Fabrication of 3D Nanofiber Scaffold by Sequential Electrospinning

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Abstract — *The generation of a 3D porous scaffold that would mimic the native ECM of tissue and organs is a topic of interest in tissue engineering. Electrospinning is a widely used technique in the area because it generates fibers of nano metric diameter with high surface, so several studies have applied it in the creation of 3D scaffolds. This research proposes a new approach that consists on the application of sequential electrospinning cycles over a rotational collector leaving a timespan between each cycle in which all the remaining solvent evaporates and the electro charges on the upper layer are dissipated. It was proved that the thickness of the scaffold increases linearly with each cycle. Additionally, when compared to continuous electrospinning the novel method generates a scaffold which presents almost double the thickness size and a similar porosity.*

Key Terms — *3D Scaffold, Porous Architecture, Sequential Electospinning, Tissue Engineering.*

INTRODUCTION

Tissue engineering is an emerging multidisciplinary field that focuses on design and development of functional, three-dimensional human tissue for medical research and therapeutic applications. One of the key research areas is to develop a scaffold that mimics the extracellular matrix to promote tissue regeneration. This is a complex arrangement of biomaterials in micro to nano scale that form a supportive structure for cells, promoting their growth, adhesion, migration and differentiation [1]. Mimicking the 3D porous architecture of the ECM is essential for the success of the scaffold, the pore network must be suitable for cellular ingrowth, promote cell to cell contact [1], and allow the diffusion of nutrients, metabolites and soluble factors [2]. It must be considered that morphologies of the ECM vary according to the function of the tissue, and even though some ECM have 2D characteristics (eg. top layer of skin), fibrous structures with 3D orientation can be found in breast, liver, bladder and lung tissues, among others [3]. 3D nanostructures with a significant thickness are increasingly being investigated for tissue engineering.

Electrospinning is a process in which polymer fibers are formed by the application of a strong electric field on a polymer solution or melt. This technique has raised great interests among tissue engineers because electro spun nanofibers present extremely high surface area, and thus work as an excellent platform to study the cell-environment interactions, effects of growth factors, drug delivery, etc., [4] [5]. One of the key requirements of scaffolds is to replicate the topographies and spatial structures of native ECM in order to facilitate cellular penetration, growth and differentiation [3] [6]. The 3D pore structure is one of the critical features for an ideal scaffold [7]. However, the electrospinning process has generated mostly 2D thin layers (usually between 200 to 1000 nm [8] of compact nanofibers with small pores thus limiting its application in tissue engineering [9].

RESEARCH DESCRIPTION

Traditional electrospinning presents difficulties to generate 3D scaffolds, in the first place, due to the repulsion generated by the accumulation of electrostatic charge between the already deposited fibers and the incoming ones [8], and secondly, because the fibers that reach the collector are wet and soft (the solvent doesn't evaporate completely during the flight) making the 3D configuration of the jet collapse into a 2D layer when deposited on the collector [10]. Several studies have addressed this issue by testing new techniques such as wet electrospinning, coarse fibers integrated electrospinning, and electrospinning with porogens [6].

To address this problem, this study will test if a thick and porous scaffold can be developed by using the sequential electrospinning technique. This work presents a novel facile electrospinning method for creating 3D nanofiber scaffolds. The scaffolds were fabricated by sequentially electrospinning the polymer nanofibers on a solidified layer. Between each electrospinning cycle, the scaffolds were left to dry in order to avoid fiber dissolution during continuous electrospinning.

