Preparation of PET/PDMS/Collagen nanofibrous membrane embedded in a microfluidic device for cell culture

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Article

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Abstract

Electrospun nanofibers can be used as membranes for cell culture applications. In addition to their high performance and cost-effectiveness, nanofibers can be made from a variety of polymers and utilized in microfluidic devices as separators, filters, and for biological assessments. In this paper, we have employed a two-pump technique to prepare a hybrid nanofiber-based membrane with a biocompatible polyethylene terephthalate (PET), polydimethylsiloxane (PDMS), and collagen. The introduction of collagen creates a suitable substrate for cell attachment and decreased hydrophobicity. To sustain collagen on the fibers, the membranes were treated with glutaraldehyde vapor and then detoxified. The membrane integrity and structure were evaluated using SEM, AFM, contact angle analyzer, FT-IR, and tensile apparatus. In addition, cell attachment capabilities and cytocompatibility were examined using SEM and AlamarBlue, respectively. The membrane was cased in a PDMS-based microfluidic device consisting of upper and lower channels. Our findings suggest that the incorporation of PET and PDMS provides a superior bonding capability and prevents unwanted leakage. Then, Human Umbilical Vein Endothelial Cells (HUVECs) were cultured on both sides of the proposed membrane inside the device. To simulate the dynamic environment and induce cellular shear stress, the culture medium was flown through the channel and the conditions were kept overnight inside an incubator. Cellular staining (Acridine Orange and Propidium Iodide, as well as DAPI) and further evaluation of cellular adhesion suggest that cells were well attached and viable inside the microfluidic device. The proposed membrane can be employed in fabrication of various biological barriers on-a-chip for the purpose of screening drugs, examining the effects of nanomaterials, and creation of in-vitro disease models.

Introduction

Cell culture is among the primary tools in biological research. It refers to the removal of cells from their natural environment followed by their growth in an artificial setting [1]. In this process, cells are cultured in controlled conditions and specialized containers for in-vitro assessments. These containers are often plasma treated to promote cell adhesion and thus cell proliferation [2]. Due to their morphology and structure, nanofibers have also been introduced to improve cell attachment and culture in recent years [3]. Nanofibers are infinitesimal threads of fibers that collectively form a mat and can be used in countless applications ranging from particle separation [4] and filtration [5], biosensors [6], cosmetics [7], fabrication of scaffolds in tissue engineering [8, 9], to means of drug loading and delivery [10], wound dressing [11] and many more. In cell culture specifically, aligned oriented nanofibers can enhance myogenesis [12], as well as neural stem cell proliferation [13]. Functionalized nanofibers are used to promote stem cell differentiation [14], advance peripheral nerve regeneration [15], and construct nerve conduits [16]. This is due to their high volume-to-surface aspect ratio, controllability and adjustable porosity, relatively easy setup, and the fact that they can be composed of a variety of materials.

Among conventional technologies developed to fabricate nanofibers [17, 18], electrospinning is one of the most popular methods. This is mainly due to its simplicity and convenience, adjustability of fiber and pore parameters, and the ability to produce a relatively large quantity of nanofibers in a short time.

Polymers, composites, ceramics, and metals can be used to produce nanofibers by electrospinning, nevertheless, the most commonly electrospun material are polymers [19].

Various polymers, ranging from natural to synthetic or hybrid blends, have been electrospun into nanofibers. By comparison, nanofibers made from natural polymers display higher levels of biocompatibility and lower immunogenicity and therefore, they have higher clinical relevance [20, 21]. On the other hand, nanofibers fabricated from synthetic polymers exhibit better mechanical properties [22]. Therefore, nanofibers stemming from a combination of natural and synthetic polymers benefit from both aspects [23], which can be essential for biological applications.

Among recent technologies that have been increasingly utilized in fundamental biological research [24], drug development [25], and diagnostics [26], are the microfluidic systems. With the help of carefully designed microchannels in these systems, processing, and manipulation of small amounts of biological fluids are possible. It is arguably thanks to this technology that the microenvironment assessment of organs have become better accessible [27]. Organ-on-a-chip is a familiar concept and a powerful tool which allows scientists to survey cellular behavior in an organ, or multiple organs [28], using a microfluidic device. Nevertheless, cell culture inside a miniaturized system is not without challenges, especially when the envisioned application is replicating a biological barrier [29]. This is because of a multitude of reasons including limited space within the device, the complex dynamics of fluids inside microchannels, and convoluted interactions between different cell types and different materials with which the device is fabricated.

Cells successfully cultured in a microfluidic device exhibit a unique behavior that can closely resemble their natural environment [30]. For this purpose, an artificial membrane is used to house the cells inside the device. In this configuration, cells are grown on top of a membrane that can easily be integrated into the device, allow diffusion of nutrients between the top and bottom microchambers, and structurally promote cellular adhesion and proliferation. Thin porous polydimethylsiloxane (PDMS) -based layers are among the commonly used membranes to assist cell culture in microfluidic devices [31]. However, it can be costly and not accessible everywhere. In this study, an alternative method based on nanofiber technology is introduced. The proposed membrane is fabricated by electrospinning of polyethylene terephthalate (PET), polydimethylsiloxane (PDMS), and collagen. This hybrid design is biocompatible, permeable, and cost-effective and allows a firm bond between the top and bottom sections of PDMS-based devices without causing any leakage. The findings suggest that PET/PDMS/Collagen nanofiber mat that is crosslinked by glutaraldehyde is a highly efficient membrane for culturing Human Umbilical Vein Endothelial Cells (HUVECs) inside a microfluidic device and can be used for replicating biological barriers.

Results

SEM analysis

A two-nuzzle configuration (Fig. 1A) was used to electrospun a nanofiber membrane consisting of PET (22%), PDMS (10%), and collagen (5%). The SEM micrographs of nanofibers before exposure to glutaraldehyde vapor and post crosslinking are depicted in Fig. 2A and Fig. 2B, respectively. According to Fig. 2C, the average mean diameters of the fibers before crosslinking by glutaraldehyde (mean ± SD) were 390.9 ± 169.6 nm, and 660 ± 199.3 nm after crosslinking (Fig. 2D).

AFM analysis

Results from AFM analysis of the surface of the nanofibers before and after crosslinking suggest that the surface average roughness is 66.9 nm and 296.7 nm, and the root-mean-square roughness (RMS) is 22.6 nm and 99.3 nm, respectively (Fig. 3A and B). The reported average size of the fibers by AFM is in close agreement with results obtained by SEM (Fig. 2C and D).

Surface characterization (1): Contact angle measurements

The contact angles of the pre-crosslinked and crosslinked nanofibers are shown in Fig. 3. The contact angle of the nanofibers before crosslinking was 105 ± 0.5°, whereas that of the nanofibers after crosslinking was 58.8 ± 0.5 °.

Surface characterization (2): FT-IR spectral analysis

The FT-IR spectra of pre- and post-cross-linked nanofibers are presented in Fig. 5. To identify the presence of functional groups, the samples were scanned between 400 and 1000 cm^{-1} (C-O-C regions) and 1,000–1,700 cm^{-1} (C-C, C-O, and C-N bonds), and greater than 2,900 cm^{-1} which is used to identify C-H bonds and other primary amine groups.

