FABRICATION OF THREE-DIMENSIONAL NANOFIBERS FOR TISSUE ENGINEERING AND ENERGY APPLICATIONS

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การประดิษฐ์เส้นใยนาโนแบบสามมิติสำหรับวิศวกรรมเนื้อเยื่อและการประยุกต์ใช้ด้านพลังงาน

นายธนพล เหมือนวาจา

วิทยานิพนธ์นี้เป็นส่วนหนึ่งของการศึกษาตามหลักสูตรปริญญาวิทยาศาสตรมหาบัณฑิต
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Suranaree University of Technology has approved this thesis submitted in partial fulfillment of the requirements for a Master's degree.

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ธนพล เหมือนวาจา : การประดิษฐ์เส้นใยนาโนแบบสามมิติสำหรับวิศวกรรมเนื้อเยื่อและการประยุกต์ใช้ด้านพลังงาน (FABRICATION OF THREE-DIMENSIONAL NANOFIBERS FOR TISSUE ENGINEERING AND ENERGY APPLICATIONS). อาจารย์ที่ปรึกษา : อาจารย์ ดร.วิวัฒน์ นวลสิงห์, 64 หน้า.

เส้นใยนาโนที่สังเคราะห์ด้วยเทคนิคอิเล็กโทรสปินนิง จะมีขนาดเส้นผ่านศูนย์กลางตั้งแต่ระดับนาโนเมตรถึงไมโครเมตร สามารถควบคุมลักษณะของเส้นใยได้โดยการปรับเงื่อนไขต่าง ๆ ที่เกี่ยวข้อง เช่น ความเข้มข้นของสารละลาย ชนิดตัวทำละลาย ศักย์ไฟฟ้า ระยะห่างระหว่างปลายเข็มฉีดสารละลายกับวัสดุรองรับ เป็นต้น นอกจากนี้ยังสามารถควบคุมให้เส้นใยนาโนเกิดเป็นแผ่นสองมิติแบบไม่ถักทอบนวัสดุรองรับได้ โดยความหนาของแผ่นเส้นใยนี้ขึ้นกับระยะเวลาการสังเคราะห์เส้นใย มีกลุ่มวิจัยหลายแห่งนำเสนอเทคนิคการควบคุมให้เส้นใยนาโนก่อตัวขึ้นในลักษณะสามมิติ เพื่อเพิ่มสมบัติให้ดีมากขึ้นและขยายขอบเขตการประยุกต์ใช้

งานวิจัยนี้ได้ศึกษาการสังเคราะห์เส้นใยนาโนให้มีโครงสร้างแบบสามมิติ โดยใช้เทคนิคอิเล็กโทรสปินนิงร่วมกับวัสดุรองรับที่เป็นอ่างบรรจุด้วยของเหลว วัสดุที่นำมาศึกษาและสังเคราะห์ให้อยู่ในรูปเส้นใยนาโนแบบสามมิติ คือ PVDF-HFP เนื่องจากเป็นพอลิเมอร์สังเคราะห์ ที่มีสมบัติความเข้ากันได้ทางชีวภาพ และสมบัติทางเพียโซอิเล็กทริค เหมาะสำหรับการนำไปประยุกต์ใช้ทางการแพทย์และพลังงาน งานวิจัยนี้ได้วิจัยหาตัวทำละลายที่เหมาะสมกับพอลิเมอร์ PVDF-HFP รวมทั้งศึกษาผลจากความเข้มข้นของสารละลาย ชนิดของของเหลวที่บรรจุในอ่างรองรับเส้นใย และเงื่อนไขที่ใช้ในกระบวนการอิเล็กโทรสปินนิง

จากผลการทดลองแสดงให้เห็นว่า พอลิเมอร์ PVDF-HFP ที่ละลายในตัวทำละลายอะซีโตนผสมกับไดเมทิลฟอร์มาไมด์ (อัตราส่วน 1 : 1) ด้วยความเข้มข้น 15 เปอร์เซ็นต์โดยน้ำหนัก เมื่อนำไปผ่านกระบวนการอิเล็กโทรสปินนิงที่ระยะห่าง 5 เซนติเมตร ศักย์ไฟฟ้า 10 กิโลโวลต์ และใช้อ่างรองรับเส้นใยที่บรรจุน้ำปราศจากไอออน จะทำให้เกิดโครงสร้างสามมิติของเส้นใยนาโนได้โครงสร้างภายนอกมีลักษณะเป็นก้อน ขนาดเส้นผ่านศูนย์กลางประมาณ 5 เซนติเมตร เมื่อศึกษาโครงสร้างภายในด้วยการใช้ใบมีดตัดในระนาบตามแนวดิ่ง จะพบว่า โครงสร้างภายในมีลักษณะคล้ายรังปลวกสายพันธุ์อะพิโค ซึ่งมีอยู่ในธรรมชาติ เนื่องจากเป็นโครงสร้างเส้นใยนาโนแบบสามมิติที่ยังไม่มีในรายงานวิจัยอื่นมาก่อน จึงเรียกว่า โครงสร้างเส้นใยนาโนแบบสามมิติที่คล้ายรังปลวก (Termite nest like 3D nanofibers structure) และเมื่อวิเคราะห์ขนาดเส้นผ่านศูนย์กลางของเส้นใยนาโนด้วยกล้องจุลทรรศน์อิเล็กตรอน พบว่า เส้นใยมีขนาดประมาณ 802 ± 23นาโนเมตร

เพื่อศึกษาความเป็นไปได้ในการนำโครงสร้างเส้นใยนาโนแบบสามมิติไปใช้ทางการแพทย์ จึงได้ทดลองเพาะเลี้ยงเนื้อเยื่อด้วยเซลล์ NIH 3T3 ทั้งนี้ก่อนทำการเลี้ยงเซลล์ ได้ศึกษาผลการปรับเปลี่ยนสมบัติการไม่ชอบน้ำของเส้นใยนาโน PVDF-HFP ด้วยวิธีต่าง ๆ จากผลการศึกษาแสดงให้เห็นว่า การใช้พลาสมาสามารถทำให้เส้นใยนาโนมีสมบัติชอบน้ำได้ ซึ่งเป็นการปรับสภาพให้เหมาะกับนำไปทดลองเลี้ยงเซลล์ ส่วนผลการเลี้ยงเซลล์ NIH 3T3 บนเส้นใยนาโนแบบสามมิติ พบว่า เซลล์สามารถยึดเกาะโครงสร้างและมีการแผ่ขยายของเซลล์ ดังนั้นโครงสร้างเส้นใยสามมิติที่สังเคราะห์ได้นี้ สามารถนำไปประยุกต์ใช้ด้านวิศวกรรมเนื้อเยื่อ

นอกจากนี้ งานวิจัยยังได้ศึกษาสมบัติเพียโซอิเล็กทริคของ PVDF-HFP ที่สังเคราะห์ให้มีโครงสร้างเส้นใยนาโนแบบสามมิติ เปรียบเทียบกับแผ่นเส้นใยนาโนแบบสองมิติ พบว่า เส้นใยนาโน PVDF-HFP ทั้งสองแบบ สามารถทำให้เกิดกระแสไฟฟ้าได้เมื่อมีการใส่แรงกระทำลงไป และผลการทดลองชี้ให้เห็นว่า โครงสร้างเส้นใยนาโนแบบสามมิติ ให้ค่ากระแสไฟฟ้าและศักย์ไฟฟ้าสูงสุดมากกว่าแผ่นเส้นใยนาโนแบบสองมิติ ดังนั้นโครงสร้างเส้นใยนาโนแบบสามมิติที่สังเคราะห์ขึ้นนี้ สามารถนำไปประยุกต์ใช้ในด้านพลังงานได้

สาขาวิชาฟิสิกส์ ลายมือชื่อนักศึกษา_____

ปีการศึกษา 2561 ลายมือชื่ออาจารย์ที่ปรึกษา_____

THANAPON MUENWACHA: FABRICATION OF THREE-DIMENSIONAL NANOFIBERS FOR TISSUE ENGINEERING AND ENERGY APPLICATIONS. THESIS ADVISOR : WIWAT NAUNSING, Ph.D. 64 PP.

3D ELECTROSPINNING/LIQUID BATH COLLECTOR/PVDF-HFP/TISSUE ENGINERRING/TERMITE NEST/PIEZOELECTRIC

A conventional electrospinning technique can fabricate nanofibers with diameters in order of nanometer and micrometer ranges. The morphology and diameter of nanofibers can be controlled by several parameters, such as solution concentration, type of solvent, applied voltage, and distance between the tip and collector. Electrospinning is a versatile technique to fabricate scaffolds for tissue engineering as they have biomimetic mechanical, chemical and biological properties. In general, conventional electrospinning fabricates two-dimensional (2D) nonwoven fibers mat structures. However, it has some limitations and difficult to fabricate nanofibers into three dimensional (3D) shapes because low controllability of porosity and internal pore shape.

This research, electrospinning has been successfully used for the fabrication of three-dimensional (3D) PVDF-HFP nanofiber structures by using liquid-bath collector electrospinning technique. The produced 3D nanostructures mimic the natural termite nest and have potential use for medical applications.

The 3D nanofibers fabricated in this work were characterized by a scanning electron microscope. The results demonstrated that PVDF-HFP nanofibers have

diameter of 802 ± 23 nm. In addition, *in vitro* culturing of NIH 3T3 was studied. Optical microscope and hemocytometer counting were used to study effect of cell proliferation by monitoring the number of cells on the 3D nanofibers scaffold. The cell culturing results showed that the 3D nanofibers scaffolds are promoting cell adhesion and migration. Therefore, it is possible to use the nanofibers 3D structure for tissue engineering applications.

Moreover, this work showed that the piezoelectric performances of the 3D nanofibers are better than their 2D counterparts. This would suggest that the PVDF-HFP 3D structure can be applied for energy applications.

School of Physics

Academic Year 2018

Student's Signature _____

Advisor's Signature _____

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CHAPTER I

INTRODUCTION

1.1 Background and motivation

Fibers scaffolds with micro- and nanoscale structures have an important role in assisting tissue regeneration, such as cell adhesion, proliferation, differentiation, and migration (Khademhosseini et al., 2006; Ovsianikov et al., 2012). Electrospinning is a versatile technique to fabricate tissue engineering scaffolds with mimic biological characteristics, chemical, and biological properties.

A conventional electrospinning setup can fabricate electrospun scaffolds, which are highly porous, high surface to volume ratio, and can be controlled diameters and fiber alignments. However, most this technique has some limitations such as the difficulty to fabricate three dimensional (3D) shapes, low controllability of porosity and internal pore shape.

This work is interested to fabricate 3D structures nanofibers by using electrospinning technique with liquid-collecting. This technique uses a liquid-bath collector instead of a conductive collector i.e., a metal sheet. Poly(vinylidene fluoride-co-hexafluoropropylene) (PVDF-HFP) was used in this work to fabricate 3D nanofibers scaffold because it is a biocompatible polymers and has a slow degradation rate (Shea et al., 2000). So, the PVDF-HFP is a material suits for tissue engineering applications such as a wound-healing.

To mimic scaffold-like extracellular matrix (ECM), collagen is used as an additive in nanofibers scaffolds in order to increase the efficiency of fibers scaffolds for cell proliferation. This work, collagen was blended in 3D fibers scaffolds by dissolving the collagen in a collecting bath and soak fibers scaffolds in the collagen solution. In this study, PVDF-HFP blended with collagen were used to evaluate the efficiency of 3D fibers scaffold for tissue engineering regeneration by NIH 3T3 fibroblast cells in the cell culture process.

Another interesting application of the PVDF-HFP is energy applications. The important property of the PVDF-HFP electrospun nanofibers is piezoelectric property. This makes it has ability for energy harvesting or scavenging. Moreover, the PVDF-HFP can be applied for other applications, such as solar cells, fuel cells, nanogenerators, lithium-ion batteries (LIBs), and supercapacitors (Shi et al., 2015). In this work, we interested in the energy harvesting applications. PVDF-HFP nanofibers can be used to generate electric energy from the piezoelectric property (Parangusan et al., 2018). This work, also testing piezoelectricity of the 3D nanofibers compared with 2D nanofibers in order to evaluate the energy harvesting applications.

1.2 Objective of this work

1.2.1 To fabricate 3D nanofibers scaffold for tissue engineering.

1.2.2 To fabricate artificial organs for medical applications.

1.2.3 To investigate the effect of liquid collagen coating on 3D nanofibers.

1.2.4 To study and develop biomedical material.

1.2.5 To study and develop 3D nanofibers for energy scavenging.

1.3 Scope of study

In this research, PVDF-HFP is a polymer used to fabricate 3D nanofibers scaffold. The 3D scaffold was fabricated by using electrospinning technique with liquid-bath collector. Collagen is an additive in 3D nanofibers scaffolds to enhance efficiency of cell adhesion and proliferation. NIH 3T3 cells were used to evaluate the efficiency of 3D fibrous scaffolds for tissue regeneration. The morphology of cells and 3D nanofibers scaffold were observed by scanning electron microscope (SEM). Optical microscope and hemocytometer counting were used to study effect of cell proliferation by monitoring the number of cells on the scaffold. The 3D PVDF-HFP nanofibers were characterized piezoelectricity performance and compared with the 2D PVDF-HFP nanofibers.

1.4 Expected results

After the research completion, we expect the following:

1.4.1 Knowledge and technique to fabricate 3D nanofibers scaffold.

1.4.2 3D scaffold with high efficiency for tissue engineering.

1.4.3 Biomedical material for regenerative medicine and surgery.

1.4.4 New 3D material for energy scavenging.

CHAPTER II

LITERATURE REVIEWS

This chapter will present a brief overview on the theoretical background of electrospinning technique and the nanofibers fabrication process. In addition, a biocompatible polymer that can be used for cell regeneration will be detailed. In section 2.3 will give more information about the electrospinning technique for fabrication of 3D nanofibers scaffold, comparison between 2D and 3D scaffold in tissue engineering applications. The final section will present applications of nanofibers for energy scavenging.

2.1 Electrospinning

Electrospinning is a technique widely used for fabrication of continuous fibers in micro- and nanoscale diameters. This technique is able to produce nanofibers of polymers, composites, semiconductors, and ceramics (Ramakrishna et al., 2005). In additional, electrospinning technique has the ability to form various fiber assemblies by nanofiber fabrication processes. (Teo et al., 2006). A conventional electrospinning setup requires a high voltage power supply, syringe, syringe pump, needle, and collector. The principle of electrospinning setup and process are shown in Figure 2.1.

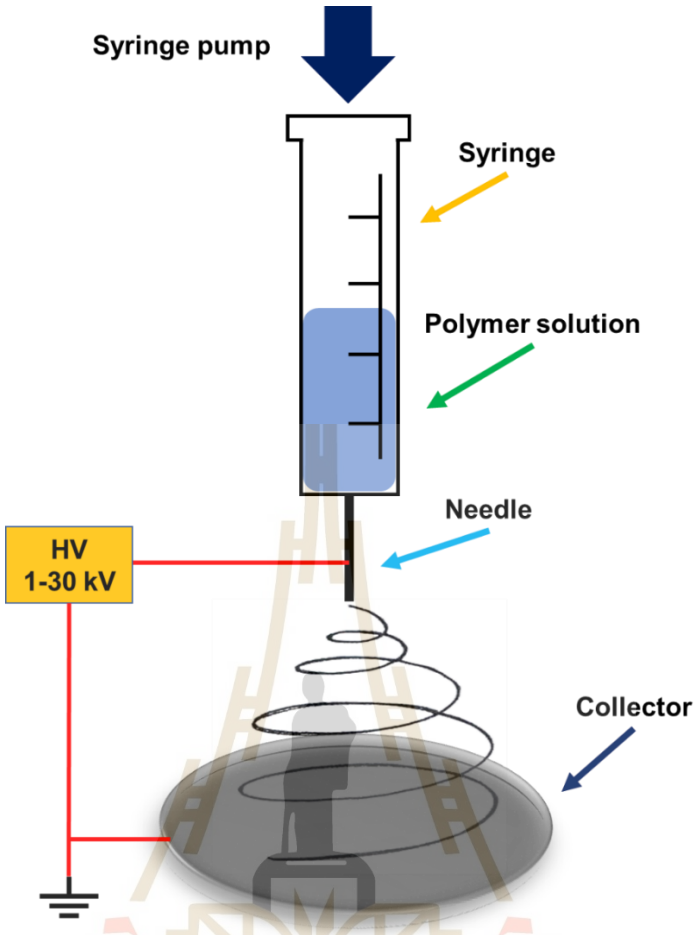

Figure 2.1 Schematic of electrospinning setup.

A high voltage (more than 5 kV) is applied to the solution at a needle tip. Electrically charged solution forms a conical shape, called a Taylor cone, at the tip of the needle. When the repulsive force within the electrically charged solution larger than its surface tension, a whipping polymer jet is ejected from the tip of the spinneret. The jet of solution is moving to the collector connected with ground. During this process, the solvent evaporates and fibers deposit on the collector in form of a randomly nonwoven mesh. The fibers fabricated by electrospinning depend on various parameters include applied voltage, flow rate, distance between needle and collector, solution (such

as concentration, viscosity, conductivity), size of the needle, and humidity. For example, reducing the solution concentration decreases the fiber diameter (Shenoy et al., 2005).

Nanofibers with high surface area to volume ratio that fabricate by biocompatible polymer can be used for biomedical applications such as drug delivery, wound dressing and scaffold for tissue engineering (Qin et al., 2011) because the electrospun fibers can mimic the nano-features of natural extracellular matrix (ECM) (Kim et al., 2011).

2.2 Electrospun PCL, PCL-collagen, PVDF-HFP nanofibers

Electrospun nanofibers scaffold fabricated from biocompatible polymers such as polyvinyl alcohol (PVA), polycaprolactone (PCL), polyacrylonitrile (PAN), and poly (vinylidene fluoride-co-hexa-fluoropropene) (PVDF-HFP) have wound healing performance (Xin et al., 2009). Many of researcher use these nanofibers in tissue engineering applications. PCL and PVDF-HFP were investigated in this work.

2.2.1 Electrospun polycaprolactone scaffold

Polycaprolactone (PCL) is nontoxicity for cells, slow degradation and low cost, so PCL has potential in tissue engineering. Chen et al. used PCL to fabricate electrospun scaffolds. They found that cells (3T3 fibroblast cell) can grow on the PCL fibers scaffolds (Figure 2.2) and reported the effect of fiber diameter in cell adhesion and proliferation on electrospun PCL scaffold. The diameter of fibers has effect on 3T3 fibroblast adhesion and proliferation (Figure 2.3). They found average diameters around 100 nm has lowest cell adhesion and proliferation because 100 nm scaffold were beaded on fibers.

