FUNCTIONALIZED ELECTROSPUN NANOFIBERS AND THEIR BIOMEDICAL APPLICATIONS

By

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To my parents
ACKNOWLEDGMENTS

After so many years, there is a long list of people to thank. I will try to do my best to narrow it down and mainly focus on my time at the Multidisciplinary nano Microsystems Group (MnM).

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FUNCTIONALIZED ELECTROSPUN NANOFIBERS AND THEIR BIOMEDICAL APPLICATIONS

By

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Chair: Yong-Kyu Yoon
Major: Electrical and Computer Engineering

This dissertation describes the design, fabrication, and characterization of biodegradable electrospun nanofibers and electrically conductive electrospun nanofibers for active cell culture, controlled drug release, in-vitro neural signal recording and analysis, fMRI compatible neural probe, and flexible strain gauge sensors for smart wearables and microfluidic lab on a Chip. Electrospun nanofibers feature a large surface-to-volume ratio and cell affinities compared with the current conventional solid materials. Various nanofiber microstructure architectures and materials properties, different fabrication methods, and packaging solutions are investigated to respond to the requirements of next-generation biomedical devices.

Some major challenges for nanofiber technology include manufacturability, micropatternability and associated resolution, and seamless packaging. Multiple advanced nanofiber fabrication methods are proposed and studied. Non-metallic low density polyethylene (LDPE) tubes are utilized for tube nozzle electrospinning (TNE) with multiple nozzles, where a linear nozzle array is micromachined by a computer numeric control (CNC) system utilized for printed circuit board fabrication. The TNE approach enables high throughput nanomanufacturing of nanofibers. Micropatterning of
electrospun nanofibers using standard Ultra-Violet (UV) light photolithography has been demonstrated using SU-8 photoresist as the nanofiber polymer. To enhance pattern resolution, oil-immersion lithography has been exploited, which enhances refractive index matching between the polymeric nanofibers and the background. High aspect ratio nanofiber microstructures have been demonstrated using the oil-immersion lithography. Additionally, the thermal reflow of the negative photoresist has been exploited to achieve self-packaged seamless integration of the nanofibers in the microchannel.

Polycaprolactone (PCL), a biodegradable material, slowly degrades in the human body with a typical degradation time constant of several months making it an appropriate candidate for 3D cell culture scaffold and long term controlled drug release applications. Different solvents and electrospinning conditions have been studied to produce various diameters of electrospun PCL nanofibers. Also, mixtures of electrospun membranes of nanofibers and microbeads have been produced, where the nanofibers and microbeads have different release duration time. A different mixing ratio can be utilized to modulate the release profile. Moreover, a sandwiched multilayer membrane consisting of nanofiber barrier layers without drug loading and a drug loaded microbead layer has been fabricated by dual syringe electrospinning. The sandwiched membrane shows an extended linear release profile. Incorporating magnetic nanoparticles in the biodegradable nanofibers has been investigated to produce mechanical stimulus wirelessly to stem cells to study their proliferation and differentiation.

Electrospun polymer nanofibers can further be electrically conductive by undergoing high temperature carbonization in the absence of oxygen, which makes the
nanofiber very useful for electrically active sensing applications. Electrospun nanofibers are inherently continuous and randomly oriented in deposition, forming a microscale spring, which enables carbon nanofibers (CNF) to form stretchable conductive composite polymers within hosting elastomers such as, polydimethylsiloxane (PDMS). A high gauge factor of 23.1 and 50% strain in room temperature is demonstrated using a CNF membrane sandwiched by flexible PDMS. Microelectrode arrays (MEAs) are commonly utilized for stimulating and recording extracellular electrical signals including both local field and action potentials in both in-vitro and in-vivo neural studies. CNF derived from photopatternable polymer nanofibers can be a potential candidate for neural science studies due to its unique extracellular matrix, high surface area to volume ratio, and electrical conductivity. High aspect ratio CNF stacks fabricated by oil-immersion lithography are proposed to be the new nanoporous detector for MEA applications. Reasonable electrical impedance, high neural interaction in in-vitro culture, and neural signal recording are successfully demonstrated. Recently, functional magnetic resonance imaging (fMRI) associated with high magnetic field and radio frequency (RF) pulse has been widely utilized for brain activity monitoring due to its precise image resolution. Unlike conventional metallic probes which are subject to significant noise associated with the RF signals and tissue damage by eddy currents and resulting hyperthermia, CNF offers an alternative solution that is electrically conductive at low frequency but RF transparent. Flexible CNF based polymer neural probes are successfully fabricated and showed no artifacts under 4.7T fMRI scanning.
CHAPTER 1
INTRODUCTION

Motivation and Challenges

With the growing need for the high performance, high-speed, and compact electronic devices, the dimension of each component has reached the microscale range and tends to move to smaller dimensions. For example, the semiconductor transistor has reached a few nanometers and will continue scaling down over the next decade. However, the dimensional scaling trend is much slower in other disciplines such as biomedical disciplines and sensors compared to CMOS semiconductors.

Electrospun nanofiber technology has gained great popularity primary due to its nanoporous morphology. Its unique physical and chemical properties differentiate it from bulk materials. Nanofibrous meshes provide large surface areas and allow nanoparticles to transfer through them. In fact, demand for nanoporous materials and nanoscale devices has been significantly increased in both academia and industry in the past decade. Figure 1-1A shows the trend of the number of publications on nanofibers in the last two decades,

![Figure 1-1A](image1.png)

Figure 1-1. Number of (A) Publications and (B) Patents on "Nanofibers" from 1994 to 2014 (Source: Google Scholar accessed).
which has been rapidly increasing in the past several years and exceeded 12,000 in 2014. Figure 1-1B shows the trend of the number of patents on nanofibers in the last two decades, which also shows a significant increase. Around a thousand patents regarding nanofibers are filed every year after 2010, and the numbers are continuously growing. Electrospinning has become a well-known fabrication method to prepare continuous fibers with diameters ranging from tens nanometers to several micrometers.

This continuous decrease in device dimensions is expected to increase the sensitivity, performance, and make the system more compact, facilitating system miniaturization. One of the limiting factors of the nanofiber technology includes the incompatible fabrication process with traditional microelectromechanical systems (MEMS) and complementary metal oxide semiconductor (CMOS) processes. Therefore, the development of advanced electrospinning nanofiber technology and its suitable applications for high efficiency, low cost, compactness, and high performance becomes an important topic.

In this dissertation, new fabrication processes are proposed towards high manufacturability, freedom of design selections, precise patterning resolution and aspect ratio, and conformal packaging. Additionally, electrospun nanofiber membranes used as the core of multiple applications ranging from biomedical to smart wearables are demonstrated.

**Research Contributions**

The main focus of this research is the development of functionalized nanofibers with advanced fabrication processes for the advancement of biomedical applications. The expected contributions from this research are:
1. Development of the multi-tube nozzle electrospinning process for nanofiber throughput enhancement.

2. Development of the dual syringe electrospinning to offer higher design freedom in materials, compositions, and functionalities.

3. Demonstration of the conformal packaging of nanofiber microstructures by utilizing polymer thermal reflow.

4. Demonstration of the electrospun multilayer sandwiched membranes with different polymer concentration solutions and morphologies using dual syringe electrospinning for extended drug delivery.

5. Fabrication and testing of magnetic nanoparticle embedded scaffolds for active cell cultures.

6. Development of carbon nanofiber based strain gauge sensors with ultra-high sensitivity for smart wearable applications.


8. Design, fabrication, and testing of carbon nanofiber based neural probes with fMRI compatibility for simultaneous imaging monitoring and in-vivo stimulation or recording.

9. Fabrication of microscale nanofibrous microchannels with nanofluidic flow characteristics.

10. Design and fabrication of low loss conductors based on the nanocomposition of aligned nanofiber and electroplated metal.

**Dissertation Organization**

This dissertation is composed of six chapters in total. In Chapter 1, the motivation, research objective, research outline, and the fundamentals of electrospinning are detailed, along with a review of the historical background, traditional electrospinning setup, and several literature publications reporting modifications of different morphologies. Moreover, the effects of solution conditions and operation parameters on electrospun membrane morphology are discussed in detail. Chapter 2 discusses the advanced electrospinning modifications and nanofiber fabrication processes. A multijet
electrospinning process is proposed for high density nanofiber production by replacing the conventional metallic needle with a micromachined non-metallic low density polyethylene (LDPE) tube having an array of micro-nozzles drilled in the sidewall. Dual syringe electrospinning is proposed to offer additional degrees of freedom in design parameters such as nanocomposite membranes consisting of two materials with different properties and sandwiched multilayer architectures. High precision nanofiber patterning can be realized through oil-immersion lithography, which replaces the air medium with an index matching medium to suppress the reflective index difference and UV light scattering effects. Since nanofibers offer microporous morphology, packaging becomes a major challenge for microfluidic applications. Conformal sealing is demonstrated by utilizing thermal reflow properties of the uncross-linked negative photoresist polymer nanofiber. Biodegradable functionalized nanofibers and their suitable applications are discussed and demonstrated in Chapter 3. An electrospun magnetic nanoparticles embedded polycaprolactone mechano-active scaffold is designed and fabricated for active cell culture. The scaffold can provide external mechanical agitation on the cultured cell through oscillating external magnetic fields. The electrospun biodegradable membranes with various morphologies for extended drug delivery are fabricated and characterized. The electrospun membrane morphology, polymer crystallinity, and membrane architecture effects on drug release profiles are studied. Chapter 4 discusses and explores the potential applications of polymer derived carbon nanofibers. A high gauge factor flexible strain sensor made of electrospun polymer derived conductive carbon nanofiber and polydimethylsiloxane (PDMS) nanocomposites is developed for smart wearables. Fabrication and optimization of
carbon nanofiber based microelectrode arrays for in-vitro neural signal recordings are detailed and discussed in depth. A flexible carbon nanofiber neural probe compliant with high magnetic field functional magnetic resonance imaging (fMRI) has been fabricated and demonstrated. Chapter 5 relates to utilizing the advanced packaging technology developed in Chapter 2 for microfluidic applications. Nanofibrous microchannels with conformal polymer sealing and nanofluidic flow characteristics are demonstrated. In Chapter 6, theoretical analysis and fabrication processes of electrospun micro/nanoscale composites conductors for conductor loss reduction at the high frequency regime are discussed in depth. Chapter 7 gives the conclusions and future work to further explore the potential of electrospun nanofibers for biomedical applications.

**Electrospinning Process**

Figure 1-2 shows a conventional electrospinning setup which consists of a mechanical syringe pump, a syringe connected with a metallic needle, a high voltage power supply, and a metallic collector plate. Electrospinning is the process of electrostatically charging a polymer solution, dispensing the charged polymer solution
with an electrostatic force, evaporating of solvent content, and collecting the produced solid fibers at a grounded collector. The highly charged solution intrinsically splits into multiple very fine and long fibers due to the electrostatic repulsion force as it traverses the distance between the tip of the needle and the collector plate.

The electrospinning process is detailed as follows. First, the polymer solution is loaded into a syringe with a hypodermic needle and pumped out at a constant flow rate using a syringe pump. Second, the needle tip is connected to the positive terminal of the high voltage supply. Third, a metallic plate is connected to the ground terminal of the high voltage supply and placed a few centimeters distance away from the needle tip. During the process, a polymer droplet is held by the surface tension at the tip of the needle and subjected to the electric field and electrostatic force. As the electric field increases, the hemispherical surface of the solution droplet at the tip of the needle starts to form a conical shape, the so-called Taylor cone. The electrospinning phenomenon occurs when the applied electrostatic force overcomes the surface tension of the polymer solution. The critical voltage can be described by Eq.(1-1) [1].

\[
V_c = \sqrt[4]{\frac{TCD^2}{L^2}} \left( \ln \frac{2L}{R} - 1.5 \right) (0.18 \cos \theta \pi R \gamma) \text{ kV} \tag{1-1}
\]

where \( V_c \), \( TCD \), \( L \), \( R \), \( \theta \), \( \gamma \) parameters are the critical voltage in kV, the tip-to-collector distance, the length of the needle, the inner radius of needle, the Taylor’s cone angle \( (49.3^\circ) \), and the surface tension of polymer, respectively.

When the electrostatic force exceeds the surface tension of the polymer, a charged jet of solution is ejected from the Taylor cone to the metallic collector. As the polymer jet travels, it continues to elongate but shrink in diameter due to its solvent evaporation [2]. The jet experiences a bending instability at a critical point between the
needle tip and collector wherein the jet starts whipping in multiple directions in a chaotic fashion [3]. As a result, a web of non-woven solid polymer nanofibers are produced on the collector.

**Modified Electrospinning Process**

The conventional electrospinning setup consists of only one metallic needle for the polymer jet, which limits its production capacity. Therefore, the single needle electrospinning (SNE) requires a very long time to produce a large volume of nanofibers and thus is very challenging to cover a large area with a uniform nanofiber distribution. To overcome the low production rate and enhance the manufacturing capability, Theron et al. proposed the use of multiple metallic needle sources in a linear array for polymer electrospinning via multiple nozzles simultaneously [4]. Since then, people from various research groups have conducted experiments and reported different multiple metallic needle electrospinning setups. Additionally, some have reported the feasibility of producing multiple blends of nanofibers with different polymer loading [5-6].

However, the jet repulsion between separate metallic needles produces large nonuniformity of deposited nanofiber stacks over a large collector area. Marquez et al. have reported the usage of a non-metallic material as the jetting source to minimize the repulsion effects, where a microfluidic channel made of a flexible polymer, polydimethylsiloxane (PDMS), is used to produce polymeric jets at the orifices on the edge of the microchannels [7]. Dosunmu et al. have used a microporous tube as the jetting source for random direction electrospinning [8]. Bellan et al. have reported a highly uniform nanofiber membrane with a very small nozzle diameter (< 5 µm) [9].

A typical electrospinning setup produces randomly orientated nanofibers due to the inherent whipping process. The challenge of controlling the spatial orientation of
electrospun nanofibers has been met with some success. Some researchers have conducted different electrospinning schemes to obtain aligned electrospun nanofibers. Li et al. have demonstrated aligned nanofibers by carefully designing the collector with a small gap [10]. The schematic setup and calculated electric field strength are shown in Figure 1-3A and 1-3B, respectively. Lee et al. have studied the effect of the

mechanically moving collector, which is shown in Figure 1-3C. The highly orientated nanofibers can be collected by carefully controlling the speed of the rotating collector [11]. Recently, Jao et al. have demonstrated a 4 collector electrospinning setup with an alternating offset voltage scheme between two electrode pairs to achieve orthogonally aligned nanofibers [12]. The schematic is shown in Figure 1-3D.

The conventional electrospinning setup consists a single syringe loaded with polymer solution, which produces a single material membrane. However, due to technology advancement, the demand for multifunction nanofibers increases. Composite nanofibers of two or more materials have been reported using co-electrospinning method with different syringe architectures. Figure 1-4a shows the modified syringe architecture with a syringe inside of another syringe for electrospun core-shell nanofibers [13]. The electrospun core-shell nanofibers are shown in Figure 1-

![Diagram of modified co-electrospinning setup for core-shell nanofibers](image-url)

Moreover, the post decomposition process can be performed to decompose the inner material for nanotubular type nanofibers [14] as shown in Figure 1-4c.

Recently, Zhou et al. proposed and demonstrated another type of syringe modification by combining 2 syringe needles together to produce a single Taylor cone and bi-phase Janus-type nanofibers [15] as shown in Figure 1-5a-b. The magnetic-fluorescent Janus-type nanofiber enhances luminescent intensity and tunability. Starr et al. reported a multiferroic Janus-type nanofiber of barium titanate and cobalt ferrite [16]. Co-electrospinning provides a promising method to produce multifunctional nanofibers for performance enhancement in real-world applications.

Electrospinning can be used to form nanoscale fibers, microscale spherical beads, or their combination, which result from differing conditions of polymer concentration, electric field strength, tip-to-collector distance, type of solvent, etc. The diameters of nanofibers and spherical beads are in the range of a few tens of nanometers to a few nm.

micrometers, which can be useful in diverse disciplines due to their nanoscopic properties such as large surface to volume ratio and nano/micro porosity. Applications of electrospun nanofibers include energy storage devices [17], sensors and actuators [18], and scaffolds for tissue regeneration [19].

**Biodegradable Nanofibers**

Tissue engineering is an interdisciplinary field that often use biodegradable materials for both in-vitro and in-vivo tissue regeneration artificial scaffolds [20]. Tissue engineers have discovered nanofibers as a promising material for cell culture because of its tunable porosities, large surface area to volume ratios, and similar physical structures to natural extracellular matrices (ECMs). In addition, the geometrical, mechanical, chemical, and electrical properties of the constituted fibers can be easily controlled to achieve the desired requirements and functionalities. Electrospun nanofibers have shown great cell viability and density enhancement due to its nanoporous morphology. Nanofibers can also serve as mechanical support for the tissue; it enables the cell to attach and communicate to surroundings. It has the potential to regulate division, migration, and shape of the cells. A significant improvement of cells proliferation on nanoporous scaffolds is reported in several literature reports [21-22]. Many studies have concluded that nanofiber scaffolding is a promising solution for *in-vivo* culturing [23-24], especially for bone cells which require a highly porous and large surface area scaffold [25]. Recent research interest has been focused on the development of functional nanofibrous scaffolds that provide various guidance cues, such as morphology [26], electrical [27], or biochemical cues [28], for specific cell types to promote cell function and tissue regeneration. Fabricating a
biocompatible nanofiber scaffold that has the nanoporous morphology is a highly demanded task in biomaterials and tissue engineering fields.

For certain applications in tissue engineering, scaffolds with aligned patterns are useful to guide cell growth with the desired anisotropy. Highly oriented electrospun nanofibers can be easily obtained using various collector designs [29]. Researchers have reported that electrospun aligned nanofibrous scaffolds can enhance the extension and direct the out-growth of bone cells [30], cardiac cells [31], vascular cells [32], neural cells [33], and skeletal muscle cells [34] due to the alignment of nanofiber morphology.

Another important application for biodegradable nanofiber membranes is drug delivery. Over the past several decades, polymer sciences have been the backbone of pharmaceutics. Currently, many pharmaceutical polymer excipients are commonly used in the development of novel drug delivery systems. Combined usage of electrospinning with pharmaceutical polymers provides novel strategies for developing unique drug delivery systems, and through the manipulation of electrospinning process, may offer flexibility for design-specific release profiles. Literature has reported embedding drugs in a biodegradable material to obtain a much slower release profile. Moreover, these materials can degrade in the human body without creating bio-hazards over time, which is an important advantage when compared with other materials.

**Electrically Conductive Carbon Nanofiber**

Electrically conductive polymers have attracted much interest in the past 20 years because they simultaneously display the physical and chemical properties of organic polymers and the electrical characteristics of metals. Electrospinning is a convenient method to produce nanofibers with controlled diameters in the range of tens to
hundreds of nanometers. The resulting nanofiber membrane is lightweight, highly flexible, nanoporous, and has a high surface area per volume ratio. Adding the electrical conductivity, a conductive electrospun nanofiber becomes a promising solution for a variety of applications, such as resistive based sensors, flexible energy storage devices, organic photovoltaics, and scaffolds for neural engineering with electrical signal recording/stimulation capabilities. However, the fabrication of electrically conductive electrospun nanofibers still remains relatively challenging compared to nanofibers of most other polymers.

A considerable amount of recent work has been reported trying to make electrospun polymeric nanofibers with embedded conductive nanoparticle polymer composites. The electrical conductivity of these nanocomposite is still a few orders of magnitude lower than that of traditional solid metallic thin films. Therefore, producing a conductive nanofiber for various applications remains as a major interest.

For neural engineering, conductive nanofibers which provide not only ECMs but also electrical stimulation and recording are important [35]. Several studies have demonstrated using these nanofibers to electrically stimulate muscle [36] and cardiac [37] cells. Experimental researches using functional conductive nanofibers have provided valuable information regarding cell responses to individual guidance.
CHAPTER 2
ADVANCED FABRICATION TECHNIQUES

Modified Electrosinning Process

Nanotechnology has drawn great attention in both academia and industry with an increasing demand for smaller and more compact electronic devices. The same phenomena is also happening with nanofiber technology, which offers unique nanoporous morphology, large surface to volume ratio, and nanoscale dimensions. However, the unique properties of nanofibers make them incompatible with both traditional CMOS and MEMS microfabrication. Therefore, to follow the trend of scaling electronic devices and take advantage of the unique properties of nanofibers, new advanced fabrication techniques are required.