Mechanical strength properties

The stress-strain diagram of pre- and post-crosslinked nanofibers are shown in Fig. 6. The results clearly indicate that crosslinking significantly increases the durability of the fibers. In addition, the Young s modulus values presented in Table 1 show an increase in tensile strength from 1.88 MPa to 1.92 MPa when nanofibers are crosslinked.

Cytocompatibility and cell attachment

To evaluate cytocompatibility and cell attachment, cells were treated with AlamarBlue and observed by SEM imaging. Figure 7A illustrates the cell viability of HUVECs cultured on crosslinked nanofiber membranes after 1, 3, and 5 days of cell seedling. Expectedly, cell viability increases from 60.88% on the first day to 84.29% and 92.38% on the 3rd and 5th day, respectively. Figure 7B illustrates the SEM images of HUVECs cultured on the nanofibers after 72h of cell seeding.

Acridine Orange / Propidium Iodide staining in the microfluidic device

To ensure the presence of living cells on top of the nanofiber-based membrane, AO and PI were used. As shown in Fig. 8A, 24 hours after cell culture inside the microfluidic device, about 98% of the cells are

emitting green fluorescence and therefore are alive. Figure 8B shows a very small number of dead cells that are stained orange on the nanofiber membrane.

DAPI staining

Once the presence of living cells on one side of the nanofiber membrane in the microfluidic device was established, DAPI staining was used to visualize the presence of attached cells on the contra lateral side of the membrane. Figure 8B indeed shows stained cells that are attached in the microfluidic device.

Discussion

Cells that are effectively cultivated in microfluidic devices can display a distinct behavior that closely mirror that of their native environment [32]. Organ-on-a-chip is a relatively novel approach that can provide both systemic and human-like characteristics to fit variety of biologically and pharmacologically relevant models. While the immediate objective of this work was to investigate a thin electrospun nanofiber mat as an alternative to commercially available membrane for cell culture in microfluidic systems, this study advances the theme of integrating nanofibers in microfluidic systems for the purpose of cost-effective fabrication of organ-on-a-chips.

Electrospinning is a relatively simple and highly controllable technique that allows fabrication of nanofibers from a wide range of polymers. By adjusting parameters such as the concentration of the polymer in the solvent and viscosity, flow rate, voltage and the distance between the deposition needle and the collector, as well as the rate at which the collector rotates, one can control the diameter, thickness, length, and porosity of the nanofibers [33]. In this study, two separate syringe pumps were used to load the hydrophobic PET/PDMS and the hydrophilic collagen to prepare a hybrid nanofiber-based membrane. Porous PET and PDMS membranes are currently commercially available for cell culture in microfluidic devices, but they can be costly and often not widely available. PET nanofibers on the other hand are cost-effective and can be easily synthesized. However, to help better incorporate the PET nanofiber mat in a PDMS-based microfluidic device, PDMS nanofibers were also added. Then, collagen which creates a suitable substrate for cell culture was added to the design. Finally, to sustain collagen on the fibers, the membrane was crosslinked by glutaraldehyde vapor and then detoxified.

After electrospinning, the membrane integrity and structure of the PET/PDMS/Collagen nanofiber mat were evaluated. SEM findings (shown in Fig. 2) indicate that most of the fibers had diameters ranging between 100–900 and 300–1200 nm before and after crosslinking, respectively. This suggests that crosslinking has increased nanofiber diameter which is in close agreement with data acquired by AFM (see Fig. 3). Surface analysis of the nanofibers by a contact angle analyzer suggests a hydrophilic characteristic after cross-linkage which is shown in Fig. 4. Generally, lower contact angle implies an increase in hydrophilicity of the surface [34]. This reduction of the measured angle by ~ 46° suggests that the crosslinked nanofibers are more hydrophilic and thus better suitable for cell adhesion. Another powerful tool for analyzing inter-molecular interactions on the surface of the nanofibers is FT-IR spectra. Evidently, the FT-IR spectra of the crosslinked nanofibers closely reflects the characteristic bonds which

are present in the non-crosslinked nanofibers. According to Fig. 5, there were slight shifts among the two samples with different relative intensities, thus indicating that the crosslinking process did not affect surface chemical structure. The transmission band at 800 cm^{-1} which corresponds to the vibration of Si-$(CH_3)_2$ groups (due to the presence of PDMS) in the sample was significantly deepened after crosslinking. The transmission band at near 2,900 cm^{-1} which corresponds to the stretching vibrations of the O-H and C-H groups [35] was slightly broadened and underwent a low-frequency shift to 2962 cm^{-1}. This characteristic is most likely attributed to the structural changes of the hydrogen bonding after crosslinking. Nevertheless, Fig. 5 is an attestation that chemically, there has not been a major structural change to the FT-IR profile once the material underwent crosslinking. Finally, the nanofibers were examined via a tensile strength apparatus. The process by which the electrospun membrane goes through to become incorporated into the microfluidic device may harm its structural integrity. Therefore, the mechanical durability and strength of the sample are significant. According to Fig. 6, treated nanofibers with glutaraldehyde vapor show an increase in endurance which is likely due to their thicker fiber diameters.

Once characterized, the nanofibers were prepared for cell culture assessments. Due to their distinctive form and unique structure, nanofibers have been previously employed to boost cell adhesion and culture [36]. The biocompatibility assessment of electrospun PET fibers has been previously reported [37], and there is strong evidence that integration of collagen in the warp and weft of the sample can provide conditions for enhanced cell attachment and diminished in-vitro toxicity [38]. On the other hand, PDMS is a well-known biocompatible and culture-friendly material. Therefore, it was expected that combination of all three would as well be seemly to house cells. For this study, an endothelial cell line (HUVAC) was chosen to culture on the membrane. For cell viability assessment, the absorbance of AlamarBlue dye, which is directly proportional to cellular metabolic activity and living cell count, was read for 5 consecutive days. Compared to control, there is a slight decrease in viability of cells cultured on the nanofiber on day one (see Fig. 7A). However, this value increases during the following days. It is suspected that during the initial seeding process, a small concentration of the crosslinking agent (glutaraldehyde) could have been released from within the fibers thereby lowering the initial viability rates on the first day. To assess attachment and morphology, SEM images of cells cultured on the nanofibers after 72h of seeding were obtained. According to Fig. 7B, the cells are correctly formed and well situated on the nanofiber substrate.

At this point, the nanofiber mat was carefully placed and bonded between the layers of the microfluidic device (see Fig. 1). To assess cell culture inside the device, HUVECs were first seeded on top of the mat and then on the contralateral side. To ensure cell survival, acridine orange (AO) and propidium iodide (PI) was used on one side of the membrane, and to ensure cellular adhesion, DAPI were used on the contralateral side. AO is a cationic dye that is able to penetrate the cell membrane and bind to the DNA of living cells where it produces green fluorescence. PI on the other hand can only penetrate the nonintact membrane of dead cells and bind to the DNA whereby it generates an orange fluorescence. DAPI (4',6-diamidino-2-phenylindole) is a simple verification dye to detect the presence of cells on the nanofiber

membrane by staining the cell nucleus. While staining techniques are very useful at assessing cellular conditions, they also stain nanofibers and that is the reason why DAPI was used on the other side of the mat. Figure 8A and 8B suggest that most cells were alive, and Fig. 8C shows number of cells that are well situated on the contralateral side of the nanofiber mat.

