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In-vitro & In-vivo studies of Electrospun Nanofibers for Biomedical Applications

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Abstract: *In this paper, the application of electrospun nanofibers towards biomaterials is introduced. From a biological viewpoint, almost all of the human tissues and organs are deposited in nanofibrous forms or structures. Different biodegradable polymers are used to form nanofibers. Poly(l-lactide) (PLLA) is used to fabricate electrospun nanofibers. The morphological properties of the nanofibrous web are investigated in vitro and in vivo states. The nanoscale fibrous scaffolds provide an optimal template for cells to seed, migrate and grow. This research will prove the ability of using the nanofibrous assembly into several biomedical applications such as skin regeneration, medical prostheses, tissue templates, wound dressing and drug delivery.*

Keywords: *Nanofibers, Electrospinning, Poly(lactide)*

1. INTRODUCTION

In the last two decades, many researchers try to manipulate materials at nanoscale [1-4]. The nanomaterials have new properties completely different from its own properties at the macroscale of the same material. Nanofibers are one of the materials that have their unique and attractive characteristics that enable them to be used in different applications [5, 6].

The common and efficient technology to form nanofibers is electrospinning. The inherent advantages afforded by the electrospinning process include an extremely high surface area-to-volume ratio and a sub-cellular scale "nanofiber" to promote fiber to fiber interactions. The generation of fibers by electrospinning was first patented in 1934 by Anton Formhals[7]. During the past decades many researchers studied the electrospinning process, simulated the behavior of fiber movement and discovered the applications of it [8-11].

In the electrospinning of polymers, an electromagnetic fields; created by high voltage source, cause polymers in volatile solvents to elongate and splay into small fibers. The fibers are drawn to finally hit a grounded surface. Figure1 shows that when the diameter of polymer fiber materials are shrunk from micrometers to nanometers, there appear several amazing characteristics such as very large surface area to volume ratio (this ratio

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for a nanofiber can be as large as 10^3 times of that of a microfiber), flexibility in surface functionalities, and superior mechanical performance compared with any known form of the material.

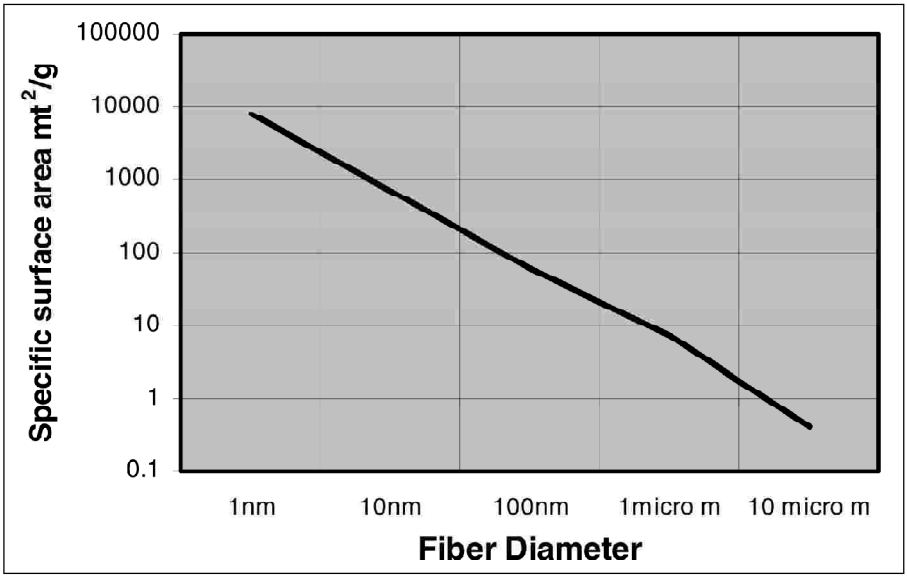


Figure 1: The High Increase of the Specific Surface Area Due to the Diminishing of the Fiber Diameters to Nanoscale

Over the past decade the nanofibers become the optimal candidate in bioapplications [12-14]. From a biological view point, almost all of the human tissues and organs are deposited in nanofibrous forms or structures. Examples include: bone, dentin, collagen, cartilage and skin. Interest in using nanofibers as biomaterials started in 1977 when proposals were first patented for the use of electrospun products as wound dressing [15].