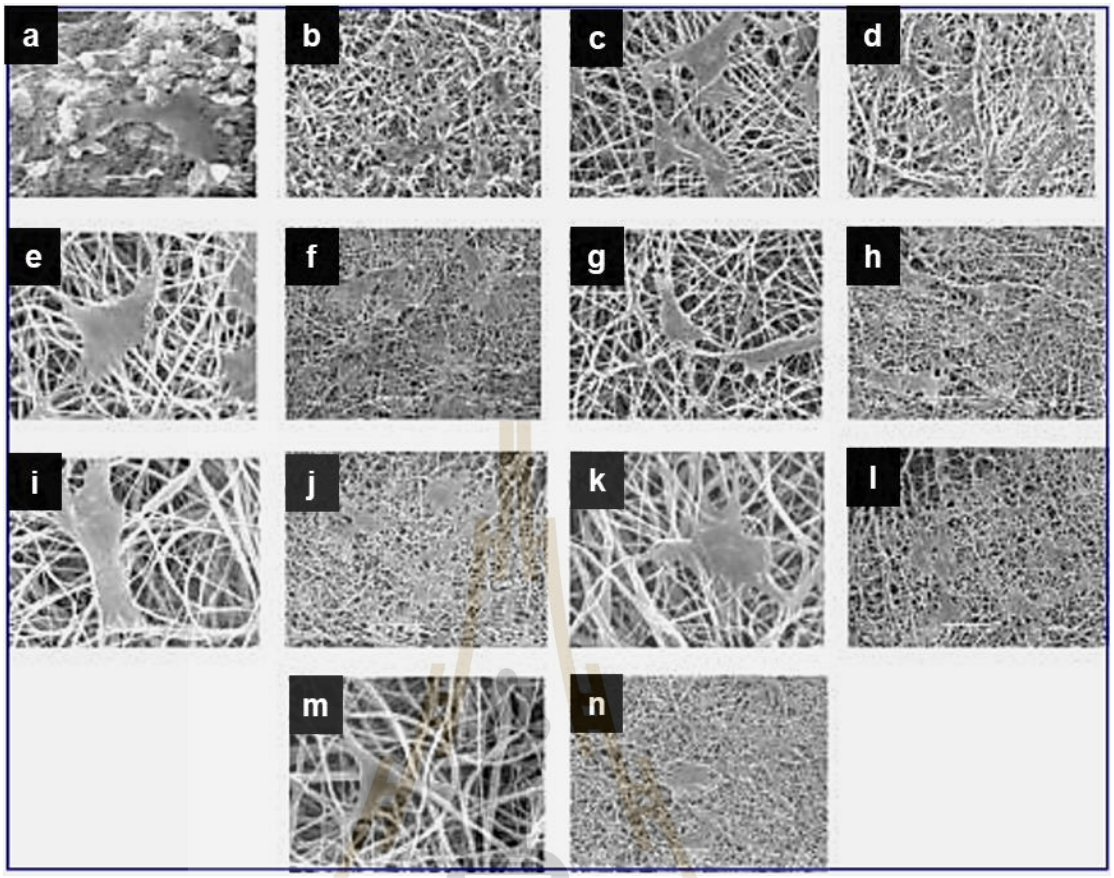

Figure 2.2 3T3 fibroblast cell morphology on different fiber diameter PCL scaffolds: (a, b) 117 nm; (c, d) 428 nm; (e, f) 641 nm; (g, h) 787 nm; (i, j) 900 nm; (k, l) 1,051 nm; (m, n) 1,647 nm. Modified from (Chen et al., 2007).

For small diameters fibers (lower than 400 nm), they have good effect for cell adhesion and proliferation. When the diameter of fiber increase (from 400 nm), growing rate of cells decreases. However, for microscale scaffold (1000 nm), the growing rate of cells increases when fibers diameter increase.

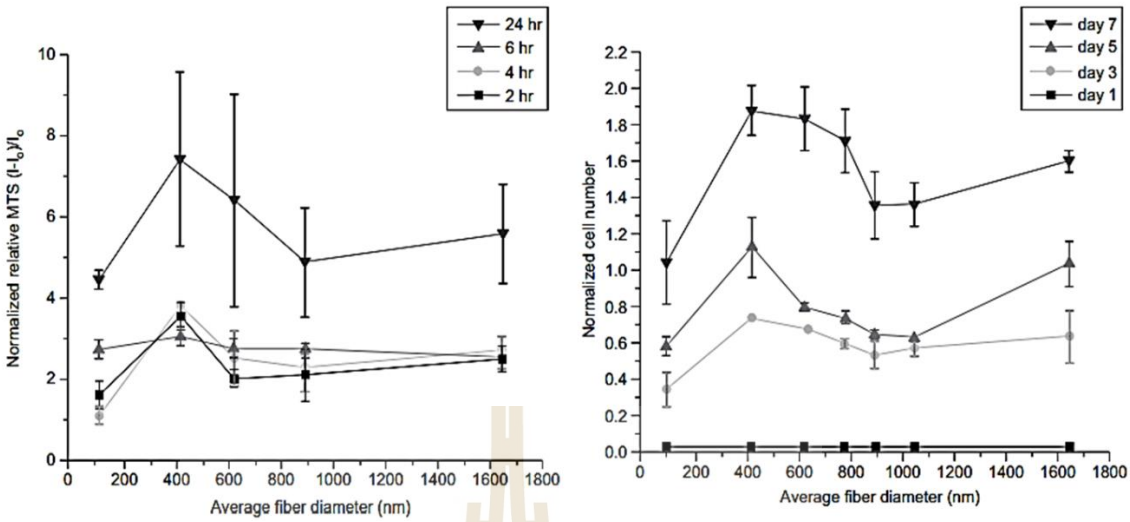

Figure 2.3 Effect of the average fiber diameter and morphology of polycaprolactone electrospun scaffolds on 3T3 fibroblast adhesion and proliferation. (Chen et al., 2007).

2.2.2 Electrospun PCL-collagen scaffold

For increasing performance of elctrospun fibers scaffolds for cell attachment and cell proliferation, natural biopolymers such as collagen, silk, and cellulose can use into electrospun nanofibers (Zhang et al., 2004). Collagen is one of component in natural extra cellular matrix (ECM), Venugopal et al. blended collagen with PCL electrospun fibers by mixing PCL and collagen type I and dissolved in 1,1,1,3,3,3-hexafluoro-2-propanol (HFP) to fabricated by electrospinning. The fibroblast cultures (Human dermal fibroblast, HDF) used to evaluate the efficiency of the PCL-blended collagen nanofibrous. The cell proliferation was monitored after 2, 4, and 6 days by colorimetric MTS assay. They reported that the proliferation of HDF was significantly increased on PCL-blended collagen nanofibers higher than that of pure PCL nanofibrous membrane Figure 2.4 and Figure 2.5.

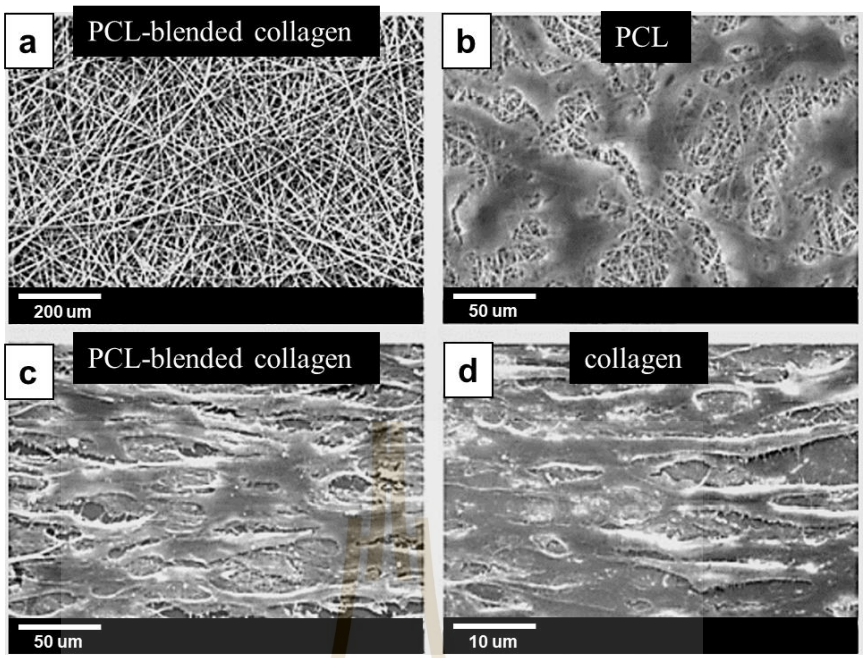

Figure 2.4 SEM images of HDF cultured for 6 days on electrospun nanofibers. (a) PCL-blended collagen nanofibers, (b) HDF on PCL nanofibers, (c) HDF on PCL-blended collagen nanofibers, (d) HDF on collagen nanofibers. Modified from (Venugopal et al., 2007).

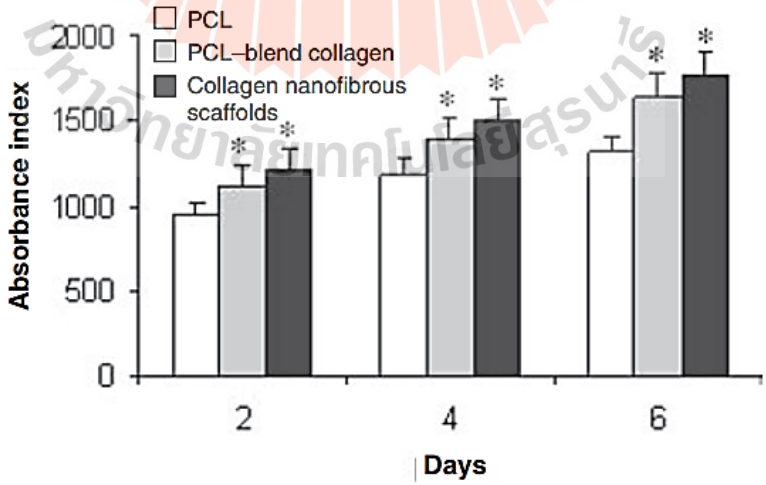

Figure 2.5 Proliferation of HDF on PCL, PCL-blended collagen, and collagen nanofibrous scaffolds. (Venugopal et al., 2007).

For using of collagen to increase cell proliferation efficiency of the electrospun nanofibers Zhang et al. found that surface coating of collagen on PCL nanofibers scaffolds have an effect on the efficiency of cells proliferation. In their work, electrospun PCL-collagen nanofibers are fabricating by two surface coating techniques to coatings collagen on PCL nanofibers.

The first technique is the coaxial electrospinning, they fabricated core shell nanofiber by the shell material are collagen and PCL was the core component. (Figure 2.6). The second technique coating collagen on PCL nanofibrous by soak PCL nanofibrous into a collagen solution overnight. The fibroblast culture used for evaluated efficiency of each nanofibrous.

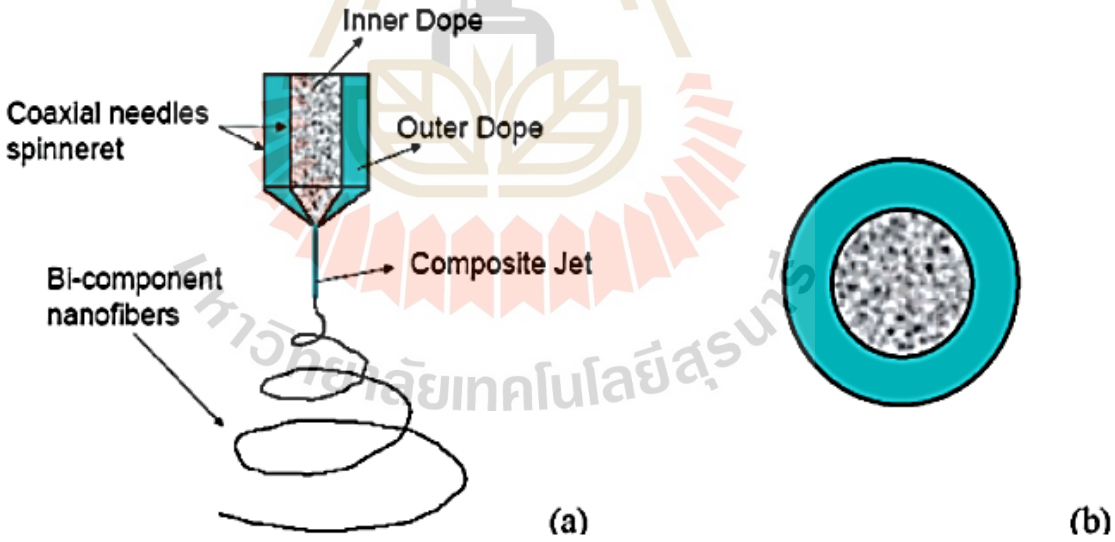

Figure 2.6 Coaxial electrospinning (a), and cross-sectional view of resultant bi-component composite fiber (b). (Zhang et al., 2005).

The result shown core-shell collagen-PCL has significantly more cells proliferation than that of the soaking collagen PCL fibers (see Figure 2.7). However,

both coating techniques have more cell proliferation than pure PCL nanofibers. Interestingly, they found the cells can penetrate beneath the core-shell collagen-PCL nanofibers. It cannot be found in pure PCL or soaking collagen-PCL nanofibrous scaffold (Figure 2.8).

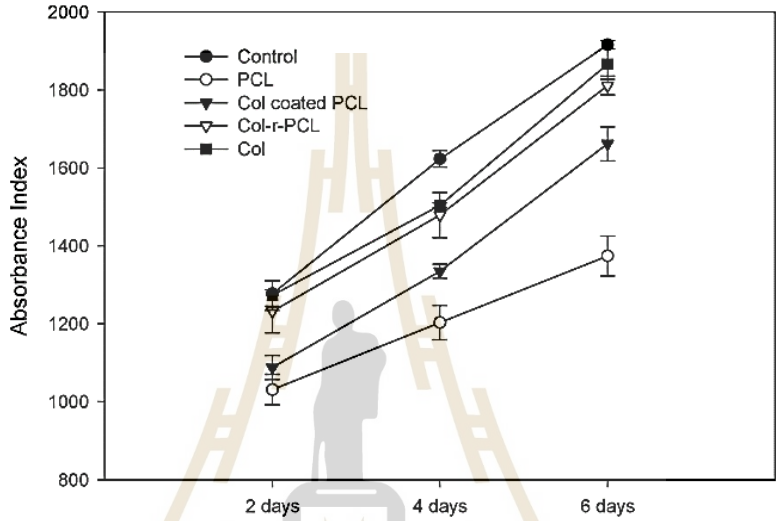

Figure 2.7 Cell proliferation of human dermal fibroblasts by MTS assay. (Zhang et al., 2005).

From above result, in our work, various techniques were tested in order to coat the collagen on the nanofibers scaffolds for study effect of surface coating for cell proliferation and then we will use poly (vinylidene fluoride-co-hexa-fluorpropene) (PVDF-HFP) to fabricate 3D nanofibers scaffold and compare it with 3D fibers scaffold that fabricated by PCL because the PVDF-HFP electrospun fibers have a better wound healing than the PCL electrospun fibers. PVDF-HFP electrospun fibers have much high porosity and air permeability (Xin et al., 2009).

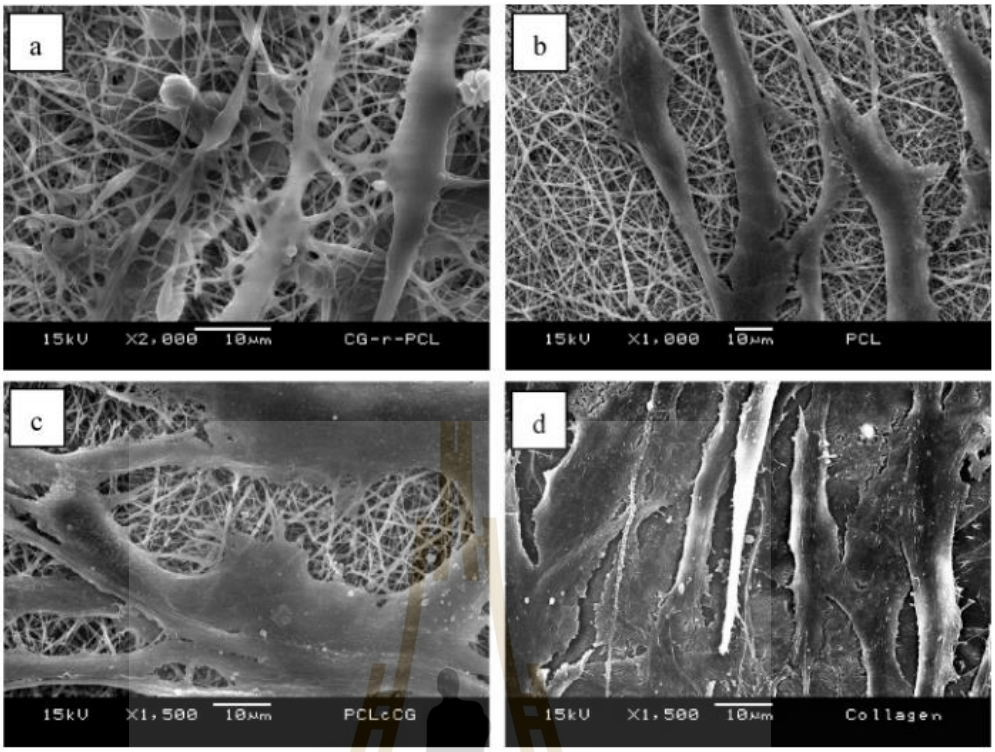

Figure 2.8 A comparison of cells ingrowth behavior on different nanofibrous scaffolds: (a) core-shell collagen-PCL (Col-r-PCL); (b) pure PCL; (c) PCL with surface roughly collagen coated; and (d) pure collagen nanofibers. (Zhang et al., 2005).

2.3 Three dimensional (3D) nanofibrous scaffold

The 3D fibers scaffolds have a highly porous structure and good mechanical stability. High porosity structural can help cells proliferation, migration and enable the exchange of nutrients between the scaffold and environment (Kim et al., 2005). Conventional two-dimensional (2D) electrospun fibers cell culture is poorly biological phenomena such as proliferation, adhesion, migration and differentiation of cells than 3D electrospun fibers cell culture, the 3D nanofibrous scaffold have more closely like natural ECM environment than 2D nanofibrous scaffold (Choi et al., 2015). Therefore, the 3D nanofibrous scaffold have high efficiency for tissue engineering applications.