In this manuscript we used a nanofiber membrane suitable for cell culture inside a microfluidic device. Here, the device was designed to uphold cells on both sides of the membrane. The resulting hybrid design is biocompatible, permeable, and cost-effective and allows a firm bond between the top and bottom sections of any PDMS-based devices without causing for leakage. The findings suggest that the nanofiber mat that is crosslinked by glutaraldehyde is a highly efficient membrane for culturing HUVECs inside the device and can be used for replicating biological barriers. SEM image of cells grown on the nanofiber showed that they are expanded and correctly attached to the surface. The designed base membrane in the microfluidic system allows researchers to evaluate permeability, viability, and functionality of various biological barriers.

Methods

All procedures were performed according to the guidelines approved by the Ethics and Experimentation Committee (No. IR.TUMS.MEDICINE.REC.1400.988) of Tehran University of Medical Sciences. All methods presented in this study were carried out in accordance with relevant guidelines and regulations, and they were reported in accordance with ARRIVE guidelines. For this study, no live animal was sacrificed for this work.

Materials

Type I collagen was manually extracted using a method explained in the following section. PET (Sky blue grade, Japan) and PDMS (Sylgard 184, Dow Corning, USA) were obtained. Tetrahydrofuran (THF), N, N-dimethylformamide (DMF), 1, 1,3,3,3 hexafluoro-2-isopropanol (HFIP), and Alamar blue were purchased from Sigma-Aldrich, Germany. Glutaraldehyde solution 25% (ready to use) was purchased from Panreac Applichem, Iran. DMEM/F12, PBS, FBS, and Trypsin-EDTA (0.25%) were purchased from Gibco, USA.

Collagen extraction

Type I collagen was extracted from rat tendons by a protocol explained in [39]. Briefly, tails of 12 previously euthanized rats (ex-vivo) were washed in 70% ethanol. The skin of each tail was removed with a razor, and the collagen fibers were extracted by pliers. The fibers were then rinsed three times with deionized water and placed on a magnetic stirrer with 0.2 percent acetic acid solution for 24 hours at 4°C. The solution was then centrifuged at 4°C for 30 minutes at 11200 RCF. The supernatant was then poured into flat containers and placed at -20°C. Then, frozen fibers were put in a freeze-dryer device (LYOQUEST-55, Telstartechnologies, Spain) for the solvent to evaporate.

Fabrication of nanofiber membrane

In this study, the electrospinning technique was used to prepare PET/PDMS/Collagen nanofiber membrane. The electrospinning device (FNM, Iran) includes two syringe pumps, two high-voltage power supplies, and a rotating collector which is covered by aluminum foil (Fig. 1A).

To construct the proposed nanofiber membrane, PET was dissolved in DMF/THF (1:1) solvent (25% wt) at 45°C in constant stirring conditions. Separately, PDMS/Curing agent (10:1) was added to DMF/THF (1:1) solvent (10% wt) on a magnetic stirrer at room temperature. Then, 5% wt collagen solution was prepared in HFIP solvent. When PET solvent reached transparency, it was left to cool down to room temperature and mixed with PDMS on a magnetic stirrer at room temperature. Then, PET/PDMS and collagen were loaded into two separate 5ml syringes with blunt 18G needles and placed inside the appropriate pumps. The distance between the needle of the syringe and the collector was maintained at 15 cm on each side and the pumping rates were set at 0.4 ml/h. The syringe loaded with PET/PDMS was connected to a power supply that provided 22 kV, and the other syringe that was loaded with collagen was connected to 20 kV. The speed of the roller collector was set to 90 rpm. The resulting woven nanofiber mat was placed inside a vacuum desiccator for 30 minutes and received Glutaraldehyde (25%) vapor to crosslink its surface.

Scanning Electron Microscopic

Nanofiber samples were coated with gold nanoparticles by an ion sputtering coater (Polaris SCM-200, South Korea) to enhance electrical conductivity for imaging by scanning electron microscopy (SEM). Gold-coated nanofibers were then placed in a high-vacuum chamber and the images were taken at 20 kV (SNE-4500M, Korea). Nanofibers were evaluated for morphology, size, and size distribution. ImageJ and Origin were used for image visualization.

Atomic Force Microscopy

Atomic Force Microscopy (AFM, Nanosurf easy, Switzerland) analysis was carried out to determine the surface roughness and morphology of nanofibers.

Contact angle measurement

To examine wettability, the angle at which water droplets became in contact with the surface of nanofibers was assessed. For this purpose, a five square centimeter nanofiber membrane was placed under a contact angle instrument (MehrTavNegar, Iran) equipped with a camera at 25℃. A small drop of deionized water (6µl) was carefully placed over the surface of the nanofiber with a pipette and images of the droplet and the surface of the nanofiber were taken at the interfaces (before and after crosslinking).

Fourier transform infrared spectroscopy analysis

Fourier transform infrared (FT-IR) spectroscopy analysis was conducted by an FT-IR analyzer (WQF-510A, China) to validate the structural constituents of the prepared PET, PDMS, and collagen nanofiber. Samples have been entirely mixed with KBr for analysis. KBr has been used as a carrier for the sample in the FT-IR analysis.

Mechanical strength properties

Universal Testing Machine (UTM, Roell z050, Zwick, German) was used to evaluate the mechanical tensile of the nanofibers before and after crosslinking at 2 mm/min and room temperature.

Cell culture

Human umbilical vein endothelial cells (HUVECs) were cultured in T-75 flasks with DMEM-F12 rich medium containing 10% FBS and 1% penicillin/streptomycin and incubated at 37℃ and 5% CO_2. Cells were cultured once reached 90% confluence (about 3 days).

Cell viability

The viability of cells cultured on nanofibers was assessed by AlamarBlue based on a procedure explained in [40]. Briefly, the nanofibers were cut into circles 1 cm in diameter, sterilized with 70% ethanol for 15 minutes, and then exposed to UV irradiation for 30 minutes. The nanofibers were then placed at the bottom of a 48 well plate where 10 mm autoclaved O-rings secured them in place. Then, 0.5×10^4 cells in 250µl F12-enriched medium, 10% FBS, and 1% penicillin/streptomycin were poured on top of the nanofibers. Cell viability was assessed after 1, 3, and 5 days of culture. For that purpose, the culture medium in each well was aspirated and replaced by 250µl of complete culture medium containing 10% AlamarBlue and incubated at 37°C and 5% CO_2 for 4 hours. Then the plate was placed inside an ELISA microplate reader (BioTek, USA) and read at 570 and 630 nm.

Cell attachment by SEM

Cells were cultured in a 48 well culture plate and placed in an incubator at 37°C and 5% CO_2 for 48 hours as explained in the last step. Then, the culture media was discarded, and the wells were rinsed with PBS. After that, the cells were fixed by adding a 1:1 solution of 2% glutaraldehyde and 2.5% paraformaldehyde and kept at 4℃ for 1 hour. The nanofibers were then exposed to 60, 70, 90, and 100% ethanol for 5 minutes to dehydrate. In the next step, the nanofibers were coated with gold nanoparticles as explained previously for SEM imaging and conformational cell attachment analysis.

