DESIGN OF PATTERNED POLYURETHANE SCAFFOLDS FOR TISSUE ENGINEERING APPLICATIONS

Shah Hosseini N. [1,2], Bölgen N. [3], Khenoussi N. [2], Ceylan S. [4], Göktürk D. [4], Schacher L. [2], Adolphe D.[2]

[1]*Génie et Matériaux Textiles (GEMTEX), ENSAIT, 2 allée Louise et Victor Champier, 59056 Roubaix, France*
[2]*Laboratoire de Physique et Mécanique Textiles EA 4365—UHA Mulhouse, Mulhouse, France*
[3] *Chemical Engineering Department, Faculty of Engineering, Mersin University, Mersin, Turkey*
[4]*Bioengineering Department, Adana Science and Technology University, Adana, Turkey*

neda.shahoseini@gmail.com

ABSTRACT

The fibrous scaffold production with desired structure plays an important role in multidisciplinary field of tissue engineering. Electrospinning is popular approach for creating nanofibrous substrates in which the filaments resembling the natural extra cellular matrix (ECM) can provide topographical cues to cells directing their growth. One of the major challenges in electrospinning is tailoring the spatial organization of the filaments. To overcome this challenge, a hybrid static collector was utilized that enabled us to form distinct filament organizations on a single substrate. The effect of the filament organization on cellular growth was studied. The engineered scaffolds are promising candidates for fabrication interfaces between various tissues.

Key Words: Electrospinning, spatial nano-filament organization, tissue interfaces, hybrid collectors

1. INTRODUCTION

In the body, the structure of the extracellular matrix (ECM) provides biochemical supportive mesh surrounding the cells and supplies a substrate to guide the behaviour of the cells. The scaffolding materials key role is to mimic the native ECM and recapitulate the cell-cell and cell-substrate interactions [1–3].

ECM structure in human body varies according to functions of target tissues and cell types in the tissues [4,5] .As a result, forming scaffolds that resemble the native ECM has been the goals of many researches and various technologies have been developed to fabricate biomimetic scaffolds [6,7]. Electrospinning is one of the most popular methods to produce scaffolds from different materials [8]. In this method, the stretching action of the jet, combined with the evaporation of the solvent, allows the fibers to be extended and deposited randomly on a flat collector of opposite polarity in a micro and nano-scales. The fiber diameter and structure can be tailored by changing the spinning parameters and the formed fibrillar architecture mimics the ECM. One of the main parameter to adjust and generate the aligned filament is using rotating collector plate or conductive meshes, which resemble the ECM in tendons, ligaments, and skeletal muscles [9–11]. It has been shown that well-structured electrospun scaffolds could improve the functions of the cultured cells [12,13].

Main objective of our study is to design a novel patterned scaffold. Therefore, these electrospun scaffolds with different filament interface, based on biocompatible polymer can improve biological behaviours that are interesting in tissue engineering applications. In this regards, the electrospun nano-filaments of thermoplastic polyurethanes (TPU) were optimized based on design of experiment and structured using hybrid patterned collectors. The produced patterned TPU scaffolds with different filament organization have been cultured by Cervix Carcinoma cells to evaluate the viability and cell growth.

2. MATERIAL AND METHOD

2.1.Polymer and solvents in electrospinning of TPU

Commercially available thermoplastic polyurethanes (TPU) (purchased from VELOX, France), dimethylformamide (DMF; purchased from Sigma Aldrich, France), and tetrahydrofuran (THF; purchased from Fisher Scientific, France) were used. A TPU solution (11 wt.%) within the mixture of DMF/THF (1:1 ratio) was prepared. A custom-built vertical electrospinning device (LPMT laboratory) with a single needle was used.