Tube Nozzle Electrospinning

To continuously advance electrospinning nanofiber technology and minimize the production cost of electrospun membranes, a high throughput manufacturing mechanism is required. A typical throughput rate of a conventional single needle electrospinning setup is about 0.01 - 0.1 gm/hr [38], which is not suitable for larger manufacturing commercial usage. Researchers have been studying scaling up the number of spinnerets to increase the nanofiber throughput rate [39-40]. However, high density metallic jet electrospinning results in bending electrospinning jets due to the electric field interaction, i.e. Coulombic interaction between neighboring jets. Due to this dispersion of nanofibers, generally low dense nanofibers are formed and therefore thin

nanofibrous membranes are produced. For high density and thickness nanofiber mats, high directionality electrospinning is necessary.

To produce high density nanofibers, the tube nozzle electrospinning (TNE) process has been proposed, where a non-metallic low density polyethylene (LDPE) tube is micromachined to have an array of micro-nozzles on the sidewall to be used as spinnerets. The non-metallic needleless tube nozzle reduces electrostatic repulsion between neighboring spinnerets and therefore adjacent polymer jets, resulting in reduced dispersion and high density nanofibers. Figure 2-1 shows the different system schematics of the single needle electrospinning (SNE) (a), single tube nozzle electrospinning (TNE) (b), and 2-dimensional tube nozzle electrospinning setup (c). All systems accommodate a precision syringe pump, a high voltage source, and a grounded collector. The only difference is that the prepared LDPE tube with multiple nozzles replaces the single metallic needle.

**Experiment Setup and Operating Condition**

The electrospinning solution consists of SU-8 2025 (Microchem Inc.) diluted in cyclopentanone (Sigma Aldrich Inc.) to obtain an optimum solid percentage of 60.87%. In SNE case, the prepared SU-8 solution was loaded into the syringe without the introduction of bubbles and capped with a 22-gauge stainless steel hypodermic needle.
(CML Supply LLC, USA). The syringe was then loaded into the syringe pump (NE-1000, New Era Pump Systems Inc.) and programmed to pump at a specified flow rate. The needle tip is then connected with a high voltage supply (603C-300P, Spellman High Voltage Electronic Co.), and the copper collector plate is connected to ground. Optimized operating conditions for electrospun SU-8 nanofiber for an average diameter of 200 - 500 nm is determined to be a needle voltage of 12.5 kV, a tip-to-collector distance (TCD) of 12.5 cm, and a flow rate of 0.2 ml/min for the SNE approach.

In the TNE case, the stainless steel needle is substituted with a low density polyethylene (LDPE) tube with an outer diameter of 0.1", an inner diameter of 0.062", and a wall thickness of 0.02" (Value Plastics Inc.), which is micromachined to obtain a linear array of nozzle holes on one side of the tube by a printed circuit board milling machine (ProtoMat S100, LPKF Laser & Electronics AG, Germany).

Multiple nozzle diameters (0.2 mm and 0.5 mm), nozzle pitch (10 mm and 5 mm), and nozzle count (2, 4, and 8) are prepared. One end of the tube is thermally melted and sealed to obtain a closed tube environment which enables the solution to pressurize inside the tube. The other end is connected to a syringe by a luer lock connector (Value Plastics Inc.). The high voltage supply is connected to a copper wire which is inserted into the tube to provide charges in the polymer solution. Since the tube has multiple micromachined nozzle holes, a higher flow rate is required to maintain the same pressure as in the SNE case for Taylor cone formation. As a result, flow rates of 0.4 ml/min, 0.8 ml/min and 1.6 ml/min are experimentally observed to be the optimized conditions for 2, 4, and 8 nozzles, respectively. It is noticed that sorely increasing flow rates without optimizing other operation parameters is insufficient to initiate the
electrospinning process. With a TCD of 10 cm, the voltages required for 2, 4, and 8 nozzle TNE to observe the Taylor cone formation and initiate the electrospinning process are 12.5, 15, and 20 kV, respectively. Figure 2-2(a-b) show the digital single lens reflex (DSLR, Canon T2i, Canon USA Inc.) images of the Taylor cones formation and polymer jets ejection in 4 and 8 nozzle TNE. The same requirement is observed for multi-TNE. Increasing flow rate and voltage is needed with increasing the number of tubes to 2 and 4. Figure 2-3 shows a DSLR image of electrospinning using 2 tube with 3 nozzles and requires a higher needle voltage of 25 kV feeding both tubes. The

Figure 2-2. Electrospinning jets on tube nozzles, (a) 4 nozzles and (b) 8 nozzles architecture (φ indicates semi vertical angle).

Figure 2-3. Multi tube electrospinning using 2 tubes with 3 nozzles each showing Taylor cone and electrospun nanofibers.
Taylor cone is seen to diverge not only between adjacent nozzles but also between adjacent tubes.

**Electric Field Simulations**

An electrostatic analysis using the finite element method was performed to understand the effect of the spatial electric field on the repulsion of the nanofibers and its relation to the semi-vertical angles. The electrostatic field distribution of the TNE architectures was performed using COMSOL Multiphysics 4.3 (COMSOL AB). Figures 2-4a shows the simulation result of the electrostatic field line of a single 8 nozzle tube with a space of 0.5 cm and a TCD of 12.5 cm. The flow of charged ions/electrons by the polymer jet to the grounded collector is indicated by the gradient of electric fields. A diverging trajectory electrospinning jet with increased angles of divergence is observed. This is attributed to the divergent fields of adjacent jets of charged particles which increase as they are further away from the jetting source.

In the 2 tube TNE, when 2 tubes are adjacent to each other, similar electrical

---

**Figure 2-4.** Electric field streamlines showing repulsion in multi-jet electrospinning in (a) a single tube and (b) dual tubes.
repulsion is observed as that of a single tube except the additional out-of-plane spatial repulsion between the 2 tubes as shown in Figure 2-4b. The divergence between the tubes is similar to that at the other edges of a single tube. This is attributed to the lack of convergent field as seen by the inner nozzles of a single tube. Due to this dispersion, the stack thickness of the nanofibers stays relatively similar to that of the single tube for the same electrospinning time. For 4 tubes, the simulation result shows that the polymer jet from the inner 2 tubes is constrained by the electrical repulsion force from the outer tubes as shown in Figure 2-5. Table 2-1 summarizes the repulsion angles of 2

![Electric field streamlines showing repulsion in multi-jet electrospinning in 4 tubes](image)

Figure 2-5. Electric field streamlines showing repulsion in multi-jet electrospinning in 4 tubes

<table>
<thead>
<tr>
<th>Pitch distance (mm)</th>
<th>Outer Angle</th>
<th>Inner Angle</th>
</tr>
</thead>
<tbody>
<tr>
<td>2 Tube TNE</td>
<td></td>
<td></td>
</tr>
<tr>
<td>5</td>
<td>25°</td>
<td></td>
</tr>
<tr>
<td>8</td>
<td>23°</td>
<td></td>
</tr>
<tr>
<td>10</td>
<td>20°</td>
<td></td>
</tr>
<tr>
<td>4 Tube TNE</td>
<td></td>
<td></td>
</tr>
<tr>
<td>5</td>
<td>28°</td>
<td>13°</td>
</tr>
<tr>
<td>8</td>
<td>24°</td>
<td>10°</td>
</tr>
<tr>
<td>10</td>
<td>22°</td>
<td>8°</td>
</tr>
</tbody>
</table>
tubes and 4 tubes with a spacing distance varying from 5 mm to 10 mm. In the 4 tube TNE case, a more compact electrospinning path and a thicker nanofiber stack are expected.

**Nanofiber Characterization**

All experiments were performed at room temperature in an acrylic chamber. Nanofibers were electrospun in periods of 30 seconds with a relaxation interval of 30 seconds for 8 cycles. The additional intermediate period between each electrospinning session was utilized to dissipate the remaining charges in the electrospun nanofibers,

![Figure 2-6. Electrospun nanofibers collected at different operating conditions using the 1 tube and 2 nozzle TNE architecture with a nozzle diameter of 0.2 mm; (a) V=12.5 kV, TCD=10 cm, (b) V=12.5 kV, TCD=12.5 cm, (c) V=15 kV, TCD=10 cm, (d) V=15 kV, TCD=12.5 cm.](image)
which reduces the repelling force between incoming nanofibers and the nanofibers that are on the collector.

Followed by the Taylor cone formation at every nozzle, the polymer droplet quickly shrinks to a very fine jet and elongates until it follows a bending instability stage where the nanofibers are chaotically whipped toward the collector and forming the nanofibers membrane. Scanning electron microscopy (SEM, JEOL 5700, JEOL Ltd.) images of nanofibers that are collected by the TNE with a nozzle diameter of 0.2 mm at different voltages and TCD conditions for the 1 tube 2 nozzles TNE architecture are shown in Figure 2-6. It is observed that the nanofiber density and diameter decrease with increasing TCD as the polymer jet experiences more stretching during the whipping process. The porosity of the nanofiber membrane can also be affected by the process parameters while the nanofiber throughput increases as increasing the number of nozzle. ImageJ software (National Institute of Health, USA) is used to determine the porosity of nanofiber membranes. As the TCD increases, the porosity of the nanofiber membrane increases. The results are summarized in Table 2-2. With increasing TCD, the polymer jet also experiences more charge repulsion during the whipping process. As a result, the porosity increases with increasing TCD. The nozzle diameter also plays

<table>
<thead>
<tr>
<th>Tip to collector distance (cm)</th>
<th>7.5</th>
<th>10</th>
<th>12.5</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Nozzle diameter = 0.2 mm</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Porosity (%)</td>
<td>29.1</td>
<td>31.0</td>
<td>35.3</td>
</tr>
<tr>
<td>Pore area (μm²)</td>
<td>0.31</td>
<td>0.52</td>
<td>0.57</td>
</tr>
<tr>
<td><strong>Nozzle diameter = 0.5 mm</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Porosity (%)</td>
<td>56.9</td>
<td>59.0</td>
<td>-</td>
</tr>
<tr>
<td>Pore area (μm²)</td>
<td>0.28</td>
<td>0.24</td>
<td>-</td>
</tr>
</tbody>
</table>
a role to determine the porosity of the nanofiber membrane. Higher porosity and larger pore size are observed at the larger nozzle size.

**Growth Rate of Nanofiber Stacks**

Nanofiber throughput is determined by the mass of nanofibers collected over a specific period of time. In the single tube 2 nozzle architecture, 16.5 mg of SU-8 nanofibers are collected in 16 cycles (each cycle has 30 sec of electrospinning and 30 sec of relaxation.) This gives a mass throughput of 0.06 g/hr per nozzle which is similar to the SNE technique. Increasing the nozzle count on a single tube from 2 nozzles to 8 nozzles gives a throughput of 0.46 g/hr. The result indicates that the throughput increases with the nozzle count in a linear fashion. While doubling the tube count on the 4 nozzle architecture, the production rate is also doubled compared to the single tube,

![Figure 2-7. Thickness of nanofiber stacks where error bars indicate height deviations when using single tube nozzle and two tube nozzles systems.](image-url)
which is 0.49 g/hr and 0.25 g/hr, respectively. However, a slightly decreased throughput rate is observed in the 8 nozzle case, where the throughput rates of 2 tubes and a single tube are 0.75 g/hr and 0.46 g/hr, respectively.

Figure 2-7 shows the nanofiber stack growth rate of single tubes with 4 and 8 nozzles, and 2 tubes with 2, 4, and 8 nozzles. In a single tube architecture, both the 4 and 8 nozzles cases show a linear increase of growth rate until 480 sec and then starts to saturate. Therefore, only the growth rate in the linear growth region (< 240 sec) is used for calculating the slope of growth rate. In the 4 and 8 nozzle cases, the growth rates are calculated as 0.16 μm/sec and 0.21 μm/sec, respectively. The saturation in height of the nanofibers is attributed to the electrostatic repulsion from the residual charge in the stacked nanofibers.

In the case of the 2 tube nozzle, a steady growth rate in all 2, 4, and 8 nozzles is observed. The growth rate of 2 tube nozzles increases as increasing the nozzle count and it reaches a maximum of 0.18 μm/sec at the 8 nozzle case. But the growth rate of 2 tube nozzles does not increase linearly as increasing the tube count. The decrease in

<table>
<thead>
<tr>
<th>Nozzle count</th>
<th>Collection time (min)</th>
<th>Nanofiber mass (mg)</th>
<th>Mass throughput (g/hr)</th>
<th>Substrate Area (cm²)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Single Tube</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>2</td>
<td>16</td>
<td>16.5</td>
<td>0.06</td>
<td>54.78</td>
</tr>
<tr>
<td>4</td>
<td>16</td>
<td>66</td>
<td>0.25</td>
<td>54.78</td>
</tr>
<tr>
<td>8</td>
<td>16</td>
<td>124</td>
<td>0.46</td>
<td>54.78</td>
</tr>
<tr>
<td><strong>Two Tube</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>2</td>
<td>16</td>
<td>72</td>
<td>0.27</td>
<td>55</td>
</tr>
<tr>
<td>4</td>
<td>16</td>
<td>130</td>
<td>0.49</td>
<td>130</td>
</tr>
<tr>
<td>8</td>
<td>16</td>
<td>200</td>
<td>0.75</td>
<td>190</td>
</tr>
</tbody>
</table>
growth rate is attributed to the lateral repulsion of nanofibers from the adjacent tubes. Unlike the single tube nozzle case, the nanofiber repulsion in the lateral direction is observed in the 2 tube nozzles case. Therefore, we observe that even though the nanofiber throughput increases with the nozzle count as observed in Table 2-2, the growth rate is limited by the repulsion of nanofibers over a larger area on the collector. The growth rate increases with the increasing number of nozzle count as the higher throughput of nanofibers is achieved with higher directionality in the multiple tube case.

**Dual Syringe Electrospinning**

Conventional single syringe electrospinning (SSE) for electrospun single material membranes has gained a lot of attention due to its unique morphology with a nanofiber diameter on the order of hundreds of nanometers. As technology advances, the demand for multifunctionalized electrospun membranes continues to grow. To adapt to the demand, the conventional electrospinning process has been modified by developing co-electrospinning techniques to produce core-shell type nanofibers and Janus-type nanofibers which have been described in the previous chapter. However, the degree of freedom of polymer solution controllability is still limited. Core-shell and Janus-type nanofibers require the two solutions have similar viscosity conditions to produce single-phase composite nanofibers. Electrospun composite membranes with more independency in the morphology still remain as a challenge. In this work, a new electrospinning scheme consisting of two syringes loaded with different polymer solutions is proposed. The dual syringe electrospinning (DSE) technique can realize an alternating hybrid multilayer or composite membranes with more independency in the selection of different materials and solution conditions such as viscosity, additive
concentration, and solvent selection. Fabrication of electrospun membranes that have two distinguished morphologies and functionalities becomes possible with DSE.

**Nanofiber Characterization**

Figure 2-8 shows the schematic of DSE which consists of two syringes loaded with different polymer solutions and concentrations, a syringe pump to provide a steady flow rate on both syringes, a high voltage power supply with the positive terminal connected to the syringe needle tips, and a grounded metallic collector. Multilayer or composite membranes with different morphologies can be obtained by alternating or simultaneous electrospinning approaches, respectively. DSE offers more degrees of freedom in design parameters such as two polymers with different degradation times and concentrations, dual-drug release profiles, and sandwiched architectures with different morphology. For a drug delivery application, we can realize a linear release by embedding drugs only in a microbead layer and using nanofiber layers as diffusion barriers and packaging layers.

**Electrostatic Repulsion**

Since two syringes are used instead of the traditional SSE system, there is a possibility in which the polymer jet repulsion between two electric fields produced from

![System schematic of a dual syringe electrospinning and potential membrane morphologies that can be obtained.](image-url)
the two needle tips results in a lack of polymer deposition on the collector plate. The electrostatic and particle distribution simulation using COMSOL Multiphysics 4.3 (COMSOL AB) were performed to understand the spatial electric field repulsion between the two needles. Figure 2-9 shows the simulated particle distribution with a various need tip distance ranging from 2.5 cm to 4 cm. Minimum charge repulsion and uniform polymer distribution can be obtained with a needle distance of 3.5 cm.

**Multilayer Membrane Fabrication**

Scanning electron microscope (SEM) images of electrospun SU-8 membranes

![COMSOL simulation of two polymers distribution](image)

Figure 2-9. COMSOL simulation of two polymers distribution with 2.5 cm (a), 3 cm (b), 3.5 cm (c), and 4 cm (d) between two needles. Insert images are simulated particle distribution.
with varying the SU-8 2025 and DMF ratio of 1:1, 2.5:1, 5:1, 7.5:1, 8.75:1, and 10:1 are shown in Figure 2-10(a-f), respectively. All samples were electrospun at an electric field of 1 kV/cm and a TCD of 10 cm. Figure 2-11a shows the diameters of nanofibers and

Figure 2-10. Various electrospun membrane morphologies that can be obtained by dual syringe electrospinning.

Figure 2-11. (a) SU-8 microbead and nanofiber diameter measurement with varying SU-8 concentration. (b) SEM image of the electrospun alternating multilayer membrane by DSE.
microbeads with varying SU-8 2025 and DMF ratios and different morphology. It is categorized in regime a, b, and c. Regime a and c are identified as dilute and semi-dilute entangled solutions, respectively, which produce only single phase membranes. In dilute regime a, the low polymer concentration solution produces a single-phase microbead membrane due to the surface tension and low viscoelasticity. The diameters of single-phase microbeads are measured ranging from 250 to 1250 nm as SU-8 2025 ratio increases from 1 to 5, respectively. Regime c produces pure nanofibers with an average nanofiber diameter of 440 nm at an SU-8 2025 ratio of 10. Regime b is the semi-dilute unentangled condition whose polymer concentrations are between regime a and c, producing a two-phase hybrid membrane consisting of both microbeads and nanofibers. As increasing polymer concentrations in regime b, the solution viscosity moves towards to semi-dilute entangled which results in decreasing the microbead volume ratio and increasing the microbead average diameter.

Figure 2-11b shows the SEM image of the multilayer membrane architecture which is realized by DSE. All samples are electrospun at an electric field of 1 kV/cm, a TCD of 10 cm. An electrospinning interval of 1 minute and a resting between electrospinning sessions of 30 seconds were used to give additional time for charge relaxation. Solutions with an SU-8 ratio of 1 and 10 were loaded in the DSE system for the alternating electrospinning process. First, the SU-8 nanofiber layer was electrospun for 8 cycles. Then, single-phase SU-8 microbeads were electrospun for 8 cycles. The process was repeated twice to obtain a membrane consisting of 4 alternating layers.

**Oil-immersion Photolithography Patterning**

An important step of microfabrication is micropatterning, which defines the dimension and shape of the microstructure. The most common and popular patterning
technique is ultra-violet (UV) light photolithography, which uses the UV light power to chemically react with a photo sensitive polymer, the so-called photoresist. There are two types of photoresist used for UV photolithography, positive and negative photoresist. Positive photoresist is a kind of photosensitive polymer which consists of base resin, sensitizer, and casting solvent. The base resin in positive photoresist will decompose as exposed in a certain wavelength of UV and is soluble in developer. On the other hand, negative photoresist is soluble in developer as is while the polymer chain will cross-link and become insoluble in developer after it is exposed to a UV light.

**Photolithography Process**

Figure 2-12 shows a typical UV photolithography process. First, the surface preparation of either a silicon (Si) or glass wafer is required to promote adhesion of photoresist to the substrate. The wafer is cleaned with acetone, methanol, and deionized water, which is also called the solvent clean, to remove any oil or organic solvents.
residues. Then RCA clean is used to remove organic residues entirely. During the process, Si is oxidized to form a thin silicon dioxide (SiO\(_2\)) layer. Hydrofluoric acid (HF) is applied to remove the thin SiO\(_2\). Si should be hydrophobic after this process.