Fabrication of the microfluidic device

A simple PDMS-based microfluidic device containing an upper and a lower channel was designed to house the nanofiber membrane (Fig. 1B). Once the masks were designed, soft lithography was used to fabricate the molds. In summary, SU8-2050 (Microchem, USA), a negative photoresist, was spread over a silicon wafer by a spin-coater (Microchem, Newton, MA, USA). Then UV light was lit through the mask over the wafer by a mask aligner (Danesh Equipping System, LSM5, Iran) allowing the SU8 to cross-link onto the surface of the wafer. Once the unbonded photoresist was washed away from the surface of the wafer, a negative imprint of the microchannel design was created. In this study, two silicon molds (upper and lower) were fabricated. To create the microfluidic device, PDMS was mixed with its curing agent (10:1) and debubbled inside a vacuum pump. The mixture was then poured inside the silicon molds and placed on a hot plate at 80°C for 1 hour. Once cast, designated inlets and outlets were created using a

biopsy punch (diameter = 3 mm). Then, the surface of each casted PDMS was washed with isopropyl alcohol and acetone (Merck, Germany). After drying, the nanofiber membrane was fused between the upper and lower sections via an oxygen plasma treatment apparatus (Harrick Plasma, Ithaca, NY, USA) and a leak-free microfluidic device was formed. To sterilize, the device was rinsed with 70% ethanol and exposed to UV for 30 minutes.

Cell culture inside the microfluidic device

To ensure free fluid motion within the device, culture media was injected into the top channel. Then, the device was placed inside an incubator for 15 minutes. Then, yellow micropipette tips were left inside the input and the output ports. Two million cells (HUVEC) were diluted in 1 ml culture media. Then, 100µl of that solution was carefully injected inside the micropipette tip pinned to the channel input port, where gravitational and capillary forces allowed the gentle flow of cells inside the channel. Under a light microscope, the flow of cells towards the nanofiber membrane and the output port was monitored. For cells to sediment and attach to the nanofiber membrane, the device was placed face-up inside an incubator for 4 hours.

Acridine Orange and Propidium Iodide staining

Once the cells were sedimented inside the device, the device was kept inside an incubator for 24 hours. Then, 50µl of a 1:1 mixture of AO and PI with a concentration of 50µg/ml was injected into the input of the top channel and quickly placed under the fluorescence microscope. AO emits green fluorescence with the maximum wavelength at 526 nm (excitation 502 nm) where PI is excited at 488 nm and, with a relatively large Stokes shift, emits at a maximum wavelength of 617 nm (orange).

DAPI staining

The microchannel was washed twice with PBS and then 50 µl of DAPI solution dissolved in deionized water (1 µg/mL) was injected into the input port and left for at least 1 minute. Then, the device was placed under fluorescence microscopy (Optika, IM-3, Italy) where DAPI was excited with ultraviolet light (358nm) and was detected through the blue/cyan filter.

Declarations

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Authors' contributions

H.M.E and and S.N.T. performed the device preparation and cell culture experiments, H.M.E. manufactured the nanofiber membrane and the Microfluidic device, F.F. and S.A.S. aided in device making, cell culturing and data analysis, S.N.T. and H.Gh. were involved in planning and supervised the work. Writing of the original draft was done by S.N.T. and H.M.E.; H.Gh. and S.A.S. edited and reviewed the manuscript. All authors discussed the results and commented on the manuscript.

Data availability statement

The datasets generated during and/or analysed during the current study are available from the corresponding authors on reasonable request.

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Tables

Table 1. Mechanical properties of nanofibers

	Nanofibers' diameter by SEM (nm)	Nanofibers' diameter by AFM (nm)	Tensile Strength, $\sigma_{at\ break}$ (MPa)	Young's Modulus, E (MPa)	Fracture strain
pre-crosslinking	390	250	1.88	0.62	1.4
post crosslinking	660	750	1.92	0.48	1.46

Figures

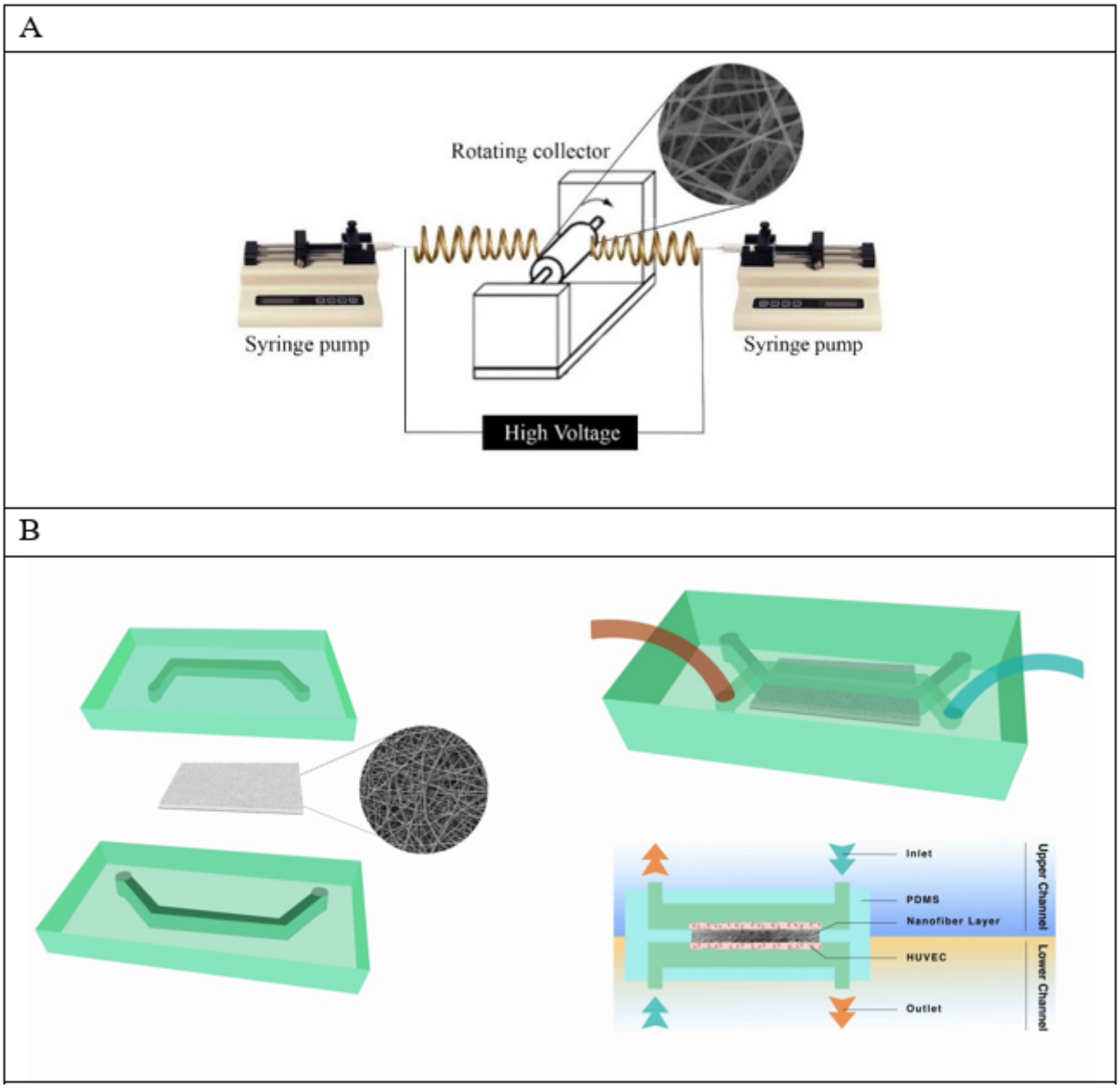

Figure 1

A) Schematic of a double-needle electrospinning set-up which was utilized for the fabrication of PET/PDMS/Collagen nanofiber membrane. B) Schematic of the microfluidic device, the porous nanofiber membrane is placed between the upper and the lower PDMS-based microchannels