The aim was firstly to find the most significant parameters, which influences the TPU nano-filaments morphology in an electrospun scaffold. Hence, a design of experiment based on Response Surface Methodology (RSM) was developped using ''Minitab 16'' software. During the electrospinning process, the solution concentration (11 wt. %) and feed rate (0.5 mL/h) have been kept constant. Contrarily, two other parameters; voltage and needle-to-collector distance have been varied. The developed DoE, based on two variables such as distance with three levels and voltage with five levels, is presented in Table 1. The TPU solution has been electrospun on an aluminum foil for duration of 5 minutes.

Table 1. DoE experiment for TPU solution with two different inputs

No.	Electrospinning parameters	Abbreviation	units	Levels
1	Distance	D	cm	16, 20, 24
2	Voltage	V	kV	10, 12, 14, 16, 18

Feed rate has kept 0.1 mL/h.
Concentration has kept 11 wt.%.
21.7° C and 24% H

2.2.Hybrid collector

In our last study [14], the conception of a novel series of embossed pattern collector with 5×5 cm^2 dimension fabricated by 3D printing machine (Objet Eden 260V, Stratasys, France) is defined. This collector contains two conductive (Aluminum) and non-conductive (resin) parts with different width of 0.9 and 9 mm, respectively. Figure 2. (a.) presents the characteristic of the hybrid collector with the respective dimensions.

2.3 Cells and culture conditions

Cell viability tests were carried out with Cervix carcinoma (HeLa, ATCC® CCL-2™) cell line. HeLa cell line was provided from Koç University, Faculty of Medicine, Bagcı Onder Laboratory and cell lines were cultured with high glucose Dulbecco's Modified Eagle's medium (DMEM, Lonza, Belgium) supplemented with 10% fetal bovine serum (FBS, Lonza) in a humidified incubator (NUAIRE, NU-5800, USA) at 37 °C and constant 5% CO2 level. The viability of the cells was evaluated by MTT (3-(4,5-Dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide, a tetrazole) assay at 3 and 5 days.

3. RESULTS AND DISCUSSION

3.1 Non-defect TPU nano-filaments

By performing fifteen experiments, four conditions has been selected as the optimized parameters of electrospinning to produce homogeneous and non-defect TPU nano-filaments on patterned collectors with various range of orientation and filament diameter. Image processing based on Fourier Transforms Method (FTM) has been performed on micrographs obtained by scanning electron microscopy (SEM) to get a numerical value to measure and compare different obtained nano-filaments orientation (more details [14]).

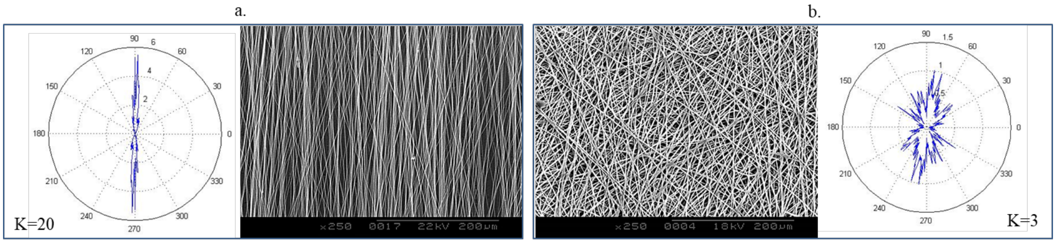

Figure 1. Different nano-filaments orientation histogram with given a numerical value of orientation (K)

As an example concerning the image processing, two kinds of orientation ratio with the different K value have been presented in Figure 1. By increasing the K orientation value from 3 to 20, nano-filaments become more and more oriented. The diameter of TPU filament has been measured between 1300-810 using ImageJ software. ANOVA analysis has shown that the TPU filament diameter decreases by decreasing the voltage and the needle-to-collector distance has a lower effect on diameter.

3.2 Structuration of nano-filaments

As it can be seen in Figure 2 (a.,b.), nano-filaments followed exactly the pattern, on both of conductive and non-conductive material within the collector surface.