Photoresist adhesion to the substrate becomes very important as the feature size gets smaller and the aspect ratio gets higher. A hydrophobic surface is required to promote photoresist adhesion and prevent microfeatures from delaminating. Next, photoresist is spin coated uniformly on the substrate to a desired thickness. After spin coating, a soft bake or pre-exposure bake is used to evaporate excess solvent from the photoresist. Insufficient baking will not remove all the solvent, which results in a poor structure pattern and profile. Third, the exposure of photoresist using a specific wavelength of UV light causes a chemical reaction so that the photoresist becomes either soluble (positive photoresist) or insoluble (negative photoresist) in developer. A typical photomask is made of glass or quartz with a pre-patterned thin chrome layer, producing transparent areas and dark areas (covered by chrome). The UV exposure time is a function of the photoresist material. Over or under exposure will decrease structure resolution. A post-exposure bake is used for negative photoresists to thermally activate and expedite the chemical cross-link process. Lastly, the exposed sample is immersed in developer to dissolve the exposed areas for positive photoresist or the unexposed areas for negative photoresist.

As an electrospun nanofiber membrane shows an effective pore size in the range of a few hundred nanometers, researchers have started to investigate the possibility of using this unique property for microfluidic applications. However, nanofibers have not been often used in micro/nano-fluidic systems so far, which is mainly due to its poor
patternability and lack of integrability with other components and devices. Previously, non-lithographic patterning techniques such as mechanical nanofiber patterning [12], laser machining [41] and deep reactive-ion etching with bonding processes [42] have been reported. These techniques are useful for patterning structures with a few hundred micron or a few millimeter scale. However, patterning structures with a few micrometer resolution still remains as a challenge. In 2009, Shi et al. proposed an indirect photolithographical patterning technique [43] to obtain nanofiber structures within microscale resolution. It is achieved with photoresist patterning on top of a stack of nanofibers and subsequent reactive ion etching (RIE) of the stack. This technique has enhanced the pattern resolution down to submicron, but it requires an additional process step and excessive process time.

Since photolithography is one of the promising technologies to make microscale structures and patterns, researchers have explored the possibility to form nanofibers out of photoresist polymers and directly perform photolithography on the stack of photoresist nanofibers for patterning. Multiple groups have demonstrated photopatterning of electrospun nanofibers using photosensitive polymers by a direct photolithographical approach [44-45]. This method has enhanced patterning resolution of a nanofiber stack to a few microns. This method opens the door for researchers to explore more potential applications using nanofibers.

**Oil-Immersion Process Simulation and Experimental Result**

In the conventional UV patterning process, the photopatternable vertical thickness of a nanofiber stack is limited due to significant optical scattering and diffraction [46]. The typical diameter of electrospun nanofibers is between 100 nm to 500 nm, in a similar size range of the wavelength of UV light source ($\lambda_{UV} = 365$ nm). Experimental
work to illustrate the diffraction effect in the nanofibrous stack has been performed, where negative photoresist, SU-8, is used with a refractive index of 1.68 for electrospun nanofibers in the air environment [47], resulting in only a limited stack height of 34.6 ± 4.1 μm. Later they have overcome this limitation by replacing the air inside the nanofiber stack with a refractive index matching medium such as canola oil. This process is known as oil-immersion lithography [48].

Figure 2-13. Oil-immersion lithography: (a, b) Schematics, (c, d) COMSOL simulation results, and (e, f) SEM images of the fabricated nanofiber microstructures. (a)(c)(e) are obtained with the air medium and (b)(d)(f) are with the oil medium.
Figures 2-13(a-b) show the schematic of oil-immersion lithography. As the air is replaced by the medium which has the same refractive index, the nanofiber stack is optically equivalent to a homogeneous solid film. Figures 2-13(c,d) show the UV intensity simulation (COMSOL Multiphysics, COMSOL Package Inc.) in the air and oil media. The simulation result shows clearly the reduced scattering effect with the oil medium. Figures 2-13(e,f) show the fabricated scanning electron microscope (SEM) images of nanofiber stacks with a UV dose of 400 mJ/cm². The oil-immersion technique significantly enhances the pattern resolution and patternable aspect ratio. By using immersion lithography, the height of the nanofibrous electrodes is increased from 25.9 ± 4.1 μm to 55.5 ± 3.3 μm with a UV dosage of 400 mJ/cm², while its diameter is decreased from 100.8 ± 24.1 μm to 73.3 ± 5.9 μm for a 60 μm mask diameter. Thus, a three times higher aspect ratio structure is obtained. This method enables more potential applications which require high aspect ratio nanoporous microstructures such as 3D artificial tissue scaffold.

**Thermal Reflow Process**

Another challenge of nanofiber devices is conformal packaging. Many applications require conformal sealing of the nanofiber microstructure without damaging the nanoporous morphology. Since the nanofiber microstructure is porous and surface roughness can be in micrometer range, conformal sealing remains as a limiting factor for nanofiber technology in many applications. One of the interesting properties of some negative photoresist polymers is thermal reflow. The portion of negative photoresist that is not cross-linked by UV light has a certain softening temperature point, the so-called glass transition temperature. This property is often used in the thin photoresist film process to flatten the film on the well leveled uniform surface. Also sometimes it is used
to fabricate smooth spherical microstructures [49]. The thermal reflow property can be also used for sealing photopatterned electrospun nanofiber structures. The process is described below.

**Fabrication and Microchannel Characterization**

The scanning electron microscope (SEM) (JEOL 5700) images of the fabricated SU-8 nanofibrous microchannels with different SU-8 nanofiber stack thicknesses are shown in Figure 2-14. The images of 10a and 10b show 45° and 90° angled views of the microchannel with an SU-8 nanofiber stack thickness of 200 µm, which shows partial sealing resulting from the thermal reflow process. As the nanofiber stack thickness increases to 400 µm or thicker, completely sealed SU-8 nanofibrous microchannels can be observed in Figure 2-14(C-F). It is clearly seen that the microchannel is completely

Figure 2-14. SEM images of (A) 45° and (B) 90° angled views of the fabricated semi-sealed nanofibrous microchannels with an SU-8 nanofiber stack thickness of 200 µm. (C) 45° angled view of completely sealed nanofibrous microchannel with an SU-8 nanofiber stack thickness of 400 µm. (D-F) 90° angled view of completely sealed nanofibrous microchannels with an SU-8 nanofiber stack thickness of 400, 550, and 670 µm, respectively.
filled with nanofibers and the channel ceiling and sidewalls are conformally covered with the reflown SU-8 solid membranes. This unique technique is very useful to seal microchannels without destroying the nanoporous morphology. The nanofibrous microchannel has a porosity of 24.37% with an average pore size of 0.97 μm² and a height to radius ratio of 0.93 based on the imaging process (Image J, Scion Co.).

Figure 2-15 shows the height and width of the microchannel versus the thickness of the electrospun nanofiber stack. Both the microchannel's height and width decrease from 34.5 μm to 28.6 μm and 73.9 μm to 46.0 μm for an initial electrospun nanofiber stack thickness of 200 μm to 673 μm, respectively. It is speculated that the thicker electrospun nanofibers have more uncrosslinked SU-8 on the nanofibrous microchannel, which pushes the reflown SU-8 inside the nanofiber stack due to gravity, resulting in a smaller microchannel dimension. Therefore, the reflow effect can be exploited for the channel construction as a process parameter to some extent. The microchannel

![Graph](image_url)

Figure 2-15. Nanofibrous microchannel height and width vs. the thickness of the electrospun nanofiber stack.
dimensions can be controlled not only by the initial channel mask dimensions and exposure dosage but also the SU-8 nanofiber thickness and reflow.

**Summary**

Non-metallic tube nozzle electrospinning (TNE) for a superior production capability with lower electrostatic repulsion force between nanofibers has been successfully demonstrated. TNE demonstrates a high nanofiber collection rate of 0.46 g/hr and 0.75 g/hr in the 1x8 and 2x8 nozzle architectures, respectively. Moreover, an average deposition rate of 0.34 \( \mu \)m/sec is obtained in TNE with the 2x8 nozzle architecture. Multi-nozzle TNE shows potential of significant electrospinning time reduction which can ultimately contribute to reducing the manufacturing cost of nanofiber based devices.

Dual syringe electrospinning (DSE) is proposed and developed to offer higher design degrees of freedom in fabricating membranes consisting of different materials, compositions, and functionalities. The optimal needle distance to achieve minimum polymer jet repulsion and uniform polymer distribution is simulated by COMSOL. Fabrication of multilayer membranes with different materials and morphologies is demonstrated using alternating mode DSE.

Index matching oil-immersion lithography is studied for nanofiber patterning with micron-level resolution. An index matching medium is used to suppress the major limiting factor, UV light scattering. Nanofiber microstructures with high precision and three times aspect ratio enhancement are achieved.

Since nanofiber membranes offer micro/nanoporous morphology, seamless packaging becomes the major challenge in the applications such as microfluidics. A simple and cost effective conformal packaging method for nanofiber microstructures has
been demonstrated by utilizing thermal reflow properties of the uncross-linked negative photoresist polymer nanofiber.
CHAPTER 3
BIODEGRADABLE NANOFIBERS MICROSTRUCTURE

In this chapter, nanofibers made of a biodegradable material are demonstrated to form the basis of biodegradable nanofibrous devices for drug delivery and active cell culture applications. To achieve the best possible results for different bio-applications, electrospinning with different biodegradable materials must be studied. The electrospun nanofiber properties also need to be analyzed since each application has its own unique constraints, where the capability of electrospinning technology with different materials becomes very critical. The electrospun nanofiber mechanical properties have been studied to provide quantification for consideration of future applications.

Developing an artificially controllable morphology scaffold is critical in advanced tissue engineering for cell culturing [50]. Electrospinning is a simple and cost-effective method for producing nanofibers; electrospun nanofibers are alignable and controllable in diameter and porosity by changing the electrospinning parameters such as electric field strength, tip-to-collector distance, and solution viscosity. Theoretically, any polymer with the right electrospinning parameters can be electrospun into nanofibers. The electrospinning nanotechnology process has opened a door to new approaches due to the possibility of producing nanofibers from many different bio-polymers.

**Drug Embedded Nanofibrous Membrane for Ocular Disease**

Micro/nanoscale functional materials in the various morphologies ranging from films, spheres, to fibers have gained large attention in many fields such as medicine, biology, electronics, optics, and energy. Recent advances in nanotechnology provide

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opportunities to develop an optimal drug delivery system. Many ophthalmic diseases require frequent regular treatments such as eye drops 4-6 times per day, and the treatment can last over a few weeks to months or even lifetime [51-52]. This high frequency of instillation is needed due to the short ocular residence time of eye drops of only a few minutes [53]. The high frequency and long-term dosing regimen leads to poor compliance [54]. For example, long term administration of dexamethasone could cause local side effects such as glaucoma, visual acuity impairment, and posterior subcapsular cataract formation [55], as well as systemic side effects such as diabetes, hemorrhagic ulcers, and osteoporosis [56]. Replacing eye drops with extended release devices could yield accurate dosing and increase drug residence time and bioavailability, which would eliminate the frequent instillations and many associated side effects [57]. Devices for extended controlled release are also useful for treatment of inner ear diseases [58] and posterior segment ocular diseases [59].

Controlled release devices which have extended release profiles to reduce treatment frequency and improve compliance with minimized overdose potential are greatly desired. Recently, many efforts on carrier device structures such as contact lens [60-61], biodegradable microspheres [62], electrospun nanofibers [63-64] have been investigated for different ocular disease treatments. However, realization of a prolonged drug release greater than a few weeks, linear release profile, membrane separation and dispersion, or sufficient drug concentration to penetrate through the cornea for posterior ocular diseases remains as a big challenge. Especially, a controlled drug delivery system with a linear release profile for months is still on demand.
Electrospinning technology shows a good potential because its micro/nanoscale diameter and high flexibility, which is critical to minimize the potential for discomfort when the devices are placed in the eye. Additionally, the morphology of electrospun membranes ranges from microscale spheres to nanoscale fibers depending on solution properties and other electrospinning parameters [65]. Pure microbead, pure nanofiber, or microbead/nanofiber hybrid membranes can be obtained when the solution is in dilute, semi-dilute entangled, or semi-dilute unentangled regions, respectively. The potential of controlling electrospun membrane morphology can be beneficial for designing devices with specific release profiles. The hybrid membranes are often considered as structural defects. However, the hybrid structure consisting of microbeads and nanofibers has an advantage coming from its different dimensions of the nanofibers and the microbeads, e.g. it can lead to differing release times of embedded drugs. Therefore, the drug release profile can be controlled by understanding the effects of solvent composition, polymer concentration, tip to surface distance and the electric field on the morphology of electrospun membrane. Moreover, the drug release profile and transport mechanism are studied and modeled based on the dimension, polymer crystallinity, and membrane architecture.

This work specifically focuses on designing a dexamethasone releasing devices with a long-term release profile which could be potentially placed into the fornix for anterior eye therapy. Two fabrication methods of microbead/nanofiber composite membranes, such as single syringe electrospinning (SSE) and dual syringe electrospinning (DSE) reported in Chapter 2, are studied. SSE produces (1) single-phase membranes which are either microbeads or nanofibers and (2) two-phase hybrid
membranes consisting of both microbeads and nanofibers. DSE is exploited for multilayer sandwiched membranes where drug is loaded in a microbead layer, sandwiched by nanofiber barrier layers without drug loading. The effects of polymer concentration and electrospinning parameters on the membrane morphology are studied by scanning electron microscope. Drug release measurements are carried out by soaking the membrane in a PBS solution at room temperature under sink condition.

**PCL Sample Preparation and Nanofibers Characterization**

Polycaprolactone (PCL) has been identified as the potential candidate for the delivery agent due to its biocompatibility, biodegradability, and long degradation time [66-69]. PCL's hydrophobicity leading to a sustained release is observed [70]. Dexamethasone (DX), an ocular drug for infection and allergies, is loaded for a controlled release study.

Polycaprolactone (PCL, average Mw = 80,000), dichloromethane (DCM, ACS reagent, ≥99.5%), dimethylformamide (DMF, anhydrous, 99.8%), dexamethasone (DX, 98%), acetone (ACS reagent, ≥99.5%), and ethanol (ACS reagent, 200 proof) are purchased from Sigma Aldrich Chemicals (St. Louis, MO, USA). All chemicals are used as received. PCL is dissolved at various concentrations in a 1:1 mixture of DCM/DMF or 1:1 mixture of acetone/ethanol. The drug DX is then added to the PCL solution at 10% of polymer loading. Then, DX loaded PCL solution undergoes a constant magnetic stirring at 60 rpm overnight at room temperature to obtain a homogeneous condition.

The PCL solution was filled in the syringe for electrospinning. The tip-to-collector distance and electric field were varied from 7.5-17.5 cm and 0.6-1.4 kV/cm, respectively,
to investigate their effects on the structure of the mats. All experiments were done at room temperature inside an acrylic chamber.

Scanning electron microscope (SEM) images of electrospun PCL membranes with varying PCL concentrations from 2 % to 18 % in mixture of DCM/DMF are shown in

Figure 3-1. SEM images of the electrospun nanofiber membranes with an electric field of 1 kV/cm, a TCD of 10 cm, and a PCL concentration of 2 % (a), 4 % (b), 6 % (c), 8 % (d), 10 % (e), 12 % (f), 14 % (g), 16 % (h), and 18 % (i) in a 1:1 mixture of DCM/DMF.
Figure 3-1(a-i), respectively. All samples are electrospun at an electric field of 1 kV/cm and a TCD of 10 cm. Figure 3-2 shows the diameters of nanofibers and microbeads with varying PCL concentrations and different morphology. It is categorized in regime a, b, and c. Regime a and c are identified as dilute and semi-dilute entangled solutions, respectively, which produce only single-phase membrane. In dilute regime a, the microbead formation is due to low polymer concentration which results in the surface tension instability and low viscoelasticity. The average diameter of single-phase microbeads at 2 % PCL is measured as 860 nm. Regime c produces pure nanofibers with average nanofiber diameters ranging from 310 nm to 670 nm as PCL concentration increases from 12 % to 18 %, respectively. Electrospun membranes with PCL concentration higher than 12% are likely in the semi-dilute regime with a relatively high viscoelasticity which prevented the growth of the surface tension instability and the formation of microbead structures. The SEM images also show that regime b contains a mix of microbeads and nanofibers. Regime b is the semi-dilute unentangled condition

![Graph showing microbead and nanofiber diameter measurement of PCL with varying PCL polymer concentration.](image)

Figure 3-2. Microbead and nanofiber diameter measurement of PCL with varying PCL polymer concentration.
located between regime a and c which produces a two phase hybrid membrane consisting of both microbeads and nanofibers. As increasing polymer concentration, the solution becomes more semi-dilute entangled which results in decreasing the microbead volume ratio and increasing the microbead average diameter. Since the drug release rate is determined by the diffusion through the amorphous region of polymer matrix, a larger diameter microbead structure is preferred to extend the release profile.

**Single-Phase Release Measurement**

Three PCL membranes with different morphology are prepared for single-phase membrane release experiments. Table 3-1 shows all three different PCL single-phase membranes prepared in this study with various solution conditions and electrospinning parameters. Figure 3-3(a-c) show the SEM images of the single-phase membranes, which are named as nanofiber membrane 1(a), nanofiber membrane 2(b), and microbead (c), explored in this study. Nanofiber membrane 2 is electrospun by 16 wt% of PCL dissolved in acetone/ethanol. The average nanofiber diameters are measured as 310 nm and 670 nm for nanofiber membrane 1 and 2, respectively. The larger nanofiber diameter obtained with nanofiber membrane 2 is due to higher solvent evaporation rate of acetone/ethanol at room temperature compared to DCM/DMF solvent mixture in nanofiber membrane 1. Nanofiber membrane 2’s solution jet is

![SEM images of electrospun single-phase membrane](image)

Figure 3-3. SEM images of electrospun single-phase membrane.
completely solidified before it reached to the collector which means that nanofibers experience less stretching during the whipping process.

Dulbecco’s phosphate buffered saline (PBS) is purchased from Mediatech, Inc. (Manassas, VA, USA). The drug release experiments are conducted by soaking 2.5 mg of dexamethasone loaded membrane in 5 ml of PBS at room temperature under sink conditions. The sink conditions were validated by ensuring that 100% of the loaded drug is released into the solution. The samples are placed on a shaker to keep the release medium well-mixed (2314 Lab rotator, Thermo Scientific, Waltham, MA, USA). The dynamic concentrations of dexamethasone in the release medium is determined periodically by measuring the absorbance spectra in the detection range of 220-270 nm using a UV-Vis spectrophotometry (Thermospectronic Genesys 10 UV, Rochester, NY, USA). The concentration of dexamethasone is determined by a least square fit between the measured and reference calibration spectra.

The DX release profile of single-phase membranes which consist of either pure nanofibers or pure microbeads are shown in Figure 3-4. The release durations of nanofibers with average diameters of 310 nm and 670 nm are approximately 2.5 hr and 6 hr, respectively. The short releasing durations are attributed to the small diameters of

<table>
<thead>
<tr>
<th>Table 3-1. Summary of the solution composition and operational parameters used in preparation of single-phase PCL membrane.</th>
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<tr>
<td><strong>Solvent</strong></td>
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<td>Nanofiber 1</td>
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<tr>
<td>Nanofiber 2</td>
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<tr>
<td>Microbead</td>
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nanofibers. The microbead with an average diameter of 860 nm has a release profile of 8 hr. Moreover, the microbeads dispersion is observed after 3 hr, which separates the membrane into smaller segments. Since the release mechanism is based on diffusion, the microbeads release duration is reduced by dispersion after which the contact area between microbeads and solution is enlarged. This indicates the release duration is affected by both the diameter of the structures and its aggregation morphology.

Two-Phase Hybrid Membrane Modeling

A hybrid architecture consisting of nanofibers and microbeads is observed when the PCL concentration is diluted to regime b resulting from reduced solution viscosity, which favored the formation of the microbeads due to the surface tension. The hybrid structure shows the potential for longer release times due to the larger diameter of

![Figure 3-4. Dexamethasone release profile of single-phase nanofibers and microbeads membranes.](image)
microbeads compared to nanofibers. Moreover, the ratio of the microbeads and nanofibers can be controlled by varying the applied voltage and TCD. To further explore this issue, a series of experiments were conducted using 4 % (w/v) of PCL dissolved in 50:50 ratio of DCM/DMF with various applied voltages and TCD. The decrease in the volume fraction of the microbead with increasing TCD is observed. With increasing TCD, the solution jet experiences a longer stretching time, which results in more nanofiber formation and a smaller microbead concentration.

