Development of Plasma Device Integrated Electrospinning System for Biofabrication

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***Abstract*— Electrospinning is a bio-fabrication method, which helps to produce highly interconnected nano-porous structure with a high surface to volume ratio by using polymer solutions. In tissue engineering applications, scaffolds composed of electrospun nanofibers are produced from synthetic polymers to resemble specific tissue. Most of synthetic polymers has high hydrophobicity since the nonpolar groups located along their backbone. Thus, it effects cell-scaffold surface interactions and cellular responses Different surface modification techniques are used for nanofiber functionalization. Plasma discharge is used an effective method for the modification of the polymer surface. In this study, plasma integrated systems were set up. Conventional electrospinning system was used as control. The parameters, such as needle-to-syringe distance, plasma application spot, type of plasma electrode, were changed and the effect of different types of setup on nanofiber alignment and diameter and wettability were analyzed.**

Keywords —electrospinning, nanofiber, DBD plasma, tissue engineering, scaffold

I. INTRODUCTION

Nanofibers can be fabricated from polymers by using template, self-assembly, phase separation, melt-blown and electrospinning methods [1]. These fibers are used in tissue engineering applications due to their high surface to volume ratio, porous and interconnected structure [2]. These unique characteristics make nanofibers desirable for advanced applications. Electrospinning methods offers ideal conditions in terms of producing highly interconnected open nano-porous structure with a high specific surface area [3]. An electrospinning device basically consists of a high-voltage power supply, a grounded collector and a positively charged syringe filled with polymer solution. Power supply creates an electric field between syringe and collector by charging liquid droplet. When the electrostatic charge becomes greater than the surface tension of polymer solution, a polymer jet is created. This polymer jet creates fibers, ranging from micro to nanometer scale diameters, by drying out and elongating due electrostatic repulsion. All fibers are collected on the collector by forming various structures with different orientations and mechanical properties[4].Various surface modification techniques for applying synthetic polymer nanofibers to tissue engineering and drug delivery are wet chemical method, surface graft polymerization, co-electrospinning and plasma treatment [5].

Plasma the fourth state of matter [6] is by far the most common phase of matter in the universe as distinct from the solid, liquid, and gas phases [7]. Plasma is generated by electrical breakdown in a gas [8]. Plasma is an ionized gas composed of charged particles (electrons, ions), excited atoms, molecules, free radicals, and UV photons. A collection of freely moving charged particles which is, on the average, electrically neutral [9].Plasma medicine that is categorized to thermal and cold [10] is among the field that has been integrated physics, chemistry, biology, and biomedical engineering and is applied plasma to medical applications [11]. Especially low temperature plasma can be used as a treatment method in medical applications. For example; hospital hygiene [12], antifungal treatment [13], dental care, skin disease, chronic wounds [14], cancer treatment, sterilization of medical equipment and surface modification of materials [15]. Dielectric Discharge Barrier plasma that is cold plasma type occurs between two electrodes separated by an insulating dielectric barrier. It is an electrified gas with a chemically reactive media. When the interaction other materials electrical field exist between DBD electrode and materials. The electrical field increases the controlled flow of ions towards between two electrodes

Plasma discharge is used an effective method for the modification of the polymer surface. In tissue engineering applications, scaffolds composed of aligned electrospun nanofibers are produced from synthetic polymers to resemble specific tissue. The nanofibers provide huge surface area for cell adhesion, migration and differentiation. As an example, poly (lactic-co-glycolic acid) (PLGA) electrospun fibers are commonly used due to its biocompatibility, biodegradability and minimal immune response. Nevertheless, PLGA has high hydrophobicity, since the non-polar groups located along its backbone. Therefore, it effects cell-scaffold surface interactions and cellular responses. Oxygen plasma can react with polymers and produce oxygen functional groups on the polymer surface, e.g., the C–O, C=O, and O=C–O groups [16]. For tissue engineering applications, surface modification of PLGA scaffolds improves their biological performance by immobilizing bioactive molecules such as growth factors, drugs, proteins and peptides. Nevertheless, after producing

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electrospun nanofibers, plasma application can be inefficient due to not penetrate inner side of 3D scaffolds. Therefore, a system, in which plasma reacts with each nanofiber in the structure, can be developed. In this way, homogenous functionalization can be occurred.