The 2D electrospun have a small pores size, so cells were difficult to migrate or proliferate into the thickness direction of the scaffold. For this reason, Lee et al. fabricate 3D nanofibrous scaffold by using direct-write electrospinning (DWES). DWES use for generation of lattice-patterned nanofibrous mats by stacking of the mats into a 3D scaffold (Figure 2.9). PCL are polymer that use to fabricated 3D nanofibrous. In they work DWES using a nozzle spinneret moving on X-Y stage to fabricate lattice-patterned nanofibrous mats and stacking it layer-by-layer for increase thickness of nanofibrous scaffold. They scaffold had a size of about 5 x 5 x 1.5 mm^3 and its mats had a grid size of 0.75 x 0.75 mm^2.

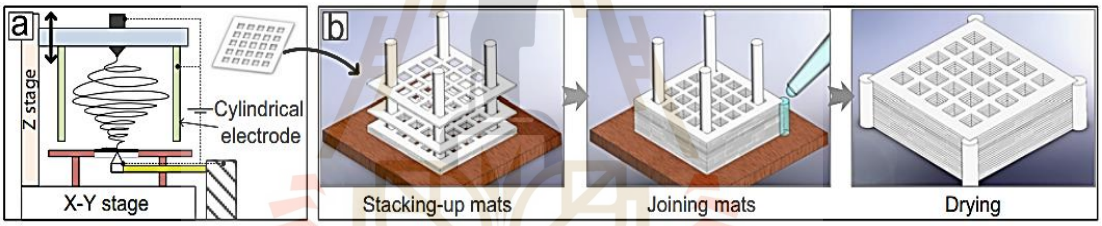

Figure 2.9 Procedure for the fabrication of the 3D nanofibrous scaffold: (a) patterned nanofibrous mat fabrication using DWES and (b) stacking of mats into a 3D scaffold. (Lee et al., 2012).

They compared DWES scaffold with stacking conventional electrospinning mats and salt leaching scaffold. (Figure 2.10). They show cells can grow and spread inside the DWES scaffold much more than the other scaffold, while only a few cells were observed inside the scaffold fabricated using conventional electrospinning and salt leaching process. (Figure 2.11). The DWES scaffold could provide more suitable environment for 3D tissue regeneration.

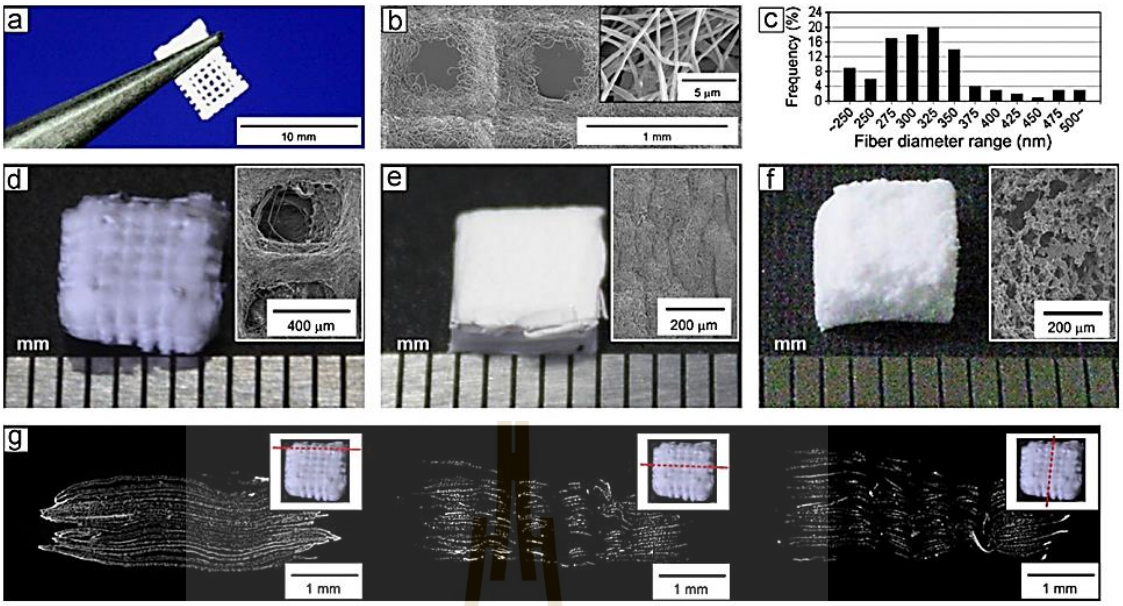

Figure 2.10 Characterization of a patterned nanofibrous mat: (a) photograph, (b) SEM images, and (c) nanofiber diameter distribution. Photographs of 3D scaffolds fabricated using (d) the proposed method, (e) conventional electrospinning, and (f) salt leaching. (g) Micro-CT images of the scaffold shown in (d). (Lee et al., 2012).

For other technique that can fabricate 3D Electrospun fibrous scaffolds Jun et al. review different electrospinning processes have been developed to fabricate 3D fibrous scaffolds such as liquid-collecting electrospinning, gas foaming, self-assembly, 3D printing electrospinning (Figure 2.12).

In our work, we are interested to fabricate 3D nanofibers by liquid-collecting electrospinning. This technique using liquid reservoirs as collectors has attracted attention as a method for preparing 3D fibrous scaffold. The utilization of liquids with low surface tension such as water, ethanol, and methanol. Kim et al. fabricate 3D nanofibrous scaffold by liquid-collecting electrospinning. They can fabricate 3D nanofibrous with highly porous structure, PCL was used to polymers for fabricate 3D

nanofibrous and 95% ethyl alcohol (ethanol or EtOH) is liquid in collecting bath (Figure 2.13).

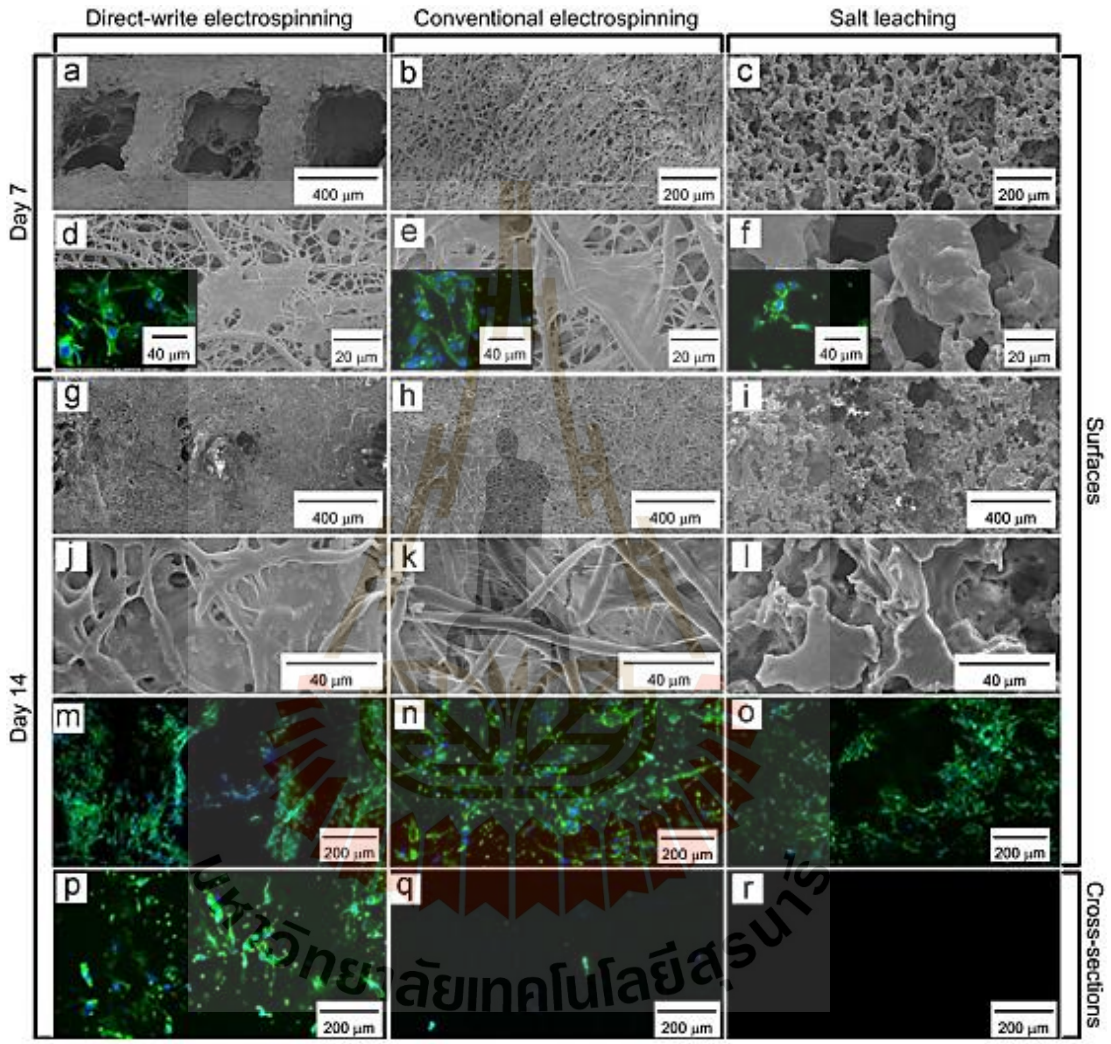

Figure 2.11 SEM and F-actin images of three types of scaffolds after (a)–(f) 7 days and (g)–(o) 14 days at the surfaces and (p)–(r) at the cross-sections. (Lee et al., 2012).

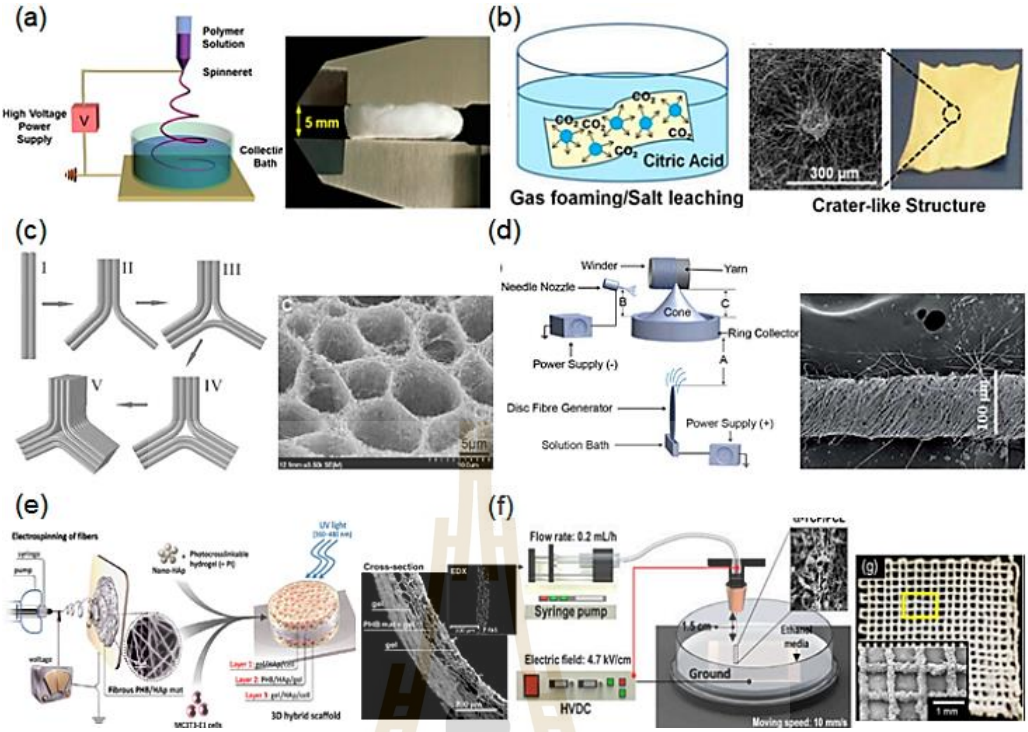

Figure 2.12 3D fibrous scaffolds using different electrospinning process: (a) liquid-collecting electrospinning; (b) gas foaming; (c) self-assembly; and (d) fibrous yarn scaffolds; (e) schematic illustration of a hydrogel-integrated fibrous scaffold; (f) a hybrid system using 3D printing and electrospinning. (Jun et al., 2018).

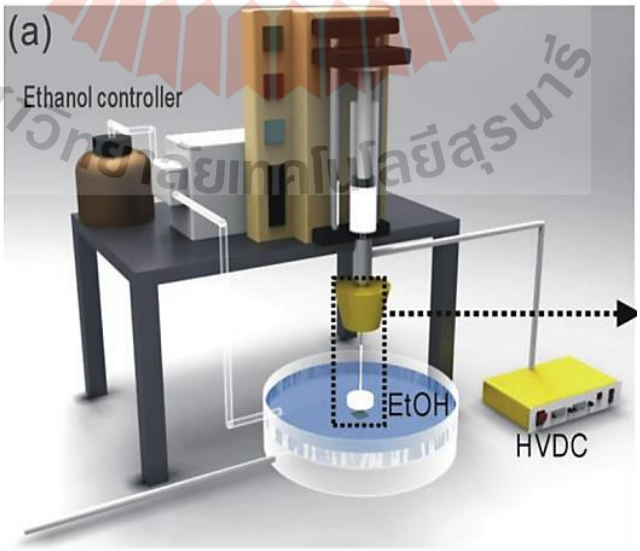

Figure 2.13 Schematic of the electrospinning process supplemented with an ethanol bath. (Kim et al., 2013).

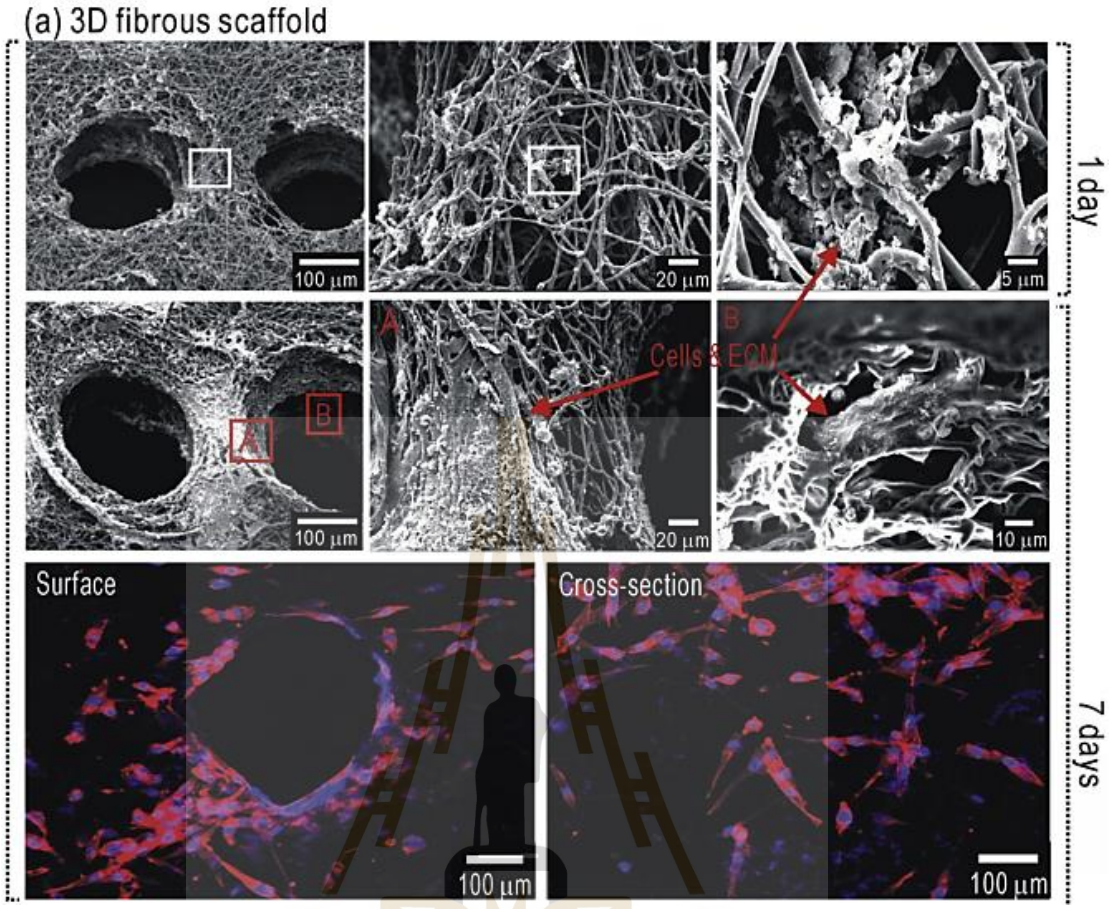

Figure 2.14 SEM and confocal microscope images showing the interaction between cells and scaffolds. In the confocal images, blue and red colors indicate nuclei and F-actin (Kim et al., 2013).

For creating a pore in the 3D fibrous scaffold, they use femtosecond laser with wavelength 800 nm and pulse duration 35 fs. The laser creates a pore size between 100 and 400 μm is enable to cell attachment and easy diffusion. (Murphy et al., 2010; Roosa et al., 2010). To observe the behavior of cell culture in the scaffolds osteoblast-like cells (MG63) were seeded in the scaffold. They have shown interactions between the cells (MG63) and the 3D fibrous scaffold (Figure 2.14). The cells were attached in the fibrous scaffold, the proliferated cells mostly covered the surface of the 3D fibrous scaffolds

and cells can penetrate in the thickness direction of scaffold. For these results, we hope that the 3D fibrous scaffolds have been a potential biomaterial for tissue engineering applications.

2.4 Electrospun fibers for energy scavenging applications

For energy scavenging applications, electrospun of PVDF and its copolymers nanofiber have a good property for fabricating piezoelectric devices, because of their have polar crystalline phases, α-phase, β-phase, and γ-phase. The β-phase have unidirectional dipole moment which represents piezoelectricity, where α-phase have random dipole moment which is a nonpolar molecule (Figure 2.15(a)). Parangusan et al. reported that PVDF-HFP nanofiber membrane, with good mechanical property, was used for portable electronic device, flexible sensors, and energy harvesting system.