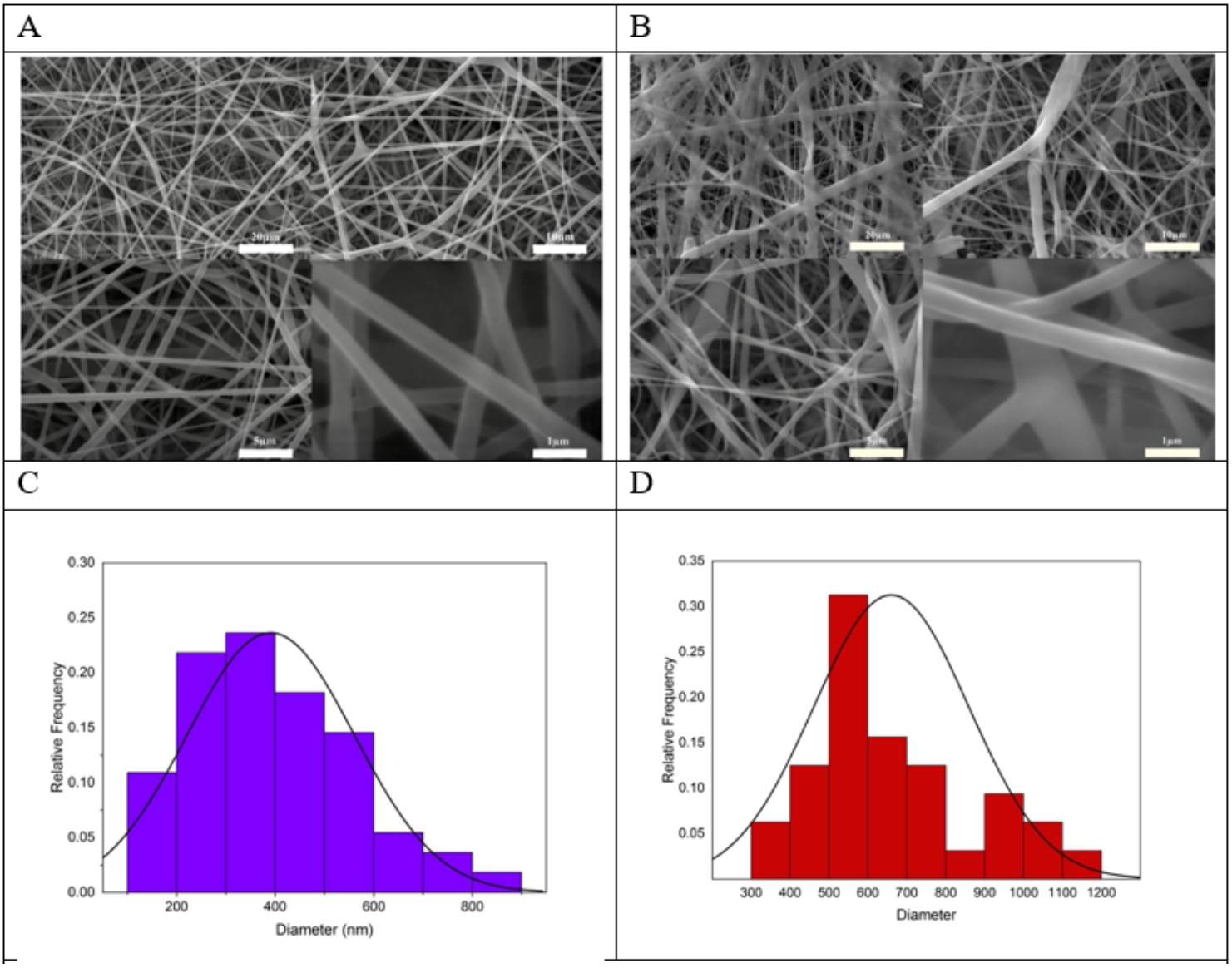

Figure 2

Upper panel: Scanning Electron Microscopy images showing morphologies of nanofibers containing 22% PET, 10% PDMS and 5% Collagen (A) before and (B) after crosslinking by glutaraldehyde vapor. Lower panel: Bar graphs showing the diameter of nanofibers before (C) (390.9 ±169.6 nm) and after (D) crosslinking by glutaraldehyde vapor (660 ± 199.3 nm).