One of the major challenge of producing electrospun nanofibers is alignment. Nanofiber with a high alignment improve mechanical properties and cell proliferation processes, such as cell migration and differentiation. In this study, plasma device was integrated to electrospinning system to provide efficient functionalization and alignment by increasing electrical field

II. METHODS

A. PLGA Fiber Spinning

A blend of 7 wt % PLGA was dissolved in 1,1,1,3,3,3-hexafluoro-2-propanol (HFIP) solvent. The polymer solution was transferred and injected from a 1-ml syringe through a 21-gauge needle by using a syringe pump. The needle was connected to the positively charged electrode of a high-voltage power supply. The aligned nanofibers are collected by an aluminum rotating drum collector, powered by a high-speed DC motor and frame collector. The previously optimized electrospinning conditions of 1.0 ml/h injection rate, 20 kV electrical potential, and needle-to-collector distance of 7.5 cm and 15 cm, and rotation speed of 1000 rpm were used to produce aligned nanofibers. Injection rate, electrical potential and rotation speed were kept as constant parameters. Collector type and needle-to-collector distance were changed depends on application

In addition to conventional electrospinning system, plasma source was designed and cold atmospheric plasma produced by using this device to form aligned nanofibers on collector by increasing electrical field. Switch circuit (Figure-2) uses 220V Alternative current (AC). 220 V AC source carries current to specific thyristor that produce short pulses to a transformer. And this transformer produces minimum 5kV to maximum 20 kV electrical voltage on 5 cm circle and 0.2 cm needle diameter copper produces minimum 5kV to maximum 20 kV electrical voltage on 5 cm circle and 0.2 cm needle diameter copper DBD electrode.

Figure 1. Set up of Plasma Integrated Electrospinning Device

By using both electrospinning and plasma devices, eight setups were used to produce nanofibers (Table 1). In Setup 1, rotating drum collector was used to keep needle-to-collector distance as 15 cm and no plasma was applied on the system. This system was used as a control group. In Setup 2, rotating drum collector was used keeping needle-to-collector distance as 15 cm. Circular shape plasma electrode was used to apply plasma. In Setup 3, needle plasma electrode was used and all the parameters were kept as the same with second setup. In Setup 4, frame collector was used as distinct from the second setup. In Setup 5, application includes needle electrode was used and all the parameters were the same with Setup 4. In Setup 6, tip of syringe was covered with plastic to prevent plasma damage on the tip of syringe and the distance was decreased from 15cm to 7.5 cm. In Setup 7, all parameters were kept the same with Set up 6, but plasma was applied directly to drum. In Setup 8, all the parameter were the same with Setup 6, but plasma angle was changed from 45° to 180º.

		Setup 1	Setup 2	Setup 3	Setup 4	Setup 5	Setup 6	Setup 7	Setup 8
Plasma Parameters	**Electrode**	Not used	Circle	Needle	Circle	Needle	Circle	Circle	Circle
	Voltage	Not used	15 kV	15 kV	15 kV	15 kV	15 kV	15 kV	15 kV
	Application Spot	Not used	Needle	Needle	Needle	Needle	Needle	Collector	Needle
	Electrode Angle	Not used	45º	45º	45º	45º	45º	180º	180º
Electrospinning Parameters	**Collector**	Drum	Drum	Drum	Frame	Frame	Drum	Drum	Drum
	Flow Rate	1 ml/h	1 ml/h	1 ml/h	1 ml/h	1 ml/h	1 ml/h	1 ml/h	1 ml/h
	Solution Amount	1ml	1ml	1ml	1ml	1ml	1ml	1ml	1ml
	Voltage	20 kV	20 kV	20 kV	20 kV	20 kV	20 kV	20 kV	20 kV
	Solution Type	%7 PLGA	%7 PLGA	%7 PLGA	%7 PLGA	%7 PLGA	%7 PLGA	%7 PLGA	%7 PLGA
	Injector Type	Metal	Metal	Metal	Metal	Metal	Plastic Covered	Metal	Plastic Covered
	Distance	15cm	15 cm	15cm	15 cm	15 cm	7,5 cm	7,5 cm	7.5 cm

Table 1. Plasma and Electrospinning Parameters

B. *Characterization of Nanofibers*

After coating with gold (QUORUM Q150 RES), the fiber mesh was attached to a stub and imaged with a scanning electron microscope (SEM; Carl Zeiss 300 V) at 10 kV accelerating voltage. Fibers were analyzed with IMAGEJ software to determine the average fiber size. The wettability of nanofibers was measured with a contact angle goniometer. 10µl drop of deionized water was dropped to the fiber surface and photographed. Contact angle was measured by One Attention software.

C. Simulation Method

Electric field analysis was performed by using ANSYS and the electric field of the plasma-assisted electrospinning system was calculated and simulated. As boundary conditions, voltage of 15 kV from the different plasma electrodes and 20 kV from the ignition were given. Ground was selected as the ground collector and the current path was loaded on the ground collector. Then, it was respectively found the total electrical field intensity, total current intensity results, and the current direction of the system.

III. RESULTS

A. *PLGA Fiber Spinning*

Average fiber diameters of PLGA nanofibers produced by using Setup 1, Setup2, Setup 3, Setup 4 ,Setup 5, Setup 6, Setup 7, Setup 8 are 337 nm, 267 nm, 576 nm, 371 nm,379 nm, 743 nm, 218 nm and 649, respectively.