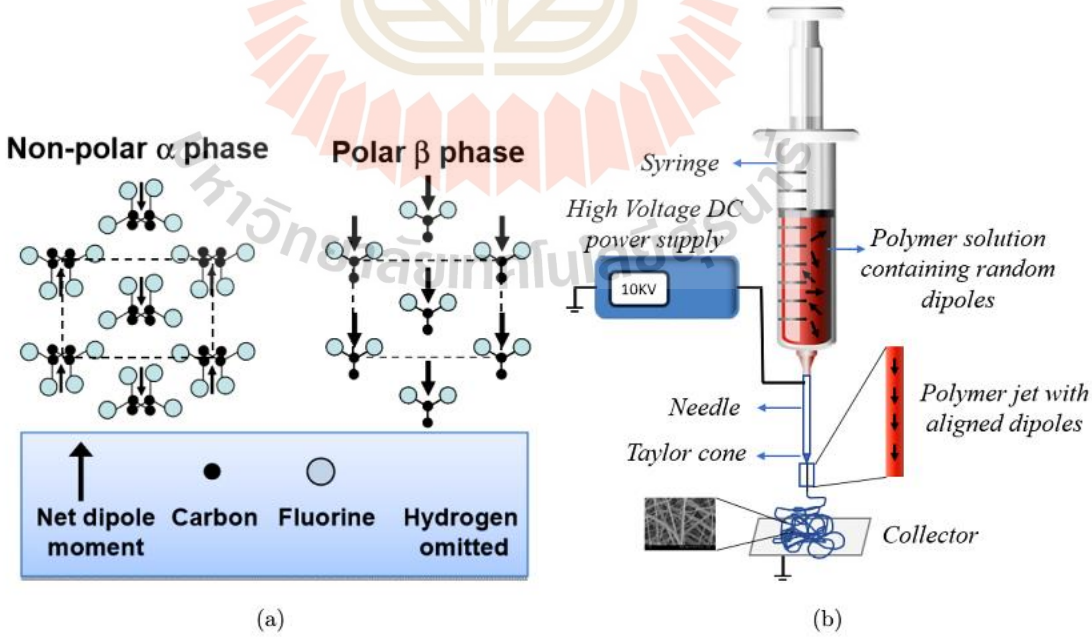

Figure 2.15 (a) Depiction of α and β phase crystalline within PVDF and (b) electrospinning setup (Tamil Selvan et al., 2019).

To produce piezoelectric nanofibers, electrospinning is one of the versatile and low-cost technique to produce self-poled piezoelectric nanofibers by high electric field from high voltage applied on solution jet (Figure 2.15(b)). For the idea to fabricate 3D nanofibers, Mandal et al. have demonstrated electrospun of PVDF nanogenerator that have piezoelectric properties. Furthermore, this research was shown the possible to connect of two electrospun nanofibers mat to either double or cancel their output voltage. They presented the output voltage of stacking electrospun nanofibers are depending on the polarity of these electrospun nanofibers (Figure 2.16). Therefore, with the result of this research, 3D nanofiber structure is possible to generate output voltage more than the 2D structure nanofibers by the 3D nanofibers have more staking fibers than the 2D structure.

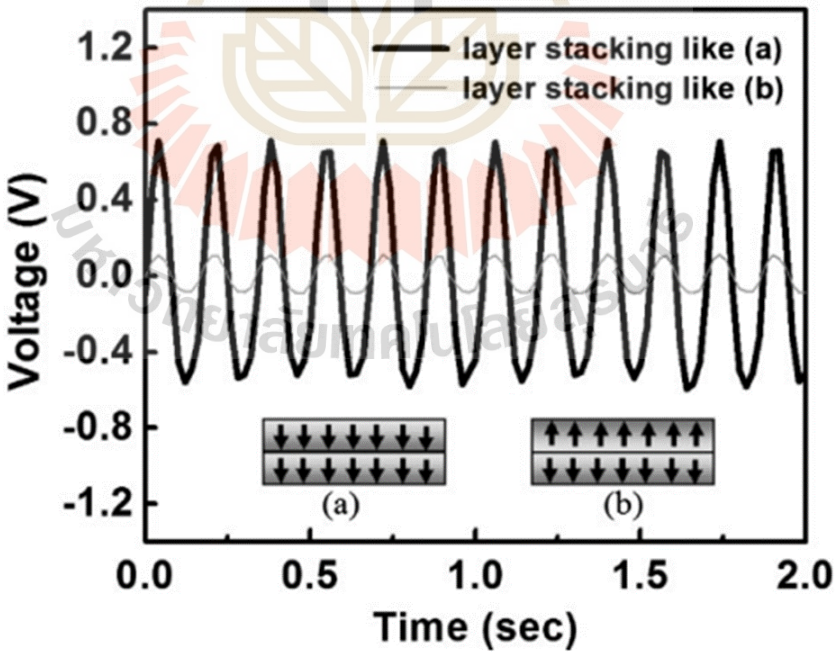

Figure 2.16 The output voltage of connecting two nanofiber mats (Mandal et al., 2012).

From the other research that was shown in this chapter, this work has a hypothesis consist of 4 parts that shows as

Hypothesis of this work

- Materials
    - PVDF-HFP
    - Solvent? (Acetone, Acetone/DMF)
    - Concentration? (10, 13, 15, 17, 20 %wt)
- Liquid-collecting electrospinning
    - Liquid? (Ethanol, DI-water)
    - Optimized electrospinning parameters? (Applied voltage, Distance)
- Biocompatible property (for tissue engineering application)
    - Surface modification? (Coated PVA, Hydrothermal, Plasma treatment)
    - *In vitro* cell culture? (NIH 3T3)
- Piezoelectric performance (for energy application)
    - 3D structure > 2D fiber mat?

CHAPTER III

METHODOLOGY

This chapter shows about the procedure of experiment in 2 sections: 1) Fabrication and surface modification of 3D nanofibers, and 2) *In vitro* cell culturing. The first section will show the process of fabrication 3D nanofibers by liquid-electrospinning technique, how to prepare polymer solution and electrospinning set-up and the process of surface modification of 3D PVDF-HFP nanofibers by 3 techniques. The last section will show the process of the *in vitro* cell culturing on the 3D fibers scaffold.

3.1 Fabrication of 3D nanofibers

Conventional electrospinning is difficult to fabricate nanofibers in 3D structure thus, in this work uses liquid bath as collector for electrospinning to fabricate 3D structure nanofibers. For liquid that use in collecting bath, this work use ethanol (from other work that shown in chapter II, and deionized water (DI water). PVDF-HFP is types of polymer that use in this work. Liquid-collecting electrospinning set-up show as Figure 3.1. In this work prepared the polymer solution (PVDF-HFP) by mixing with acetone (AC) and dimethylformamide (DMF) in weight ratio 1:1 for use to fabricate nanofibers with varies concentration of the solution as 10, 13, 15, 17, 20 %wt. For each case of liquid (Ethanol/DI- water) that use in collecting bath, we changed condition by varies parameter of electrospinning to study the occurrence of 3D nanofibers as follows.

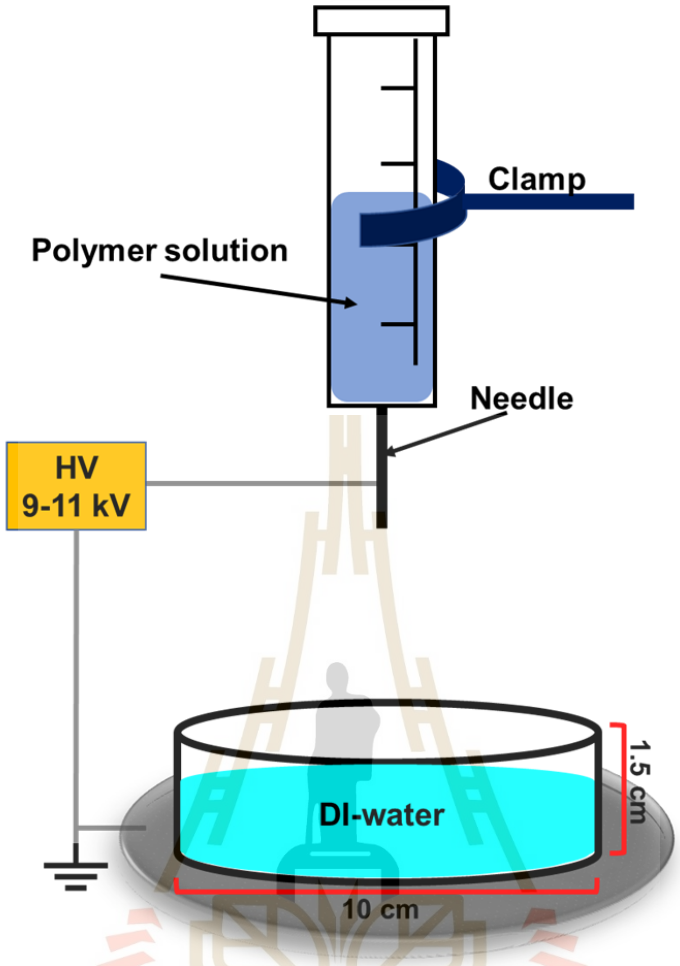

Figure 3.1 Schematic of the liquid-collecting electrospinning.

Liquid-collecting electrospinning setup and parameters

- Collector: glass petri dish, diameter = 10 cm, Height = 1.5 cm
- Fill 70 ml ethanol/DI-water in glass petri dish
- Distance between needle with measured from the water surface: 3, 5, 10 cm
- High voltage: 7 - 16 kV
- 10 mL syringe and 18G×1½ needle
- Flow rate without a syringe pump. Instead, gravity was used.

When 3D fibers can be fabricated, next step is finding solution to make 3D fibers scaffold able to absorb the solution which contains water as a component for coating collagen and can use to *in vitro* cell culturing process because PVDF-HFP is hydrophobic property polymer. In this work was modified surface of 3D nanofibers by 3 techniques as follows.

1.) Hydrothermal process at by soaked fibers in 10 %wt of PVA solution at 150 ℃ for 1-hour (Sheikh et al., 2016)
2.) Coated PVA by soaked 3D nanofibers in 12 %wt of PVA solution at 100 ℃ and wash in DI water at 80 ℃ for 3 hours
3.) Oxygen plasma treatment (D.M. Correia et al., 2015)

After finished each process, the 3D nanofibers will be measuring the contact angle of water drop on fibers to confirm the hydrophobic changed to hydrophilic property.

3.2 *In vitro* cell culture

Before *in vitro* cell culturing process, the 3D nanofibers scaffolds were sterilized under ultraviolet light for 30 minutes thereafter the scaffolds were soaked in 70% ethanol for 30 minutes and then rinse with phosphate-buffered saline (PBS) for 30 minutes, repeat 3 times and soaked in cell culture medium overnight to facilitate cell attachment on the 3D nanofibers scaffold. For coating collagen on PVDF-HFP 3D nanofibers scaffolds, collagen type A was dilute with PBS at ratio 1:10 (collagen:PBS) from collagen type A 0.5 mg/ml. Pre-wetting PVDF-HFP 3D electrospun scaffolds were soaked in 1/10 collagen/PBS for 1 hour. For cell culture process, Pre-wetted 3D electrospun scaffolds were place in 24-well plates, thereafter the NIH 3T3 cells were

seeded on each scaffold (pristine PVDF-HFP, PVDF-HFP coated collagen, and glass cover slip) at density of 1 x 10^4 cells/cm^2 and incubated at 37 ℃ with 5% humidified CO_2 for 3 days (Figure 3.3).

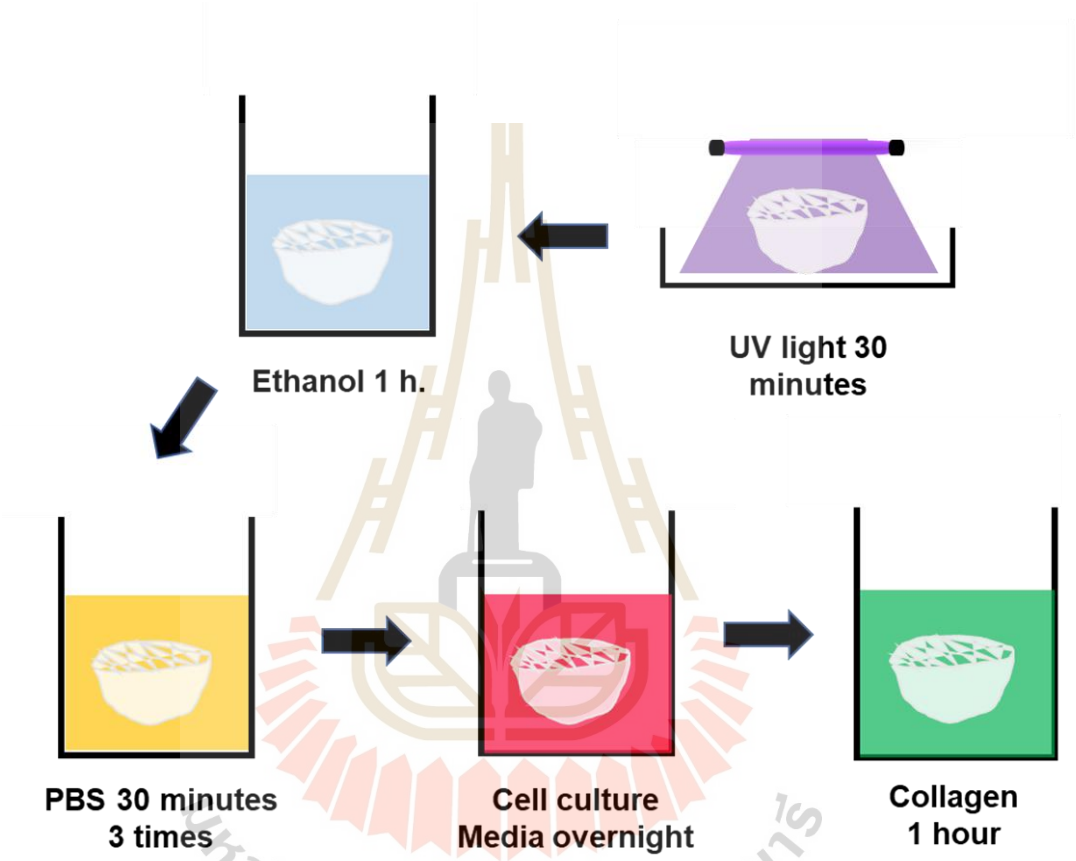

Figure 3.2 Preparation the 3D nanofibers scaffold for *in vitro* cell culturing process.

After 3 days of cell culture, the 3D electrospun scaffolds were wash with PBS for 10 minutes 2 times and then fixed cells on scaffold with 2.5% glutaraldehyde for 1 hour at room temperature after that wash the cells with PBS for 10 minute 2 times and dehydrated cells through a alcohol series by soaked in ethanol 50%, 70%, 80%, 90%, 95% respectively for 15 minute at 4 ℃ and 100% of ethanol for 15 minute at 4 ℃ for 2

times, finally critical point dried (CPD) to maintain the cell morphology. The fixed cells on 3D scaffold were sputter coated with gold by gold sputtering machine (JEOL Model JFC-1100E). Then morphologies of the 3D nanofibers and cells on the fibers were observed by the SEM machine (JEOL Model JSM-6010LV). The SEM images use to counting number of cells on the 3D nanofibers scaffold and measured diameter of fibers by imported SEM images into analysis software (ImageJ software) and the number of cells were counted manually.

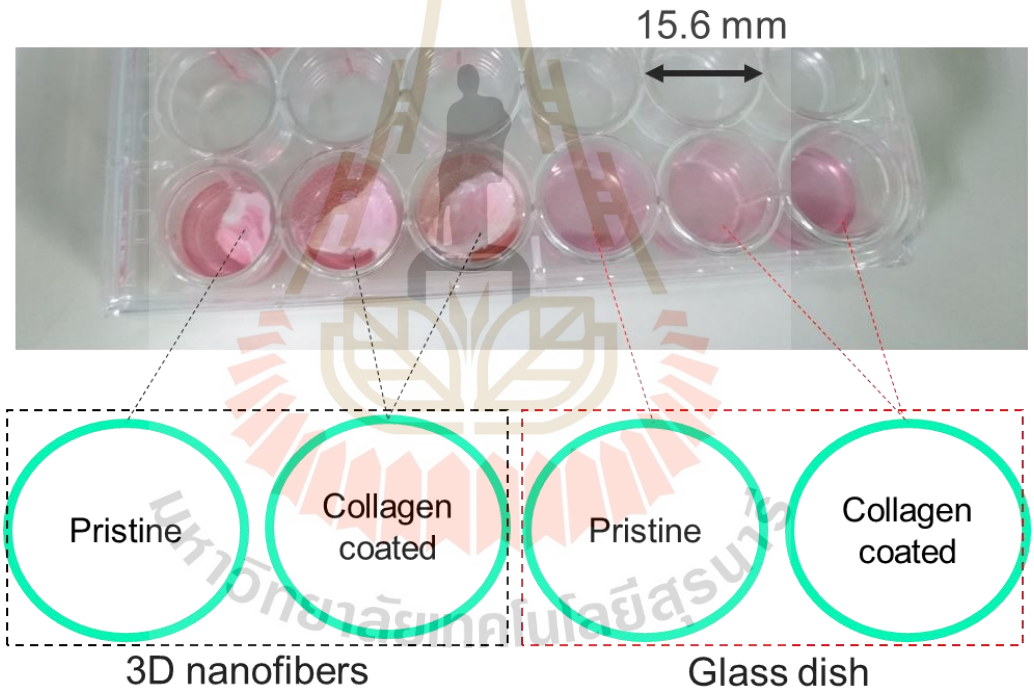

Figure 3.3 The 3D nanofibers scaffold and glass dish place in 24-well plates for *in vitro* cell culturing process.

CHAPTER IV

RESULTS AND DISCUSSION

This work contains the fabrications of 3D nanofibers scaffolds for tissue engineering. This chapter will show the result of the 3D nanofibers scaffolds that fabricated by using liquid-collecting electrospinning. There are five main results in this work:

- Result I: Effect of the liquid (Ethanol, DI-water),
- Result II: Effect of solution concentration varies by 10, 13, 15, 17, 20 %wt,
- Result III: Cross section of 3D nanofibers structure,
- Result IV: Surface modification of 3D PVDF-HFP fibers (from hydrophobic to hydrophilic property), this result showed the effect of surface modification by using three difference methods, method 1 Hydrothermal, method 2 Coated with PVA, and method 3 Plasma treatment,
- Result V: Cell adhesion on the 3D nanofiber. Using difference coating materials, the 3D scaffolds have difference efficiency for cell attaching and cell proliferation. This section will discuss and show the results of each coated scaffold.

4.1 Result I: Effect of the liquid-bath collector

For electrospinning process, the PVDF-HFP has to prepared in a solution form. The PVDF-HFP powders were dissolved in a mixing of DMF and AC (ratio 1:1) with

a concentration of 15 %wt. The liquid-bath collector was filled with ethanol and DI-water in order to compare the effect of liquid. The result of liquid types that effect to nanofibers fabrication with electrospinning technique will be presented in the following.

4.1.1 Liquid-bath collector with ethanol

In case of ethanol 99.5% was used for liquid collecting of electrospinning, the experiment setup with parameter: distance between needle with collector equal 5 cm, solution drop without syringe pump and high volt adjust due to polymer solution ejected without a drop of solution for this case used apply high voltage around 9-11 kV. When the fibers reach on the ethanol surface, fibers will sink into the ethanol but the fibers still floating in ethanol (Figure 4.1).

Figure 4.1 The PVDF-HFP fibers in ethanol bath.