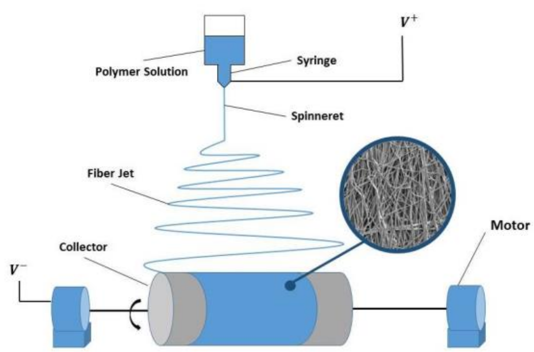

Figure 1
Electrospinning with a Cylinder Collector

METHODOLOGY

An eight-layer scaffold was built using sequential electrospinning technique, with a start point of 10 mm and a travel distance of 250 mm. Each layer was produced in one hour long electrospinning cycles maintaining the parameters indicated in Table 1. To let the layers properly dry and the dispersion of electro charges, there was at least a 3 hours long pause in between the cycles. Before starting a new cycle one small sample (1 cm x 5 cm app) of mat was peeled off the collector. The cycle was repeated 8 times, which mean 8 hours of electrospinning were completed. Each sample was named after the amount of layers it includes, e.g. to generate sample 3 three cycles of sequential electrospinning where completed. Also, a control mat was built using the traditional electrospinning method. The travel distance used for this setup was 63 mm and the duration of the electrospinning was 2 hours. Notice the ratio travel distance vs. process time is almost the same for both setups.

Material

Poly vinyl alcohol (10wt%, PVA, Tongli Tech, China) water solution was used as the nanofiber material. Fabrication of 3D Nanofiber Scaffold by Sequential Electrospinning. [11]

Electrospinning Setup

A stainless steel cylinder collector with a diameter = 100 mm, length = 300 mm was installed inside the Electrospinning Robotic Platform (TL-Pro-BM, Tongli Tech, China). The collector was located perpendicular to the spinneret to create a lineal fiber jet travel distance; this collector was connected to a negative voltage and rotated at speed of 1000 rpm. A stainless-steel needle was assembled on the spinneret with a 23 gauge (0.33 mm inner diameter) which is connected to positive voltage supplier. The tip of syringe had a fixed distance of 122 mm to the collector with a fixed angle of 45°. It was moved parallel to the collector at speed of 5 mm/s. The configuration of the machine is presented in Figure 1, and the parameters described in Table 1.

Table 1
Fixed Parameter for the Electrospinning Configuration

TTD (mm)	PV (kv)	NV (kv)	MPR (ml/h)	RS (rpm)	SSS (mm)
122	10	10	2	1000	5

Attributes Measurement

The attributes of interest for this study are thickness, diameter size, porosity, and orientation, to compare the actual process calling continuous electrospinning with the proposal one, called sequential electrospinning. The focus in this research will be the thickness and porosity. For each sample the thickness was measure with a thickness gauge (Mitutoyo, Japan) and direct with the SEM images. Images of samples 1, 8 and control were obtained with the scanning electron microscope (Hitachi S4300SE/N). The magnifications used were 700k and 1500k for plane view, and 800k for cross sectional view. The images were then analyzed to obtain the required attributes with ImageJ open source software.

The thickness of the scaffold was measure manually from the image. Porosity, orientation and diameter size were calculated by the Diameter J and Porosity J plugin program.

ANALYSIS & RESULTS

Firstly, the electro spun samples were visually examined to distinguish observable attributes such as color or hardness. Photo A is the layer 1 with sequential electrospinning, photo B is a new the layer over dry electro spun and this create layer two.

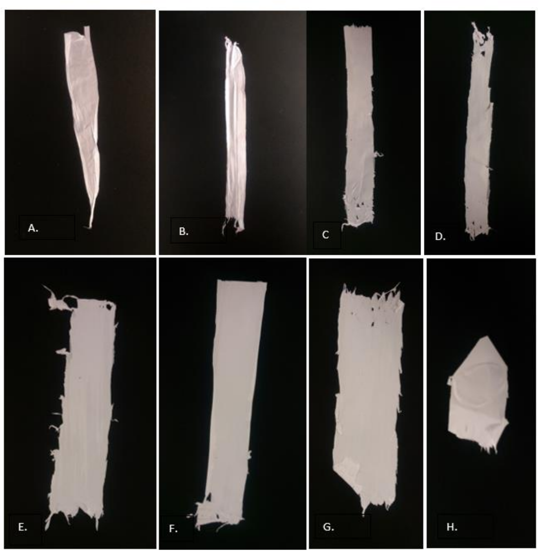

Figure 2
Eight-Layer Sample after the Process of Sequential Electrospinning

Thickness

The thickness values of the samples obtained with the gauge are presented as a scatter plot adjusted to a linear curve (Figure 3). It is clear that the points follow a linear tendency with an R2 value of 98%. Each additional sequential electrospinning cycle is adding approximately 0.0045 μm to the final scaffold.