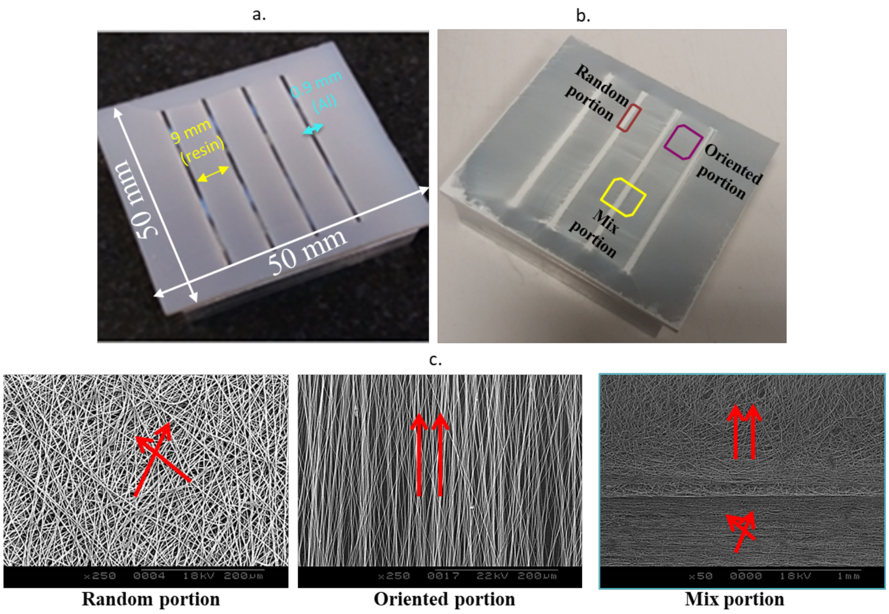

Figure 2. a.) characteristic of the hybrid collector with the respective dimensions, b.) fabricated patterned electrospun scaffold on the collector, c.) three different organization of nano-filaments on the collector

By starting the electrospinning process, firstly Al thin zone of collector surface fills by random organization of nano-filaments because of higher conductivity. After covering completely the conductive part, the effect of Al reduces by the time. Therefore, the nano-filaments will be aligned and deposited along the resin zone in the form of longitudinal oriented nano-filaments.

3.3 In-vitro study

In this part, different filament organizaitions within the flat membrane of Polyurethane has been evaluated biologically by using Cervix carcinoma (HeLa) cells. In this regard, three categories of oriented, random and mix filaments interface fabricated by a optimized conditions of electrospinning were cut and prepared, as it is presented in Figure 2. (b.). Thus, the portion of approximately 1 cm from oriented nano-filaments (deposited on resin part of the flat collector) was prepared for the first ''Oriented'' group. Since the width of the random zone is less than 0.5 cm, it was not possible to take random portion of 1 cm for the second group membrane. For this reason, ''Random'' group have been taken from the produced electrospun TPU membrane containing random nano-filaments using conventional collector. The third group of 1 cm ''Mix'' portions containing oriented and random nano-filaments have been prepared by keeping the random zone in the middle of each specimen. All the SEM micrographs are presented in Figure 2 (c.). As a preliminary study, the effects of the filaments organization in electrospun scaffold on response of the HeLa cells have been investigated by MTT and SEM analysis. . Results of the biocompatibility evaluation of scaffolds are figured out in Figure 3.