The fibers after fabricated finish as showed in Figure 4.2, the fibers can fabricate in 3D structure, while the fibers wetted with ethanol, the fibers high around 1 cm and have diameter around 1 cm. The fibers are soft and unstable shape it can be retracted. When the fibers are dried, the fibers will shrink and collapse in a smaller size (Figure 4.2). The fibers are a harder and more stable structure, but the fibers have pore size that are not large enough for cell migration.

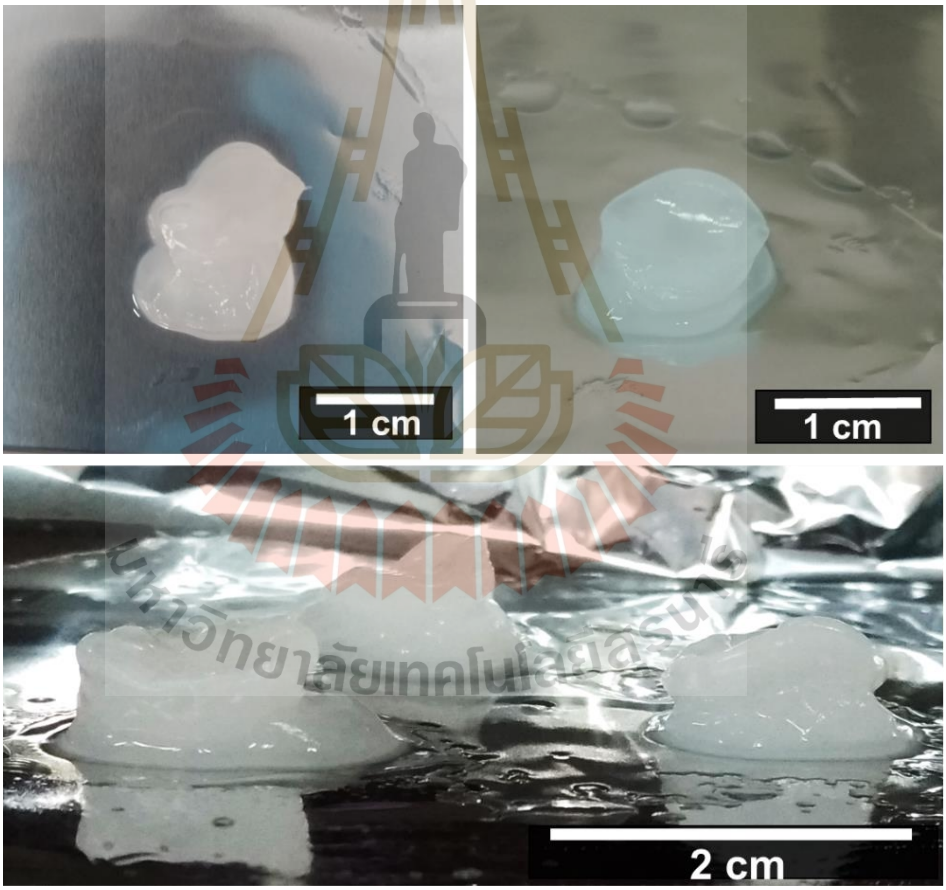

Figure 4.2 The PVDF-HFP fiber after the electrospinning process (distance between needle and collector of 5 cm).

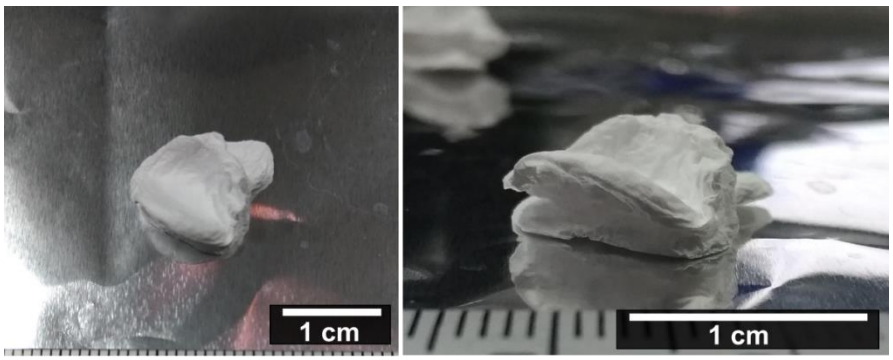

Figure 4.3 The PVDF-HFP fibers after dried at room temperature (distance between needle and collector of 5 cm).

For the case of ethanol bath with distance between needle and collector is 10 cm, the fibers have larger size than case of distance 5 cm. When the fibers dried, the fibers collapse in flat form like a 2D structure (electrospun mat) see in Figure 4.4, but this has large pore inside the fibers, see the cross section of this fiber in Figure 4.5, for this structure although it is 3D but we can fabricate this structure by wrap the 2D nanofiber, so this not objective of this work.

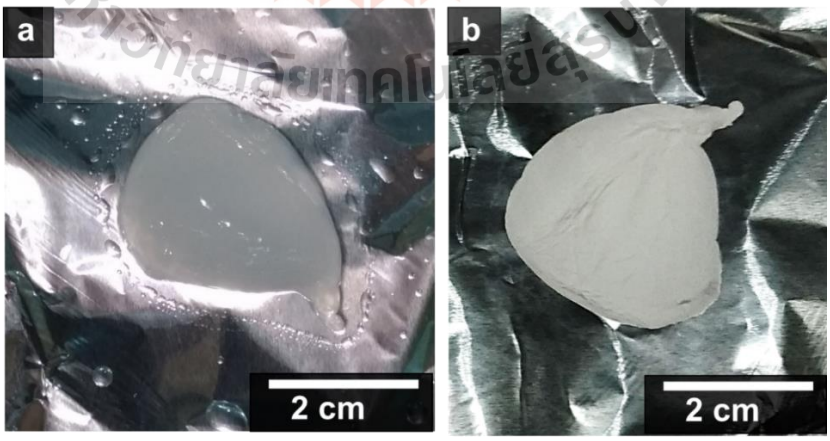

Figure 4.4 The PVDF-HFP fibers (a.) after electrospinning and (b.) dried in room temperature (distance between needle and collector of 10 cm).

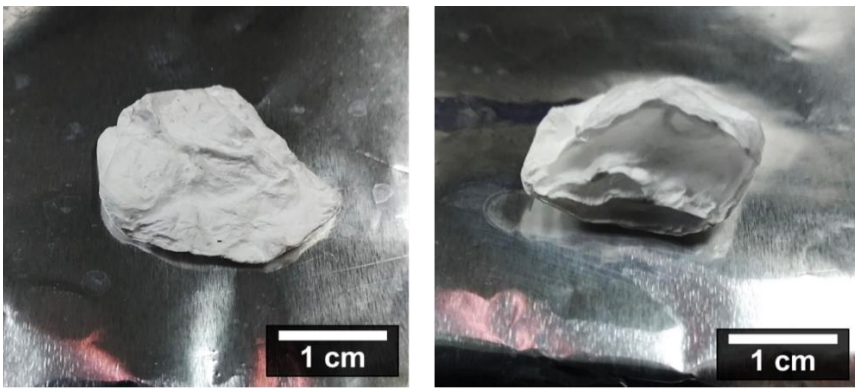

Figure 4.5 Cross section of the PVDF-HFP fibers after dried at room temperature (distance between needle and collector of 10 cm).

4.1.2 Liquid-bath collector with DI- water

When thinking about liquid, water is a basic liquid and important to our life. Thus, DI water was used in this experiment for fabrication of 3D PVDF-HFP nanofibers. Electrospinning process in this setup is the same as the previous experiment (4.1.1). For the case of DI-water bath, this work was fabricated with the same condition as the case of ethanol bath with 15 %wt solution concentration. Electrospinning with DI-water bath can fabricate 3D structure nanofibers as shown in below. In case of PVDF-HFP 15 %wt solution concentration with DI-water bath, during electrospinning, when fibers reach to the water surface, the fibers not dip into water because of PVDF-HFP is a hydrophobic polymer and water has surface tension, which is a force that resists fibers not to be dipped in water. Therefore, the fibers were collected as a sheet floating on the water surface at the beginning of electrospinning process (Figure 3(a)). After the fibers can be collected as an object, floating on the water surface. As the fibers stack layer-by-layer on the water surface, the weight of fibers on the water surface will

steadily increasing, and at one point the force by the fiber structure weight overcomes the surface tension of water then the structure is gradually pushed into the water (Figure 3(b)). This way, it is possible to form fibers as 3D structures (see Figure 3(c, d, e)).

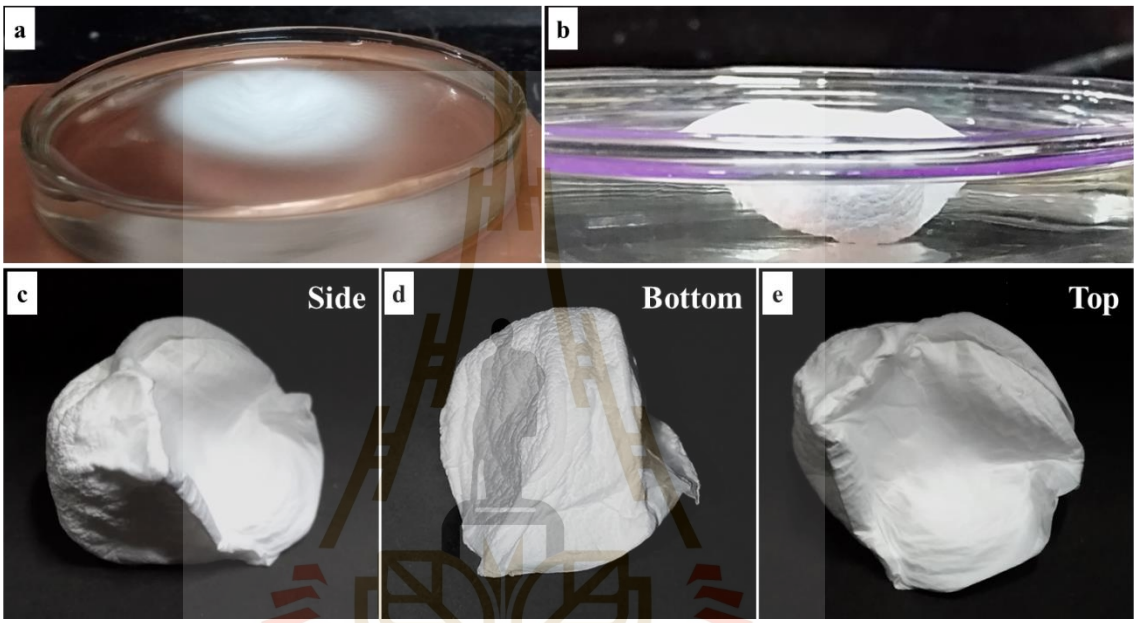

Figure 4.6 The PVDF-HFP fibers (a) floated on the water and slightly collapsed, (b) were pulled down into the water by the weight of stacking fibers, (c), (d) and (e) show the 3D nanofibers scaffold from each side.

The fibers can keep the shape, these have stable structure and flexible, the fibers can recover the shape when the fibers are changed its shape by pressing or squeezing. The height and width of this fiber is around 1.5 x 1.5 cm and the structure is float on water. The previous result this technique can be fabricated 3D structure nanofibers so, for more to study about the fabrication of this fiber structure, the electrospinning

parameters will change like a case of ethanol bath, the changed parameter is distance between needle with collector from 5 cm changed to 10 cm, the result shown in Figure 4.7. The fibers have spread more than case of 5 cm, when the polymer solution ejected from the tip of needle, therefore the fibers are stick at the edge of glass dish and cover the surface of water, thus the fibers can't dip into the water cause the fibers unable to fabricate the 3D structure.

Figure 4.7 Fibers cover on the water surface at distance between needle with collector of 10 cm.

4.2 Result II: Effect of solution concentrations

Furthermore, this work study about the parameters that effect to produce the 3D structure nanofibers, that is concentration of PVDF-HFP solution. Thus, this work was

varied solution concentration for 10, 13, 17, 20 %wt to study the effect of concentration to fabricate the 3D structure of nanofibers. The first result of the effect of solution concentration will show in case of 10 %wt, the result was shown that the fibers can't produce to 3D structure, the deposited fibers spread with a large area on the water surface and the fibers not sink into the water. Figure 4.8 show that the fibers have average diameter side around 441 ± 18 nm and this fiber have more beads inside, maybe because of the existence of the beads inside the fibers to make the fiber floated on the water surface by the structure of the beads these look like a sphere, so the beads can increase the area that against the force from water surface tension like a buoy on the water. However, this concentration can't produce the 3D structure of PVDF-HFP nanofibers.

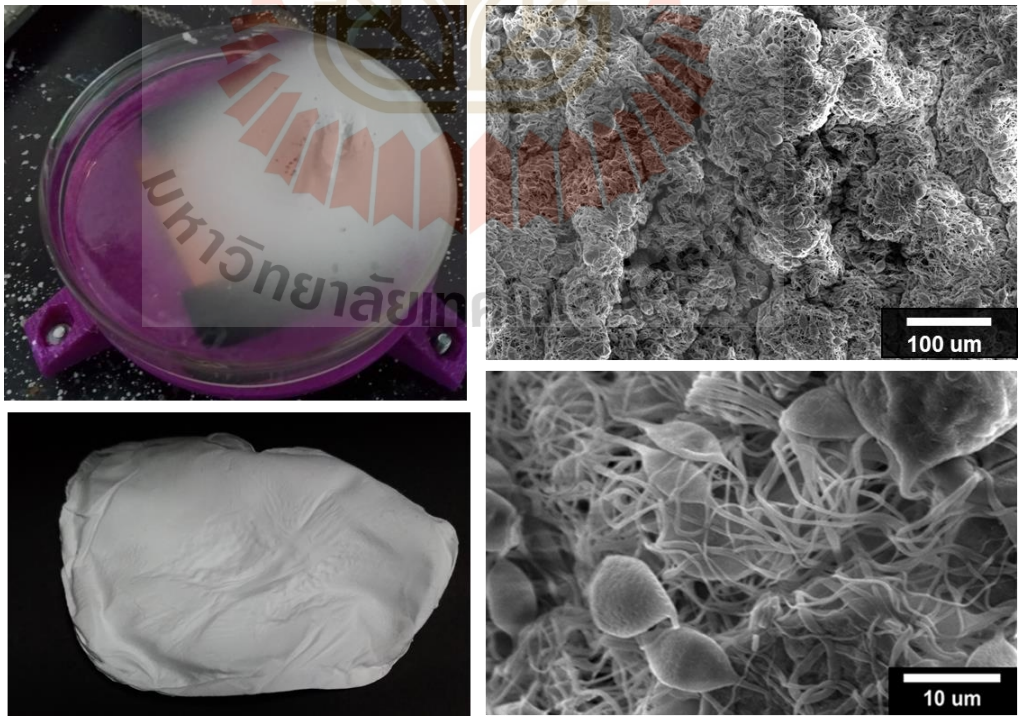

Figure 4.8 Optical and SEM images of PVDF-HFP dissolved at 10 %wt.

In case of increasing concentration up to 13% wt of PVDF-HFP solution, this concentration is still cannot produce the 3D structure. The fibers can a little bit collapse into the water at a center of fibers sheet. But, it's not enough to produce a 3D structure. The fibers that produce by 13 %wt concentration still have beads inside but lower than the fibers that produce by 10 %wt concentration and have similar average diameter size 451 ± 16 nm see in Figure 4.9. Two case of low concentration 10 and 13 %wt of PVDF-HFP solution are have beads inside and can't produce the 3D structure nanofiber so, maybe we can conclude that if the fibers have beaded, the fiber can't produce a 3D structure with this method.

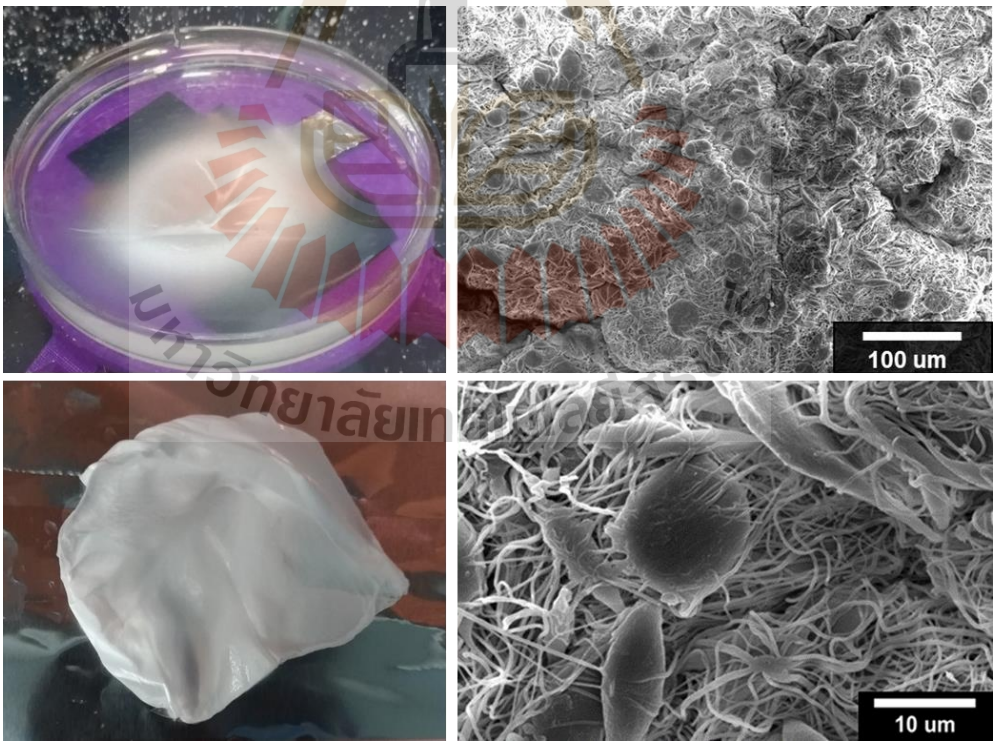

Figure 4.9 Optical and SEM images of PVDF-HFP dissolved at 13 %wt.

In case of 15 %wt solution concentration, this work has successes to produce 3D nanofiber structure that showed in result 1, the fibers that produced with this concentration can form to 3D structure in the DI-water bath. The SEM images of this fiber show that the fibers of this concentration don't have a bead inside the structure and these fibers have average diameter size of 802 ± 23 nm (Figure 4.10). For the process of the mechanism of liquid-electrospinning to produce 3D nanofibers structure and the cross-section of this fiber will show in the next result (result 3).