Figure 3-5A shows that the volume percentage of microbeads increased from 25 % to 92 % as the TCD is reduced from 17.5 cm to 7.5 cm, which indicates that the microbead volume percentage increases with the reduction of polymer jet solidification time. Similarly, Figure 3-5B shows that the volume percentage of the microbeads varies from 26 % to 65 % as the applied voltage decreasing from 17.5 kV to 7.5 kV with a fixed TCD of 12.5 cm. This is because lower voltages result in lower electric field and electrostatic force, and thus, less stretching.

Figure 3-5. Changes in the volume percentages of microbeads with (A) TCD at a constant electric field of 1 kV/cm and (B) the applied voltage at a fixed TCD of 12.5 cm.
With the experimental release profile, a mathematical model is developed for the microbead diameter prediction of the electrospun hybrid membrane. The model is modified from the model for the electrospun single-phase nanofiber membrane, which is developed by J.J. Feng [71]. The diameter of microbeads can be modeled by using the volume conservation and fluid-dynamics equations. Figure 3-6a shows the scheme of the dimensional elements used in two-phase electrospinning modeling. The volume of the polymer can be expressed as the summation of the volume of the nanofiber and the volume of the microbead, which is given as Eq. (3-1).

\[ \frac{4}{3} \pi r_{MB}^3 + \pi r_{NF}^2 L_{NF} = V \]  \hspace{1cm} (3-1)

where \( r_{MB} \), \( r_{NF} \), \( L_{NF} \) and \( V \) are the microbead radius, nanofiber radius, nanofiber length, and polymer volume. The polymer solution flows through the needle assuming that

Figure 3-6. (a) Scheme of the dimensional elements used in two-phase electrospinning modeling. (b) Illustration of pressure gradient and force balance in the polymer.
solution is incompressible Newtonian flow, two-dimensional fully developed laminar flow, constant circular cross-section, and has negligible surface roughness and gravity effects. The volumetric flow rate can be express as the following,

\[ Q = \frac{V}{t} = A_n \frac{L_n}{t} = A_n v_n \]  \hspace{1cm} (3-2)

where \( Q, t, L_n, A_n, \) and \( v_n \) are the flow rate, solution travel time, length of the needle, cross-section area of the needle tip, and velocity of the solution. The pressure gradient of solution can be derived by Hagen-Poiseuille equation as the following [72],

\[ \frac{dp}{dL} = \frac{8 \mu Q}{\pi r_n^4} \]  \hspace{1cm} (3-3)

where \( \frac{dp}{dL}, \mu \) and \( r_n \) are the pressure gradient, solution viscosity, and needle radius. The acceleration of polymer jet can be derived using Coulomb's law and Newton's second law.

\[ E = \frac{F}{q} = \frac{ma}{It} \]  \hspace{1cm} (3-4)

where \( E, F, q, m, a, \) and \( I \) are the electric field, electrostatic force, electric charge, mass of the polymer, acceleration of polymer, and current inside the polymer. The Eq. (3-4) can be simplified as the following,

\[ \frac{m}{t} = \rho \frac{V}{t} = \rho Q = \rho A_n v_n \]  \hspace{1cm} (3-5)

\[ E = \frac{ma}{It} = \rho A_n \frac{a}{t} \]  \hspace{1cm} (3-6)

where \( \rho \) is the density of polymer jet. The final velocity of polymer jet at collector can be described by Torricelli's equation as the following [73],

\[ v_f^2 = v_i^2 + 2a d_{TCD} \]  \hspace{1cm} (3-6)
where \( v_f, v_i, \) and \( d_{\text{TCD}} \) are the final velocity of polymer jet, the initial velocity of polymer, which is the same as \( v_n \), and the TCD distance. The flow rate and total cross-section area of polymer jets at collector can be described by Eq. (3-7).

\[
Q = A_{\text{PCL}} v_f = \pi r_{\text{PCL}}^2 v_f
\]  

(3-7)

where \( A_{\text{PCL}} \) and \( r_{\text{PCL}} \) are the effective PCL cross-section area and radius right before it reaches the collector. Figure 3-6b shows the pressure difference and internal force balance of the polymer during the whipping process. The electrostatic force \( (F_1) \) is balanced by surface tension force \( (F_2) \). The pressure gradient between the microbead and nanofiber can be derived from surface tension force [74].

\[
\frac{d\rho}{dL} = \frac{2\sigma}{r_{MB}(\frac{L_{NF}}{2} + r_{MB})}
\]  

(3-8)

where \( \sigma \) is the solution surface tension. The final equation Eq. (3-9) can be obtained by substituting Eq. (3-8) into Eq. (3-3). The values of \( \mu \) and \( \sigma \) are obtained from the solution condition, and the values of \( v_f \) and \( r_{\text{PCL}} \) are extracted from Eq. (3-6), and Eq. (3-7), respectively.

\[
Q = \pi r_{\text{PCL}}^2 v_f = \frac{\pi r_{\text{PCL}}^4 d\rho}{8\mu dL} = \frac{\pi r_{\text{PCL}}^4}{8\mu} \frac{2\sigma}{r_{MB}(\frac{L_{NF}}{2} + r_{MB})}
\]  

(3-9)

Figure 3-7 shows the microbead diameter as a function of TCD of the experimental result and the analytical prediction. The experiments are conducted using 4 % PCL solution with an electric field of 1 kV/cm. The derived analytical prediction shows good agreement with the experimental data measured by ImageJ with the assumptions of the same solution flow rate throughout the pumping and whipping process and constant electric field conditions.
Two-Phase Release Measurement

Figure 3-8(a-b) show the two-phase hybrid membrane 1 and 2, with a PCL concentration of 4 wt%, an electric field of 1 kV/cm, and a TCD of 12.5 cm and 7.5 cm, respectively. The nanofiber and microbead diameters for hybrid membrane 1 and 2 are $108 \pm 51$ nm and $1560 \pm 600$ µm, and $92 \pm 28$ nm and $3680 \pm 1500$ nm, respectively. The volume percentage of microbeads in hybrid membrane 1...
and 2 are 40.15 % and 91 %, respectively. Table 3-2 shows the detailed solution conditions and electrospinning parameters of hybrid membrane 1 and 2.

Figures 3-9 show the dexamethasone release profiles from two-phase hybrid membrane 1 and 2. The release durations of single-phase membranes, either nanofibers or microbeads, are less than 8 hours. The short releasing duration is attributed to the small diameter of the nanofibers/microbeads. The release duration times for two-phase hybrid membrane 1 and 2, which consist of both nanofibers and microbeads, are greater than 8 hours.

Table 3-2. Summary of the solution composition and electrospinning parameters for two-phase hybrid membrane 1 and 2.

<table>
<thead>
<tr>
<th>Solvent</th>
<th>PCL Concentration w/v %</th>
<th>Tip-to-collector distance (cm)</th>
<th>Electrical Field (kV/cm)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Hybrid 1</td>
<td>DCM/DMF</td>
<td>4</td>
<td>12.5</td>
</tr>
<tr>
<td>Hybrid 2</td>
<td>DCM/DMF</td>
<td>4</td>
<td>7.5</td>
</tr>
</tbody>
</table>

Figure 3-9. Dexamethasone release profiles of two-phase hybrid structures with different average microbead diameters of 1.5 μm and 3.68 μm. Insert shows an SEM of the hybrid structure.
microbeads, are about a month or longer. Moreover, two-phase hybrid membrane 1 and 2 show a release burst in the first few hours, then a much slower and steady release. This is observed due to the hybrid structure consisting of nanofibers and microbeads. The first stage of release, the burst, is suspected to be due to the nanofiber, and the following second stage is due to larger diameters of the microbeads.

**Diffusion Mathematical Modeling**

With the experimental release profiles of single-phase and two-phase membranes, a mathematical release model is developed which consists of microbeads and nanofibers. Assuming the drug transport is diffusion-controlled, the cumulative drug release from the spherical and cylindrical devices can be described by the following equation [75]:

\[
\frac{M_t}{M_\infty} = 1 - \frac{6}{\pi^2} \sum_{n=1}^{\infty} \frac{1}{n^2} \exp \left(-\frac{D n^2 \pi^2 t}{R_s^2}\right) \tag{3-10}
\]

\[
\frac{M_t}{M_\infty} = 1 - \frac{32}{\pi^2} \sum_{n=1}^{\infty} \frac{1}{q_n^2} \exp \left(-\frac{q_n^2}{R_c^2} \Delta t\right) \cdot \sum_{p=0}^{\infty} \frac{1}{(2p+1)^2} \exp \left[-\frac{(2p+1)^2 \pi^2}{H^2} \Delta t\right] \tag{3-11}
\]

where \(M_t\) is the cumulative drug release at time \(t\), \(M_\infty\) is the total mass of drug loaded, \(D\) is the effective diffusivity of the drug, \(R_s\) is the radius of the microbeads, \(R_c\) and \(H\) are the radius and height of the nanofibers, and \(q_n\) is the roots of the zero-order Bessel function of the first kind. Eq. (3-11) considers the drug release from both the curved surface of the cylinder as well as the two circular ends. Since the cylinders in the electrospun mats are connected to the spheres, we can neglect the drug release from the circular ends to simplify Eq. (3-11) to the following

\[
\frac{M_t}{M_\infty} = 1 - \sum_{n=1}^{\infty} \frac{4}{q_n^2} \exp \left(-\frac{q_n^2}{R_c^2} \Delta t\right) \tag{3-12}
\]
The complete release model of hybrid membrane 1 and 2 consisting of both nanofibers and microbeads can thus be described by combining Eq. (3-10) and (3-12),

\[
\frac{M_t}{M_{\infty}} = 1 - \left\{ V_c \cdot \left[ \sum_{n=1}^{\infty} \frac{4}{q_n^2} \exp \left( -\frac{q_n^2}{R_c^2} Dt \right) \right] + V_s \cdot \left[ \frac{6}{\pi^2} \sum_{n=1}^{\infty} \frac{1}{n^2} \exp \left( -\frac{Dn^2\pi^2 t}{R_s^2} \right) \right] \right\}
\]  

(3-13)

where \( V_c \) and \( V_s \) are the volume fractions of the nanofiber and microbead, respectively. Note that Eq. (3-13) is only valid under the assumption that the drug is uniformly distributed and the releases of dexamethasone from the nanofiber and microbead are independent of each other. The equation also assumes that the release profiles are controlled by the radial diffusion through the fiber and the beads, while the transverse diffusion in the mats is rapid.

The drug release from nanofiber membrane 1 and 2 can be described by Eq. (3-12). The values of \( R_c \) and \( R_s \) are obtained from SEM images analysis, and the unknowns in Eq. (3-12) and (3-13) including \( D, V_c, V_s \) are determined by fitting the experimental data to the equations using the function “fminsearch” in Matlab®. The

Figure 3-10. Dexamethasone release profiles of (a) single phase and (b) two-phase membranes. The solid lines are the best fit results based on the diffusion control model. “Drug release %” is calculated as the ratio of cumulative release at any time and the total dexamethasone mass loaded in PCL. Data are presented as mean ± SD with \( n = 3 \).
best fitting curves are plotted with the experimental data as shown in Figures 3-10. The mathematic model is in good agreement with experimental data, which confirms the release mechanism is due to drug diffusion inside the PCL polymer. Since PCL has a degradation profile about a year, no significant degradation is observed during the experiment. Additionally, all release data fit a constant diffusivity model, which further suggests that degradation has not impacted the drug transport. Table 3-3 lists the dimension and chemical ratio of the samples obtained from the image analysis software, ImageJ.

Table 3-3. Summary of the structural characterization (diameters and volume fractions) and drug diffusivity in single-phase and two-phase hybrid membranes.

<table>
<thead>
<tr>
<th></th>
<th>Nanofiber 1</th>
<th>Nanofiber 2</th>
<th>Hybrid 1</th>
<th>Hybrid 2</th>
</tr>
</thead>
<tbody>
<tr>
<td>Diameter of fiber (nm)</td>
<td>300</td>
<td>670</td>
<td>108</td>
<td>92</td>
</tr>
<tr>
<td>Volume % of fiber</td>
<td>100</td>
<td>100</td>
<td>59.85</td>
<td>8.70</td>
</tr>
<tr>
<td>(Experimental)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Volume % of fiber</td>
<td>100</td>
<td>100</td>
<td>64.26</td>
<td>43.32</td>
</tr>
<tr>
<td>(Model prediction)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Diameter of bead (nm)</td>
<td>N/A</td>
<td>N/A</td>
<td>1500</td>
<td>3680</td>
</tr>
<tr>
<td>Volume % of bead</td>
<td>N/A</td>
<td>N/A</td>
<td>40.15</td>
<td>91.30</td>
</tr>
<tr>
<td>(Experimental)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Volume % of bead</td>
<td>N/A</td>
<td>N/A</td>
<td>35.74</td>
<td>56.68</td>
</tr>
<tr>
<td>(Model prediction)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Diffusivity in fiber</td>
<td>725.5 ± 111.0</td>
<td>1286.5 ± 77.1</td>
<td>18.07 ± 7.20</td>
<td>2.59 ± 0.48</td>
</tr>
<tr>
<td>($10^{-11}$ mm²/hr)*</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Diffusivity in bead</td>
<td>N/A</td>
<td>N/A</td>
<td>14.27 ± 1.65</td>
<td>97.34 ± 11.53</td>
</tr>
<tr>
<td>($10^{-11}$ mm²/hr)*</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

*The data are presented as mean ± SD, with n = 3.
As a consistency check, the values of Vc and Vs obtained from data fitting were compared to that obtained directly from the SEM images for hybrid membrane 1 and 2. For hybrid membrane 3, the volume percentage of the fibers and beads predicted by the sink model were in good agreement with SEM image analysis. A discrepancy of about 30% was observed in hybrid membrane 2 between the model prediction and the SEM image analysis suggesting that the drug concentration may not be uniform. Alternatively, the differences could be due to the errors in estimation of the volume fractions directly from the images because the relative volume of the fibers was small. Interestingly, there were considerable differences between the fitted diffusivity for various samples and also between the fibers and the beads in the same sample.

For instance, the fitted diffusivity for nanofiber membrane 1 was significantly larger than that for nanofiber membrane 2, and the diffusivity of the fibers in hybrid membrane 1 was significantly lower than that of nanofiber membrane 1 and 2. The diffusivity values for the fibers and the beads from hybrid membrane 1 are comparable, but the diffusivities for the fibers and the beads differ significantly for hybrid membrane 2. This could potentially be an artifact of errors in measuring the fiber sizes from the SEM images because the relative volume of the fibers was small. Since all materials considered here are PCL matrices, the differences in diffusivity suggest that there are differences in the polymer alignment or equivalently the degree of crystallinity across these samples. The differences in crystallinity could arise in electrospun mats due to the significant stretching during the electrospinning process.

**Effect of Crystallinity on Drug Diffusivity**

The polymer crystallinity and orientation of the electrospun membrane could depend on several processing parameters [76], and it may affect the drug diffusivity. X-
Ray powder diffraction (XRD, PANalytical XPert Powder) is used and operated at 40 kV and 20 mA for the measurement. X-ray diffraction profiles are measured in the transmission mode and fitted by using the XPert HighScore Plus software. The degrees of crystallinity are analyzed with full width at half maximum (FWHM) calculations. We hypothesized that the electrospun membranes are more crystalline compared to spin cast films due to the high electrostatic stress during the whipping process. For comparison, the degree of crystallinity and drug transport in spin cast PCL film is also measured. The spin cast PCL thin film is prepared by spin coating the 16% (w/v) PCL in 1:1 mixture of DCM and DMF on a glass substrate. The spin cast film is dried at room temperature for 24 hrs to evaporate the solvents.

Figure 3-11 shows the XRD measurements of nanofiber membranes, hybrid membranes, and a 100 μm thick spin cast PCL film. The height of the peaks cannot be directly compared but the degree of crystallinity can be inferred from the number of

![XRD diffractogram for the electrospun single-phase and two-phase membranes compared to the spin cast PCL film.](image-url)
peaks and the width of the peaks as higher crystalline samples exhibit narrower peaks. The electrospun mats exhibit more and narrower peaks which is an evidence of higher crystallinity. The calculated full width at half maximum (FWHM) values for the two main surfaces (110 and 200) are listed in Table 3-4 for nanofiber membranes, hybrid membranes, and the spin cast film. The data clearly shows that the electrospun membranes are more crystalline compared to the spin cast film. The crystallinity of the electrospun membranes can be attributed to the shear stress generated in the processing that can align the polymer. Table 3-4 shows that nanofiber membrane 1 is more crystalline than nanofiber membrane 2, which explains the difference in effective

<table>
<thead>
<tr>
<th></th>
<th>Spin cast PCL film</th>
<th>Nanofiber 1</th>
<th>Nanofiber 2</th>
<th>Hybrid 1</th>
<th>Hybrid 2</th>
</tr>
</thead>
<tbody>
<tr>
<td>PCL_{110}</td>
<td>1.207</td>
<td>0.334</td>
<td>0.450</td>
<td>0.269</td>
<td>0.377</td>
</tr>
<tr>
<td>PCL_{200}</td>
<td>1.912</td>
<td>0.376</td>
<td>0.393</td>
<td>0.371</td>
<td>0.527</td>
</tr>
</tbody>
</table>

Figure 3-12. Dexamethasone release profile of 100 µm thick PCL spin cast film. The solid lines are the best fit results based on the sink model. “Drug release %” is calculated as the ratio of cumulative release at any time and the total dexamethasone mass loaded in the film.
diffusivity. Nanofiber membrane 2’s solution jet, which has a higher solvent evaporation rate, is solidified completely during the whipping process, resulting in lower crystallinity due to less stretching by electrostatic force. Moreover, XRD results and FWHM calculations of hybrid membrane 1 and 2 show even better crystallinity compared to nanofiber membrane 1 and 2, which is consistent with the reduced diffusivities. These results suggest that the electrospinning process can enhance polymer crystallinity and orientation which results in reducing the effective diffusivity.

Since the PCL spin cast film was less crystalline compared to the electrospun membranes, we hypothesized that the drug diffusivity should be significantly higher in the spin cast films. The drug release profile of the PCL spin cast film is shown in Figure 3-12. The spin cast film can be viewed as a homogenous porous polymer film. The extracted effective diffusivity of spin cast film is $3.67 \times 10^{-6} \text{ mm}^2/\text{hr}$, which is roughly 350 times larger than that of Nanofiber membrane 1 and 2.

**Effect of Nanofiber Barrier Layer**

A multilayer membrane consisting of a drug loaded microbead layer sandwiched by nanofiber barrier layers without drug loading is fabricated by DSE to study the effect of the nanofiber barrier layer. 18 % PCL and 2 % PCL with DX are loaded in DSE for the alternating electrospinning process. Before PCL electrospinning, an electrospun polyvinylpyrrolidone (PVP) nanofiber layer is deposited on a silicon substrate as a sacrificial layer. Note that PVP is water soluble. The sandwiched PCL multilayer structure is easily released from the substrate when PVP is dissolved in a PBS solution. After depositing a sacrificial PVP layer, a 40 μm thick PCL nanofiber layer is electrospun at 1 kV/cm with a TCD of 10 cm. Then, single-phase PCL microbeads with DX loaded are electrospun at 1 kV/cm and a TCD of 10 cm for 2 mg of microbeads deposition.
Finally, another 40 μm thick PCL nanofiber layer is deposited, which serves as the top barrier layer with the same electrospinning condition.

Figure 3-13 shows the release profile of the sandwiched multilayer membrane. The inserted images show the device schematic and SEM image of the multilayer architecture. The release profile shows a reduced burst release but highly linear steady release compared to both single phase and two phase membranes. The small burst release is caused by the hydrophobicity of the PCL nanofiber barrier layer, which results in the delay of water penetration, and thus the diffusion of the drug through the barrier layer into the PBS is retarded. After an initial period, drug was released in a much slower and more linear fashion, where the release rate is determined by the diffusion of the drug through the porosity of the nanofiber barrier layers. The linear steady release is ideal for a drug delivery system to provide an appropriate dosage throughout the entire

![Figure 3-13. Dexamethasone release profile from the sandwiched multilayer membrane. The solid lines are the best fit results based on the sink model. “Drug release %” is calculated as the ratio of cumulative release at any time and the total dexamethasone mass loaded in the film.](image)
release duration without potential over dosage, toxicity, and side effects. DX release time has been greatly increased, e.g. extended up to 80 days by the additional nanofiber barrier layers.