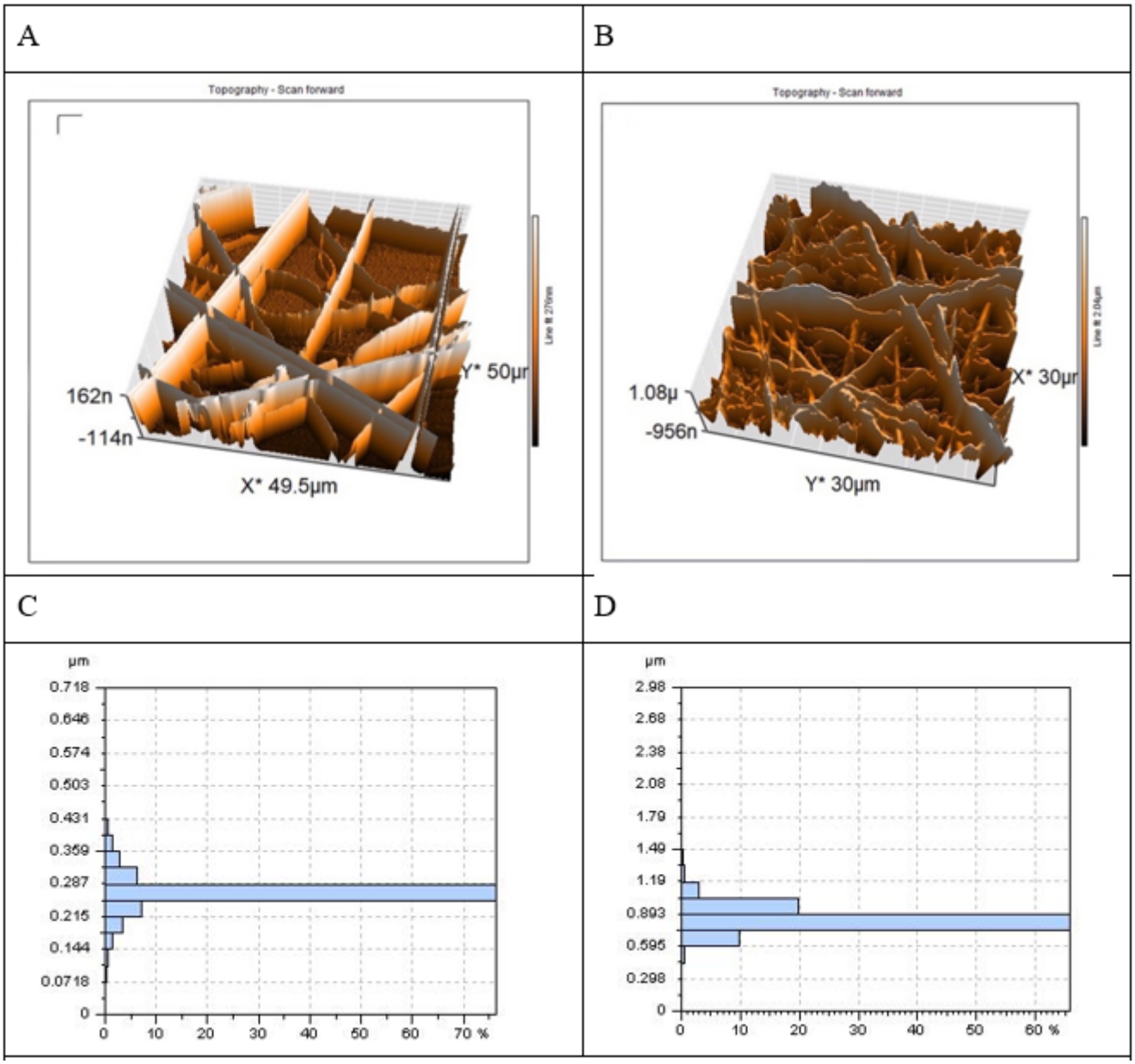

Figure 3

Upper panel: AFM scanning of the electrospun nanofiber samples. The topographical representation of the fibers shows the difference between the height and the orientation of the fibers before(A) crosslinking by glutaraldehyde vapor and (B) after crosslinking. Lower panel: A histogram representation suggests that the size of the nanofibers (C) before crosslinking (approximately 250 nm) grew larger (D) after they were crosslinked (approximately 750 nm).

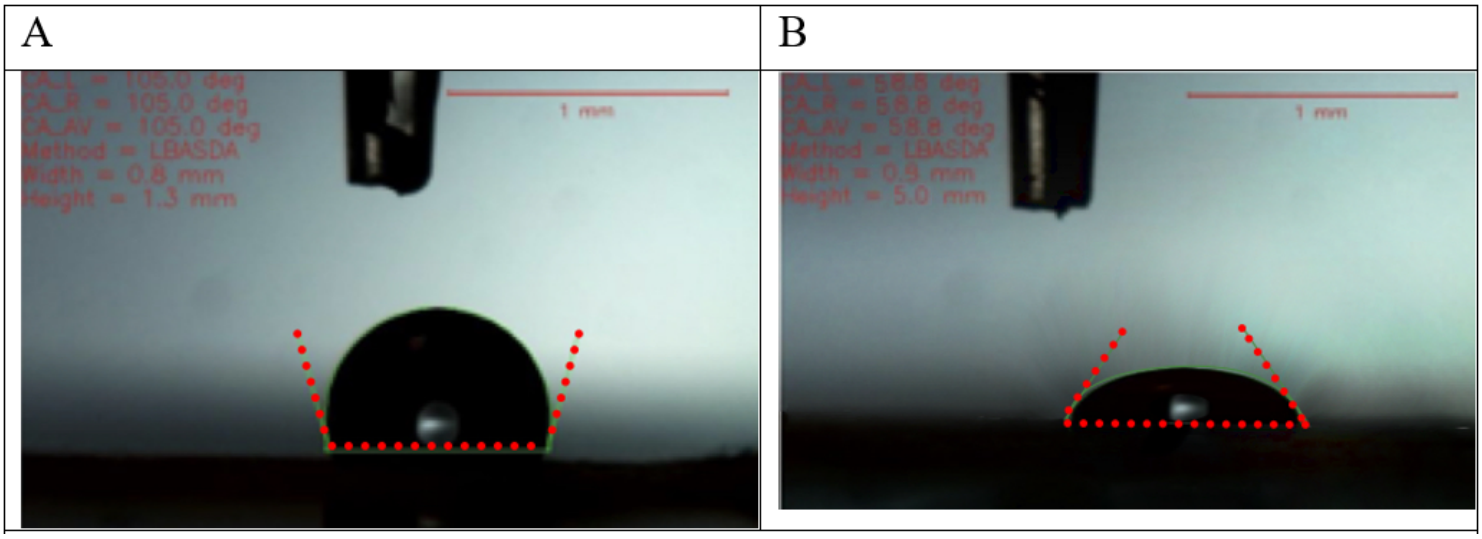

Figure 4

Water contact angle measurement of pre- and post-crosslinking of nanofiber by glutaraldehyde vapor. Photographs of a water droplet on the pre- (A) and post crosslinking nanofiber (B).

