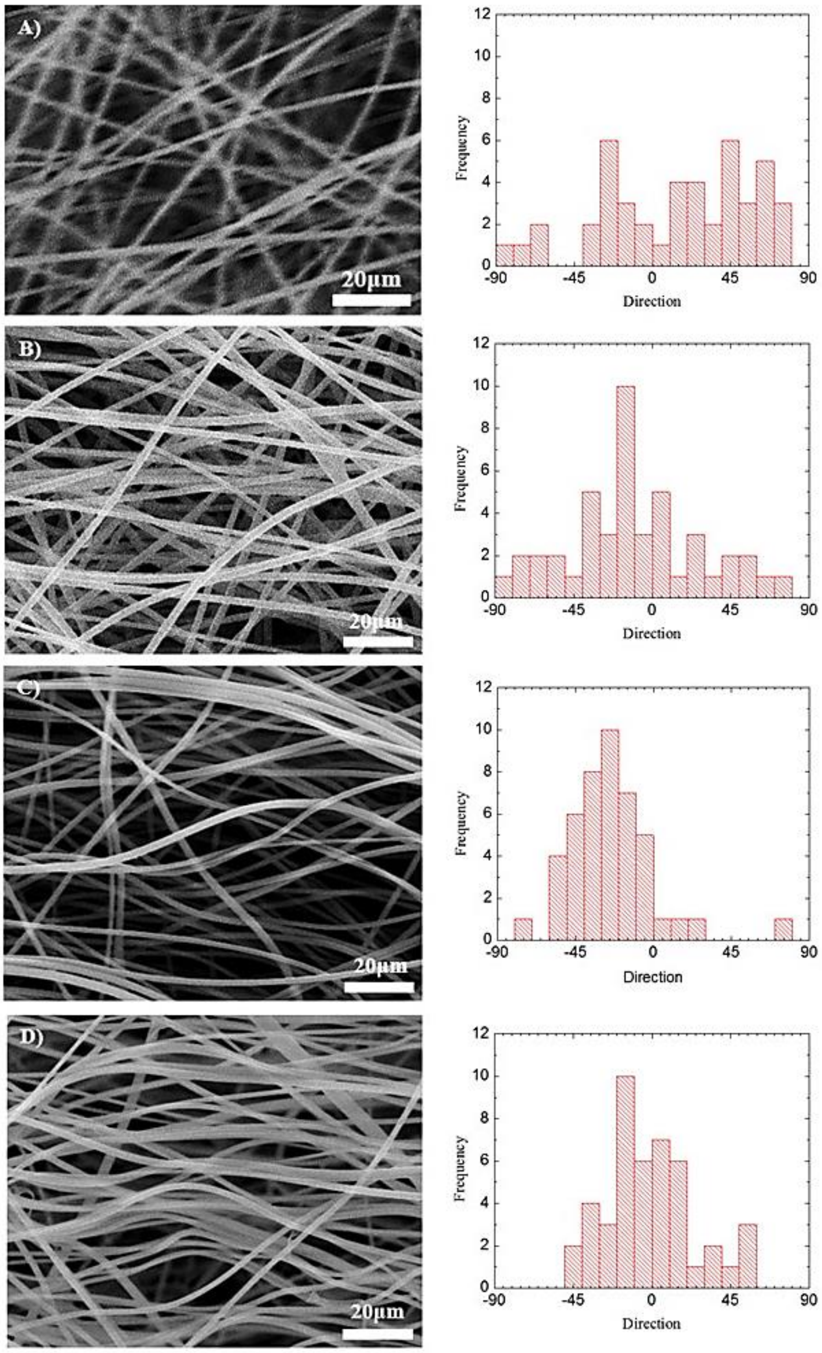

Figure 2. SEM images and directionality histogram of the electrospun gelatin fibrous scaffolds collected by A) the static plate, and the rotating drum at the speed of B) 1000rpm, C) 2000rpm and D) 3000rpm

Figure 3 shows fiber diameters of gelatin fibrous scaffolds produced by static plate and rotating drum at the rotating speeds of 1000 rpm, 2000 rpm and 3000 rpm. The type and speed of collector had little influence on fiber diameters. Moreover, there had no beads formed in the fibrous scaffolds with these three rotation speeds. This indicates that the electrospinning ability of 1000rpm, 2000rpm and 3000 rpm was excellent with the process parameters set.

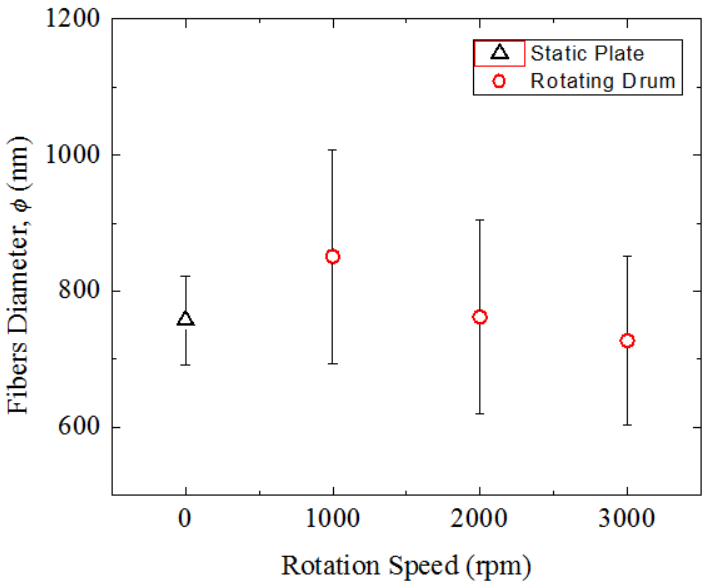

Figure 3. Fiber diameter against static plate and rotation speed of rotating drum

Conclusion

The rotating drum was designed and fabricated, and aligned gelatin nanofibers were successfully produced using a rotating drum. All electrospun scaffolds show the microstructure of fibrous networks without the existence of bead. The results show that the rotation of the drum increased the alignment degree, but have little influence on fiber diameters. The highest alignment degree of gelatin nanofibers with 727±125 nm was produced successfully at 3000rpm of rotating drum which is the optimum rotating speed. Therefore, it is shown that the rotation speed of the rotating drum was an essential parameter to control the degree of alignment of nanofibers. The scaffolds presented in this study have the potential to mimic fibers in the extracellular matrix of some native tissues.

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Author Contributions

All authors were involved in the study, Ham Wen Jun led the fabrication and visualization of gelatin scaffolds, data analysis and manuscript writing; Koh Ching Theng is the principal investigator and is responsible for the overall direction of the project.

Disclosure of Conflict of Interest

The authors have no disclosures to declare.

Compliance with Ethical Standards

The work is compliant with ethical standards.

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