Table 3-5 shows the diameter and release duration comparison of single phase, two-phase, and sandwiched multilayer membranes. Single phase membranes for both nanofibers and microbeads have the rapid and short release profiles such as a few hours due to smaller diameters. Two-phase hybrid membranes show an extended release duration of 30 days. However, two different release rates are observed due to the different morphology of nanofiber and microbead. Sandwiched multilayer membranes have the longest release duration with a microbead diameter of only 0.86 μm. The PCL nanofiber barrier layers serve as a limiting factor for PBS solution intake and diffusion of DX to achieve an extended and linear release profile.

The PCL based extended drug delivery systems with single-phase, two-phase, and sandwiched multilayer structures have been implemented using the SSE and DSE methods. PCL solutions with various concentrations and TCDs are studied to modulate the release properties.

Table 3-5: Summary of nanofiber and microbead average diameters with corresponding release time for all samples.

<table>
<thead>
<tr>
<th></th>
<th>NF diameter [μm]</th>
<th>MB diameter [μm]</th>
<th>Release time (80%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Nanofiber 1</td>
<td>0.31</td>
<td>N/A</td>
<td>0.75hr</td>
</tr>
<tr>
<td>Nanofiber 2</td>
<td>0.67</td>
<td>N/A</td>
<td>1.92hr</td>
</tr>
<tr>
<td>Microbead</td>
<td>N/A</td>
<td>0.86</td>
<td>3.70hr</td>
</tr>
<tr>
<td>Hybrid 1</td>
<td>0.11</td>
<td>1.52</td>
<td>3.40day</td>
</tr>
<tr>
<td>Hybrid 2</td>
<td>0.09</td>
<td>3.68</td>
<td>15.9day</td>
</tr>
<tr>
<td>Sandwiched</td>
<td>0.61</td>
<td>0.86</td>
<td>46.7day</td>
</tr>
</tbody>
</table>
the morphology of electrospun microbeads and nanofibers. The DX release profile and duration time can be designed and controlled by the morphology and architecture of the electrospun membrane. The two-phase hybrid membrane shows a two-phase release profile with a rapid release in the first few hours and a slow steady release for 30 days. This can be used for applications that require higher dosage throughout the process. The sandwiched multilayer membrane shows an extended release profile with a low burst release, a long release duration of 80 days, a highly linear release rate, and no dispersion throughout 80 days. The two-phase hybrid and sandwiched multilayer membranes could be used as an infrequent treatment alternative to satisfy different application needs. While the results are encouraging, further in-vivo animal studies are suggested.

**Mechano-Active Nanofibrous Scaffold**

The primary goal of nanofiber technology for tissue engineering is producing an in-vitro cultured tissue that can be implanted in-vivo. Many passive nanofibrous scaffolds which only provide mechanical support have already shown improvement in cell proliferation [77]. However, estimations of thermal, electrical, or mechanical stress to the target cell still remain as a challenge to implement with these passive scaffolds. An active nanofibrous scaffold is the potential solution for tissue engineers to study the effects of external stresses.

An active tissue scaffold with various external stimulation modalities has attracted great attention for advanced tissue engineering research [78-79]. Especially, these scaffolds may be the key to understanding differentiation conditions of stem cells. Understanding the influence of different active stimuli and controlling cell viability and differentiation are critical for future biomedical stem cell research. Alternating external
electric fields at low frequency have also been studied to enhance neural cell viability due to electrokinetically driven flow. Electrokinetic driven flow can also either enhance or suppress neural cell differentiation [80]. An European research group has reported that the efficiency of stem cell harvesting is maximized with 91.6-95 % cell viability when cultured in a condition of a vibrating frequency of approximately 5 Hz [81].

In this work, a mechano-active nanofibrous scaffold system, which can provide mechanical stress on culturing cells by oscillating external magnetic fields, is demonstrated. The system consists of iron oxide nanoparticles embedded in an electrospun PCL nanofiber membrane, a membrane holder, and an external electromagnet producing alternating magnetic fields. Iron oxide nanoparticles are good candidates due to their clear response to magnetic fields and because they

![Figure 3-14](image)

Figure 3-14. (a) Schematic of the proposed architecture of a magnetic nanofibrous scaffold placed above and the response to the magnetic field generated by an electromagnet. (b) Circular plate with uniform load and edge clamp.
are bio-friendly. The nanoparticles have a controllable size of less than 50 nm, which is less than 5 vol% of most of cell sizes (1–100 μm) [82]. Due to their nanoscale size, magnetic nanoparticles can be incorporated in the bio-polymer, polycaprolactone, to increase the interaction with a biological entity such as tissue cells. Polycaprolactone is well known as a biodegradable polymer and has been studied for tissue engineering for many years [83]. The magnetic electrospun nanofiber scaffold is a feasible method for tissue engineering. The system will operate at 4.5 Hz and a nominal displacement in an order of 100 nm.

**Design Principle**

Figure 3-14a shows the schematic of an active magnetic nanofibrous scaffold placed 2 cm above an electromagnet which actuates the scaffold by generating a magnetic field. The magnetic nanofibrous membrane oscillation frequency can be controlled by controlling the AC magnetic field frequency generated from the electromagnet.

The magnetic nanofibrous membrane can be considered as a thin circular membrane with uniform thickness \( l \) and radius \( R \), where the thickness is much smaller than the radius. The magnetic nanofibrous membrane will undergo a mechanical bending when a magnetic field is applied as shown in Figure 3-14b. Once the magnetic field is turned off, the membrane bends back to its original form. This property gives us the ability to oscillate the membrane by applying an AC signal to the electromagnet to create an oscillating field. The displacement \( U(r, z, t) \) of the membrane in z-direction is described by the Kirchhoff-Love's theory [84].

\[
D \nabla^4 U + \frac{1}{1-\nu} \nabla^2 M_T + \rho l \frac{\partial^2 U}{\partial t^2} = 0
\]  

(3-14)
where \( t, l, \rho, \) and \( \nu \) are the time, thickness, density, and Poisson ratio, respectively. The flexural rigidity \( (D) \) is defined by
\[
D = \frac{E l^3}{12(1-\nu^2)}
\]
(3-15)

where \( E \) is the Young's modulus. The resonant frequency of a thin membrane is defined by:
\[
f_n = \frac{1}{2\pi} \sqrt{\frac{D}{\rho l}} \left( \frac{\lambda_n}{R} \right)^2
\]
(3-16)

where Young's modulus can be defined from rigidity and resonate frequency equations.
\[
E = 48\pi^2 (1 - \nu^2) \rho \left( \frac{f_n}{l} \right)^2 \left( \frac{R}{\lambda_n} \right)^4
\]
(3-17)

The force on the membrane can be modeled as point charge due to the electromagnetic field and Lorentz force theorem can be applied [85]. The Lorentz force for a 2-D planar structure can be defined as,
\[
F = IA \times B = m \times B
\]
(3-18)

where \( IA \) can be replaced by magnetic moment, \( m \). The pressure is defined as the force per unit area.
\[
P = \frac{F}{A}
\]
(3-19)

Considering the uniform pressure and edge fixed membrane, the maximum principle stress happens at the center of the membrane.
\[
\sigma_m = \frac{3(3+\nu)Pr^2}{8lt^2}
\]
(3-20)
\[
d_m = \frac{Pr^4}{64D} = \frac{Pr^4}{64} \frac{12(1-\nu^2)}{Et^3} = \frac{3Pr^4(1-\nu^2)}{16Et^3}
\]
(3-21)

where \( \sigma_m \) is the principle stress, \( d_m \) is the membrane deflection displacement at the center point, \( r \) is the radius, \( t \) is the thickness, and \( p \) is the pressure on the membrane.
The residual stress can be calculated by measuring the load deflection of the membrane [86]. The residual stress of the membrane can be defined as,

\[
\left(\frac{E t}{r^2}\right) d^3 + \left(\frac{1.66 t d}{r^2}\right) d = 0.547 p
\]  

(3-22)

\[
\sigma = \frac{r^2}{1.66 t d} \left[0.547 p - \left(\frac{E t}{r^2}\right) d^3\right]
\]  

(3-23)

where \(\sigma\), \(E\), \(d\), \(r\), \(t\) is the residual stress, Young's modulus, membrane deflection, radius, and thickness. Pressure \(p\) is determined in Eq. (3-23). The Poisson ratio \((\nu)\) of the PCL is 0.46 and the density \((\rho)\) of the nanofibrous membrane is 572.85 kg/m\(^3\).

The displacement and resonate frequency is simulated by COMSOL Multiphysics 4.3 (COMSOL Package Inc.). The magnetic flux is designed to be less than 10 gauss to prevent any possible field damage to cell. The target displacement is 100 nm, and designed frequency is 4.5 Hz. With design parameters, the magnetic nanofibrous membrane dimension is simulated to have a diameter of 2 cm and a thickness of 50 \(\mu\)m.

**Fabricated Process**

PCL (Sigma Aldrich Inc.), a biodegradable polymer, is dissolved in dimethylformamide (DMF, Sigma Aldrich Inc.) and dichloromethane (DCM, Sigma Aldrich Inc.) for 24 hr to give a solution concentration of 16 wt/vol\%. Then, 5 wt\% of iron oxide nanoparticles (Sigma Aldrich Inc.) is mixed with polycaprolactone solution. Iron oxide nanoparticles have an average diameter less than 50 nm, which may also enhance surface roughness of the PCL nanofibers and result in better cell attachment. The magnetic nanoparticle embedded polymer solution was prepared by mechanically stirring by a DC motor at 500 rpm in an air tight bottle. Figure 3-15 shows the fabrication process of the active magnetic scaffold. First, a 2 inch Si wafer was cleaned by immersing in 6:1 buffered oxide etchant for 1 min, and then rinsed with DI water to
remove the natural silicon oxide insulation layer to give a better nanofiber collecting rate. Magnetic nanofibers were electrospun for 240 sec to collect a 50 \( \mu \)m thick membrane on the Si wafer. The magnetic PCL solution with DMF and DCM was electrospun with a flow rate of 1 ml/min, a tip-to-collector distance of 12.5 cm, and a voltage of 15 kV. Second, an O-ring shape membrane holder was micromachined from a PCB Prototyping machine (ProtoMat S100. LPKF Laser & Electronics AG, Germany) with an inner diameter of 2 cm and an outer diameter of 5 cm. Non-toxic water soluble glue was used to attach the O-ring holder on the Si wafer to a 50 \( \mu \)m thick magnetic nanofibrous membrane. Third, the O-ring holder and Si wafer were placed inside a vacuum chamber for 2 hrs to evaporate the excess solvents in the magnetic nanofibers and the non-toxic glue. After the drying process, the O-ring holder with the magnetic nanofibrous membrane was separated from the Si wafer, and the membrane is sandwiched.

**Figure 3-15.** Fabrication process of the active magnetic nanofibrous scaffold with another O-ring holder to increase stability and handle ability.
The fabrication process is scalable, manufacturable, and cost effective. A 7 days in-vitro cell culture analysis on the magnetic nanofibrous scaffold was performed using mouse cells and cultured with Dulbecco’s Modified Eagle Medium/Ham’s F-12 (DMEM/F12) and 10 % phosphate buffered saline (PBS) solution.

**Fabricated Magnetic Nanofiber Scaffold**

The optical images of the fabricated scaffold and the scanning electron microscope (SEM) (JEOL 5700) images of the magnified magnetic nanofiber (MNF) are shown in Figure 3-16. The color of magnetic nanofibers changes from white to dark orange and is evidence of iron oxide nanoparticles. The SEM image shows a rough magnetic nanofiber surface evidently supporting the iron oxide nanoparticles distributed in nanofibers. Electrospinning has been performed in different voltage and TCD conditions ranging from 12.5 kV to 17.5 kV and 12.5 cm to 17.5 cm, respectively.

Figure 3-17 shows the nanofiber diameters from 583 ± 36 nm to 742 ± 42 nm with different electrospinning conditions. The density of iron oxide (Fe$_2$O$_3$) is 5,242 kg/m$^3$.

![Figure 3-16. Photography of the fabricated scaffold. The insert shows SEM picture of the electrospun nanofibers with embedded iron oxide nanoparticles.](image)
and the density of PCL is 1,145 kg/m$^3$. The nanofibrous membrane has a total volume of $1.5708 \times 10^{-8}$ m$^3$ with a density of 572.85 kg/m$^3$, which contains air, PCL, and Fe$_2$O$_3$. The ratio of PCL and Fe$_2$O$_3$ after both DMF and DCM evaporated during the electrospinning and vacuum drying processes is 22 wt% of Fe$_2$O$_3$ in PCL with the density of 2,046.34 kg/m$^3$. The mass of the magnetic nanofiber in the membrane is $8.998 \times 10^{-6}$ kg and calculated nanofibers volume is $4.397 \times 10^{-9}$ m$^3$. The air volume can be calculated by subtracting the magnetic nanofibers volume from the total membrane volume, where the air volume is $1.13 \times 10^{-8}$ m$^3$. Then the porosity of the nanofibrous membrane can be determined by the percentage of the nanofiber volume in the total volume of membrane, which is 72%.

Magnetic and Mechanical Characterization

The magnetic hysteresis loops of magnetic nanofibrous membrane were measured by Vibrating Sample Magnetometer (ADE technologies) with the magnetic field aligned perpendicular to the membrane, which is out of plane. Figure 3-18 shows the nanofiber diameter variation at different electric field strengths.

![Figure 3-17. Nanofiber diameter variation at different electric field strengths.](image)
the magnetic moment vs. magnetic field curve. Multiple property parameters such as effective relative permeability and magnetization can be extracted from the hysteresis loop. The relative permeability can be calculated by magnetization and magnetic field strength.

\[
B = \mu_o(H + M) = \mu_o(H + XH) = \mu_o(1 + X)H \tag{3-24}
\]

\[
\mu_r = 1 + X = 1 + \frac{M}{H} \tag{3-25}
\]

The calculated effective relative permeability varies from 1.05 to 1.07 for the fibers collected in an electric field of 1.16 kV/cm to 0.83 kV/cm, respectively. With increasing electric field strength, the nanofiber diameter decreases and the porosity of the membrane increases. Therefore, fewer iron oxide nanoparticles are embedded in the membrane as the electric field increased.

Figure 3-18. Hysteresis loops of 3 different membranes with nanofibers collected in different electric field strengths: sample 1:1.16 kV/cm, sample 2:1 kV/cm, sample 3:0.83 kV/cm.
The magnetic field is generated by a round electromagnet (APW Inc.) with a diameter of 1.75 inches in this experiment. However, this electromagnet does not provide a uniform magnetic field across the membrane. Therefore, a better understanding of the non-uniform magnetic field distribution effect becomes an important factor so COMSOL Multiphysics 4.3 (COMSOL Package Inc.) simulation is performed. The displacement of the center of the membrane to uniform a DC magnetic field is simulated and calculated as shown in Figure 3-19a. The displacement at the center point of the membrane shows a linear relationship as the magnetic flux density varies from 15 mT to 35 mT.

Figure 3-19b shows the simulated, calculated, and measured displacement response for a non-uniform DC magnetic field scenario. The maximum displacement also shows a linear relationship, but displacement is reduced to 5% due to the total magnetic field reduction on the membrane. The measurement results show a similar trend and match with the non-uniform field numerical calculation and simulation results.

Figure 3-19. Simulated, numerically calculated, and measured DC response of the magnetic nanofibrous scaffold, (a) uniform magnetic field across membrane, (b) non-uniform magnetic field applied.
A laser vibrometer has been used for the AC displacement and self-resonate frequency measurement. Figure 3-20 shows the fabricated magnetic nanofibrous membrane has an AC response with a self-resonant frequency of 4.43 Hz, a maximum displacement of 91 nm, and a Q-factor of 14 in an air environment. The effective Young's modulus at the first resonant mode is calculated as 0.127 MPa. The magnetic nanofibrous membrane is also simulated in typical cell culture environment, which is

![Graph showing AC response of the fabricated magnetic nanofibrous membrane](image)

Figure 3-20. Simulated and measured resonance frequencies of the nanofibrous membranes compared to the simulated resonance frequency of a solid PCL membrane.

Table 3-6. Mechanical characteristics of the nanofibrous and solid membranes

<table>
<thead>
<tr>
<th></th>
<th>Nanofiber membrane</th>
<th>Solid PCL membrane</th>
</tr>
</thead>
<tbody>
<tr>
<td>Density</td>
<td>572.85 kg/m$^3$</td>
<td>1145 kg/m$^3$</td>
</tr>
<tr>
<td>Porosity</td>
<td>72%</td>
<td>0%</td>
</tr>
<tr>
<td>Relative Permeability</td>
<td>1.05-1.07</td>
<td>1</td>
</tr>
<tr>
<td>Self-Resonant Frequency</td>
<td>4.5 Hz</td>
<td>80.5 Hz</td>
</tr>
<tr>
<td>Effective Young's Modulus</td>
<td>0.127 MPa</td>
<td>90 MPa</td>
</tr>
</tbody>
</table>
25 °C of phosphate buffer solution (PBS) with pH level of 7.2. Since at 25°C, PBS with 7.2 pH level has a density of 0.995 g/ml, which is much higher than air environment, the self-resonant frequency shifts down to 3 Hz. The measurement results are well matched with the designed and simulated results. The nanofibrous membrane has a lower density, self-resonant frequency, and effective Young's modulus due to its nanoporous morphology. The mechanical properties of the nanofibrous and solid membrane are characterized and summarized in Table 3-6.

**In-Vitro Cell Culture Result**

An *in-vitro* cell viability test has been performed with mouse adipose stem cells

![Images of the mouse cell viability test](image)

Figure 3-21. Images of the mouse cell viability test for 5 days: (A) on PCL nanofibers (2x) (B) on MNP embedded nanofibers (2x) (C) on PCL nanofibers (40x) (D) on MNP embedded nanofibers (40x). MNP embedded nanofibers show superior cell viability to PCL nanofibers. Blue: nuclei, Green: cytoplasm.
from mouse fat tissue for 7 days using the PCL nanofibrous membranes with and without MNP embedded. The external magnetic field was not applied in either case. Mouse cells are pre-cultured in Dulbecco's Modified Eagle Medium with Nutrient Mixture F-12 (DMEM/F12) and 10% PBS solution for 3 days to stabilize the condition and prepare to be seeded. Then, cells are labeled with Dil (Life Technologies cat # V22889) and seeded on both nanofibrous membranes. After a 7 day culture in DMEM/F12 and 10% PBS solution, cells are fixed and fluorescent stained with 4',6-diamidino-2-phenylindole (DAPI) and 488 fluorochrome for cytoplasm. Cells attached on both the pure PCL nanofibrous membrane and the one with magnetic nanoparticles embedded. The magnetic nanofibrous membrane shows much higher cell attachment and cell viability results as shown in Figure 3-21.

**Summary**

Electrospun biodegradable polycaprolactone (PCL) membranes for active cell cultures and extended drug delivery applications have been demonstrated in this chapter. Magnetic nanofibrous membranes have been fabricated by electrospinning with PCL solutions with 5 wt% of iron oxide nanoparticles dispersed. The nanofibrous membrane is designed and fabricated using a simple, cost effective, and scalable process. The self-resonate frequency and mechanical properties of the nanofibrous membrane have been characterized and compared with bulk PCL. The numerical calculation results show great agreement with simulation and measurement results. The active membrane dimensions are optimized to resonate at 4.5 Hz for maximum stem cells proliferation. Increased cell viability is observed on the fabricated magnetic nanofibrous scaffold compared to a conventional PCL nanofibrous scaffold.
Electrospun drug embedded PCL membranes have been studied for extended drug delivery applications. Various membrane morphologies such as single-phase, two-phase, and sandwiched multilayer structures have been fabricated using the conventional electrospinning and dual syringe electrospinning setups. Electrospun membranes with various PCL concentrations and tip-to-collector distance are characterized and studied. The solution conditions for single-phase nanofiber, two-phase hybrid, and single-phase microbead morphologies are identified. The drug release duration and profile can be designed and controlled by the modulating electrospun membrane morphology and architecture. An analytical model for microbead diameter prediction has been developed and the results show great agreement with measurement results. The two-phase hybrid membranes show release profiles consisting of a rapid release in the first few hours and a slow steady release up to a month due to the morphology difference. The two-phase membranes can be used for treatments that require a higher dosage initially then a lower dosage for an extended period of time. An extended release profile with a low burst release, a long release duration of 80 days, a highly linear release rate, and no dispersion throughout 80 days is achieved with sandwiched multilayer membranes. The release profiles suggest that electrospun two-phase hybrid and sandwiched multilayer membranes can be alternative candidates for infrequent treatment applications.
CHAPTER 4
ELECTRICALLY CONDUCTIVE NANOFIBER MICROSTRUCTURE

As electrospinning nanofiber technology rapidly advances, researchers have discovered ways to produce electrically conductive nanofiber. Electrically conductive nanofiber has gained popularity for many different applications in electrical engineering such as energy storage devices, sensors, and smart wearables.