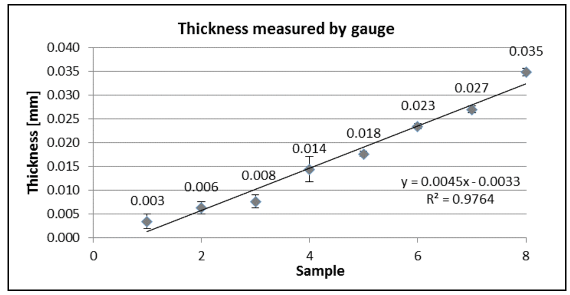

Figure 3
Sample Thickness Measured by Gauge

The thickness of sample 1, 8 and control was also measured using the SEM cross sectional images presented in Figure 4. The difference in thickness is observable in the photographs. To have a better precision of the thickness it's was measure five different parts in the layer to calculate the average for each one. These values are compared to those obtained with the gauge. It can be observed that using the latter tool results in consistently smaller thickness values compared to those by SEM.

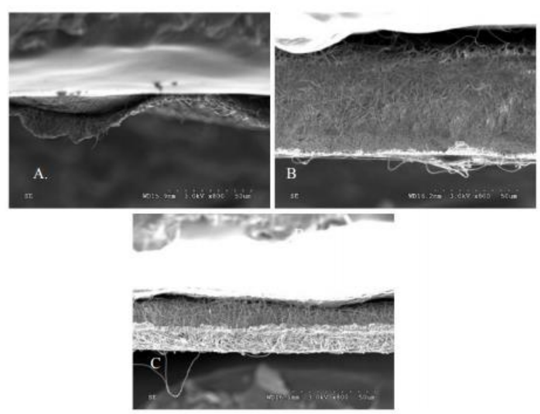

Figure 4
Cross section of Mat Samples
A. Sample 1. B. Sample 8. C. Control sample.

Diameter and Porosity

The images obtained with the SEM are presented in Figure 5. It can be observed that the control sample has the smallest fiber diameters and the fibers have no orientation. Sample 4, on the other hand, has larger pores. In order to validate this observations, porosity, fiber diameter and orientation were measured in the images 1500x of magnification by the program plugins Diameter J and Orientation J.

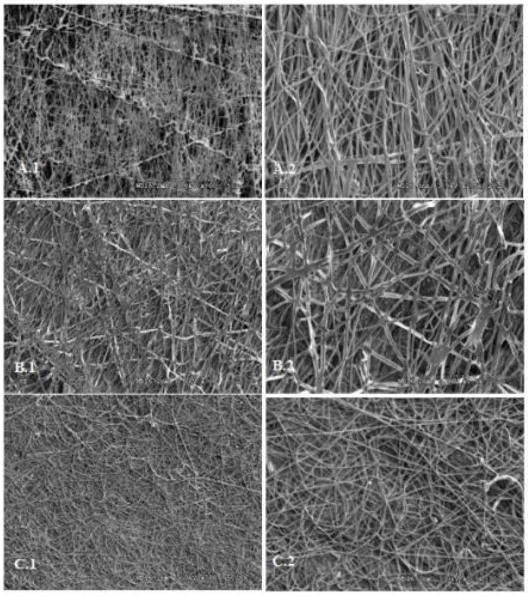

Figure 5
Scanning Electron Microscopy (SEM) Images
Lines: A. Sample 1. B. Sample 8. C. Control sample
Columns: 1. 700x. 2. 1,500x.

Porosity percentages are presented in Figure 6. The most porous sample is control sample, followed by sample 8.

