

## **Fabrication of Nanofibers for Tissue Engineering and Regenerative Medicine**

**Dr. Jafar F. Al-Sharab, Northwestern State University**

Dr. Jafar Farhan Al-Sharab has recently joined the faculty in the Engineering Technology Department at Northwestern State University as an assistant professor. Jafar F. Al-Sharab received BS in Industrial Engineering from the University of Jordan, MS in Metallurgical Engineering from the Science University of Malaysia, and PhD in Materials Science and Engineering from Vanderbilt University/Nashville, TN. Prior to joining NSU, Professor Al-Sharab was an Instructional and Research Faculty at Rutgers University. At Rutgers, he was heavily involved in research and teaching at both graduate and undergraduate levels. In the period of 2011-2014, Dr. Al-Sharab was a visiting professor in the Department of Mechanical and Aerospace Engineering at New York University Polytechnic School of Engineering. In addition to his work with academic institutions, Dr. Al-Sharab was a consultant of various technological companies especially in the areas of structure-property-correlations and advanced characterizations. Dr. Al-Sharab's research interests are in the areas of Nanotechnology, Electron Microscopy, Structure-property correlations, synthesis and characterization of energy related materials (harvesting and storage), flame retardant polymers, nanocomposite materials, and advanced materials characterization. He is an active member of professional organizations related to his research interests. He has authored more than 40 technical papers.

**Dr. Mohammed Benalla, Northwestern State University**

Mohammed Benalla obtained in 1993 an associate degree with a double major in physics and Chemistry then a bachelor in 1995 majoring in physics from The University Mohammed V, Rabat – Morocco as well as a first Master in 2001 in Electro-technics & Industrial Electronics from Mohammadia School of Engineering, Rabat-Morocco. The second Master was obtained in 2007 from City College of New York, CCNY, majoring in biomedical Engineering. The Master Thesis was Blood Vessel Wall Permeability and Endothelial Cells Interconnectivity. In 2012, Dr. Benalla achieved his PhD from the Biomedical Engineering Department CCNY in Biomechanics. The PhD thesis was the Determination of the Lacunar-Canalicular Permeability of Human Cortical Bone Using Physiological Loading. After his PhD, Dr. Benalla worked as a research associate with the Biomechanics Laboratory in CCNY with a cooperation with the Orthopedic Department of Mount Sinai Hospital and the Graduate Center of New York. In addition to his research Dr. Benalla taught as Adjunct Assistant Professor in different Colleges in New York. The different colleges Dr. Benalla taught in are New York City College of Technology, Brooklyn, LaGuardia Community College, Long Island City, Hostos Community College, Bronx and CCNY, Manhattan. In August 2013 Dr. Benalla joined Georgia Southern University as a full time faculty with the Department of Mechanical Engineering where he taught as a Visiting Assistant Professor for two years. Presently, Dr. Benalla works as Assistant Professor with the Engineering Technology Department of Northwestern State University of Louisiana. The different Classes Dr. Benalla has taught during his academic carrier are Signal Processing, Digital Electronics, Electrical Circuits, Tissue mechanics, Poroelasticity, Biomaterial, , Thermodynamics, Fluid Power, Engineering Design, Statics and Strength of Materials. Dr. Benalla has published more than seven papers. All his research and publications are related to the skeletal tissue mechanotransduction phenomenon and human bone Dynamic permeability at the lacunar-canalicular porosity on the osteonic level.

# **Fabrication of nanofibers for tissue engineering and regenerative medicine**

## **Abstract:**

There is a continuous need for new organs and tissues due to lack of donor organs necessary to help combat some the debilitating diseases. To close the gap between demand of organs and those who are in need, regenerative medicine and tissue engineering must be utilized. In our research we utilize electrospinning of nanofibers as a method of forming scaffolds. Due to the high charge density of polymers under the influence of electrospinning, it lends itself to the idea that a magnetic field has the capability of controlling and aligning the high-charge density fibers to the field, forming regular, aligned and thus effective scaffolds for the engineering of tissue. In this paper we will be presenting examples related to the effect of process parameters on the various properties of the fabricated fibers. In addition to the scientific merit of the research topic, the paper describes important educational modules/experiments, which can enrich participated students with significant information on nanofabrication, and characterization techniques.