**Nanofiber Composite Strain Gauge Sensor**

The demand for flexible, stretchable, and wearable sensors which can be easily integrated on or in clothing and apparel or directly attached on the skin continues to grow. One major application is motion detection, which requires highly stretchable and sensitive sensors. Usage of elastomer/conductor nanocomposites with carbon nanotubes [87], graphene nanoribbons [88], and silver nanowires [89-90] was reported. However, these sensors either lack stretchability (< 10%) or show poor sensitivity (gauge factor < 3) as the nanoparticle/ribbon and short segmented nanowires in the elastomer are not stretchable, resulting in a limited working span. Electrospun nanofibers are inherently continuous and randomly oriented in deposition, forming a microscale spring. As the continuous tangled electrospun nanofibers provide high stretchability and piezoresistivity, the resistivity of the carbonized nanofiber can be utilized for a strain gauge application. Carbonization of the electrospun nanofibers enables the formation of stretchable conductive polymers able to be embedded in elastomers.

_______________________________
Design Concept and Working Principle

Figure 4-1A shows the working principle of a carbon nanofiber composite strain gauge sensor for smart wearables. The strain gauge sensor is flexible and compact enough to be integrated with wearable systems. Figure 4-1B shows the proposed architecture of the composite strain sensor, consisting of CNFs embedded in PDMS. As the sensor experiences mechanical strain, the CNF/PDMS nanocomposite stretches like a microspring. The core fabrication steps include electrospinning, photolithography, carbonization, and polymer embedding.

Fabrication Process and Device of Nanofiber Composite Sensor

Figure 4-2 shows the fabrication process of a CNF/PDMS nanocomposite strain gauge sensor. First, a clean silicon (Si) wafer is prepared by solvent cleaning and Piranha cleaning. Second, SU-8 nanofibers are electrospun using the standard electrospinning setup with an electric field of 1 kV/cm, a distance between the needle tip to collector of 15 cm, and a polymer flow rate of 1 ml/min directly on a silicon wafer. Third, the SU-8 nanofibers are patterned using oil-immersion lithography and exposed to UV light from the top. After oil immersion lithography, the uncross-linked SU-8

![Figure 4-1. Schematic of the wireless smart wearables (A). Proposed carbon nanofiber (CNF) strain sensor with flexible PDMS (B).](image)


nanofibers are developed. Fourth, high temperature carbonization is performed at 1000 °C with an optimized ramp rate of 3 °C/min under a forming gas environment with a flow rate of 13 slm. After carbonization, the elastomer, PDMS, in liquid form is prepared and poured onto the sample and heated at 80 °C for 2 hrs to cure the PDMS. Then the CNF/PDMS composite is separated from the Si wafer and silver epoxy is applied to connect the wire. Finally, PDMS is applied to the backside to completely seal the CNF/PDMS composite.

Figure 4-2. Fabrication process of CNF/PDMS composite strain sensor.

Figure 4-3. (A) Microscope image of fabricated CNF strain sensor. SEM images of (B) SU-8 nanofiber, (C) carbon nanofiber, and (D) cross-section of a strain sensor with sandwiched CNF by PDMS.
The microscope image of the fabricated sensor is shown in Figure 4-3A. The CNF is completely packaged by PDMS and 2 wires are for external circuitry connections. Figures 4-3(B-C) show SEM images of the photopatterned SU-8 nanofibers before and after high temperature carbonization. Nanofiber diameter shrinkage during carbonization is observed. The SEM image of the cross-section view of the CNF/PDMS composite is shown in Figure 4-3D. The image shows that CNF is completely embedded in PDMS and sandwiched by PDMS which not only serves as electrical insulation layers, but also as mechanical supporting layers.

**Measurement Results and Discussion**

Figure 4-4 shows the measured I-V curves for the strain sensors using CNFs carbonized at 900 °C and 1000 °C with the measuring setup shown in inset. The extracted sheet resistances (Rs) of the fibers carbonized at 900 °C and 1000 °C are 830kΩ/□ and 50kΩ/□, respectively. Figure 4-5 shows a measured resistance change.
per strain range and shows a near linear response. The measured maximum gauge factor \( GF = \frac{\Delta R}{R} / \epsilon \) of 23.1 occurred at 50% strain at room temperature. The strain range can be further improved by optimizing the PDMS thickness. Table 4-1 shows the comparison of this work with other state of the art strain sensors. This work shows a significant increase in sensitivity (gauge factor) while maintaining high stretchability. The measurement results indicate that CNF and PDMS composites have strong piezoresistivity with about 12-30 times higher gauge factor than the graphene or carbon nanotube based nanocomposite sensors. The result agrees with our hypothesis that CNF/PDMS composite structures remain intact under the high mechanical strain, which is attributed to its unique architecture consisting of continuous and tangled electrospun nanofibers. The low cost, manufacturable, high sensitivity CNF/PDMS sensors are demonstrated to be highly useful for smart wearable motion monitoring in fitness, sports, and medicine in the future.

Figure 4-5. Measured relative resistance change to the applied strain at room temperature.
Table 4-1. Comparison of stretchability and gauge factor of this work and others.

<table>
<thead>
<tr>
<th>Composite Material</th>
<th>Maximum Strain</th>
<th>Gauge Factor</th>
</tr>
</thead>
<tbody>
<tr>
<td>CNF in PDMS (This work)</td>
<td>50%</td>
<td>23.1</td>
</tr>
<tr>
<td>SWCNT on PDMS [87]</td>
<td>280%</td>
<td>0.82 (&lt;40% strain)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>0.06 (&gt;60% strain)</td>
</tr>
<tr>
<td>Graphene nanoribbon [88]</td>
<td>3%</td>
<td>1.9</td>
</tr>
<tr>
<td>Silver Nanowire [89]</td>
<td>20-60%</td>
<td>2-6</td>
</tr>
<tr>
<td>Printed Silver Nanowire [90]</td>
<td>60%</td>
<td>5</td>
</tr>
</tbody>
</table>

**Carbon Nanofibrous Microelectrode Arrays**

Microelectrode arrays (MEAs) are commonly utilized for stimulating and recording electrical signals for both *in-vitro* and *in-vivo* neural studies. Unlike microelectrode neural probes, MEAs are non-invasive planar devices for measuring the distributed electrical sensitivity of neural networks. In order for MEAs to detect the neural signal which ranges from 10 to 100 μV, MEAs must have an impedance lower than 500 kΩ at 1 kHz [91]. Recent efforts focus on better integration of electrodes with neural tissue, improvements in electrical characteristics, and optimization for long-term electrode viability. A variety of innovative neural electrode materials have been reported such as

Figure 4-6. (A)Schematic of the carbon nanofibrous (CNF) MEAs with conductive carbon film traces. 1st CNF MEA design layout (B) and optimized design layout (C).
porous platinum [92], tungsten, silicon [93], ceramic [94] and flexible polymers [95]. However, the electrical properties of these materials are strongly dependent on the dimensions of the electrodes. Typically to decrease the electrode impedance, the exposed area must increase, which results in a loss of selectivity and increases in tissue damage. Recent studies have been more focused on porous conductive materials to lower the impedance while increasing selectivity and sensitivity. It has been shown that the topographical surface of the electrode has a strong influence on cell attachment and migration [96]. Three-dimensional extracellular matrix surface textures at the scale of several nanometers to micrometers have been identified as important determinates of cell adhesion and interaction [97].

Materials with nano-topography that may enhance interactions between electrodes and neuron surfaces have become the potential candidate for future MEAs. Carbon nanofiber (CNF) was identified as a potential candidate with novel nanoporous topography for this application. Electrospun polymer nanofiber which can be photolithographically patterned and thermally converted to electrically conductive CNF is identified as a potential alternative candidate. High aspect ratio microelectrode can be achieved using oil-immersion photolithography, mentioned in Chapter 2.

**Microelectrode Design and Fabrication Process**

Figure 4-6A shows the design concept of carbon nanofibrous microelectrode arrays which consists carbon nanofiber (CNF) pillars for neural signal detection, and carbon thin film (CTF) traces as the transmission lines and contact pads connecting to external circuitry. The first design layout is shown in Figure 4-6B, where the average length of CTF, width of CTF, and diameter of CNF are 23 mm, 10 µm to 250 µm taper width, and 30 µm, respectively. However, the working electrode yield rate of the 1st
design is below 10% due to the large thermal expansion mismatch between CTF and quartz substrate during high temperature thermal annealing, which results in large stress and CTF delamination. Dimension optimization is performed and the 2\textsuperscript{nd} design is shown in Figure 4-6C, where the average CTF length and taper width are 9.6 mm and 80 to 230 µm, respectively.

Quartz is selected as the substrate for the CNF MEAs due to its particular benefits including its transparency for use in microscopic studies, backside UV exposure capability, electrically nonconductive property, and non-toxicity for cell culture. Figure 4-7 shows the fabrication process of CNF MEAs, which consist of electrospinning, immersion lithography, carbonization, and passivation. First, 300 nm of chrome is deposited on a quartz substrate by DC sputtering. Second, 2 µm of positive resist, Shipley S1818, is spin coated, photolithographically patterned, and developed, and then the chrome is etched to leave 30 µm diameter transparent holes serving as the mask for electrodes in later step. Third, SU-8 2005 is spin coated at 3000 rpm to yield a 5 µm thin film electrode.

![Figure 4-7. Fabrication process of the CNF MEAs.](image-url)
film and UV patterned as the microelectrode traces and contact pads using a mask aligner, Karl Suss MA6. Fourth, SU-8 nanofibers are electrospun using the standard electrospinning setup with an electric field of 1 kV/cm, a distance between the needle tip and the collector of 15 cm, and a polymer flow rate of 1 ml/min directly on the patterned quartz substrate. Fifth, the SU-8 nanofibers are immersed in an oil index matching medium and exposed to UV light from the backside of the quartz where the 30 μm diameter holes from the first step act as the electrode mask. Then high temperature carbonization is performed at 1000 °C with an optimized ramp rate of 3 °C/min under a forming gas atmosphere (4 % hydrogen and 96 % nitrogen) at a flow rate of 13 slm. It should be noted that the dimensions of the SU-8 thin film traces and carbonization ramping rate are critical to avoid significant thermal shock which may cause carbon structure to delaminate due to the coefficients of thermal expansion mismatch. The MEAs yield rate is improved from initial 10% of the first design to over 95% with the 2nd optimized design and process parameters mentioned above. Finally, a 2 μm SU-8 thin film is spin coated and patterned as a top electrical insulation layer.

**Fabricated Device and Electrical Characterization**

Figures 4-8(A-B) show the optical microscope images of the fabricated CNF MEAs system with the 1st and 2nd optimized design layouts, respectively. The PDMS reservoir is assembled after the CNF MEA fabrication for *in-vitro* cell culture. In the 1st design, multiple CTF traces are delaminated during high temperature carbonization at 1000 °C due to thermal stress. Figure 4-9A shows the SEM image of the CTF trace delamination. The gap between the CTF trace and quartz substrate creates electrical leakage, which results in losing neural signals. Figure 4-9B shows the leakage test; the CNF MEAs are immersed in tap water and a 0.8 V is applied on each electrode. Any
electrical leakage results in ionizing the water and creating oxygen bubbles. Multiple leakage spots are observed due to delamination. However, after the dimensional optimization, no electrical leakage is observed when the same test is performed as shown in Figure 4-9C. The result shows that well insulated CNF MEAs are fabricated with the optimized design. Scanning electron microscope (SEM) (JEOL 5700) images of the CNF MEAs with a top view and a side view are shown in Figure 4-8(C-D), respectively. Nanofiber pillar heights and diameters were measured from cross-sectional SEM images using ImageJ imaging software. The CNF electrodes’ average height and diameter are measured to be 20.7 µm and 23.6 µm using 400 mJ/cm² exposure dosage for 30 µm diameter electrode patterning. The CTF traces have an average length of 9.6 mm, a taper width from 230 µm to 80 µm, and a thickness of 2.5 µm after the carbonization process. Approximately 40.8% vertical

Figure 4-8. Optical microscope images of a fabricated CNF MEAs system with 1st design (A) and optimized design (B). SEM images of a MEAs system at top view (C) and side view (D).

Figure 4-9. SEM images of delamination of CTF in the 1st CNF MEAs design (A). Optical microscope images of the MEAs leakage test of the 1st design (B) and the optimized design (C).
shrinkage and 21.4% lateral shrinkage in size for the SU-8 nanofiber structures are observed while the overall pattern is preserved.

The electrochemical measurements are performed with a two-electrode schematic setup. The CNF MEAs serve as the working electrode, a large Ag/AgCl wire is used as a reference electrode, and phosphate-buffered saline (PBS, pH 7) is applied as the electrolyte solution. Electrical characterization of the CNF MEAs is performed using an impedance analyzer (4294A, Keysight Inc.). Figure 4-10 shows the measured impedance and phase of the fabricated CNF MEAs with optimized dimensions, original CNT MEAs, and commercial stock Titanium Nitride (TiN) MEAs (MultiChannel System). The basic elements of the experimental setup are the electrode material resistance, the solution resistance, the charge transfer resistance, and the double layer capacitance. The average measured CNF MEA impedance is 25 kΩ with a phase angle of -16.9° at 1 kHz. At the same frequency, the original CNT MEA and commercial stock MEA show impedances of 27.6 kΩ and 30.6 kΩ with phase angles of -35.9° and -72.6°, respectively. The CNF MEAs show lower impedance compared to other MEAs at lower
frequency ranges. As the CNF MEAs show less double layer capacitance than the commercial and CNT MEAs, it shows higher impedance at a high frequency regime.

The measured resistivities of the CTF and CNF using a 4 point probe setup are 0.01 $\Omega\text{•cm}$ and 0.247 $\Omega\text{•cm}$, respectively. The theoretical resistances of the CTF trace, CNF pillar, and CNF microelectrode are 1,297.5 $\Omega$, 34.9 $\Omega$, and 1,332.4 $\Omega$, respectively. However, the theoretical resistance does not agree with the extracted resistance from the impedance analyzer measurement, which is 23.92 k$\Omega$. The difference is due to the impedance analyzer only measuring either a series or parallel RC model but not the combination of series and parallel RC work. Therefore, a more accurate mathematical model is required.

Figure 4-11 shows the cell-electrode modeling based on the assumption that the potential across the cytoplasm of neurons and the PBS solution is constant [98], and the $R_m$ and $C_m$ are based on neuron cell conductivity and cell capacity and remain constant.

Figure 4-11. Schematic of electrical model for microelectrode and neuron interface [98].
Table 4-2. All variables for constructing the electrical model for microelectrodes and neuron interfaces [98]

<table>
<thead>
<tr>
<th>Variable</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>$V_M$</td>
<td>Cell potential</td>
</tr>
<tr>
<td>$V_I$</td>
<td>Voltage at the cell-electrode interface</td>
</tr>
<tr>
<td>$V_S$</td>
<td>Detection signal</td>
</tr>
<tr>
<td>$R_{S1} = R_{S2}$</td>
<td>Electrolyte resistance</td>
</tr>
<tr>
<td>$R_{S3}$</td>
<td>Counter electrode resistance</td>
</tr>
<tr>
<td>$C_{hd}$</td>
<td>Cell-electrolyte interface capacitance</td>
</tr>
<tr>
<td>$Z_{CPA} = C_P$</td>
<td>Interface capacitance in parallel fashion</td>
</tr>
<tr>
<td>$R_{ct}$</td>
<td>Charge transfer resistance</td>
</tr>
<tr>
<td>$Z_{load}$</td>
<td>Electrode resistance</td>
</tr>
<tr>
<td>$R_{S'}$</td>
<td>Interface resistance in series fashion</td>
</tr>
<tr>
<td>$C_S$</td>
<td>Interface capacitance in series fashion</td>
</tr>
</tbody>
</table>

Table 4-2 shows all the variables used in the electrical modeling. The total resistance and phase angle can be calculated as the following equations:

Total resistance: \[ R_{TOT} : Z_{load} + R_{ct} \] (4-1)

Phase angle: \[ Z_{CPA} = \frac{1}{j\omega C} \] (4-2)

The actual electrical modeling of the microelectrode consists of interface impedance which can be modeled by parallel RC connects with the electrode's total resistance.

Figure 4-12A shows the electrical model from impedance analyzer, which consists of the resistance of the CTF and CNF in series connected with an interface impedance connected in series. The more practical model is identified as the charge transfer resistance in series with the interface capacitance and resistance as shown in

![Electrical Model Diagram](image_url)

Figure 4-12. Electrical model for the microelectrode based on (A) impedance analyzer and (B) real in-vitro environment.
The series interface RC network can be converted back to a parallel RC network with the following equations.

\[
Z_S = R_{St} + \frac{1}{j\omega C_s} \quad (4-3)
\]

\[
Y = \frac{1}{Z_S} = \frac{R_{St}}{R_{st}^2 + (\omega C_s)^2} - j\frac{1}{\omega C_s R_{st}} = G - jB \quad (4-4)
\]

\[
Q_s = \frac{1}{\omega C_s R_{st}} = Q_p \quad (4-5)
\]

\[
R_{ct} = \frac{1}{G} = \frac{R_{st}^2 + (\omega C_s)^2}{R_{st}} = R_{st}(1 + Q_s^2) \quad (4-6)
\]

\[
\omega C_p = \frac{1}{B} = \frac{R_{st}^2 + (\omega C_s)^2}{\omega C_s} = \omega C_s \left(1 + \frac{1}{Q_s^2}\right) \quad (4-7)
\]

The extracted interface resistance and capacitance in parallel from measurement are 26.48 kΩ and 2.49 nF, respectively.

**In-vitro Cell Culture Result**

A 7 day *in-vitro* analysis on the fabricated CNF MEAs was performed using E18 rat cortical neurons from BrainBits LLC, which follows all National Institutes of Health (NIH) guidelines for animal use. The MEAs were treated with oxygen plasma for 30 seconds to increase surface hydrophilicity, followed by 0.1 % polyethylenimine (w/v) for supporting long term cell growth. Cell growth was analyzed via Calcein AM staining. In a previous study, neurons migrate to the CNF, resulting in a higher cell density than on the substrate. Figure 4-13 shows color enhanced SEM images of neural growth on a fabricated SU-8 nanofiber (SNF) microelectrode after a 7 day *in-vitro* culture and showed similarity in size between the nanofibers and neurons/neurites. High interaction between neurites and the SNF, where neurites grew not only onto but also into the unique micropores in SNF, is clearly observed. This result proves that nanofibers can draw cells to adhere due to its extracellular matrix surface texture. This phenomenon
could potentially yield an electrode which is capable of encouraging neural growth on and into the electrode area, resulting in the enhancement of recording signal strength.

In order to further understand the neural interaction with carbon/polymer nanofibers, neurons were also cultured for 7 days in-vitro on both CNF and SNF membranes which were suspended between PDMS structures shown in Figure 4-14A. The average pore diameters of the CNF and SNF membranes are measured by the software ImageJ and are 2.40 µm ± 0.23 µm and 0.87 µm ± 0.07 µm, respectively. Figures 4-14B and 4-14D show the fluorescent microscope images of the top side of the membranes where neurons were seeded. Lateral growth of neurites and interconnects between neurons are observed. Figure 6C and 6E show fluorescent microscope images of the bottom of the membranes. The images indicate that only neurites are capable to grow through these nanoporous membranes. The neuron cell bodies have an

Figure 4-13. SEM image of 7 day in-vitro neural growth on a SNF electrode.
approximate diameter of 10 µm, which is significantly larger than both the CNF and SNF pore size. Higher numbers of neurites grow through SNF than CNF due to SNF having nearly 3 times larger pore size than CNF. Larger pore sizes allow neurites to grow through more easily. This result indicates that neurites are not only capable but also tend to grow into porous CNF electrodes which can enhance both neuron adhesion and electrode sensitivity. Moreover, the average neurite length per area on CNF is measured as 22 mm/mm², which is 1.5 times longer than literature reported under same experimental condition on CNT [99].