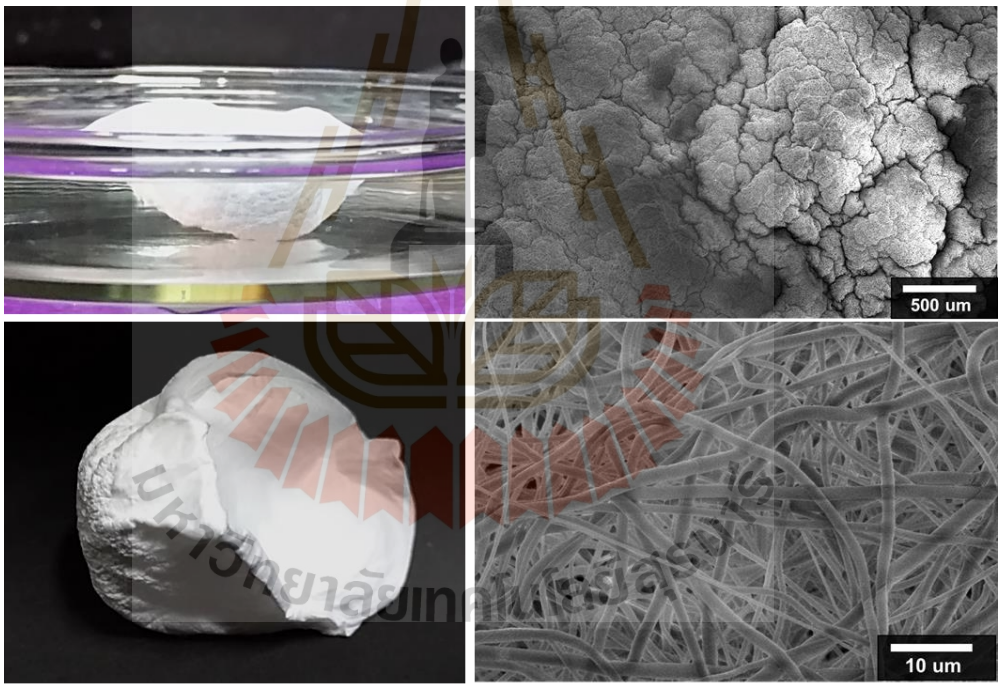

Figure 4.10 Optical and SEM images of PVDF-HFP dissolved at 15 %wt.

In this work, the 3D structure nanofibers can produce with 15 %wt concentration and can't produce with a lower concentration than this condition. Therefore, this work was studied if the concentration is higher than 15% wt. How will

this be able to create 3D structures? Frist start with concentration of 17 %wt , the fibers can be produce in to a 3D structure like a case of 15 %wt (Figure 4.11), the external structure is not different but if we look at the cross-section of this fiber, it maybe difference from the case of 15 %wt, the result will show in next chapter. And likewise, if the concentration of the polymer solution increases, the average diameter of these fibers will increase. So, these fibers have lager diameter than the 15 %wt 3D fibers (these fibers have average diameter = 1256 ± 43 nm).

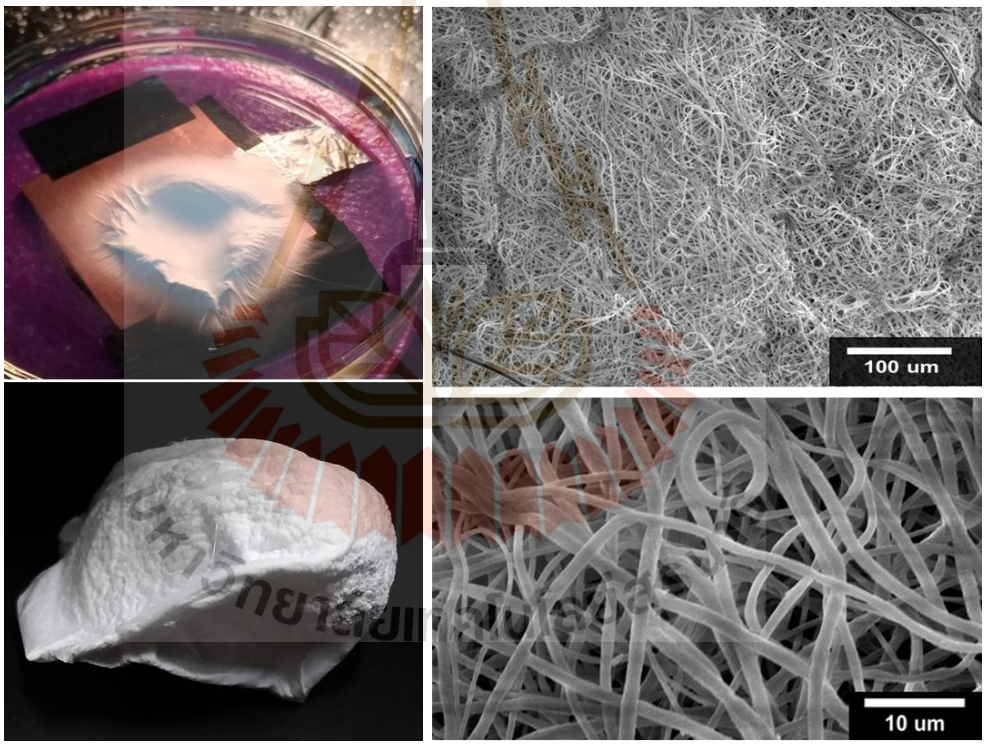

Figure 4.11 Optical and SEM images of PVDF-HFP dissolved at 17 %wt.

For increasing concentration this work have one more condition, 20 %wt, this concentration can't be producing the 3D nanofibers structure (Figure 4.12). these fibers have lager area than case of 15 and 17 %wt. During the electrospinning process, the

fibers can collapse into the water but it did not stack the layers of fibers, the fibers will packing to one sheet. The average diameter of these fibers are similar to the case of 17 %wt. Even though fibers have same diameter size but, in this case, why cannot produce the 3D structure. To explain this, we notice that from the electrospinning process, the solution with

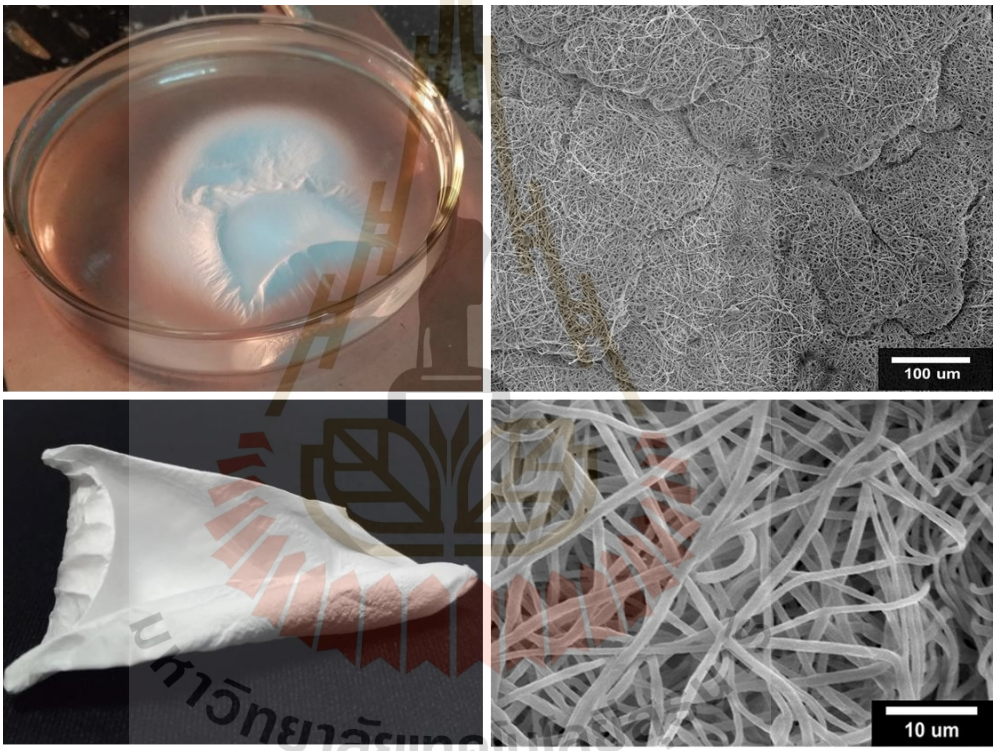

Figure 4.12 Optical and SEM images of PVDF-HFP dissolved at 20 %wt.

higher concentration will have higher viscosity so, the flow rate of deposited solution is slower than a lower solution concentration and this electrospinning set-up for this condition is using lower applied voltage than the case of 15 and 17 %wt for proper condition of electrospinning. With the lower flow rate and lower applied voltage (low

electric force) therefore, the whipping jet that happens during the electrospinning process is having a low area of spinning and the fibers that stack like a sheet on the water surface slowly collapse into the water. Therefore, such as 15% wt process does not occur in this 20 %wt condition.

From the result of varies concentration to fabricated 3D structure of nanofibers. We can find that, for this method, with the solution concentration are lower than 15 %wt the beads occur in fibers structure and the 3D structure is cannot produce. And when the concentration of the solution is higher than 17 %wt the 3D structure of nanofiber is also can't produce. For the fabrication of 3D nanofibers structure of this technique, the concentration of the solution is a key parameter to produce the 3D structure.

4.3 Result III: Cross section of 3D nanofibers structure

The cross- section of the fibers show that the fibers have many pores inside, we can see the structure inside this fiber in cross-section images from Figure 4.13. The structure of this fiber is look like the termite nest in nature (Figure 4.13(d)). Explanation of the construction of these 3D fiber structures starts from the fibers reach to water surface and stack the fibers sheet layer by layer until the of fibers sheet increase more than the surface tension of water, then the fibers are pulled into the water, thus making the fibers have curves like half sphere. The fibers that ejected later will stick at the edge of one side to the other side of curvature of the fibers sheet, therefore the fibers were a gap in the structure and when this process continues, the structure like a termite nest will be fabricated (Figure 4.14).

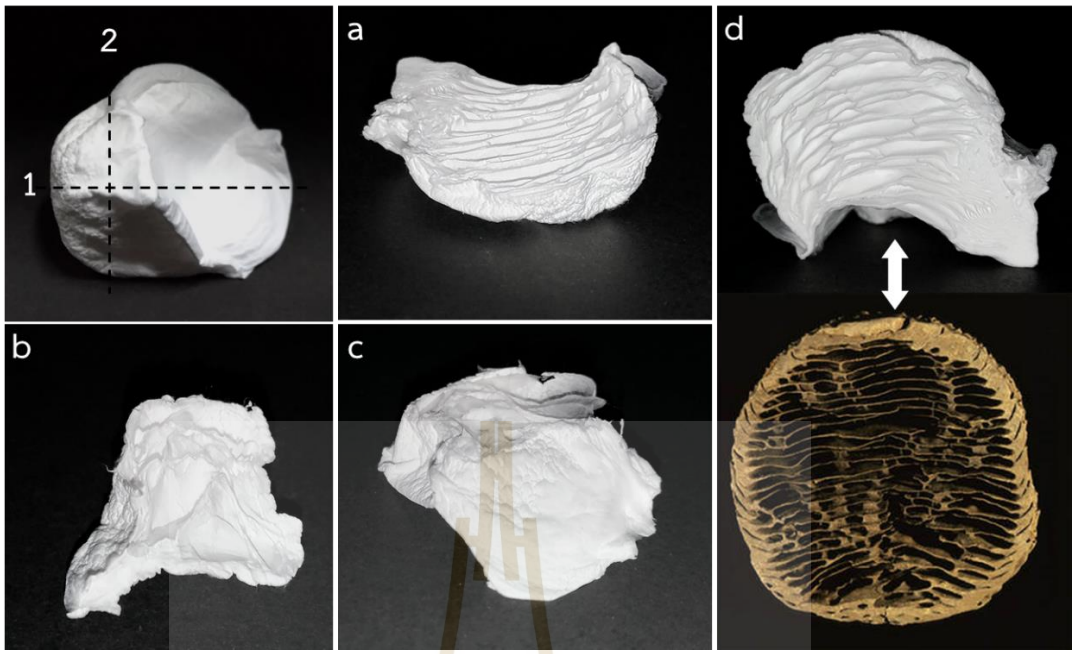

Figure 4.13 Cross-section of 3D nanofibers scaffold cut through (a) line number 1, (b) and (c) line number 2, (d) compared with a termite (Apicotermes) nest. (Mermet G., 2019 from http://www.savoirs.essonne.fr).

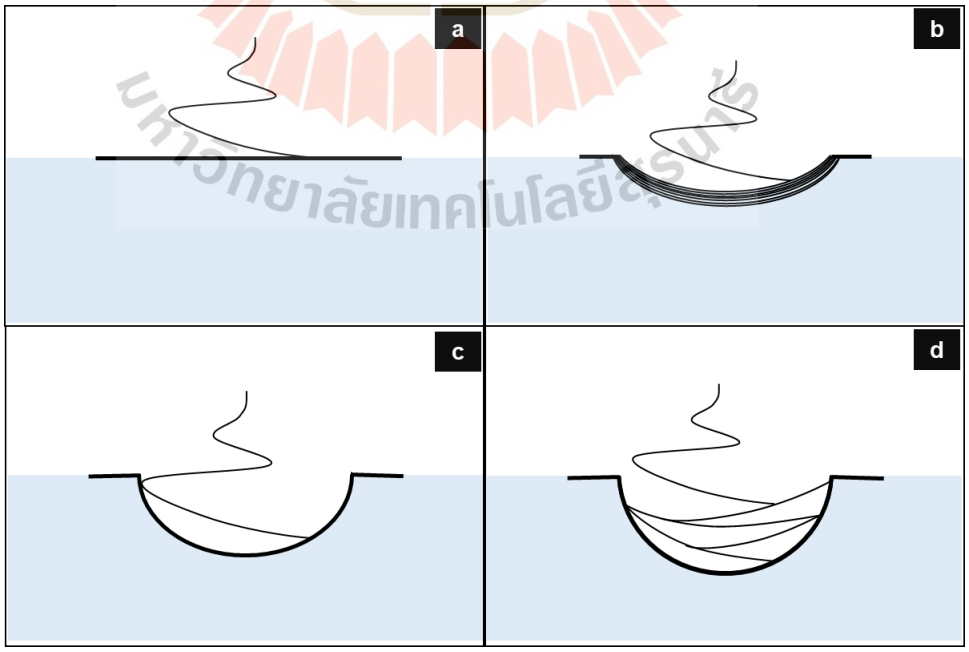

Figure 4.14 Schematic of the 3D nanofibers structure formation.

For the cross-section of 3D nanofibers that produce by 17 %wt of PVDF-HFP solution concentration, the fibers structure has a larger pore inside the structure than case of 15 %wt 3D fibers and the pores have a longer length than it too (Figure 4.15).

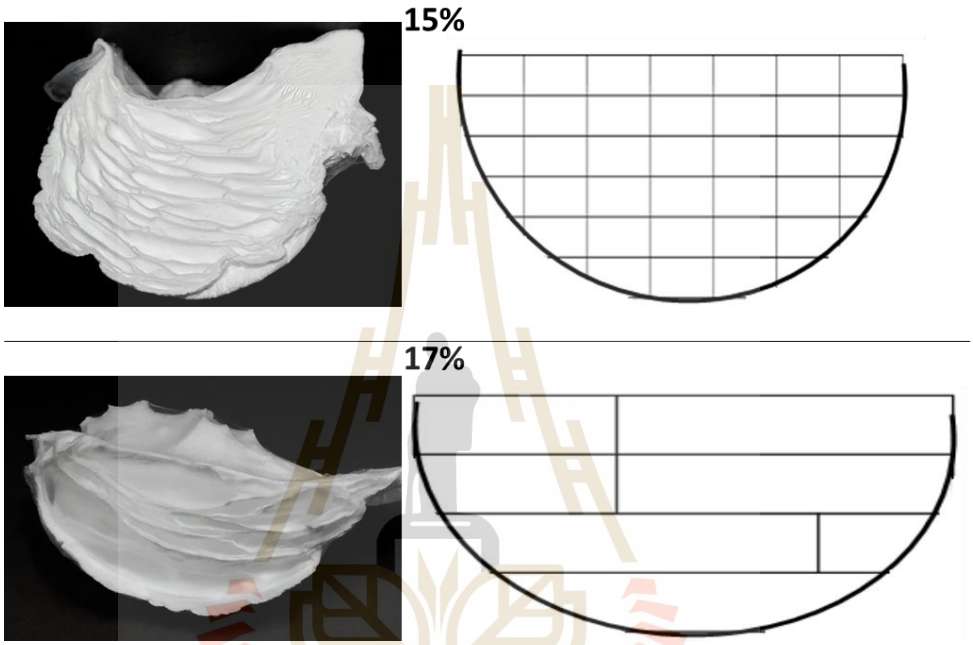

Figure 4.15 Comparing of 3D structures between the PVDF-HFP dissolved at 15 %wt and 17 %wt, with their illustrated cross-section structures.

4.4 Result IV: Surface modification of the 3D nanofibers

In this work, the 3D nanofibers will be testing the efficiency of medical applications by *in vitro* cell culturing. For the *in vitro* cell culturing process is necessary to let the fibers scaffold sink into the cell culture media but this produced the 3D nanofibers scaffold from PVDF-HFP which this polymer have a hydrophobic property, this property will make the fibers cannot sink into the cell culture media. So, this work

was studied to modify the property of these fibers before using it for the *in vitro* cell culturing process.

4.4.1 Method 1: Hydrothermal

the first method that used in this work to a modified surface of the 3D nanofibers scaffold is a hydrothermal method. Follow the condition of Sheikh et al. research, the 3D nanofibers putted in the hydrothermal reactor with 10 %wt of PVA solution at 150 ℃ for 1-hour. The result of this method shown in Figure 4.16. The 3D nanofiber after finishing the hydrothermal process, the fiber was shrinking to a smaller size also, the pore size of this 3D fiber was shrinking to small size. The 3D fiber changed color and have the hardness than pristine 3D fiber because the 3D fiber was coated with the PVA. However, this 3D fiber after modified by this method cannot use for *in vitro* cell culturing.

Figure 4.16 Hydrothermal reactor and 3D nanofibers after treated by hydrothermal method.

4.4.2 Method 2: PVA coating

When the modified 3D nanofiber is not good for this work. We will try to use another method but still using PVA to coat the fibers. 12 %wt of PVA solution that dissolve in DI-water are used to coat the 3D nanofibers by soak the 3D nanofibers in PVA solution at room temperature for 1 hour. After soaking process this fiber was washed with DI-water by stirring for 3 hrs. Figure 4.16 show that the 3D fibers can absorb the water after finishing this process. Therefore, this 3D scaffold was used to *in vitro* cell culturing for evaluate the efficiency of medical applications.