## **Introduction:**

The World Health Organization recognizes the pandemic of chronic diseases and the severe lack of donor organs necessary to help combat some of these debilitating diseases. In fact, one can see that the gap between those who need organs and are currently on the wait-list and those who are donating and transplanting organs is growing and has grown 5 times as much, since 1989<sup>1</sup>. The need is very dire and comes from an increasingly aged population. Currently, the only way to reduce this gap between organ donors and those who need organs is to encourage more people to register. However, with the new field of regenerative medicine and tissue engineering, we can apply engineering principles to alleviate the problem<sup>2-5</sup>. With tissue engineering, the growth of new tissue and appropriate cells allow for patient-specific therapy, one that is sustainable and capable of closing the gap between those who require new organs and donors available. Literature has shown how when creating the scaffold for engineered tissue, proper alignment and topography is a necessity. These factors help to form tissues that are much more robust and therapeutically effective. Electrospinning of nanofibers is a common method of forming scaffolds. Due to the high charge density of polymers under the influence of electrospinning, it lends itself to the idea that a magnetic field has the capability of aligning the high-charge density fibers to the field, forming regular, aligned and thus effective scaffolds for the engineering of tissue<sup>1</sup>.

The chosen method to create bio-mimetic scaffolds in this study is electrospinning. One of the factors that makes electrospinning so attractive is its cheap and simple setup. An electrospinning setup can be constructed with a syringe feeder, a syringe, high-voltage source, and ample supply of polymer. All these components are affordable it is simple to control scaffolds. The fabricated fibers that are laid down by electrospinning organic and biocompatible materials create scaffolds that can be used for tissue engineering. The scaffolds tend to have a large amount of porosity<sup>6-9</sup>. The porous media protects the vivacity of the embedded cells in the tissue and facilitate the

communication between the cell seeding the cell line, which is required for the resorption and the remodeling of the tissue. One final factor that allows electrospinning to be a useful method for tissue engineering is the ability to functionalize the fibers with bioactive molecules. The bioactive molecules can act as chemical cues while the topology of the nanofibers can act as mechanical cues all at the same time. This is a very powerful modification as it allows for less time and effort when inducing the cells to differentiate and form tissues. This bioactive scaffold can theoretically allow for scaffolds where only cells and media are necessary to grow tissues and organ supplements. With this amount of control over the method, it is easy to control the porosity, fiber diameter, and side-chain substituents to create scaffolds that are more useful for tissue engineering.

### **Experimental:**

An in-house electrospinning setup was developed using HI-voltage generator to provide the necessary currents for Electrospinning. A syringe feeder (Pump II +, Harvard Apparatus, PHD2000) was obtained to induce appropriate flow rates at a static pressure. Solutions of polyethylene oxide (PEO) were prepared using multiple solvents and co-solvents. Polyethylene oxide was prepared in 1% w/v solutions. The solvents used were chloroform and ethanol. Solutions were mixed and lightly heated (35° C) over the span of 2 days to allow for homogenous mixing and solute-solvent interactions. Solutions were then kept sealed at room temperature and in a hygroscopic environment.

All fibers were spun using 1% (w/w) PEO/CHCl<sub>3</sub>. Fibers were spun onto a microscope slide and measurements were done with a confocal microscope. Measurements of fiber diameter were done with computer software (SPOT v4.0.9, Diagnostic Instruments Inc.) Statistical analysis was done using GraphPad Prism.

### **Concentration as a Process Parameter for Fiber Diameter**

Three solutions were formulated with different concentrations. It was observed that increasing the polymer concentration beyond 1% would be counter-intuitive to optimize and reduce fiber diameter. With large molecular weight, it would not be optimal to have a higher concentration, as this would most definitely lead to a higher surface tension and viscosity, preventing appropriate Taylor cone formation and impeding the Electrospinning process. These solutions were then electrospun under similar conditions: 15kV voltage, 12 cm distance, 3.0ml/hr and no additional inorganic salts. Images were taken and analyzed using SPOT analysis and statistical analysis was performed by GraphPad Prism.