A 14 day in-vitro culture was performed and neural signals and action potentials were recorded on the CNF MEAs. Figures 4-15A show a signal correlation between 2 adjacent electrodes. The neurons at the electrode in the bottom plot are activated by the

Figure 4-14. (A) Cross section schematic of a testing membrane suspended by PDMS, Top and bottom fluorescent images of neural growth on a CNF membrane (B-C), and on a SNF membrane (D-E).
action potential of neurons at the top electrode. The neural signal waveforms from different electrodes are shown in Figure 4-15B-C, respectively.

Carbon nanofiber microelectrode arrays have been fabricated and optimized to solve delamination issues due to thermal stress during carbonization. Reasonable impedances of high aspect ratio CNF MEAs is measured, validating the use of CNF. Cell cultures on the fabricated MEAs demonstrate high neurite interaction and increased neurite length per area on 3D CNF electrodes from that on CNT and 2D commercial TiN electrodes. Neurite growth through both CNF and SNF membranes is observed. Neural signals and action potential waveforms are recorded on CNF MEAs for the first time. The results show that CNF is a promising material for future MEAs.

![Figure 4-15](image)

Figure 4-15. (A) Neural signal correlation between 2 adjacent electrodes. (B-C) Action potentials of neural signals on 2 different CNF MEA electrodes.
Functional MRI Compliant Neural Probe

Approximately 65 million people worldwide are affected by an epileptic condition. Epilepsy is a brain disorder involving repeated, spontaneous seizures of any type. Although surgical removal of the epileptic tissue is possible, it is sometimes very difficult to identify the specific region of tissue. Therefore, a more detailed analysis tool is required for developing a more effective treatment.

Common brain imaging techniques include microelectrode analysis, macroelectrode analysis, structural imaging, and functional imaging. Microelectrode analysis is an invasive technical which surgically implants the electrode for extracellular or local potential recordings [100]. Macroelectrodes such as electrocorticography (ECoG) and electroencephalogram (EEG) can record large neural activities in an either invasive or non-invasive fashion [101-102]. Non-invasive computed tomography (CT) and magnetic resonance imaging (MRI) are the common examples of structure imaging [103-104]. Finally, functional imaging is a non-invasive method to provide additional activity information by measuring the changes of blood flow compared to structure imaging [105].

Functional magnetic resonance imaging (fMRI) is associated with high magnetic fields and radio frequency pulses and has been widely utilized for brain activity monitoring with precise resolution. Conventional metallic neural probes are subject to significant artifacts due to Eddy currents introduced on the high conductivity electrode surfaces and magnetic susceptibility. Magnetic susceptibility is the extent of material becomes magnetized in relation to a given magnetic field. Conventional metallic neural probes are based on materials such as platinum (Pt) and tungsten (W), which have magnetic susceptibilities of $193 \times 10^{-6}$ cm$^3$/mol and $53 \times 10^{-6}$ cm$^3$/mol, respectively [106].
These undesired artifacts lower the accuracy and credibility of fMRI. Moreover, the generated Eddy currents cause unintentional stimulation and thermal tissue damage. Low magnetic field MRI compatible neural probes have been reported using carbon nanotube and titanium based electrodes [107-108]. For high resolution imaging, fMRI with magnetic fields higher than 3 Tesla is required. However, high magnetic field fMRI compatibility still remains as a challenge.

In the previous section, CNF has been investigated and reported to be chemically and biologically inert with great neural proliferation due to its nanoporous morphology, which is expected to potentially replace metallic neural probes. Moreover, negative magnetic susceptibilities of carbon materials such as diamond (-5.9x10^{-6} \text{ cm}^3/\text{mol}) and graphite (-6x10^{-6} \text{ cm}^3/\text{mol}) have been reported [109-110]. Conductive CNF with diameters less than one skin depth and potential negative magnetic susceptibility could be an alternative material for in-vivo neural stimulation and recording studies.

The research goal of this work is to fabricate a neural electrode which integrates electrically conductive CNF along on a flexible substrate with functional magnetic resonance imaging (fMRI) compatibility for understanding brain network circuitry.

![Diagram of Young's modulus of common materials for neural probe and target tissues.](image)

Figure 4-16. Young's modulus of common materials for neural probe and target tissues.
4.7T fMRI has been performed to examine the high frequency magnetic field compatibility of the CNF neural probe in agarose gel and a rat's brain.

**CNF Neural Probe Design**

Precise neural activity monitoring with spatiotemporal resolution requires that the electrodes are in proximity to recording targets. Non-invasive surface recording techniques such as EEG can acquire superficial and large scale neural activity. However, EEGs have been unable to provide useful diagnostic measures due to low spatial resolution [111]. Neural probes with submicron resolution electrodes provide superior spatial resolution for clinical studies. Microelectromechanical systems (MEMS) technology has been introduced for high precision microelectrode fabrication. Several MEMS based neural probes consisting of one or multiple planar thin silicon shank structures, stimulation or recording electrodes, interconnect traces, and bonding contact pads have been reported [112-115].

Figure 4-17. Proposed CNF *in-vivo* neural probe architecture with four electrical conductive carbon electrodes.
However, rigid silicon based neural probes can cause major brain tissue damage and low reliability due to the large mechanical mismatch between brain tissue and rigid substrate [116-117]. Figure 4-16 shows the Young's modulus spectrum of common substrate materials and soft brain tissue. Literature has reported the Young's modulus of brain tissue is around 3 kPa [118], which is 6 orders of magnitude softer than rigid silicon [119]. Recently, large interests have been focused on developing the flexible neural probes based on softer biocompatible polymers such as polyimide [120] and parylene [121]. However, having sufficient mechanical stiffness without additional assistance remains as a challenge for biocompatible polymer based flexible neural probes.

Figure 4-17a shows the schematic of the proposed CNF neural probe, where the CNF electrodes interact with neural tissue, carbon thin film traces electrically connect the electrodes and the external circuits, and the SU-8 polymer layer provides electrical insulation and sufficient mechanical strength for surgical implantation. Four different probe designs with varying trace widths, electrode diameters, electrode trace pitches, and total lengths are designed and detailed dimensions are shown in Figure 4-17b and Table 4-3.

Table 4-3. Detailed CNF neural probe dimensions.

<table>
<thead>
<tr>
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<th>Design 1</th>
<th>Design 2</th>
<th>Design 3</th>
<th>Design 4</th>
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<td>(d_{\text{probe}}) [µm]</td>
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<td>(W_{\text{trace}}) [µm]</td>
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<td>(P_{\text{trace}}) [µm]</td>
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<td>(W_{\text{shanks}}) [µm]</td>
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<td>900</td>
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Insertion Forces Analysis

One mechanical challenge of neural probe design is to have sufficient mechanical strength to withstand buckling. The buckling can be observed during axial insertion into the brain and potentially breaks the probe which leads to serious brain damage. Therefore, sufficient mechanical strength is necessary when designing the neural probe dimensions. Literature has reported that buckling force higher than 1mN is required for surgical implantation [122]. The buckling force of the proposed neural probe can be calculated by the Euler's bucking equation as the following,

\[ F = \frac{\pi^2EI}{(KL)^2} \] (4-8)

where \( E, I, L, \) and \( K \) are the Young’s modulus of the SU-8 insulation layer, the area moment of inertia, the length of the shank, and the length factor. \( K \) is determined based upon the boundary condition at each end of the probe. For insertion, the probe contact with thick SU-8 is fixed by the insertion apparatus and the sharp tip is pinned while making contact with the brain, which means the tip cannot move laterally but is free to rotate. In that condition, the \( K \) is reported as 0.6999 [123]. The area moment of inertia is defined by,

\[ I = \frac{bh^3}{12} \] (4-9)

where \( b \) and \( h \) are the width and thickness of shank. The final buckling force related to the probe dimensions can be obtained by substituting eq.(4-9) into eq.(4-8) as the following,

\[ F = \frac{\pi^2Ebh^3}{5.88L^2} \] (4-10)
The buckling forces of the proposed CNF neural probes with 50 μm thick SU-8 insulation is calculated to be about 3.36 mN, 5.25 mN, 1.68 mN, and 2.62 mN for design 1 to 4, respectively. The buckling force of the proposed CNF neural probes with 50 μm SU-8 insulation are sufficiently larger than the tissue force of 1 mN. Moreover, the design can be further optimized to meet specific geometry requirements to stimulate or record different part of the brain. For the comparison, the buckling force of conventional 15 μm thick silicon probe is calculated to be 2.90 mN, which is large enough to be inserted into a brain [124].

COMSOL Multiphysics 4.3 (COMSOL Package Inc.) simulation of CNF neural probes’ buckling forces with various designs along with different probe thickness are performed and shown in Figure 4-18. In order to ensure the buckling will not happen during the surgical insertion, the minimum buckling force of 2 mN is designed. The SU-8 insulation thicknesses satisfy the goal of 2 mN for design 1, 2, 3, and 4 are 42 μm, 36 μm, 53 μm, and 46 μm, respectively.

![Figure 4-18. COMSOL simulation of the buckling force with various CNF neural probe dimensions.](image)
Fabrication of CNF Neural Probe

The core fabrication process includes electrospinning of SU-8, oil-immersion lithography, carbonization, passivation, and chemical releasing. The electrospun nanofiber can be lithographically patterned in oil media to enhance the achievable aspect ratio of the electrode interface. Figure 4-20 shows the detailed fabrication process of the CNF neural probe. First, a thin layer of silicon dioxide (SiO₂) was thermally grown on Si substrate. Second, 300 nm chromium (Cr) was deposited as an adhesion layer. Third, SU-8 2005 was spin coated at 3000 rpm to yield a 5 μm thickness and UV patterned for the electrode traces and contact pads. Fourth, electrospun SU-8...
nanofibers were deposited at an electric field of 1 kV/cm, a tip to collector distance of 15 cm, and a flow rate of 1 ml/min. Fifth, oil-immersion lithography was performed to pattern the SU-8 nanofiber electrode, followed by the high temperature carbonization at 1000 °C under forming gas environment to convert SU-8 nanofiber to CNF with a desired conductivity which serves as a conductor at low frequencies and is transparent at higher frequency. After carbonization, excess Cr was etched and 5 µm of SU-8 is patterned to provide electrical insulation. In order to improve handleability, a 500 µm thick SU-8 layer was patterned on at the contact pad area. Finally, the CNF neural probe was released from the Si wafer by soaking a diluted buffered oxide etchant (BOE) for 48 hrs.

Figure 4-20. (a) Optical image of the fabricated CNF probes with varying dimensions. SEM images of the CNF probes with an electrode diameter and trace width of 50µm (b-c) and 100µm (d-e), respectively.
Figure 4-20a shows the optical image of all 4 designed CNF neural probes. The total thickness of the shank can be controlled from 25 µm to 100 µm. SEM images of the CNF neural probes with an electrode diameter and trace width of 50 µm and 100 µm are shown in Figure 4-20(b, d). Figure 4-20(c, e) show the close-up SEM images of CNF electrode site with a diameter of 50 µm and 100 µm. The CNF electrode provides an ideal nanoporous morphology for neural interface compared with planar electrodes and suppresses Eddy currents generation at high etic field.

![Graph showing impedance measurement](image)

Figure 4-21. Impedance measurement of the CNF neural probe: (a) magnitude and (b) phase.

![Graph showing Eddy current generation](image)

Figure 4-22. COMSOL simulated Eddy current generation of tungsten (a) and CNF (b) at 200 MHz corresponding to 4.7T.
**CNF Neural Probe Electrical Characterization**

Figure 4-21 shows the CNF neural probes impedance measurement in the frequency ranging from 40 Hz to 10 kHz. The impedance at 1 kHz for CNF neural probe design 1, 2, 3, and 4 are 8.95 kΩ, 12.7 kΩ, 29 kΩ, and 13.9 kΩ, respectively. Figure 4-21b shows the phase angle of all 4 designs vs. frequency. The CNF neural probes show similar phase angle around -36° at 1 kHz, indicating dominantly resistive impedance.

Eddy current generation by AC magnetic field is simulated using COMSOL Multiphysics 4.3 (COMSOL Inc.). Figure 4-22 shows the simulated result of CNF compared to a conventional electrode material, Tungsten. CNF shows a significant Eddy current suppression, which will minimize Joule heating and tissue damage. Ex-vivo implantation into rat’s brain was performed on CNF probe and no buckling was observed.

**fMRI Compatibility Test**

Lower magnetic susceptibility materials with diameters less than a skin depth are desired for minimizing potential Eddy current generation and imaging distortion.

![Figure 4-23. (a) T1- and (b) T2- weighted images of the Pt/Ir based neural probes implanted in a rat's brain under 3T scanning [107].](image)
Figure 4-23(a-b) show the referenced T1- and T2- MRI images of the platinum/iridium (Pt/Ir) based neural probes implanted in a rat's brain *in-vivo* under 3T scanning [107]. The images show significant MRI artifacts and distortion caused by Eddy current generation. Tools such as fMRI utilize higher magnetic field and can increase image distortion and tissue damage. However, higher magnetic fields are necessary for precise spatial resolution.

In this work, a fMRI with a magnetic field of 4.7T is utilized to study the MRI compatibility of various CNF neural probes. First, the 5 μm and 100 μm thick CNF neural probes are implanted in agarose gel and imaged with 4.7T in spin echo and gradient echo modes. Figure 4-24 shows the recorded artifact free fMRI images of the 2 under (a-c) and gradient echo (d-f) modes. The CNF neural probes with a thickness of

![Figure 4-24. fMRI images of the 100 μm and 25 μm thick CNF neural probes implanted in agarose gel under the sping echo (a-c) and gradient echo (d-f) modes.](image)
25 μm observe minor bending during insertion due to their insufficient buckling forces.

Figure 25(a-b) shows the recorded artifact-free fMRI images of the 50μm thick CNF neural probes implanted in a rat's brain ex-vivo with no buckling under 4.7T in spin and gradient echo modes, respectively. Figure 4-26 show the recorded fMRI images of CNF neural probe with read-out direction perpendicular to the probe. The images show no artifact dependency to the read-out direction for the CNF neural probe.

The CNF based neural probe has been developed as a potential alternative approach for neural recording and stimulation. 4.7T fMRI recorded ex-vivo images show no artifact and distortion with CNF neural probes. While the results are encouraging, further in-vivo animal studies of probe deflection and tissue heating are suggested.

Figure 4-25. 4.7T fMRI images of the CNF probes implanted in a rat's brain under the spin echo(a) and gradient echo(b) modes.

Figure 4-26. 4.7T fMRI images of the CNF probes implanted in a rat's brain in a lateral direction under gradient echo mode.
Summary

Electrical conductive carbon nanofiber has been utilized and investigated for smart wearable sensor and neural engineering applications. A highly sensitive and flexible strain sensor based on carbon nanofiber (CNF) embedded polydimethylsiloxane (PDMS) nanocomposite is proposed and developed for advanced wearable applications. The CNF is sandwiched by PDMS and the flexibility of sensor can be controlled by modulating the thickness of PDMS. A very high strain gauge factor of 23.1 is measured at 50% strain in room temperature, which is about 12-30 times higher than that of other literature reports. The sensor can be easily integrated with clothes or even directly attached on the human body.

The design and fabrication of the CNF based microelectrode arrays (MEAs) have been optimized to solve delamination issues and improve the yield rate to over 90%. Electrical characterization of CNF MEAs is performed and the result shows a resonable impedance. In-vitro cell culture on fabricated CNF MEAs demonstrate higher neural interaction and proliferation compared to planar and carbon nanotube based MEAs. In-vitro neural signals and action potential waveforms recorded CNF MEAs are demonstrated for the first time.

The CNF based flexible neural probes for in-vivo neural recording and stimulation have been designed and fabricated. The polymer insulation layer has been designed to obtain a sufficient mechanical stiffness for surgical implantation. The fabricated CNF neural probes show an electrical impedance ranging from 8.95 kΩ to 29 kΩ at a frequency of 1 kHz. COMSOL simulations show significant Eddy current suppression on CNF compared to conventional materials such as Tungsten. Successful implantations of CNF neural probes in agarose gel and a rat's brain ex-vivo with negligible bending have
been demonstrated. 4.7T fMRI is performed on fabricated CNF neural probes which shows no artifact or image distortion under both spin echo and gradient echo modes. *In-vivo* animal studies of probe deflection and tissue heating are suggested for further fMRI compatibility studies.
CHAPTER 5
MICRO/NANO INTEGRATION CHANNEL

Micro/nanofluidic systems have emerged as an important discipline of science and engineering because of their prospects in a wide range of biological and chemical applications, including micro total analysis systems (μTAS). The miniaturized systems take advantage of reduced sample size, increased surface to volume ratio, laminar flow, parallel processing, and reduced process and analysis time [125-126]. Moreover, nanofluidic systems provide new opportunities to investigate fundamental nanoscale transport phenomena and their applications for analysis tools of ion mass spectrometry, gas and liquid chromatography, pH meters, and capillary electrophoresis [127-129]. In a nanofluidic system, a nanochannel or nanoporous microchannel is a key component while its efficient fabrication and its cost-effective integration with other fluidic parts remain as big challenges.

The nanofabrication processes often rely on the bottom up approach such as self-assembly, which is usually more cost effective than the top down approach. Previously, various innovative fabrication techniques have been reported to produce nanoscale resolution structures for nanofluidic applications, including laser machining [130], electron beam lithography [131], atomic force microscopy lithography [132], and x-ray lithography [133]. In this chapter, a novel fabrication process for seamless integration of a nanoporous microchannel is demonstrated. The core fabrication process includes electrospinning, self-limiting photolithography patterning, and polymer thermal-reflow packaging processes.

Design and Theory

A schematic of the nanofibrous microfluidic structure is shown in Figure 5-1(b), which consists of a glass substrate with a patterned chrome layer, an SU-8 nanofibrous microchannel, an SU-8 thin film for a channel wall, and two PDMS O-ring shape reservoir walls. The SU-8 thin film microchannel is designed to have a length of 7 mm, a width of 75 μm, and a height of 35 μm. The microchannel is filled with SU-8 nanofibers to provide nanoporosity. The PDMS reservoir walls, which have an inner diameter of 2 mm and an outer diameter of 4 mm, are placed at both ends of the channel and are intersect with the pumping system and the channel.

A theoretical model of fluidic flow in a microchannel with a semi-circular profile driven by the capillary force and gravity has been demonstrated [134-135]. For the simplification of the analytical model, several hypotheses are used as follows. The fluid forms incompressible Newtonian flow, two-dimensional fully developed laminar flow, and has negligible end effects, surface roughness, and gravity. The volumetric flow rate can be derived as in Eq. (5-1) [134].

\[ Q = \frac{\pi r_{eff}^2}{128\eta K} \frac{dP}{dL} \]  

(5-1)

Figure 5-1. Schematic and geometry of an SU-8 nanofibrous microchannel: (a) Perspective view, (b) Cross-section view.
\[ K = \frac{5.299}{k^{2.56}} \]  

(5-2)

where \( r_{\text{eff}} \), \( \eta \), \( k \) are the effective radius of a microchannel, the viscosity of liquid, and the ratio of the height and radius of the semi-circular profile, respectively. The pressure gradient along the microchannel can be determined using Eq. (5-3) [135].

\[ \frac{dP}{dL} = \frac{1}{L} \left( \rho g H + \frac{2 \sigma \cos \theta}{r_{\text{eff}}} \right) \]  

(5-3)

where \( L \), \( H \), \( \sigma \), \( \theta \), are the length of the microchannel, the height of the liquid in reservoir, the surface tension of the liquid, and the contact angle, respectively. The flow time in the nanochannel can be calculated using Eq. (5-4).

\[ t = \frac{64 \eta KL^2}{r^2 \left( \rho g H + \frac{2 \sigma \cos \theta}{r_{\text{eff}}} \right)} \]  

(5-4)

**Microchannel Fabrication Process**

Designed nanofibrous microchannels are fabricated using SU-8 electrospinning, self-limiting back-side UV lithography, and thermal reflow processes as shown in Figure 5-2. First, a 120 nm thick chrome layer was sputtered on a glass substrate, followed by UV lithography with a positive resist, Shipley S1818, and subsequent chrome etching to form a photomask for the microchannel (A). An SU-8 electrospinning solution was prepared by diluting SU-8 2025 (Microchem Inc.) with Dimethylformamide (DMF) to give a concentration of 90 % SU-8 2025. The solution was then carefully loaded into a syringe without introducing air bubbles and capped with a hypodermic needle. A syringe pump was used to maintain a steady state flow rate of 1 ml/hour with the needle tip was connected to a high voltage power supply. Then, SU-8 nanofibers were electrospun to have a nanofiber stack thickness of 400 \( \mu \)m using the standard electrospinning setup with an electric field of 1 kV/cm and a distance between the needle tip to collector of 15
cm (B). Third, SU-8 nanofibers were dried in a vacuum oven for 24 hours to remove excessive solvent and then back-side UV exposed (i-line: 365 nm) with 400 mJ/cm² (C). The back-side UV exposure in the air environment results in low optical dosage on the top portion of the SU-8 nanofiber stack due to the large scattering and diffraction. This self-limiting exposure enables the top portion of the SU-8 nanofiber stack as well as the unexposed SU-8 nanofibers to reflow during the post exposure bake to form conformal channel walls around the nanofibrous microchannel. It should be noted that the cross-section of the microchannel shows a semi-circle, which is attributed to the light diffraction effect and would be advantageous in many microfluidic systems in contrast to a microchannel with a rectangular cross-section as the circular one has fewer corners and dead zones. This self-packaged conformal channel approach enables seamless

Figure 5-2. Fabrication of nanofibrous microchannels.
integration between the nanofibers and the microchannel. The channel walls can be polymerized after further UV exposure and post exposure baking (D).