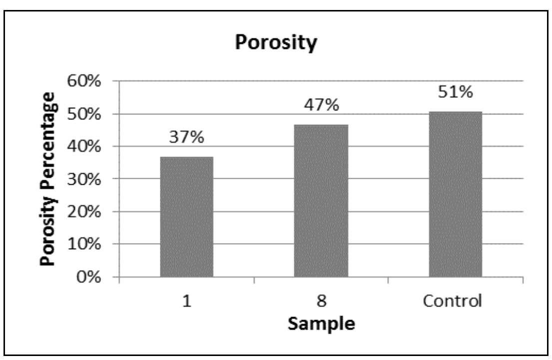

Figure 6
Diagram of the Porosity Percentage

The porosity values don't vary widely between each other, with the proposal as being 47% and control as 51%, the error marge between then is 7%. The fiber diameter values are displayed in Figure 7. In this case there's more difference between the samples, as Sample 1 has almost double fiber diameter than sample control as had stipulate that it would be the expect value.

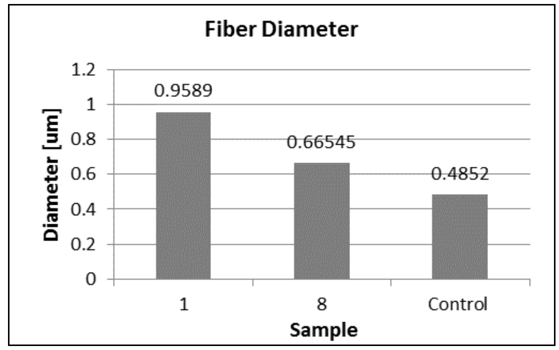

Figure 7
Diagram of the Fiber Diameter

Fiber Orientation

Orientation J software generates a histogram of the orientation angle of the fibers for each image analyzed. These images are presented in Figures 8, 9 and 10 for sample 1, 8 and control, respectively. The orientation histogram for sample 1 has a concave up shape, with most fibers having a -90° or 90° orientation. This means that fibers are mostly oriented vertically. For sample 8, the tendency can be detected but it's not as clear as with sample 1. On the other hand, the histograms for the control sample are completely random therefore the fibers have no constant orientation, demonstrating the observation made before with the SEM image.

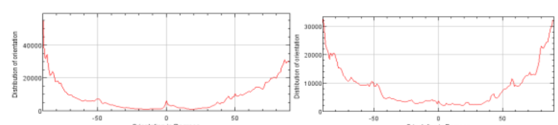

Figure 8
Fiber Orientation of Sample 1
Two Images taken with SEM x1500

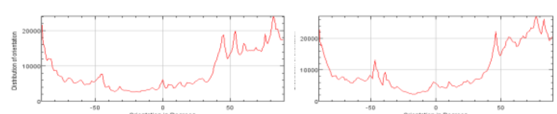

Figure 9
Fiber Orientation of Sample 8
Two Images taken with SEM x1500

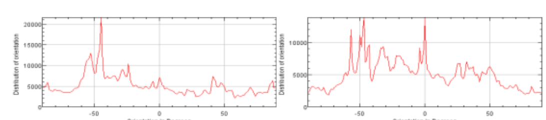

Figure 10
Fiber Orientation of Control Sample
Two Images taken with SEM x1500

Statistical Analysis

One way Anova was performed in Minitab to compare the different data sets and significance was accepted when the p-value was less than 0.05. As the result, the P value of the hypothesis was computed and the p-value for the coefficient is less than 5%, close to 0, thus it is significant impact in the results.

Also the average and standard deviation was calculate for each attribute to know how much the values can moved away from the average and the probability that the data move up or down according to the general process. The data does not deviate to much between them, this conclusion is very important because it is a medicinal process and the closer the data are between them, the less error will be processed in order to obtain a better homogeneity.

The proposed margin of error is 5%, this will help us to understand that the data is not precise or exact, we don't choose a grater margin of error because we are working on human observations and we need precision data, also to have a smaller percentage of error the sample needs to be larger. Also with a confidence level of 95% we get a Z of 1.96, with this number we calculate the sample size. The sample size helps us to understand better how much quantity of sample we need to collect to avoid the bias in the interpretation of the data, as it helps us reduce costs and time. The greater sample size we get was for the continuous electrospinning (traditional one) for the diameter size with an N of 47, this tells us that to create a better scaffold diameter should be done 47 samples which indicates that it would be 47 hours of continuous work. But with the sequential electrospinning for the sample of diameter only need a sample size of 27.