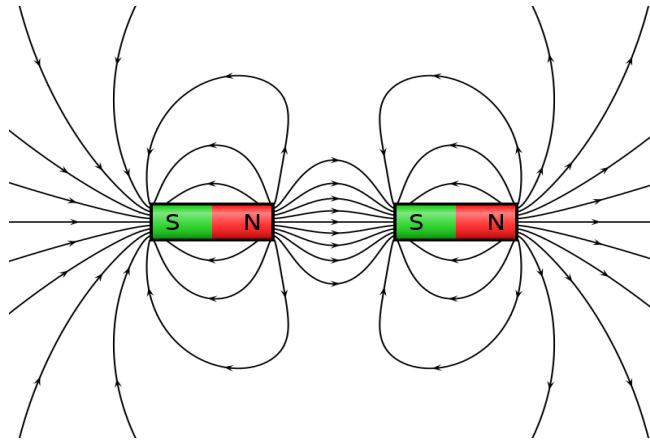
### **Addition of a co-polymer.**

Co-polymers have been shown to be a boon in electrospinning of fibers for scaffolds and filtration systems. When two polymers are spun together in a similar solution, they impart properties to the final fiber scaffolds that are not found in the single polymer fiber. For example, it has been shown that poly (caprolactone), when spun with poly(lactic acid) is capable of increasing cell attachment and proliferation. This is most likely due to the fact that poly

(lactic acid) is a much more bioavailable polymer than poly (caprolactone). This also lead to an increase of mechanical strength but unfortunately, a decrease in porosity. PEO is a bioavailable polymer, but the ability to increase cellular attachment and proliferation by mixing with PLA is a very useful property to impart to our electrospun scaffolds. Another useful property would be reduction of the fiber diameter, as more PLA would help to reduce the intrinsic PEO concentration of the solution and allow for less mass transfer at the needle tip. 5 solutions of copolymers of PEO and PLA were spun. The solutions were spun under the same process parameters, which were 14kV, 12 cm needle to collector distance, 3.0ml/hr flow rate, and no inorganic salt addition.

### Magnetic Field Effect Studies

Alignment of fibers used as scaffolds for tissue engineering leads to better viability of cells and tissue growth. In this research, the effect of magnetic field on the alignment of the synthesized fibers was investigated. A pair of permanent bar magnets was used to generate a magnetic field. Atypical interaction field is shown in **Figure 1**.



**Figure 1.** Diagram of vector force lines of attraction between two bar magnets.

Maxwell's laws show us the interaction of electricity and magnetism<sup>10</sup>. Mathematically, it is shown that the distance between two magnetic poles, affects the strength of a magnetic field force. The strength of force between two bar magnets is modeled by the following Equation.

$$F = \frac{B^2 A^2 (L^2 + R^2)}{\pi \mu L^2} \left( \frac{1}{x^2} + \frac{1}{(x+L)^2} - \frac{2}{(x+L)^2} \right) \quad (\text{Equation 1})$$

Gilbert's model of magnetostatics is used to derive the force between two bar magnets. B – magnetic flux density near each pole (Tesla), A – area of each pole (m<sup>2</sup>), L – length of magnets (m), R – radius of cylindrical bar magnets (m), μ - permeability of space (N \* Ampere<sup>-2</sup>), and x – distance between two magnets.

Equation 1 uses Gilbert model of magnetostatics, where it is assumed that magnetic monopoles exist. Electrostatic equations can be directly converted to magnetic analogs. The Gilbert model and it's assumptions work correctly when used with classical distances. However, it falls apart

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at very close distances and the Ampere model must be used. The Gilbert model was selected due to its less mathematical complications and it operates as a good assumption at our distances.

In this research, fibers mats were spun with varying distances between the neodymium magnets. Fiber mats were spun at 1.8 ml/hr at 14 kV with a needle/collector distance of 12 cm. Fiber mats were spun at distances of 3.8 cm, 6.4 cm, and 10.2 cm distances. Samples were collected using these parameters and were observed using microscopy techniques. Fibers were determined to be aligned if they were  $\pm 50$  of the Y-axis vertical. Measurements were done with SPOT Analysis software. Image analysis was conducted using ImageJ and GIMP. Statistical analysis was done with GraphPad Prism.