In Alexandria University; particularly NNREL lab, nanofibrous materials for the first time are used as scaffolds for tissue engineering and the morphology of these fibers were investigated for in vitro and in vivo study.

2. MATERIALS AND METHODS

2.1 Materials

Poly(L-lactide) PLLA polymer was purchased from Birmingham Polymers with inherent viscosity 0.94 dL/g and dissolved in $CHCl_3$ to form solutions varying from 9% to 12 % concentrations. The solution was mixed by "*Rosi1000 incubator shaker Thermolyne-USA*" for 30 min. till we achieved a homogenous solutions.

2.2 Electrospinning

A custom-designed system was built to house the electrospinning station, in order to control the safe release of solvent and prevent turbulent air from disturbing the collection of fibers. Figure 2 illustrates the electrospinning station used in the NNREL lab.

As shown a high voltage DC power supply was used to generate potential differences between 5-30 KV. Poltential differences were applied to a vertically positioned blunt-ended metal needle. Polymer solution was fed from a hypodermic syringe (Perfekum Micro-Mate) of gauge 20cc with the precision glide; blunt-ended needle. The fibers were collected onto a metal screen covered with aluminum foil for further handling as illustrated in Figure 3, which shows the non-woven web that will be used as scaffolds for the cell culture.

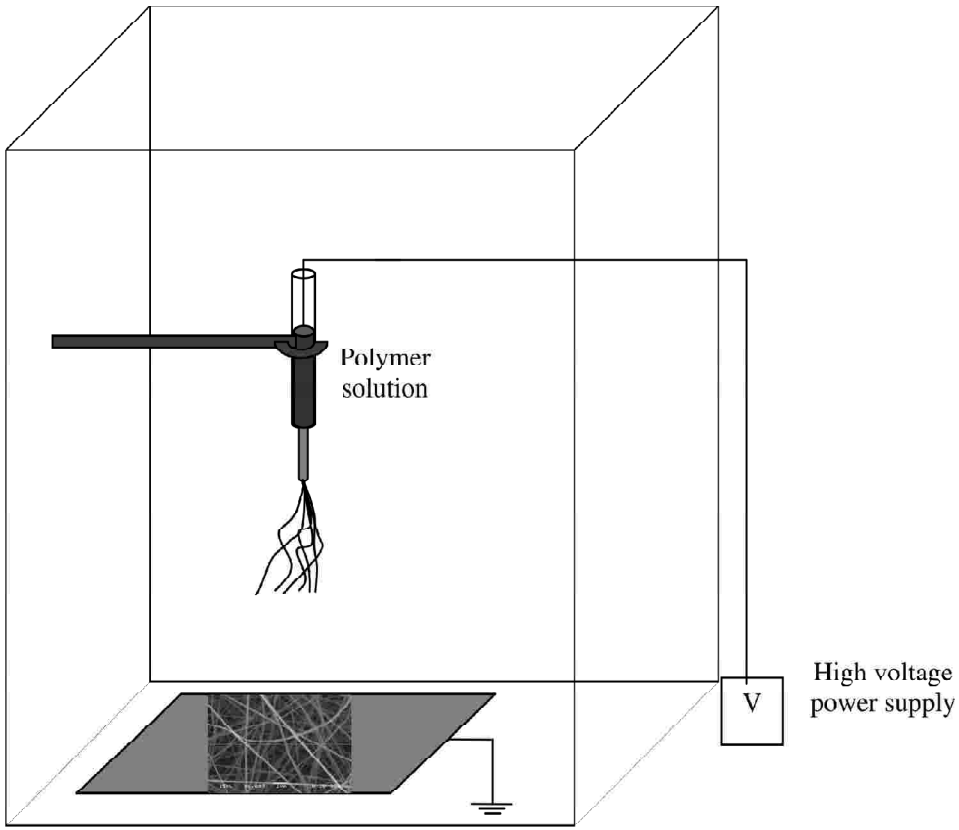


Figure 2: The Electrospinning Set-up. Polymer Solution is Electrospun through a Charged Metal Needle from which Fibers are Ejected and Collected on a Metal Earthed Plate