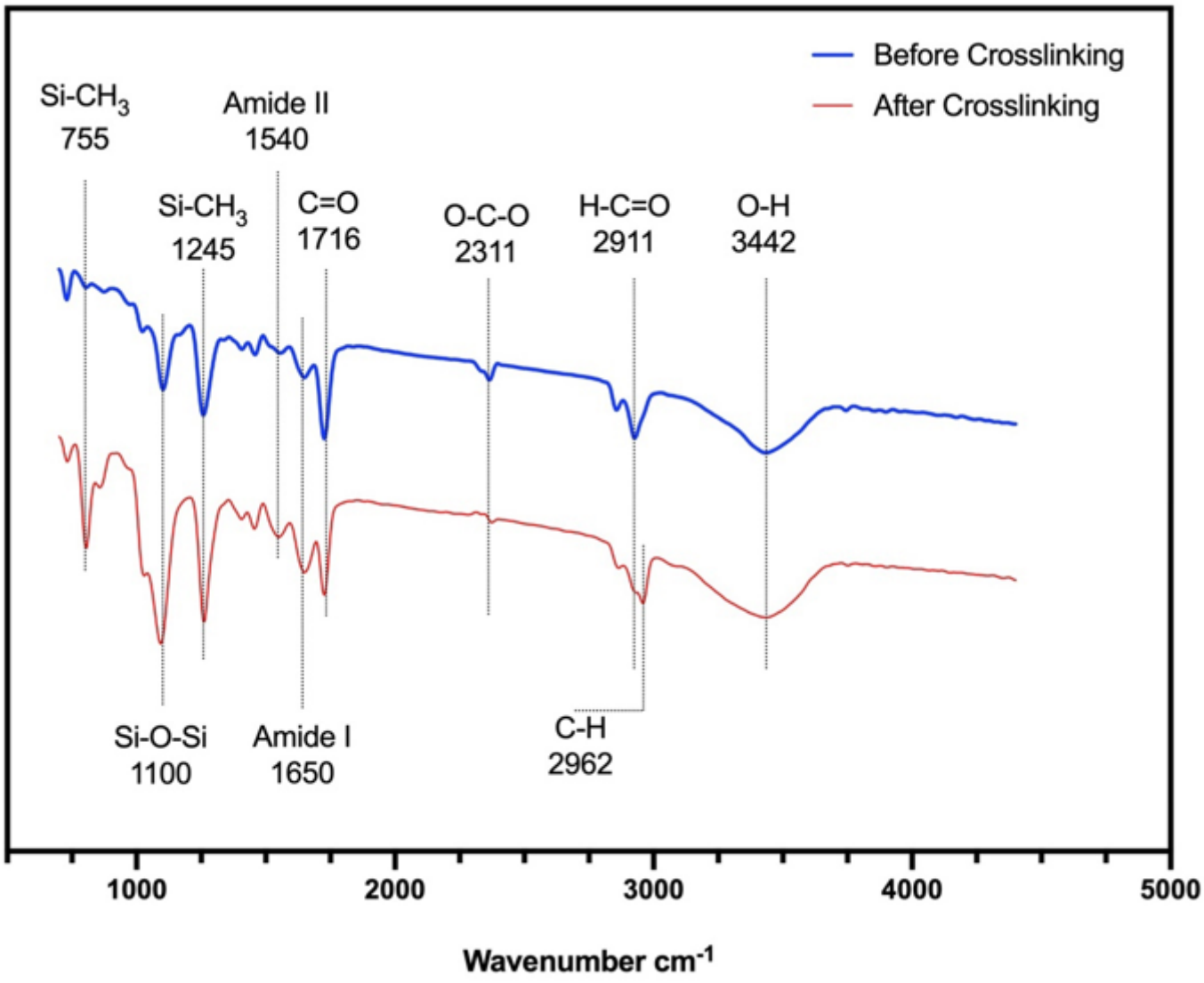

Figure 5

FT-IR spectra analyses of before and after crosslinked nanofibers. The appropriate functional groups are also identified on the graph.

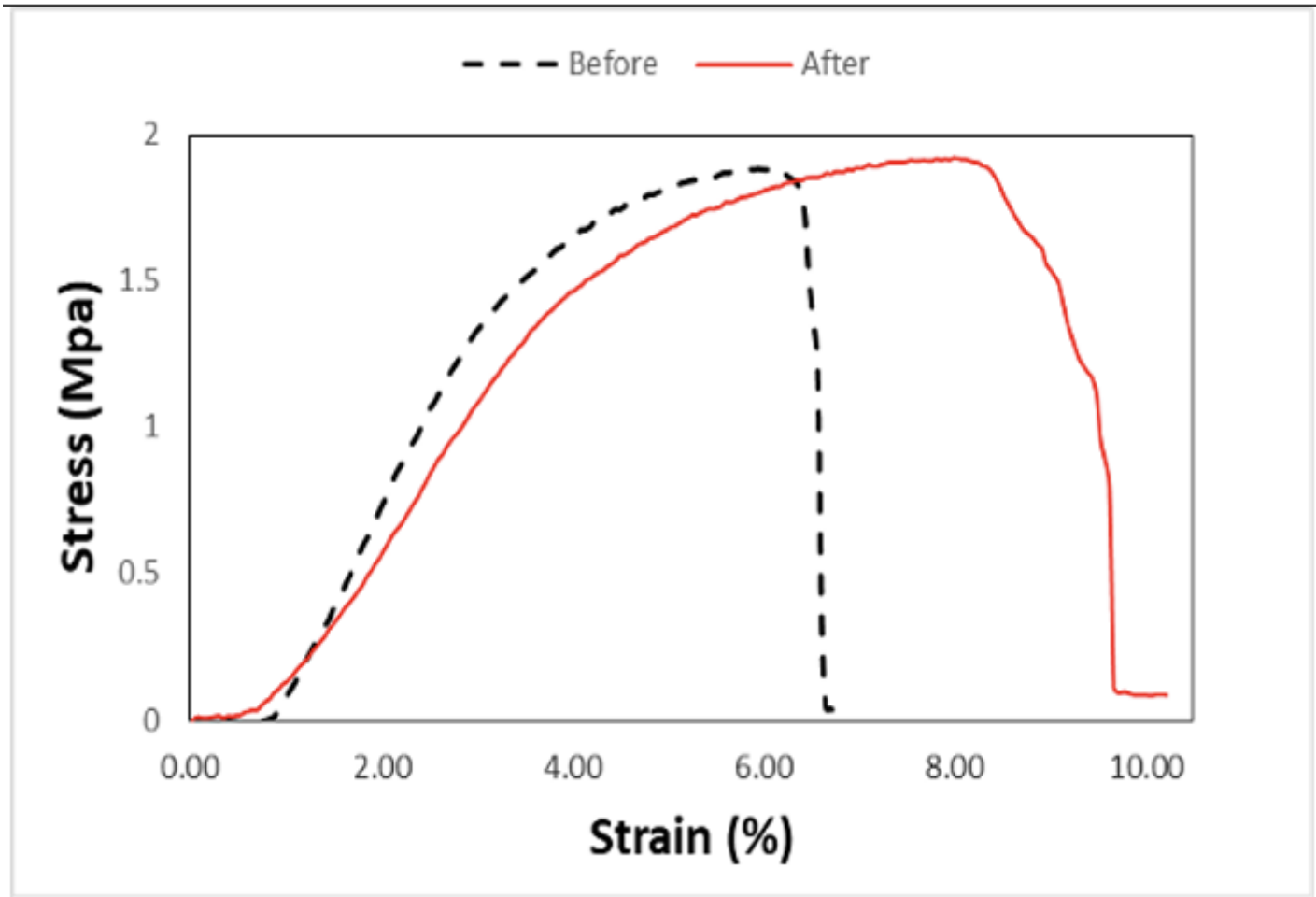

Figure 6

Stress–strain diagram of nanofibers before and after crosslinking treatment.