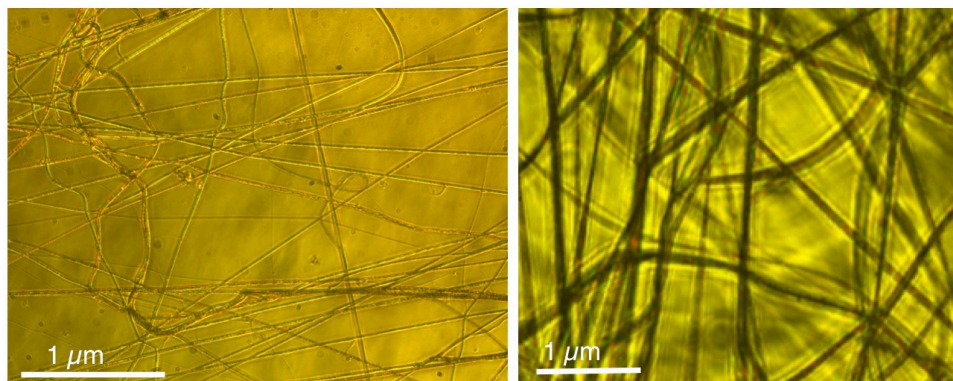
## Results

In this research, a series of fibers with differing flow rates to in-house optimize the fiber diameter and generate a simple linear regression correlating fiber diameter to flow rate of Electrospinning. The first series was from 24ml/hr to 3 ml/hr, at regular intervals of 3ml. The first series of flow rate studies showed a significant difference between fiber diameters and morphology. These fibers were 1% (w/v) PEO/CHC<sub>13</sub>:C<sub>2</sub>H<sub>5</sub>OH (7:3) and spun using 14kV applied current and a 10 cm distance between needle tip and collector plate. The optimum value of 1% ratio was determined based on parametric study. Fibers were successfully collected onto SiO<sub>2</sub> microscope plates for optical observation and measurement. Fibers were observed to collect on surfaces that were also not the collecting plates, due to the whipping effect. Fibers diameters were first examined in morphology. **Figure 2** shows a morphological trend that exists as flow rate changes during Electrospinning. As flow rate remains low (<10ml/hr), discharging fibers that are contain much more homogenous surfaces or nanometer level defects. The fibers are much more optically permeable and allow for translucent observations. As the flow rate increases, the fibers become more and more imperfect. The defects in morphology are much more pronounced and optical observation becomes harder, as we can see in **Figure 2**. Another issue is how fiber morphology becomes less and less homogenous. The standard deviation of fiber distribution becomes larger and larger, as the flow rate increases, **Table 1**. It has been hypothesized that this effect is due to the large mass flow. Without increasing the applied voltage, the surface charge density is lowered, and electrorepulsive forces are not enough to consistently overcome the surface tension of the polymer solution. As flow rate increases, Taylor cone integrity is compromised and there is larger heterogeneity in fiber morphology and diameter.

**Table 1 Fiber Diameter and Standard Deviations**

Flow Rate	Mean Fiber Diameter ( $\mu\text{m}$ )	Standard Deviation
3.0ml/hr	9.912	4.27
15.0ml/hr	37.126	20.68
24.0ml/hr	79.882	36.78

Fiber diameters are considered in **Table 1**. Average diameter and standard deviations are included. A linear regression model was constructed to better display how electrospun fiber diameters are controlled by flow rate. Regression model and multiple frequency diagrams confirm the increase in heterogeneity of fibers as volumetric flow rate increases.



**Figure 2.** Fibers spun with the addition of an inorganic salt. Image on the right shows more tendency for alignment due to the application of magnetic field

Eventually, the polymer flow rate was too slow to overcome the surface tension effects of the high molecular weight polymer. Even though a longer retention of polymer solution in needle tip increase solution charge density, the solvent evaporates in the needle tip, creating an impermeable block, halting the Electrospinning process

### **Solvent Effects on Solution Properties**

Along with process parameters, internal parameters constitute factors that control fibers. Internal parameters include surface tension, viscosity, conductivity, and permittivity. These are controlled with choice of solvent for the specific polymer. In these experiments we observed how changing and adding solvents of PEO and relative proportions of solvent and co-solvent manipulate end fiber diameter. Co-solvents used in this study were chloroform and ethanol. The amount of the co-solvent was varied from 0 to 30%. It was observed that the fiber diameter of the spun fibers was reduced by ~77 times when ethanol content is increased by 30% in the solution.

### **Cell Culturing of Tissues**

To test cellular viability of the fabricated fibers, multiple fiber mats were generated. These fiber mats examine two factors: fiber diameter and alignment. Cells (PC-12 neuronal cell line) were first thawed from N2 and plated in a T-25 flask. Cells were made to attach and proliferate over the span of 1 week, immersed in media. After, cells were spun with a centrifuge and counted. Cells were evenly divided, and plated onto the synthetic scaffolds. Scaffolds were fixated to petri dishes using alginate sutures. The scaffolds themselves were made viable and sterile through exposure to UV. Cells were incubated and were fed with sustenance media for 1 week. Work is still underway with this test and due to time limitation, results will be presented at the ASEE meeting or in other publication.