Figure 3: Non-woven Poly(L-lactide) Nanofibrous Web could be Easily Peeled off the Aluminum Foil

2.3 Scanning Electron Microscope SEM

Electrospun fibers were sputter-coated with gold for 2min at 20 mA. Fibers were imaged using an *Environmental SEM JEOL 5510* (USA). Micrographs were taken of 5 random areas of each scaffold at 10kV between 100 and 30,000 times magnification. SEM was used to determine the cell growth on the nanofibrous scaffolds.

2.4 Cell Culture

Undifferentiated mesenchymal stem cells were derived and maintained from rabbit bone marrow (BMSC). 2x2 cm sheet of non-woven nanofibrous mat were sterilized and pre-wetted (soaked for 30 minutes in 100% ETOH then washed with Pbs for 1 hour while the solution changed every 15-30 minutes). BMSC were seeded onto the previous nanofibrous mat while all half of 2x2 sheets returned into the culture media in a separate flask wells.

The unseeded and the seeded nanofibrous sheets were then incubated at 37C, 0.5% carbon dioxide and 95% humidity and for 2 days. The samples were all prepared for SEM examination according to the following protocol:

The samples were washed in PBS then fixed for 24 hours at 4C, then washed and proceed for alcohol dehydration subjected to (30, 50, 75, 95 and 100%) ethanol and left for air drying then examined under low and high vacuum for cytocompatibility.

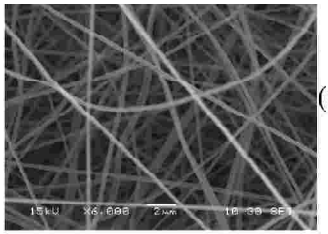
2.5 Optical Microscope

Electospun nanofibers were studied; after in vivo implantation, for tissue compatibility using Optical stereo microscope and capturing the images by using attached camera for snap shots.

3. RESULTS AND DISCUSSION

3.1 Electrospun Nanofibers Characterisation

The PLLA polymer was transformed into nanofibers using the electrospinning technique. The blending solution was subjected to a strong electric field generated by a high voltage about 1.5 KV/cm. The drop at the end of the capillary tip elongated from a hemispherical shape into a cone shape (Taylor cone). The applied voltages resulted in a jet being initiated near the end of the capillary tip. Prior to deposition on the collector, the jet showed fluid instability, the rapidly whipping jet, that leads to accelerated solidification of the fluid jet and the formation of submicrometer diameter solid fibers.