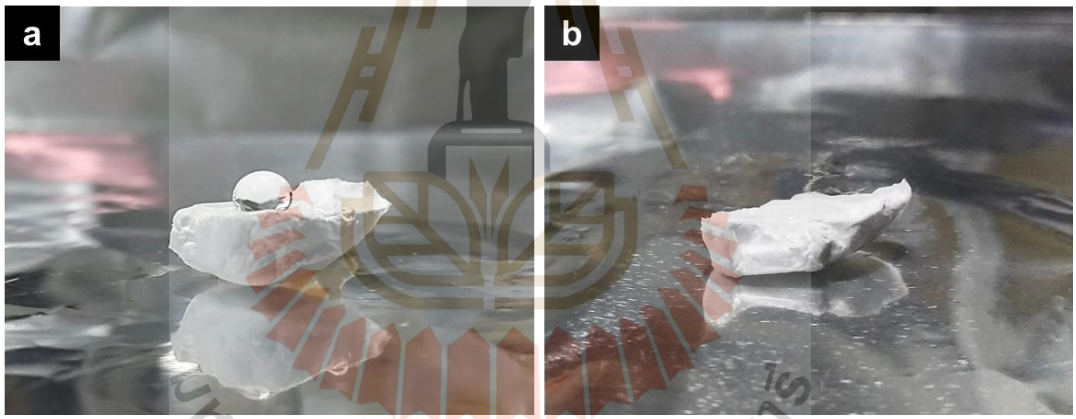

Figure 4.17 A water drop on 3D nanofibers, (a) before, and (b) after coated with PVA.

4.4.3 Method 3: Plasma treatment

One more method that used to modify the 3D nanofiber scaffold, this method is plasma treatment. The plasma of Oxygen is changing the hydrophobic property of PVDF to hydrophilic property by destroyed C-F or C-H bond in PVDF molecule and then, when the molecule interacted with oxygen in atmospheric, the hydroperoxides

group will bond in this molecule, the hydroperoxides are hydrophilic group. Therefore, this plasm treatment can be made the 3D fibers scaffold to absorb the water like a case of coating with PVA that showed in previous result. The morphology of the fibers after plasma treatment remains the same (Figure 4.18). The 3D nanofibers scaffold that modified by using oxygen plasma is able to use for tissue engineering.

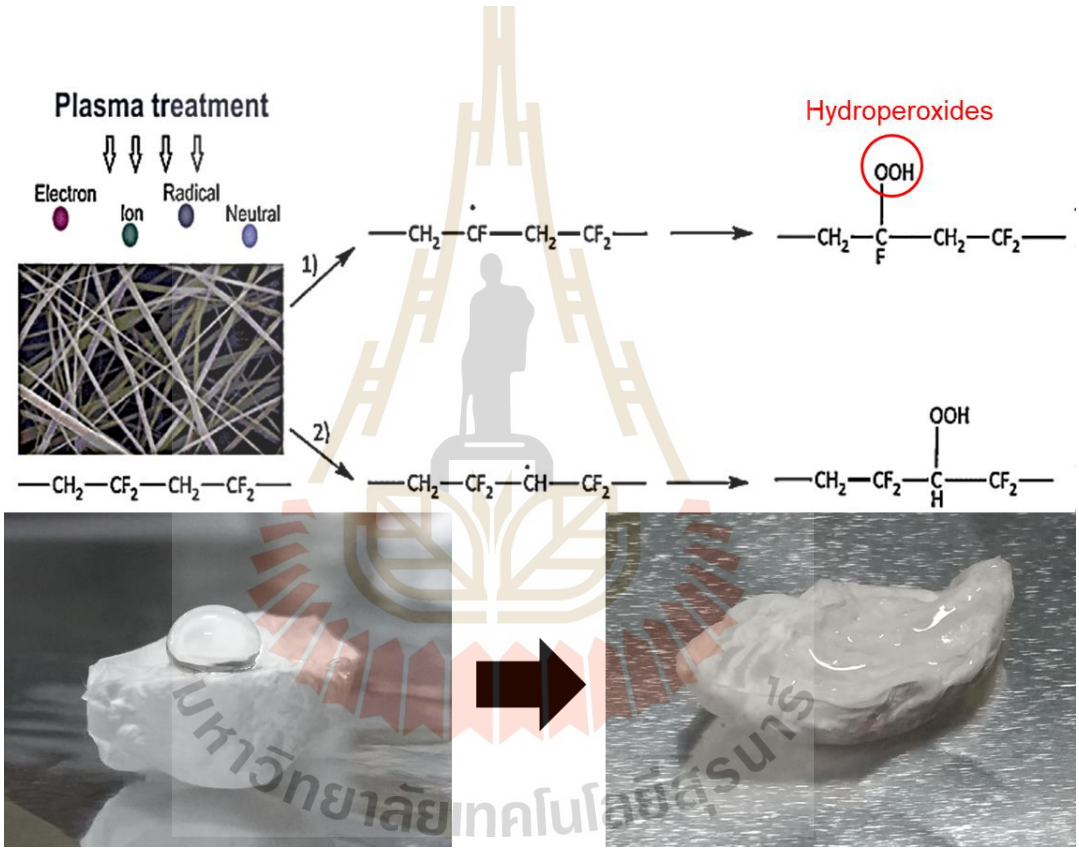

Figure 4.18 The process of oxygen plasma changed the molecule of PVDF to bond the hydroperoxides group and 3D nanofibers before and after plasma treatment.

4.5 Result V: Cell adhesion on the 3D nanofibers

4.5.1 3D nanofibers modified by PVA coating

The 3D nanofibers scaffold that modified by using PVA coating is able to use for tissue engineering. Figure 4.19 shows SEM images the morphology of NIH3T3 cells after 3-day *in vitro* culturing to observe interactions between the cells and the 3D nanofibers scaffold. The images show that the coating of PVA on these fibers are cover the fibers like a film on fibers surface, it is change the morphology of the fibers surface and the coating of PVA did not homogenous, some region of the 3D scaffold is don't have the film of PVA but some region have a film of PVA. However, the images show that these cells can attached on both region of fibers, film of PVA and PVDF-HFP nanofiber but the number of cells that can attached on this scaffold are quite low.

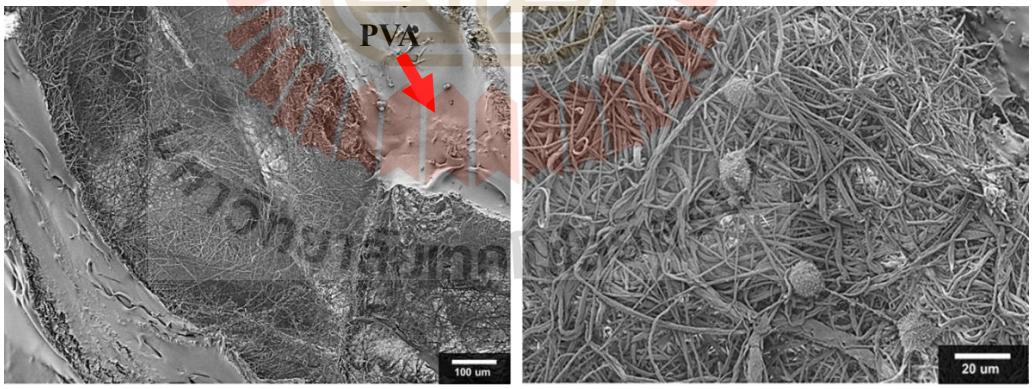

Figure 4.19 SEM images of cells on 3D nanofiber scaffold coated with PVA.

For increase efficiency of cell adhesion on 3D nanofibers scaffold, the collagen was used to coat on the 3D scaffold that mentioned in chapter 3. The result of collagen coating on the 3D fibers scaffold as show in Figure 4.20 the collagen was not dissolved,

the collagen still forming in fibers structure and these stuck and cover on the fibers surface. The number of cells on this scaffold is not significant difference from the pristine scaffold, it's quite low. We clearly to see the PVA coating of this method is not good for cell adhesion, the PVA was not coated following the structure of fibers, it was covering the fibers and changed morphology of fibers surface. therefore, this scaffold has to improve surface modification in the next step.

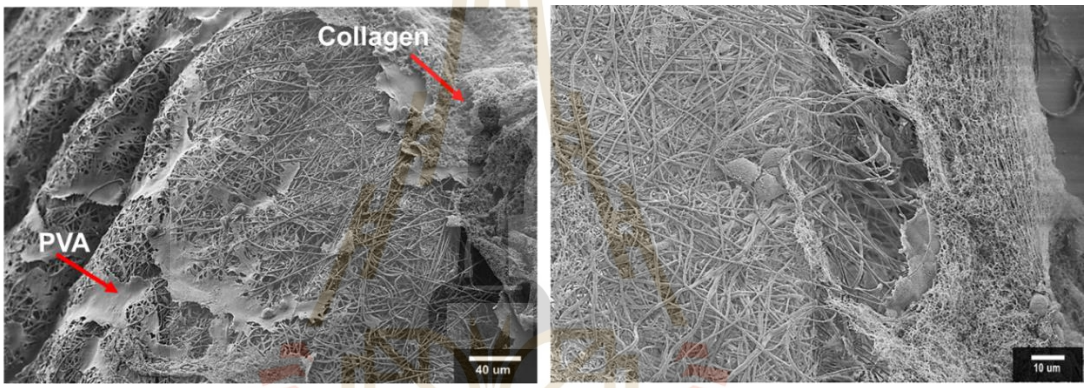

Figure 4.20 SEM images of cells on 3D nanofiber scaffold coated with PVA and collagen.

The previous result showed the 3D fiber scaffold is not good for cells adhesion because the coating of PVA is cover the fiber surface and the fibers morphology. Therefore, this work tries to improve the coating of PVA to eliminate the PVA film on the fibers surface by increase the washing time and speed of stirring water after the soaking process of coating PVA. The fibers after improved surface modification, it can be eliminating the PVA film on surface of fibers. The fibers morphology can still be preserved, see in Figure 4.21a. Even though the fibers still maintained the morphology,

but the cells cannot attach on the fibers. The reason why this scaffold is not good for cell adhesion it may cause the nanoparticle of PVA on each fibers surface, these particles made the fibers have roughly surface. The nanoparticle of PVA looks like a dust on the surface. It is decreasing the adhesion of cell on fibers. Therefore, the cells cannot attach on this scaffold. Same as the fibers that coated with collagen, the result is similar to the previous result, the fibers of collagen still stack like a sheet cover on the fibers surface (see Figure 4.21(c) and (d)).

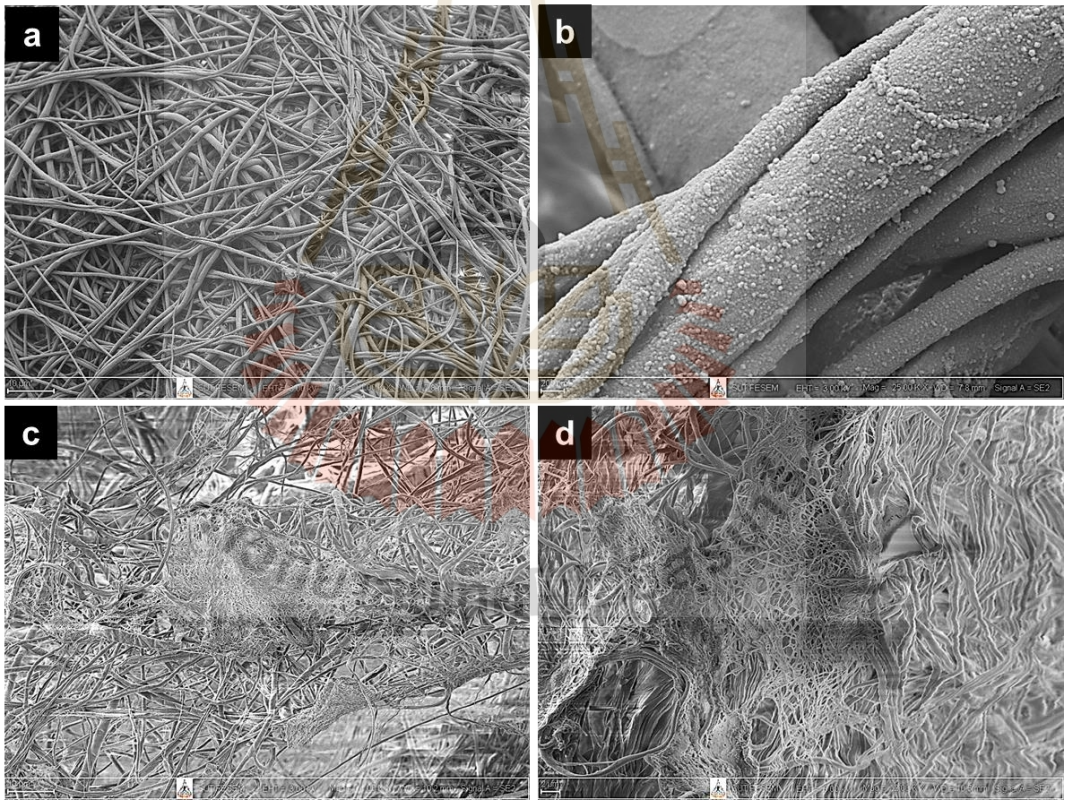

Figure 4.21 SEM images of (a) 3D nanofiber scaffold coated with PVA, (b) PVA nanoparticle on PVDF-HFP fibers, (c) and (d) collagen fibers on 3D nanofibers scaffold.

4.5.2 3D nanofibers modified by plasma treatment

The morphology of the fibers after plasma treatment remains the same. The 3D nanofibers scaffold that modified by using oxygen plasma is able to use for tissue engineering. Figure 4.22 shows SEM images the morphology of NIH3T3 cells after 1-day *in vitro* culturing to observe interactions between the cells and the 3D nanofibers scaffold.

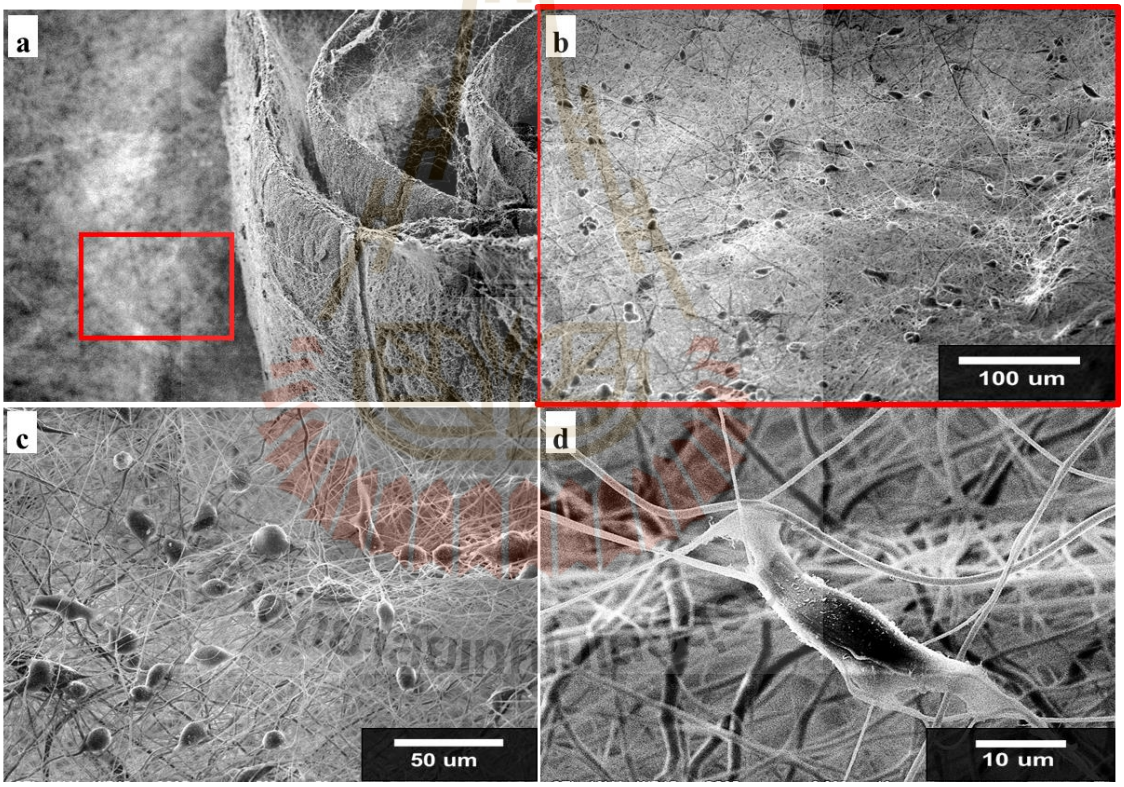

Figure 4.22 SEM images of cells attach on 3D nanofibers scaffold (a) and (b), the cell can attach on floating fibers of the scaffold (c).

The images show that these cells can spread and attached on fibers inside the pore regions of the scaffolds more than case of the fibers modified by coated PVA. For this scaffold structure, some regions have fibers that don't stick to sheets with other fibers, but the cells can attach and proliferated on these fibers (Figure 4.22(d)). The 3D nanofibers scaffold that has a structure like a termite nest can support cell adhesion and have the ability for medical application.

4.6 Piezoelectricity of the PVDF-HFP nanofibers

From the PVDF-HFP have piezoelectric property so, in this work will testing the piezoelectric property when the PVDF-HFP in the form of 3D structure by compare with the 2D PVDF-HFP nanofibers (flat nanofiber). The PVDF HFP 2D mats were cut and assembled in a generator as shown in Figure 4.23(a). The area of the fiber mat used as the core for the generator was 12 cm^2 (W = 2 cm, L = 6 cm). Copper wire was attached to each electrode and the generator was then coated in a flexible silicone rubber. The 3D PVDF-HFP fibers were cut with a surgical blade into structures that had a roughly a 1 cm^3 volume and connected with copper sheet electrode. Then, these were placed within a mold that was filled up with silicone rubber. Samples were adjusted so that the PVDF fibers would not sink to the bottom. The Figure 4.23(b) and (c) show an example of how this was done. The generator was connected with various resistive loads. This test involved dropping a 100g weight onto the generator from a height of 5 cm and recording the voltage and current responses. All data captured has been done with a Rohde & Schwarz digital oscilloscope (RTB2004).