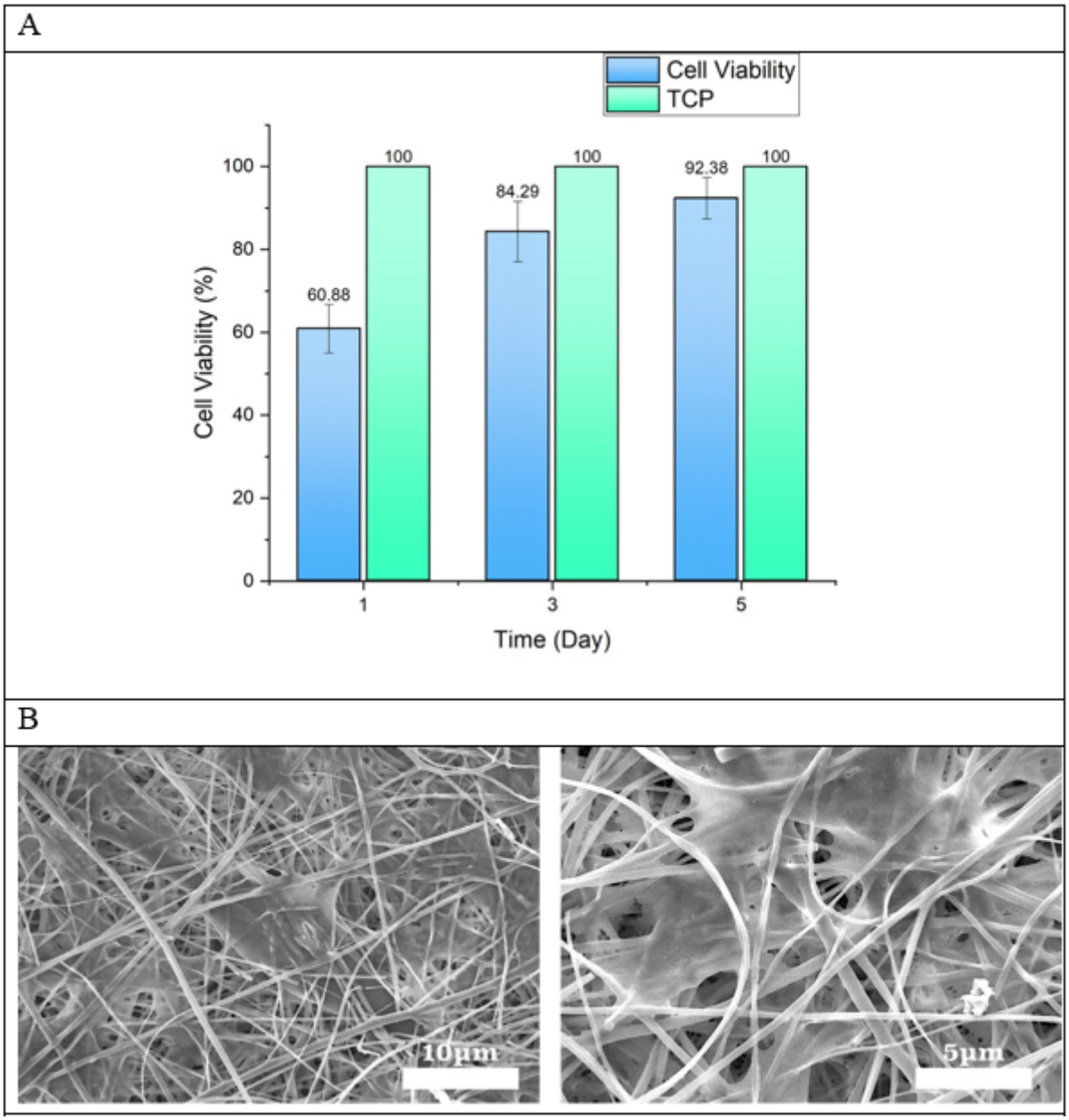

Figure 7

A) Cell viability (percentage) cultured on the crosslinked electrospun nanofiber membrane by Alamar Blue assay. TCP is tissue culture plate B) Cellular attachment and cellular morphology visual assessment by SEM

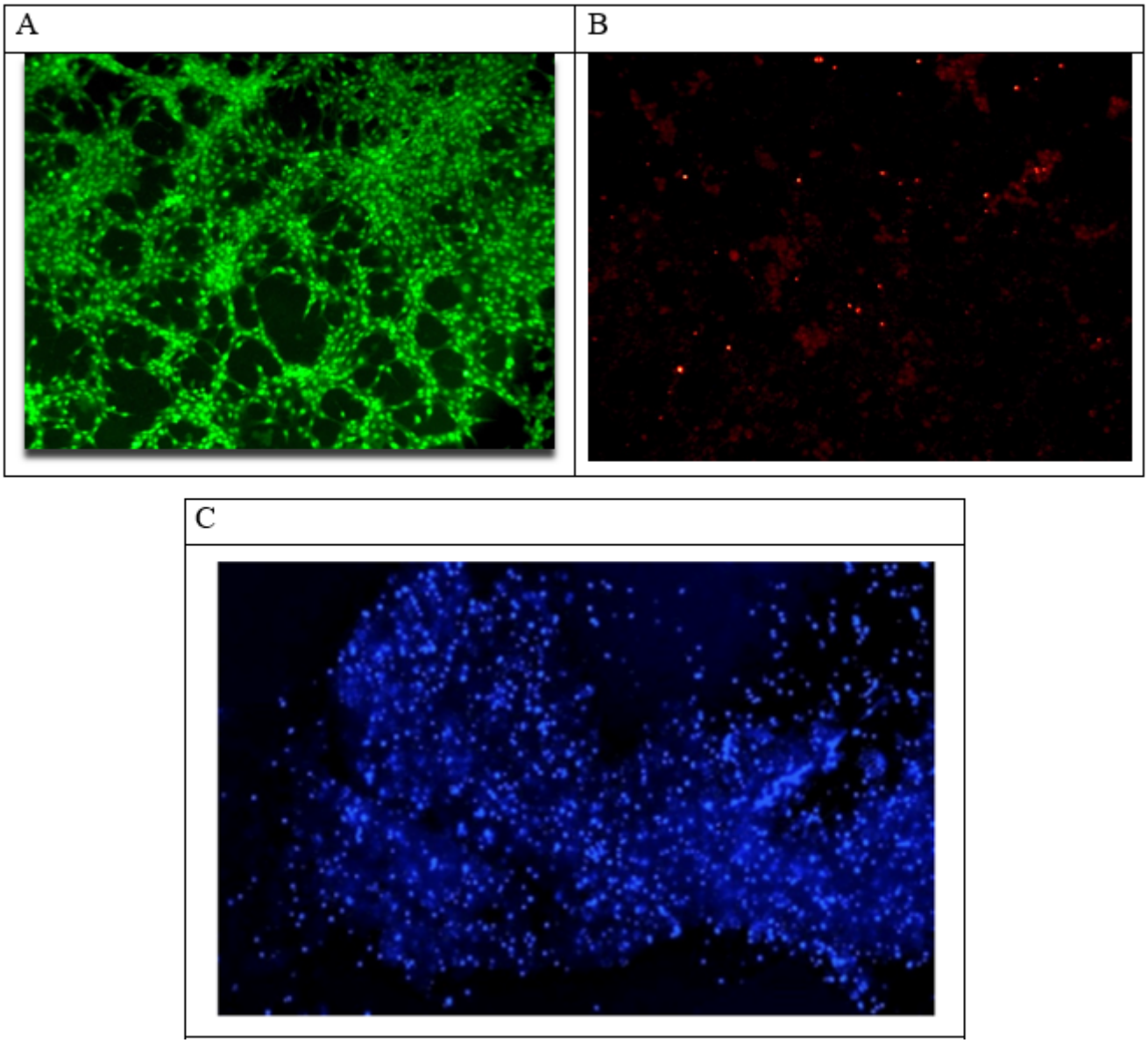

Figure 8

PI (green) and OA (orange) staining of A) living and B) dead cells on the nanofiber membrane in the microfluidic device, respectively. As shown in the image, majority of cells are alive and attached to the nanofiber. C) DAPI staining of the cells on the contra lateral side of the nanofiber membrane in the microfluidic device. It is evident that the cells are well attached on both sides of the membrane.