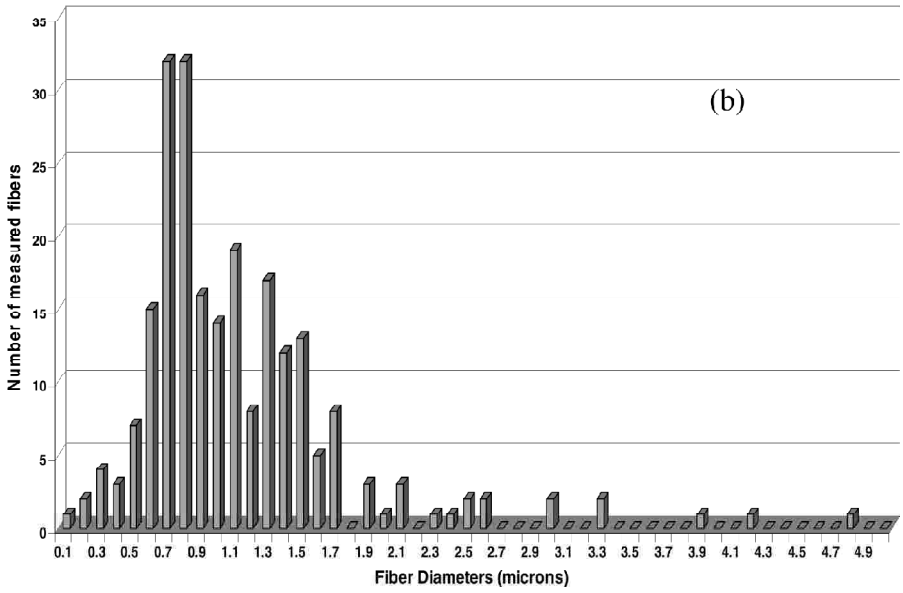


Figure 4: (a) The SEM Micrograph of the Electrospun PLLA Nanofibers. (b) The Diameters Distribution of the Electospun Nanofibers

Figure(4-a) shows the typical SEM image of nanofibers and the smooth surface of the electrospun surface. A statistical study was carried out to calculate the average of the fiber diameter which found to be around 150 nm as shown in Figure 4-b.

3.2 In Vitro Cell Growth and Cytocompatibility Characterisations

Figure 5 (a-f) contains SEM micrographs of BMSC seeded on electrospun scaffolds along 3 weeks in culture. The scaffold surfaces were almost completely covered by multiple layers of cells and/or new extracellular matrix. The cells/extracellular matrix and the nanofibers formed an interconnected network. The association between the fibers and cells/extracellular matrix can be observed (Fig. 5). The high surface area coupled with high porosity and pore tortuosity of the elecrospun fiber scaffolds provides a three-dimensional structure. The pores in the electrospun fiber structures were formed by randomly oriented flexible fibers. The arrangement of SEM images show clearly the tendency of cell growth along the days and how cell migrate and growing to cover the electrospun fibers.

3.3 In vivo Tissue Compatibility Characterization

All animal experiments were performed according to the international ethical protocol. To evaluate the degradation and tissue compatibility characteristics and long term systematic toxicity, a 2x2 mm of the non woven mat was implanted at each side of rabbit back under the skin as illustrated in Figure 6. The animal was observed for mortality and signs of overt toxicity once daily after the implantation throughout the study. No behavioural changes or visible changes of physical impairment indicating systemic or neurological toxicity were observed during the post-operative examination or at the time of sacrifice.

By the help of Optical Stereo Microscope for the in vivo state of nanofibrous mat, the images of tissue compatibility are shown in Figure 7. In two weeks survival time animal obviously shows inflammatory reactions beneath the muscle band. While in the 4 weeks survival time animal the inflammatory reactions have greatly decreased showing wide areas of normal connective tissues(CT) with remaining few inflammatory cells in some spots beneath the muscle band. Histologically, there was a minimal inflammatory response initially which subsided over time. Low magnification (Fig. 7-a) is showing the location of the implanted nanofibers under the muscular band, where inflammatory infiltration is evident at the site of implantation as a common reaction to foreign material. The higher magnification (Fig. 7-b) demonstrates the high density of the inflammatory cells for the same sample. Another specimen (Fig. 7-c) where the nanofibers were implanted for 4 weeks is showing obvious reduced inflammatory reaction compared to the 2 weeks survival period. This section is showing an interesting matrix distribution as following a particular pattern of filaments distribution [probably the nanofilaments arrangement pattern].