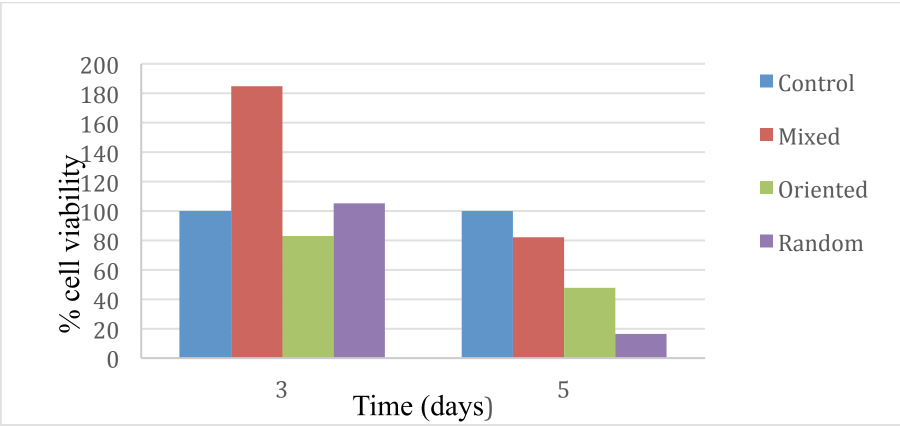

Figure 3. Viability results of cell culture groups at 3 and 5 days

The results of the MTT assay for HeLA cells culture after 3 days indicates that all scaffolds lead to increased cell number, except for oriented filaments. However, by increasing the cell culture time to 5 day, the HeLA cells viability decreased.

It can be concluded that TPU scaffolds supported cell proliferation and higher cell number have been obtained on the mixed (HeLA) TPU scaffolds. The MTT assay results indicate that the proliferation rate of cells mainly related to structure of nano-filaments and type of cells within the scaffold where they were attached.

Figure 13 shows the HeLA cell attachment on scaffolds. Cell morphology on TPU scaffolds was studied by SEM after 5 days of the culture on random, mixed, and oriented scaffolds with two cell line types. HeLA cell lines have displayed normal tumor cell line circular morphology in SEM micrographs shown below. The MTT assay and SEM images results, have confirmed the ability of TPU membrane scaffolds to support and grow HeLA cells.

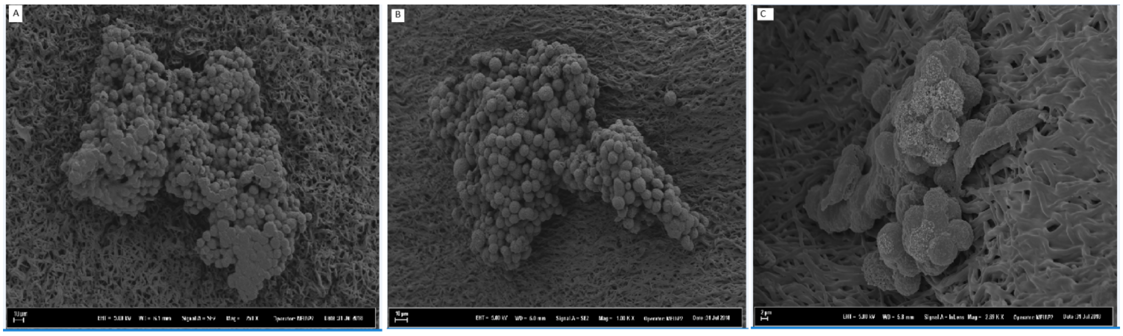

Figure 4. SEM images of oriented membranes with HeLA cells. (Random, mixed, oriented; A :750, B: 1k, C: 2.69k)

4. CONCLUSION

In this study, polyurethane has been used due to its large numbers of researchs and demanding applications, especially for the purpose of tissue engineering. Optimizing of electrospinning condition of TPU was performed based on RSM DoE. Afterward, according to the morphological characterization, the better conditions of electrospinning have been selected to be spun on the patterned collector. The obtained results from this study could provide the effective method to produce electrospun patterned scaffold, which has a high potential for medical application.
The produced electrospun scaffolds, which can provide an appropriate surface for cell growth were evaluated for the in-vitro performances. Three different categories which are oriented, non-oriented and mix structures took form in the shaped of 1 cm square. The effects of the nano-filaments' organization in electrospun scaffold on response of the HeLA cells have been investigated. MTT assay results have indicated that the viability percentage of cells was related to the alignment of the nano-filaments in the electrospun scaffold.

5. REFERENCES

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