## Educational component

This research described in this paper utilizes modern concepts in nanotechnology synthesis and nanofabrication along with polymer science to produce nanofibers used in *nanostructured scaffolds*. The process produces fibers with well-controlled properties (microstructure, chemistry, and alignment) via electrospinning processes to be used as functional scaffolds for regenerative medicine. In addition to the scientific merit of this research, it has with an important educational component. The above mentioned equipment and laboratory processes teaches students and train them on-state-of-the-art fabrication techniques, advanced characterization methods, and control of process parameters.

The paper describes the utilization of basic scientific concepts such as electric field, magnetic field, and viscosity in order to control the fabrication of nanofibers used in the regenerative medicine. A various educational modules have been developed in order to educate students basic science and correlate that with nanofabrication and nanotechnology. These modules are implemented in laboratory activity and will compose of five modules or experiments. The experiments will be preformed simultaneously by rotating teams of two students each. Each team will rotate through the set of five experiments, with at least two weeks devoted to each of the labs. Below is a brief description of these modules/experiments.

### ***Process parameters module:***

In this module students will be exposed to the application of programmable logic controllers (PLC) in real life application. Students will be allowed to control, flow rate, magnetic field, electric field, distance between the feeder and collecting plate in order to observe their effect on the morphology of the synthesized fibers.

**Table 2 A matrix to study the effect of process parameters on the characteristics of the synthesized fibers**

Parameter	Morphology	Fiber diameter	Porosity %	Chemistry of fibers	Adhesion
Flow	Surface defects Roughness Agglomeration	Measurements of fiber diameters will be done using SEM	Analysis of porosity will be estimated from processed images of SEM micrographs	Chemistry and elemental mapping of the synthesized fibers will be conducted using EDS and XRF	Standard ASTM adhesion tests will be performed
Needle-collector distance					
Magnetic field					
Electric field					
Viscosity					

### ***Materials Characterizations module:***

In this module, students will be utilizing various characterization techniques in order to correlate process parameters with characteristics of the synthesized fibers. Students will be observing and investigating the synthesized fibers via different microscopy and spectroscopy techniques. These techniques may include light microscopy, confocal microscopy, and Scanning Electron Microscopy (SEM). Students will also learn chemical analysis using Energy Dispersive Spectroscopy (EDS) attachment on the SEM and x-ray fluorescence (XRF). Exposing students to different techniques will allow them understand the capabilities, operating principle, and resolution limit of each technique.

### ***Nanotechnology and Nanofabrication module:***

The research topic in this paper falls under the umbrella of nanotechnology which is very attractive and on high demand. Microstructure of scaffolds is a major factor in guiding tissue development. Fabricated scaffolds need to be rigid enough to support tissues, but porous enough to allow oxygen and nutrients diffusion. Scaffolds with too small or too large pore size are not biocompatible and will not support growth of tissue cells. Understanding all aspect of the laboratory activity will increase students awareness and knowledge about the ***nanotechnology***. Students will be able to identify significant parameters to fabricate functional scaffolds.

This module will allow students to be exposed to nanofabrication and its applications in real life. Students will have better understanding of the ***nano*** term with direct interaction with fabrication and characterization techniques. As a part of the laboratory procedures, student will be able to fabricate nano materials, functionalize them and and investigate them. This laboratory activity requires students to submit a high standard laboratory report with similar format of a technical paper. Such an activity will enhance students technical writing skills and will allow them to exercise them literature survey.

### **Summary and conclusion:**

In this paper we demonstrated the fabrication of electrospun fibers. Morphology and properties of the fabricated fibers are highly affected by the process parameters. Fiber diameter was proportionally related to the flow rate. An 88% reduction in fiber diameter was achieved when flow rate reduced from 24ml/hr to 3 ml/hr. Fiber diameter of the spun fibers was reduced by ~77 times when ethanol content in the solution was increased by 30%. Magnetic field tends to have affect on the alignment of the spun fibers. The paper described educational modules/experiments, which enrich students' knowledge on nanofabrication, advanced characterization, and regenerative medicine. Work is underway to test the growth of the culture cells.

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