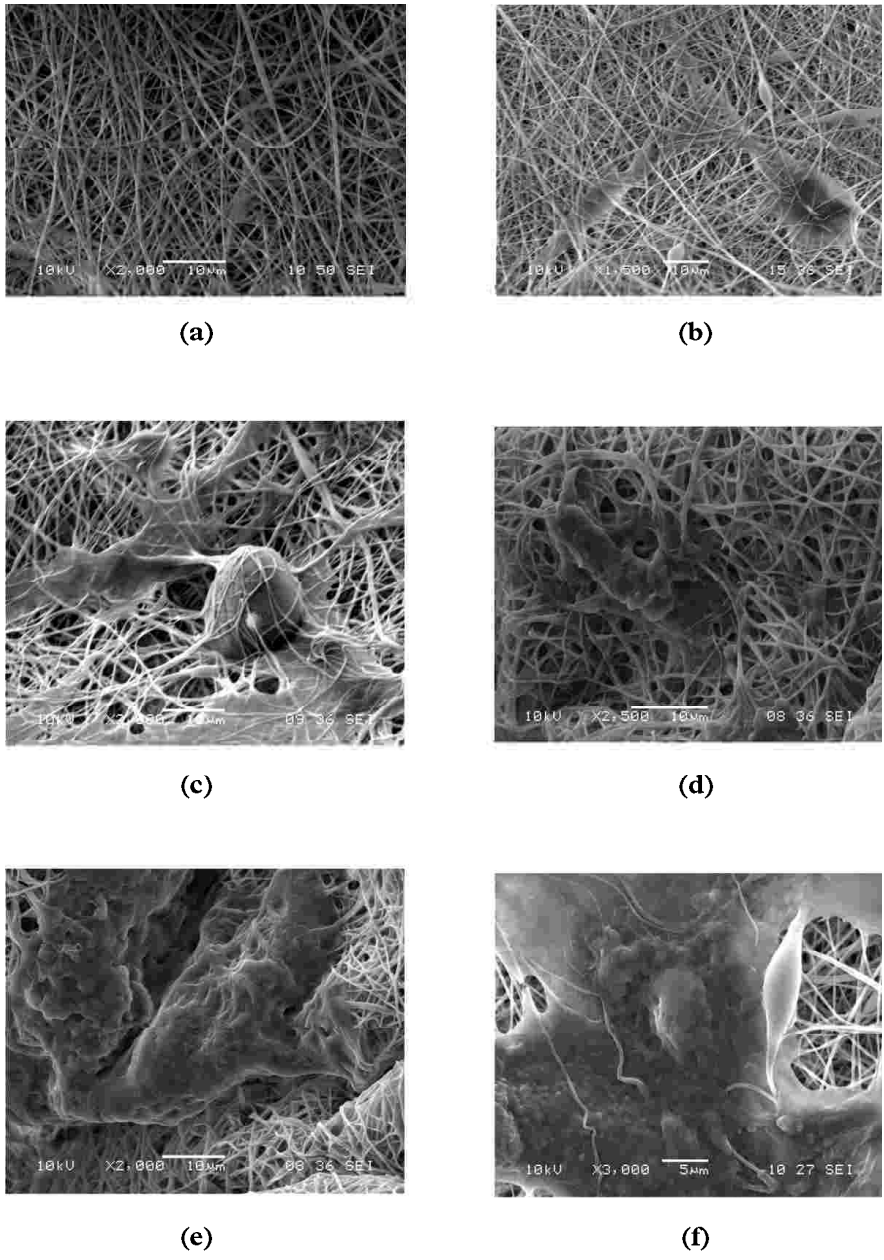


Figure 5: Non woven-Nanofibers Seeded with BMSC along 3 Weeks

(a) Control sample unseeded (2 days). (b) Seeded sample for 2 days.

(c) Seeded sample for 9 days. (d) Seeded sample for 12 days.

(e) Seeded sample for 25 days. (f) Seeded sample for 25 days.