Limitation

The total thickness of the mat built by sequential electrospinning reached only the tens of microns, when in tissue engineering a functioning scaffold must have a 3D structure with at least a millimetric thickness. It was discovered that the thickness added to scaffold behaves in a lineal way, according to this results, the desire thickness can be obtained by repetition of cycles. However, it is encouraged to realize the experiment with an additional number of cycles to prove that the thickness curve maintains its linearity. Another drawback of using the technique proposed is the time it takes to fabricate the scaffold, considering that before starting with the new layer the last one has to be dried. If each cycle only adds 10 microns of thickness then to be able to reach 1 mm around a 100 cycles must be completed. In addition to the polymer used that has to be ordered from China and the shipment takes a time.

DISCUSSION

Several studies in the field of tissue engineering have focused on the construction of a scaffold with a 3D pore architecture utilizing the electrospinning technique. One of the main approaches consists in the variation of the collector plate from a 2D to 3D shape, which can vary in complexity from parallel plates to other custom-built configuration [12]. Additional studies have used a liquid collector. Its working principle is immersion precipitation, fibers solidified instantly as they are deposited in a non-solvent liquid [13]. Another method used to obtain thicker layers is post process of the traditional scaffold, either by stacking layers together [14] or by folding them into a tubular shape [15] [16]. To improve the porosity of the scaffolds porogen particles and micrometer-sized fibers have been introduced during the electrospinning process thus increasing the void between the nanofibers. These approaches have had positive results, however, they add materials and steps to the traditional electrospinning process, and some may have difficulties with the preservation of the mat morphology during the removal from the collector [17].

Sequential electrospinning, as proposed in this paper, consists on the successive application of electrospinning over dried electrospun layers thus creating a thicker layer with each cycle to generate 3D porous scaffolds. It varies from traditional electrospinning as the latter is a continuous process, while in the former, a new electrospinning cycle is only started when the last layer deposited has already dried. With the study it was verified that the thickness of the scaffold increases linearly with each sequential cycle. Additionally, it was confirmed that realizing the traditional method of electrospinning, maintaining the same parameters and time-travel distance proportion, generates a thinner scaffold. For this specific study the scaffold built by sequential electrospinning reached almost double the thickness and increases linearly with each cycle of the one by continuous electrospinning. It was expected to obtain a thinner and densely packed continuous electro spun scaffold. However, the control sample presented a higher porosity than the sample obtained by sequential electrospinning. This indicates that the control sample has less amount of polymer deposited. One possible explanation of this phenomenon is that the accumulation of electrocharge on the collector, as the electrospinning process continues, weakens the charge density between needle and collector.

CONCLUSIONS

A novel approach for electrospinning was presented to generate 3D porous scaffolds. Sequential electrospinning consists of the repetition of electrospinning cycles on the same collector separated by a drying time gap. It was proved that the thickness of the scaffold increases linearly with each cycle. Scaffolds built with the sequential and the continuous methods were compared. The thickness obtained using the novel method almost doubles the traditional one. Although the porosities of both scaffolds are similar, the traditional method achieved a bigger porosity percentage. It would be interesting to continue further studies to improve the porosity and increase the thickness achieved. It would be interesting to continue further studies to improve the porosity and increase the thickness achieved.

RECOMMENDATION

Based on the results, the final recommended is a study focused in determining what is the maximum amount of material applied in each cycle is proposed [18]. Although the porosity of the mat wasn't affected with the repeating cycles, as stated in the objected, it is still not enough for tissue engineering application. To correct this, the study could be repeated using two polymer solutions which dissolve in different solvents. After building the electro spun scaffold one of the polymers is dissolved, leaving a larger pore size. Different pump rates should be experimented with to obtain different porosities. It would be interesting to continue further studies to improve the porosity and increase the thickness achieved [19].

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