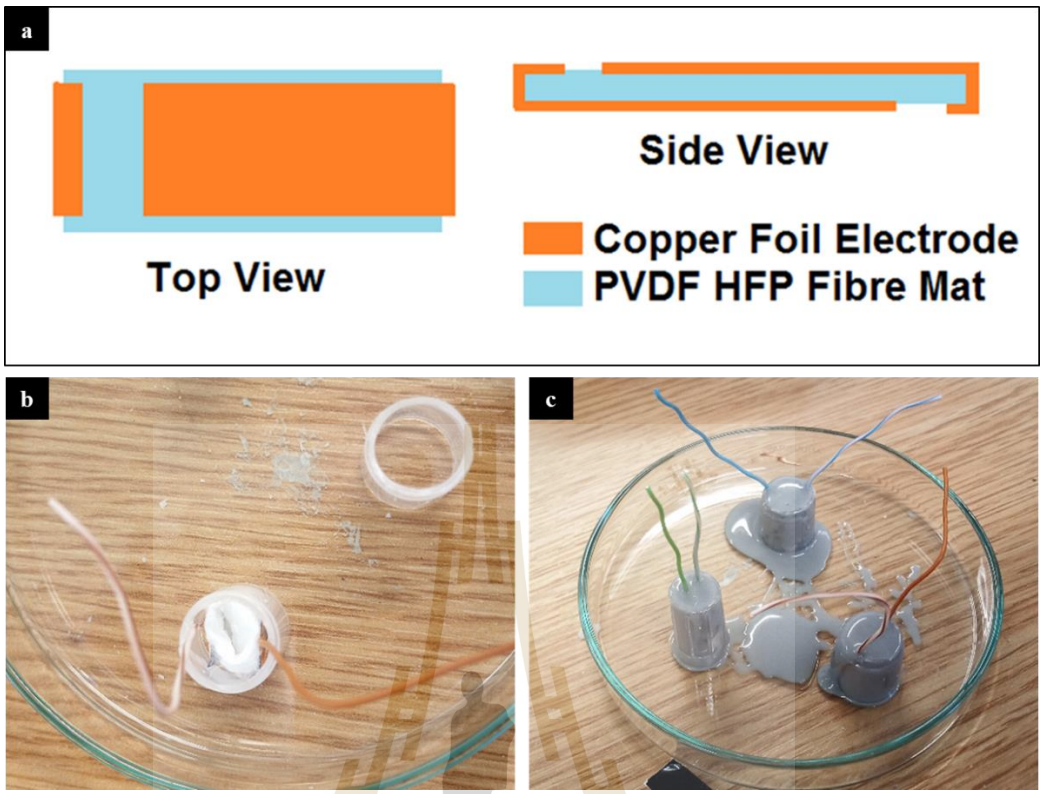

Figure 4.23 Preparation of the 3D PVDF-HFP fibers sample for piezoelectric testing.

4.6.1 Voltage and current output of the 2D nanofibers mat

The generator was connected in series with a variable resistive load that ranged from 1.5 MΩ to 10.5 MΩ in 1.5 MΩ increments. A trans-impedance amplifier was used for conditioning the current signal obtained from the trials. This method involved dropping a 100 g weight onto the generator from a height of 5cm and recording the voltage and current responses. The recorded responses of 1.5 MΩ are shown in Figure 4.24. For the result recording the voltage and current responses of another resistive load are not show, these have the result look like a 1.5 MΩ load but have a different value of the peak for each resistive load. All maximum and minimum output voltage and current of 2D flat nanofibers are shown as a graph in Figure 4.25.

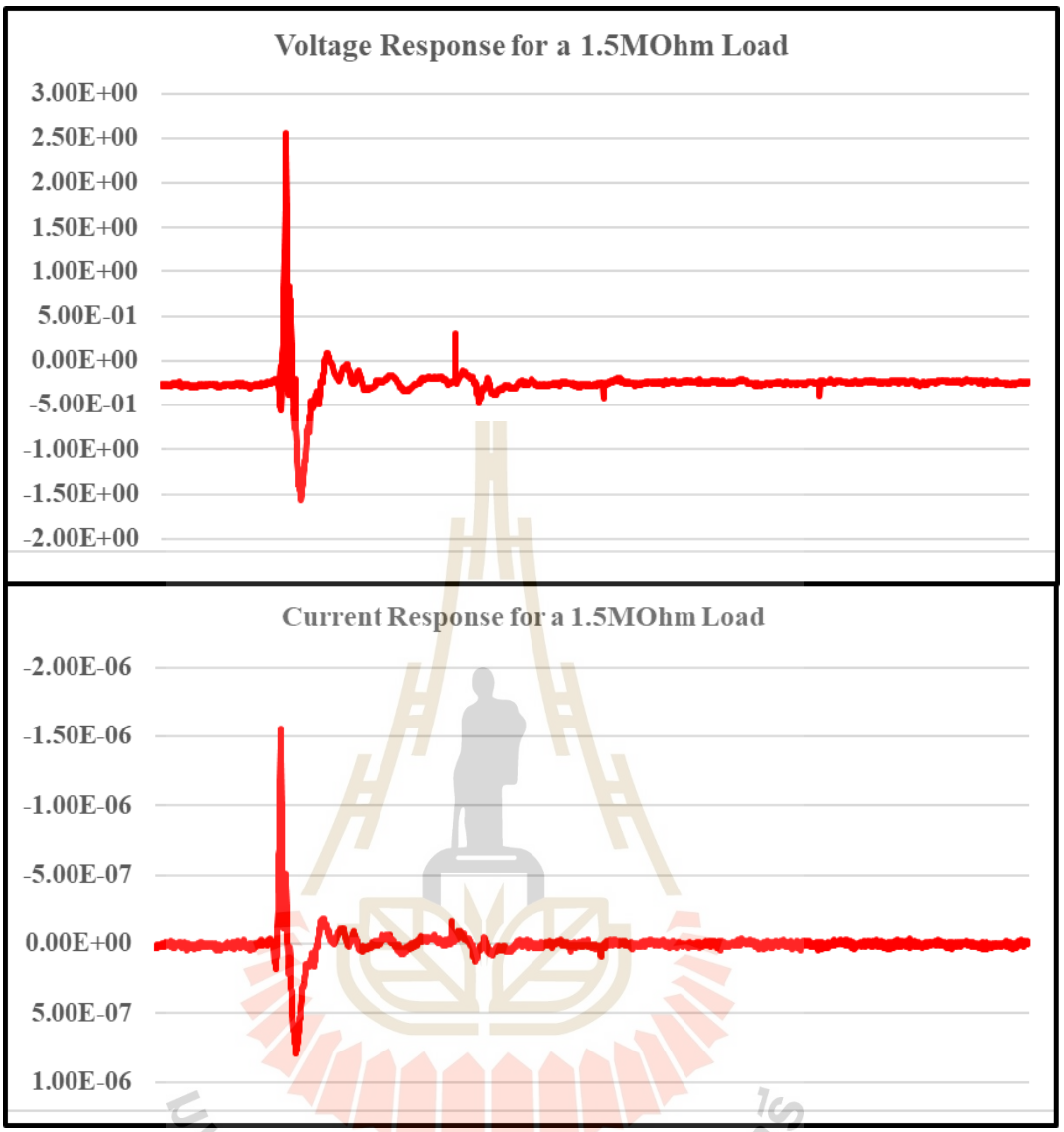

Figure 4.24 Output voltage and current of the 2D flat nanofibers generator with 1.5 MΩ resistive load.

4.6.2 Voltage and current output of the 3D nanofibers structure

With the nanofiber being 3D structure, as for the electrodes, 3 different methods for attaching electrodes to 3D material were tested in a preliminary study to identify the most suitable one to use. The tested setups consisted of using 0.1 mm copper tape with

an adhesive coating on one side as the basic electrode material. In case that the adhesive coating was not optimal to bond the fibers to the electrode, an additional layer of sputtered gold (12 and 15 nm) or conductive silver paint between the copper sheet and the sample was deposited. The Figure 4.26 show the response recorded when the samples were struck with a free-falling 100 g weight dropped from a height of 5 cm.

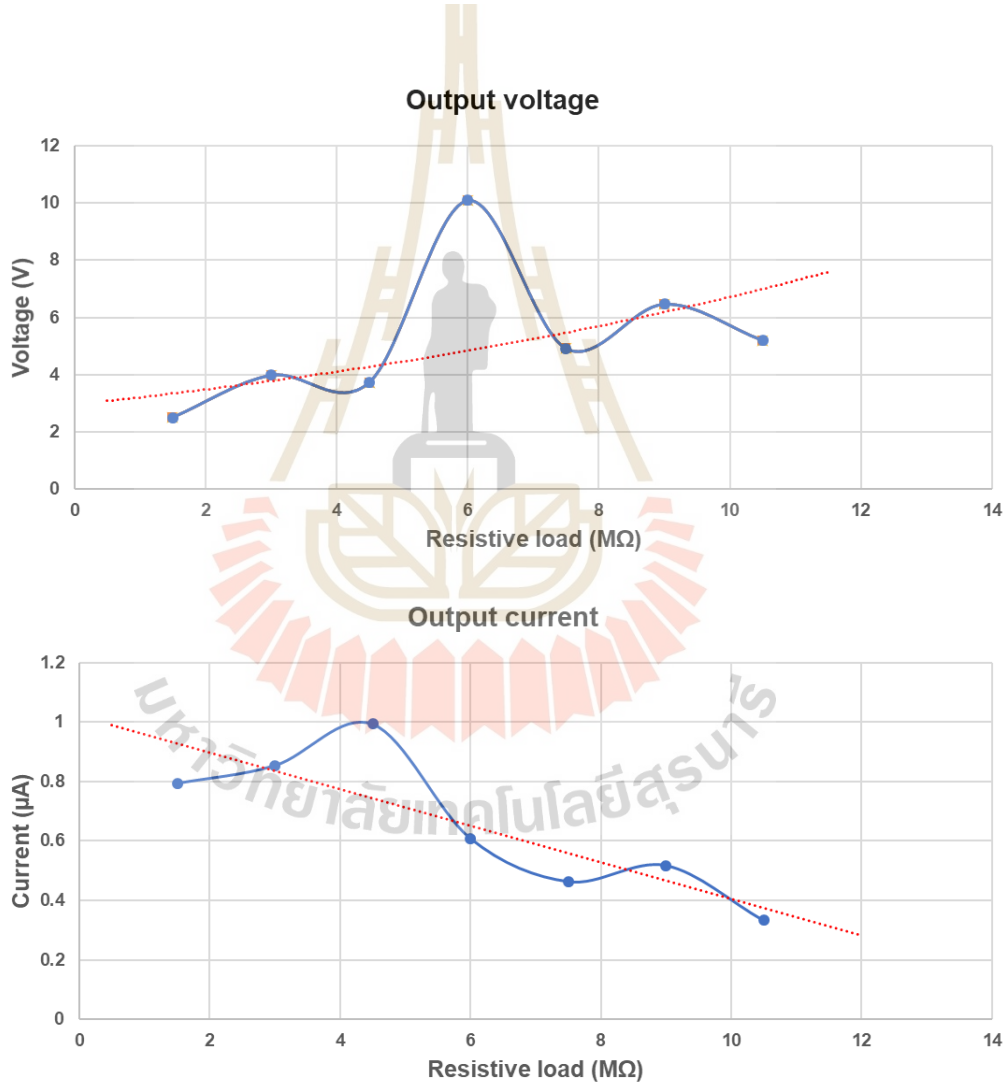

Figure 4.25 Maximum voltage and current output of the 2D flat nanofibers varies with resistive load for piezoelectric testing.

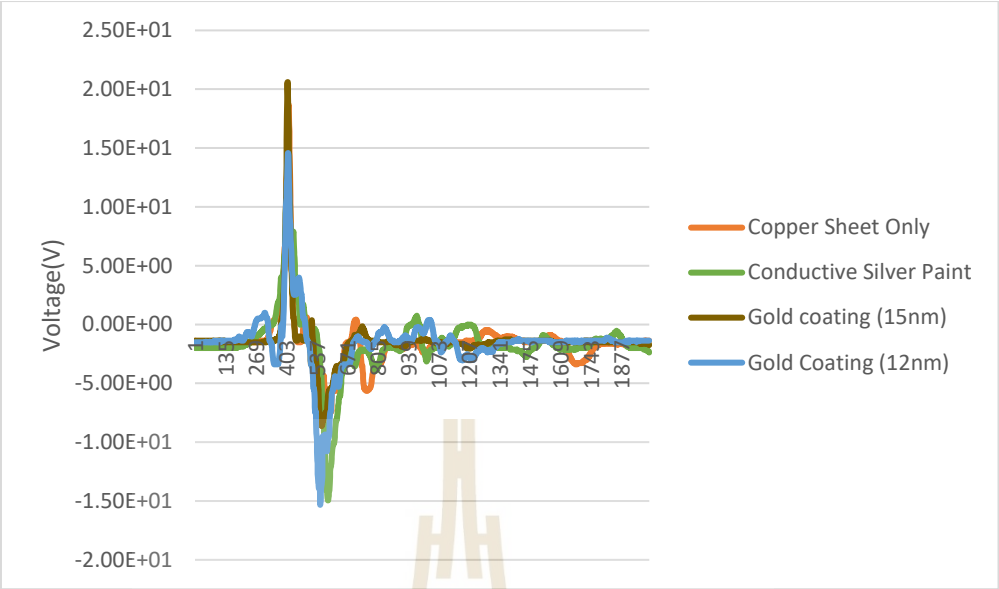

Figure 4.26 Open voltage response as a function of electrode attachment to the piezoelectric fibers.

From the recorded response, it was possible to conclude that adding a conductive silver paint or covering the area upon which the electrodes would be attached with sputtered gold did not have any significant effects on the response. The waveforms observed all have very similar shape, which could be interpreted as an indication that the electrode and material interaction is consistent. Thus, it was decided that the copper tape by itself would be chosen as the electrode material.

The process of releasing the weight onto the sample was kept reasonably similar between trials. However, these results would benefit greatly from having the means to ensure that the distance and positioning of the load before releasing is the same for all trials. However, for now, at least it was possible to observe that, as expected, the output current decreases as the resistive load is increased in most cases. Also, the output voltage increases as the load increases. Waveform shape was also found to be

reasonably similar when for each device for most loads. The test uses 3 MΩ and 6 MΩ resistive load to compare the performance of the generators with made by the 3D nanofibers (15 %wt and 17 %wt) and the generator made with the 2D flat nanofibers. The results show in Figure 4.27. From this result, it is possible to draw some conclusions from the data that was extracted from these tests. As expected from a higher PVDF concentration, the 17 %wt sample had the better maximum instantaneous output voltage figures observed and 3D nanofibers structure have better piezoelectric performance than 2D fibers mat.

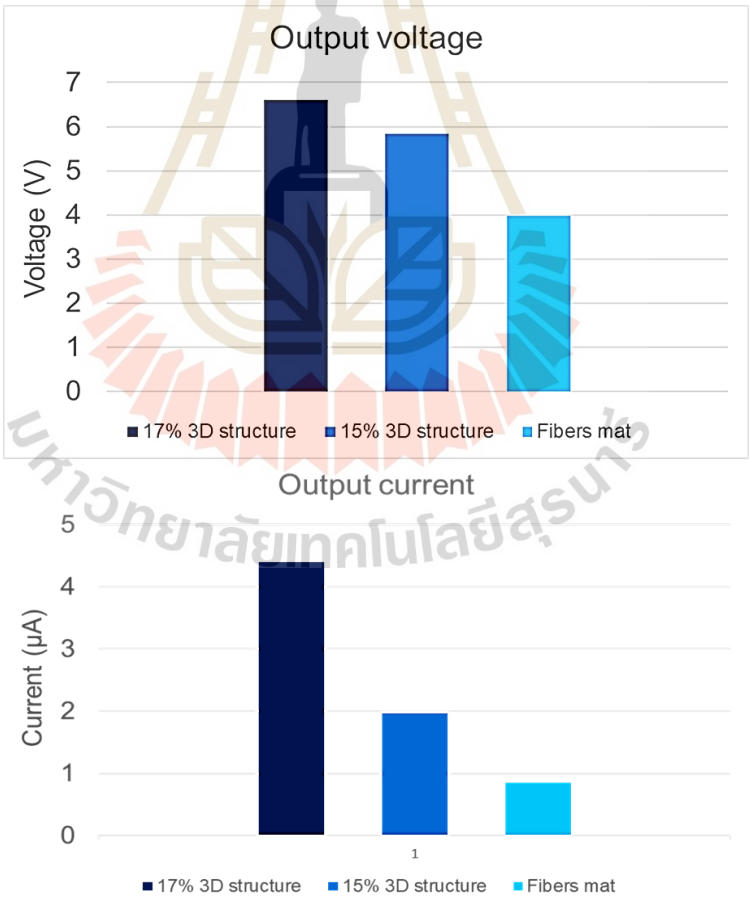

Figure 4.27 The recorded responses of various generators made by the 3D nanofibers (15 % and 17 %) and made with the 2D flat nanofibers.

CHAPTER V

CONCLUSIONS

In this work, 3D nanofiber structures that mimic the natural termite nest have been investigated and tested their piezoelectric performance. When the process parameters were optimized, electrospinning with liquid (DI water) bath collector technique can fabricate 3D structures of PVDF-HFP nanofibers successfully.

The concentration of the solution is the key parameter for producing the 3D structure. The effect of the solution concentration and other parameters are concluded and shown in Table 5.1. The optimization conditions and parameters for producing the termite nest 3D structure in this work are summarized in the following.

- Material is a biocompatible PVDF-HFP polymer
- Solvent is mixing of acetone and DMF with ratio of 1:1
- Solution concentration is 15 %wt
- Liquid bath collector has to fill with DI-water
- Optimized electrospinning parameters are applied voltage of 10 kV and distance from the tip to liquid surface of 5 cm

Table 5.1 Conclusion for the production of PVDF-HFP 3D nanofibers structure.

Concentration (%wt)	HV (kV)	Diameter (nm)	Beads	3D structure
10	15.5	441 ± 18	Yes	No
13	12.8	451 ± 16	Yes	No
15	10	802 ± 23	No	Yes
17	9.2	1256 ± 43	No	Yes
20	7.1	1277 ± 32	No	No

Cell adhesion and migration are important properties for tissue engineering. The cell interaction with the 3D nanofibers scaffold was investigated. The *in vitro* results demonstrated that the NIH 3T3 cells can attach and migrate the 3D nanofibers structure. In addition, wettability of the 3D nanofibers is also important. If the 3D nanofibers structure has hydrophobic property (characteristic of PVDF-HFP), the cell adhesion property is quite poor on it. Therefore, the surface modification is necessary to change its wettability to hydrophilic. In addition, one more important for cell adhesion is the feature of surface. Smooth surface has better adhesion than rough surface, which is shown in the effect of PVA coating. The surface modification results are summarized in Table 5.2.

Table 5.2 Conclusion for the surface modification and cell culture of PVDF-HFP 3D nanofibers structure.

Method	Structure Stability	Hydrophilic	Cell adhesion
Hydrothermal	No	Yes	No
Coated PVA	Yes	Yes	No
Plasma treatment	Yes	Yes	Yes

Moreover, the piezoelectric property of the PVDF-HFP 3D nanofibers structure was evaluated and compared with the 2D structure. The results demonstrated that the piezoelectric performances of the 3D nanofibers are better than their 2D counterparts. This show the possibility of using PVDF-HFP 3D nanofibers structure for energy applications, such as piezoelectric devices for energy harvesting or scavenging.

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