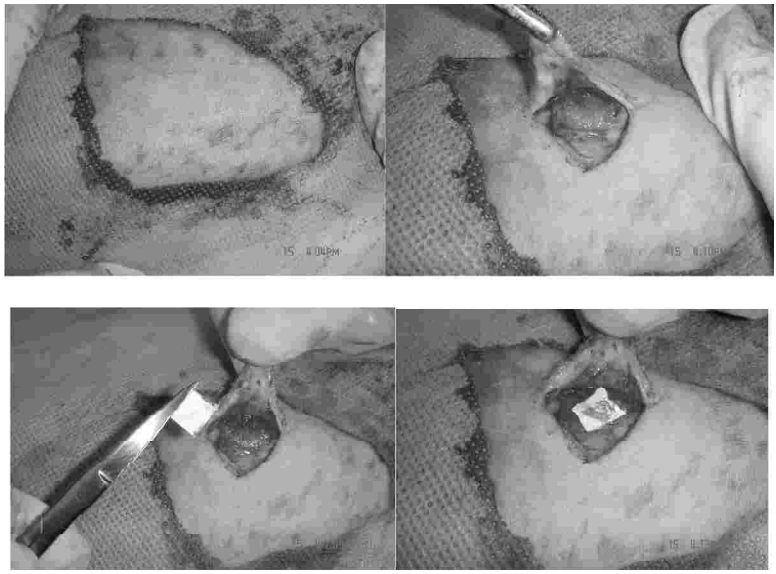


Figure 6: The in Vivo Implantation of Nanofibrous Scaffolds at the Rabbit Back Carried out by the Lab Surgeon

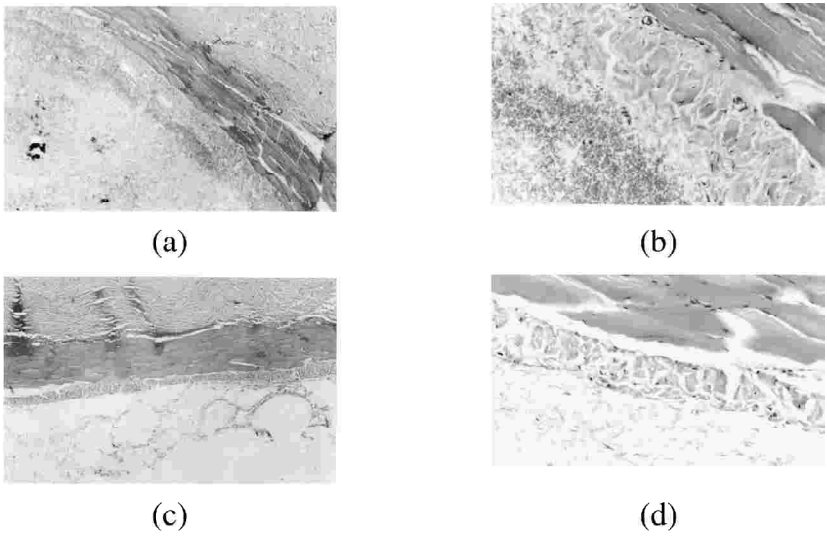


Figure 7: Photos of the Optical Microspcope showing the biocompatibility of nanofibers.

(a) Low magnification showing the location of the implanted nano-fibers under the muscular band.

(b) Specimen at higher magnification showing high density of the inflammatory cells.

(c) Nanofibrous sample implanted for 4 weeks showing obvious reduced inflammatory reaction compared to the 2 weeks survival period.

(d) Photomicrograph of a section from the same specimen (c) at a higher magnification showing the CT area under the muscular band from the inflammation.

CONCLUSIONS

Polymeric nanofibers were successfully electrospun at the NNREL lab, Alexandria University. The nanofibrous structure is the optimal candidate for biomaterials used in different applications such as tissues templates and scaffolds. This study demonstrates the promising future of using electrospinning to fabricate nanomaterials towards medical applications from drug delivery, wound dressing to prostheses. In this study, BMSC was assessed in vitro showing the attractive surface of nanofibers for cell growing. The in vivo implantation of nanofibrous mat proves the biocompatibility of nanofibers and its ability for other applications. The nanofibers have a potential promising future for several applications. Continuing with the research in Nanotechnology at NNREL, nanofibers will be formed into 3D scaffolds for further applications in biomedical applications.

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