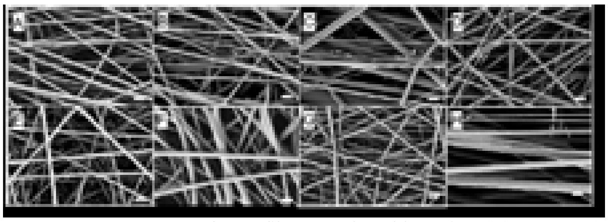

Figure 2. SEM Images of nanofibers produced by using (A) Setup 1 (B) Setup 2 (C) Setup 3 (D) Setup 4 (E) Setup 5 (F) Setup 6 (G) Setup 7 (H) Setup 8. Scale bar represents 2µm

The effect of different setups on wettability of the nanofibers is shown in Figure 3. The minimum water contact angle was observed in Setup 6 (80,24°±2,52) among setups and the maximum water contact angle was observed in Setup1(124,83°±2,23) which is used as control group in this study.

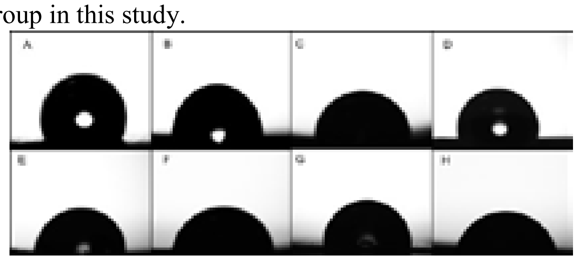

Figure 3. Shape of a water droplet on PLGA nanofibers produced by using (A) Setup 1, (B) Setup 2 (C) Setup 3 (D) Setup 4 (E) Setup 5 (F) Setup 6 (G) Setup 7 (H) Setup 8

B. *Simulation*

ANSYS Simulation Driven Product Development is used to simulate the electrical field intensity between plasma electrode, nozzle, and ground collector. Different electrode diameter changes the electrical field on ground and affected with nozzle (Figure 4 and Figure 5). When diameter of plasma electrode respectively decreases electrical field on ground collector increases. The interaction between plasma electrode and the nozzle has electrical charges and then it makes the electrical force and intensity. This intensity produces electrical force on the ground collector.

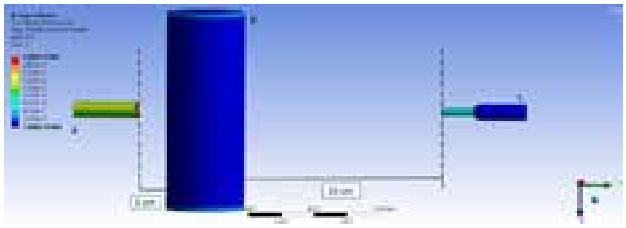

Figure 5. 15 kV 5 cm diameter Circle Plasma Electrode, b) Ground Collector 6 cm diameter, and c) 20 kV Nozzle

Figure 6. 15 kV 0.2 cm diameter Circle Plasma Electrode, b) Ground Collector 6 cm diameter, and c) 20 kV Nozzle

IV. DISCUSSION

Aligned and randomly oriented nanofibrous mats were produced by using plasma modified electrospinning with different types of setups as shown in Table 1. Fiber angle was measured by Image J. It was determined that more aligned fibers were produced with Setup 8. When Setup 8 is compared with Setup 6, the only parameter changed is plasma electrode position changing angle from 45º to 180º. To our knowledge, changing electrode position ends up with changing in electrical field and this electrical field increases fiber alignment. Even though alignment was increased by using Setup 8, median fiber diameter was maximum compared to other setups. The median diameter of nanofibers produced by using Setup 2 (239 ± 100) and Setup 7 (218 ± 103) have smaller diameter compared to other groups. When Setup 2 is compared with Setup 6, distance can be considered the parameter which effects median fiber diameter. One of essential characteristics of nanofibers for tissue engineering applications is wettability. Higher wettability after plasma application indicates that increase in COOH and OH groups. When we compare the nanofibers produce by Setup 5 and Setup 6, the water contact angle decreased to 80,24°±2,52 (Figure 3E) from 92,58°±4,19 (Figure 3F). When the parameters are compared within these two setups, it is seen that plastic covered syringe was used in Setup 6 while metal syringe was used in Setup 5. During the experiments, the tip of metal syringe was damaged due to plasma application. Therefore, it is considered that plasma can change the characteristics of syringe. After covering the tip of metal syringe, decrease of contact angle was observed. Hence, it might be considered that plasma effect on polymer was increased after covering the needle with plastic.

V. CONCLUSION

In this work, optimal nanofiber scaffolds were fabricated by integrating plasma on electrospinning for tissue engineering applications. The outcomes of this study would help our ongoing study which is designing custom made plasma integrated electrospinning device.

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