**Channel Characterization**

De-ionized (DI) water colored with food dye is used for a flow time measurement. The DI water's density, viscosity, surface tension, and contact angle are 1000 kg/m$^3$, $8.9 \times 10^{-4}$ Pa·s, 0.00729 N/m, and 60°, respectively [45]. The average length of the fabricated nanofibrous microchannel is 7 mm, and the measured time for DI water to pass through the microchannel is 232 seconds. Figure 5-3 shows the optical microscopy images of colored DI water with blue food dye flowing through the nanofibrous microchannel for 240 seconds, where each image is captured every 40 seconds. The calculated effective height and radius of the nanofibrous microchannel from Eq. (5-4) is 800 nm and 857 nm, respectively. The nanofibrous microchannel shows identical performance obtained from the capillary flow measurement as the semi-circular nanochannel and it is suitable for nanofluidic systems with the nanoporous morphology made of nanofibers.

![Image of optical microscopy images](image-url)

**Figure 5-3.** Optical microscopy images of De-ionized water flows through a nanofibrous microchannel for 240 seconds with each image taken in every 40 seconds.
A simple, controllable, cost effective, self-packaged, integrable, and manufacturable method to fabricate a hybrid micro/nano fluidic device with microscale channel dimensions and nanoscale channel characteristics has been demonstrated. The nanofibrous microchannels have been fabricated by electrospinning with diluted SU-8, followed by the standard photolithography and thermal reflow processes. The seamless microchannels filled with SU-8 nanofibers have been successfully demonstrated. The microchannel dimension is mainly governed by the initial photomask pattern dimensions, the self-limiting UV lithography in non-homogeneous nanofibrous media while the final dimension can be tuned in part by the thickness of the electrospun nanofibers and reflow.

**Proposed Potential Application: Microheater**

Temperature control is an important factor in microfluidic devices and lab-on-a-chip devices. Effective heat transfer results in improved control of chemical reagent conditions. Bio/chemical reactions are typically optimized in a certain temperature range, such as polymerase chain reaction (PCR) [136] for DNA studies. In order to avoid any failure due to lack of temperature control during liquid transport, precise temperature control is required. On the other hand, functional nanofibers have attracted a lot of attention in the past decades. In 2012, Huang et al. reported embedding 20% iron oxide nanoparticles in polymer nanofibers could generate thermal energy as the result of an external AC magnetic field [137]. They demonstrated the magnetic nanofibers could heat 1mL of water from 23 °C to 83 °C in 180 seconds with an AC magnetic field of 33.9 kA/m at 232 kHz. The magnetic nanofiber shows a linear thermal response, which can be used for cancer treatment through hyperthermia. This unique function also enables magnetic nanofibers to be used for microfluidic applications such as a microheater. The
nanofibrous microchannels integrated with iron oxide nanoparticles during polymer synthesis could serve as nanoheater integrated nanofibrous microchannels. It can precisely control the final liquid temperature as it is transported through. It is a great example of high integrability and multifunctionality of the demonstrated nanofibrous microchannel. This new microchannel architecture can be further modified for different applications.
CHAPTER 6
LOW LOSS NANOCOMPOSITE CONDUCTOR

These days, intrinsic and functional properties of porous materials have gained a lot of interests in fundamental research and industry applications such as on-chip interconnects [138], sensors [139], impact insulators [140], and heat sinks [141]. A porous metallic conductor provides advantageous properties such as being lightweight, having large surface area, good permeability, vibration damping and good heat dissipation capabilities.

Moreover, the concept of metal matrix composites (MMCs) has been heavily developed to overcome the inadequate mechanical strength and stiffness of conductive metal microstructures. Ceramic materials are often used in MMCs to enhance mechanical strength and ductility. Ceramic introduced aluminum matrix composites have been reported with improved mechanical properties for automotive applications [142]. Recently, efforts have been focused on incorporating novel materials such as carbon nanotubes (CNT) in iron, nickel, and aluminum to reinforce the strength, stiffness, abrasion, and chemical and thermal stability [143-145]. Carbon nanotube-copper (CNT-Cu) nanocomposites with high electrical conductivity and low coefficient of thermal expansion has been reported for interconnect application [146].

From the electrical property viewpoint, the conductive nanocomposites would be advantageous in high frequency applications due to the possibility of suppressing the skin effect, by which radio frequency (RF) electrical resistance can be reduced. For example, copper is a well known and highly utilized conductor in electronics due to its high conductivity, precised deposition thickness controllability, and low manufacturing cost. However, its high conductivity degrades with increasing operating frequency
because of the skin effect. Recently, superlattice metaconductors have been reported to achieve Ohmic loss reduction in RF by introducing intermediate ferromagnetic layers [147], which often requires a highly uniform and precise alternating deposition method, adding to manufacturing difficulty and cost. Development of a fabrication method for conductive nanocomposites with precise patterning, high manufacturability, and good mechanical characteristics while maintaining good electrical properties will be highly beneficial.

Electrospinning is an effective way to produce a micro/nanoscale porous membrane in an either highly oriented or random fashion. The electrospun nanofiber diameter and pore size can be controlled by polymer solution concentration and electrospinning process parameters. Moreover, the morphology of electrospun micro/nanostructure can be controlled such as spheres with a diameter of few microns, nanofibers with a fiber diameter of hundreds of nanometers, or the combination of the two [148]. Electrospun polymer nanofiber can further be lithographically patterned in oil media to obtain microstructures with a few micron resolution and high-aspect-ratio [149]. The polymer nanofiber can have ferromagnetic properties by adding corresponding precursors with a high temperature synthesization process. Literature has reported electrospun ferromagnetic nanofibers such as cobalt (Co), nickel (Ni), and nickel ferrite (NiFe$_2$O$_4$) nanofibers [150-151].

Electroplating is a process that utilizes electric current to form a thin coherent metal coating on a conducting surface. The metal deposition rate is highly dependant on electric current density. In this work, the electroplating process is utilized to fill out the pore area of nanoporous membranes with conductive metal, copper (Cu). A
nanocomposite metallic microstructure can be obtained either with or without the polymer fibers by an additional polymer removal step.

**Theory and Simulation**

The conductor loss in RF is attributed to the conductor's effective cross-section area, the so-called skin depth ($\delta$) which is given as,

$$
\delta = \sqrt{\frac{2}{\omega \mu_0 \mu_r \sigma}}
$$

(6-1)

where $\omega$, $\mu_0$, $\mu_r$, and $\sigma$ are the angular frequency, the magnetic permeability of air, the relative magnetic permeability of the conductor, and the electrical conductivity of the conductor, respectively. The skin depth decreases as the operating frequency increases, which increases the conductive loss. Several papers have reported the suppression of conductor loss by introducing ferromagnetic elements into the conductor to realize a superlattice metaconductor, consisting of alternating ferromagnetic and non-ferromagnetic layers [152-153]. The ferromagnetic materials have a unique property that the effective relative magnetic permeability becomes negative above the

Figure 6-1. The schematic of the designed nanoporous conductor with electrospun nanofibers embedded. Inset shows the cross-section view and dimension parameters.
ferromagnetic resonance frequency, which induces a counter current that can cancel out the eddy current generated by the non-ferromagnetic conductor. The effective magnetic permeability ($\mu_{\text{eff}}$) of the superlattice metaconductor is given as,

$$\mu_{\text{eff}} = \frac{\mu_{\text{NF}}t_{\text{NF}} + \mu_{\text{F}}t_{\text{F}}}{t_{\text{NF}} + t_{\text{F}}}$$  \hspace{1cm} (6-2)

where $\mu_{\text{NF}}$ and $\mu_{\text{F}}$ are the permeability of non-ferromagnetic and ferromagnetic materials, respectively. The thickness of each ferromagnetic and non-ferromagnetic layer is denoted as $t_{\text{F}}$, and $t_{\text{NF}}$ in eq.(6-2). According to eq.(6-1), an infinite skin depth of the conductor at any given operating frequency can be achieved when $\mu_{\text{eff}}$ is 0. Therefore, the thickness of the non-ferromagnetic and ferromagnetic layers can be carefully designed to obtain $\mu_{\text{eff}} = 0$ to enlarge the skin depth.

Theoretical analysis of the nanocomposited metallic interconnect is discussed and electrical simulation is performed by a high frequency structure simulator (HFSS v. 15, ANSYS Inc.). Figure 6-1 shows the schematic of the proposed nanoporous conductor, which consists of aligned ferromagnetic nickel iron (NiFe) nanofibers surrounded by electroplated copper (Cu). The inserted cross-section image of the conductor in the Cartesian coordinate system where the $d_{\text{N}}$ and $d_{\text{F}}$ are the width of the Cu slot and the NiFe nanofiber in the ferromagnetic layer. The conductor can be simplified for theoretical analysis into a multilayer conductor with alternating non-ferromagnetic (Cu) and ferromagnetic/non-ferromagnetic (NiFe+Cu) layers. The magnetic reluctance of the ferromagnetic/non-ferromagnetic layer (NiFe+Cu) is given as,

$$R_{\text{F}} = \frac{l_{\text{F}}}{\mu_{\text{F}}A_{\text{F}}}$$  \hspace{1cm} (6-3)

$$l_{\text{F}} = l_{\text{Cu}} + l_{\text{NiFe}}$$  \hspace{1cm} (6-4)
where \( l_F \) and \( A_F \) are the total width and cross-section area of the ferromagnetic layer.

The total reluctance can be also represented as the summation of Cu and NiFe reluctance as shown in eq.(6-5).

\[
\mathcal{R}_F = \frac{l_{Cu}}{\mu_{Cu} A_{Cu}} + \frac{l_{NiFe}}{\mu_{NiFe} A_{NiFe}}
\]  

The effective permeability of the ferromagnetic layer can be determined by combining eq.(6-3) and eq.(6-5) and is given as,

\[
\mu_F = \frac{l_F}{l_{Cu} + l_{NiFe}} = \frac{l_{Cu} + l_{NiFe}}{\mu_{Cu} + \mu_{NiFe}}
\]  

Electromagnetic simulation is performed by HFSS (ANSIS Inc.) and all structures have the same overall thickness of 1.75 \( \mu \)m. The reduction of RF conduct loss is calculated by the resistance differences between the reference Cu conductor and nanoporous conductor. Figure 6-2a shows the resistance of the nanoporous conductors

![Figure 6-2a](image)

**Figure 6-2.** (a) Simulation result of the nanoporous conductor resistance spectra vs. frequency in the microwave region compared to solid Cu with different thickness ratios of ferromagnetic/non-ferromagnetic (NiFe+Cu) and non-ferromagnetic (Cu) layers. (b) Simulated nanoporous conductor resistances with varying width ratios of NiFe and Cu in the ferromagnetic/non-ferromagnetic layer.
as a function of the frequency with a varying thickness ratio ($r_1$) ranging from 0.5 to 4, which is also compared with the resistance of solid Cu. For $r_1$ of 1.5, a loss reduction of 20% has been observed. The loss reduction effect degrades with decreasing thickness of Cu layer and when self confined eddy currents occur within a thickness greater than approximately 1 skin depth. Figure 6-2b shows the simulation of Cu and NiFe ratio ($r_2$) effect in the ferromagnetic layer. The frequency with the lowest resistance shifts to the lower frequency as $r_2$ decreases, where the larger negative value of $\mu_{\text{NiFe}}$ is needed to ensure eddy current cancellation. The magnetic field can no longer be confined in the ferromagnetic layer when $r_2$ is larger than 1 resulting in individual field looping around NiFe nanofibers and the resistance increases.

Figure 6-3. The proposed microfabrication process flow for the nanoporous metallic interconnect.
Fabrication Process

Figure 6-3 shows the fabrication process of the nanocomposited conductor. First, a substrate is cleaned and a Ti/Cu/Ti seed layer with each layer thickness of 30nm/300nm/30nm is sputter deposited. Second, a 40 µm thick SU-8 nanofiber stack is electrospun on the substrate with an electric field ranging from 1 kV/cm to 1.5 kV/cm to control the nanoporous morphology. After Ultraviolet (UV) light exposure, the exposed SU-8 nanofiber is cross-linked by a post-exposure-bake (PEB) at 95 ºC for 5 min. Please note that unexposed SU-8 nanofibers will undergo the reflow process during the PEB to form a layer of SU-8 thin film which serves as an electroplating mask. Then the sample is electroplated to form a nanocomposited metallic interconnect. Finally, the uncross-linked SU-8 thin film is removed during development and a series of metal etchings is performed to remove the seed layer.

Alternating Electrospinning

The effect of different voltage polarities of the needle tip and collector on the nanofiber growth rate are investigated. In conventional positive electrospinning, the positively charged nanofibers are solidified before the charge is fully dissipated at the

![Figure 6-4. SEM images of conventional positive electrospun nanofibers with a constant electric field of 1 kV/cm and a TCD of (a) 12.5 cm, (b) 15 cm, and (c) 17.5 cm.](image)
collector plate, which results in electrostatically repelling the subsequently incoming nanofibers and saturating the nanofiber membrane thickness. By switching the voltage polarity of the needle and collector, charged nanofibers with a different polarity can be electrospun, resulting in electrostatic attraction. Alternating electrospinning with intervals of 1 min or 30 sec electrospinning and 30 sec resting were performed to study the polarity and charge accumulation effects.

Positive electrospinning was performed with 1 min electrospinning and 30 sec resting period between electrospinning sessions to give time for charge relaxation.

Figure 6-4(a-c) show the SEM images of electrospun nanofibers with a constant electric

![Figure 6-4](image)

Figure 6-5. Nanofiber diameter distribution for positive electrospinning with a constant electric field of 1 kV/cm and a TCD of (a) 12.5 cm, (b) 15 cm, and (c) 17.5 cm. (d) Summary plot of average nanofiber diameter and porosity.
field of 1 kV/cm and a TCD of 12.5 cm, 15 cm, and 17.5 cm, respectively. Figure 6-5(a-c) show the distribution of the nanofiber diameter at each operating condition. The distribution of nanofibers is skewed toward the smaller diameter as TCD increases, which is due to longer electrostatic force stretching time. Figure 6-5d shows the nanofiber average diameters and porosities of all 3 operating conditions. As the average nanofiber diameter decreases, higher porosity is observed which can be utilized to control metal concentration of the fabricated interconnect. The results indicate that the ratio of the electrospun nanofiber and electroplated metal can be designed to achieve the lowest conductor loss for a desired operating frequency.

Alternating electrospinning is performed by switching polarities of the supply and collector between each cycle. Intervals of 1 min and 30 sec are used to study the charge repulsion effect. Figure 6-7 shows the measured nanofiber thickness using positive and alternating electrospinning methods. During positive electrospinning, the collected nanofibers without fully dissipating charges repel the incoming charged
nanofiber. As a result, the thickness of the nanofiber stack saturates around 40 µm. In case of alternating electrospinning, higher growth rates of 3.8 µm/min and 5 µm/min for 1 min and 30 sec interval are observed. Moreover, the saturation thickness has been significantly improved from ~40 µm to more than 80 µm by minimizing the electrostatic repulsion effect.

**Dynamic Electrospinning**

Figure 6-7a shows a standard electrospinning setup which produces randomly orientated nanofibers. As the applied voltage exceeds the surface tension of the solution, the solution extruded from the needle tip forms a Taylor cone and ejects a jet. The ejected polymer jet undergoes a whipping process during which the solvent evaporates, leaving behind a charged solid polymer fiber, which lays itself randomly on a grounded metallic collector plate [154].

The dynamic electrospinning setup which consists a DC motor (Mitsumi Ltd., Japan) connected to a cylindrical mandrel with 1" in diameter and an array of grounded metallic blades to direct the electricstatic force for the electrospun nanofibers is shown.

![Diagram](image.png)

Figure 6-7. Schematics of (a) a static electrospinning setup and (b) a rotating mandrel integrated dynamic mode setup, for randomly orientated and aligned nanofibers, respectively.
in Figure 6-7b. The rotating mandrel provides additional mechanical force to collect uniaxial orientated nanofiber. The speed of mandrel is controlled by DC motor and calculated by multiplying the circumference and rpm of the mandrel.

For alignment analysis, the frequency component is extracted from a gray scale SEM image using the fast Fourier transform (FFT) function in ImageJ. The FFT transform implements the 2D Fast Hartley transform to generate a power spectrum depicting frequency information in terms of pixel intensity in a radial plot. Then, the ‘Oval Profile’ plugin was used for pixel summing along an oval path on the image. The orientation of nanofibers is quantified with the summation of pixel intensities along a straight line originating from the center to the edge at every angle from 0° to 360°. The detailed ImageJ procedures for the analysis can be found in previous papers [155-156].

SEM images of electropsun nanofiber alignment for positive and alternating with 1 min and 30 sec intervals are shown in Figure 6-8(a-c), respectively. All samples were performed with an operating voltage of 15 kV, a TCD of 10 cm, and a constant mandrel rotating speed of 5.05 m/s. Nanofiber orientation improvement with alternating electrospinning technique is observed due to the reduction of charge repulsion.

Figure 6-8. SEM images of aligned nanofiber with different electrospinning modes: (a) positive, (b) alternating with 1min cycle, and (c) alternating with 30 sec cycle.
Figure 6-9(a-c) show the FFT intensity plots of pure positive and alternating with different time intervals electropun nanofibers collected with a mandrel rotational speed ranging from 2.66 m/s to 5.05 m/s. In all electrospinning methods, the alignment of nanofibers increases as the mandrel rotating speed increases. As a result, alignment of nanofibers is therefore dependent not only on the electrostatic charge repulsion but also mandrel rotating speed.

**Electroplated Metal Through Nanofiber Stack**

A non-ferromagnetic material, Cu, is electroplated through the nanofiber stack using a current density of 10 mA/cm$^2$. SEM images of the cross-section view of electroplated nanoporous conductors is shown in Figure 6-10a. Cu has been successfully electroplated and it fills out the pore area inside the nanofiber membrane. Figure 6-10b shows the measured Cu thickness vs. time. The dash line in the plot indicates the nanofiber stack thickness. Theoretically, electroplating Cu with a current density of 10 mA/cm$^2$ should yield a Cu deposition rate of 13.3 μm/hr. However, a slower Cu overall deposition rate and two distinguished deposition rates are observed. In the first 4 hours, a low deposition rate of 1.78 μm/hr and 3.9 μm/hr are observed in

![Figure 6-9. FFT analysis of nanofiber alignment with varying mandrel rotating speeds and electrospinning methods: (a) positive, (b) alternating with 1min cle, and (c) 30 sec cycle.](image-url)
positive and alternating electrospun nanofibers, respectively. The low deposition rate is due to the nanoporous morphology of nanofibers which makes it difficult for Cu electroplating solution to flow in and refill the Cu ions. It should be also noted that the positive electrospun nanofiber yields a lower deposition rate due to residual non-dissipated positive charges inside the nanofiber, which ultimately repels Cu ions in the solution. After approximately 20 μm of Cu is deposited, the nanoporous nanofiber height reduces and Cu ions can diffuse in and refill much easier, resulting in an increasing deposition rate. Therefore, the electroplating rate for a nanoporous template is limited by the non-dissipated charges and the morphology of nanofiber.

Figure 6-10. (a) SEM images of electroplated Cu through the nanofiber stack to form a nanoporous conductor. (b) Electroplated Cu thickness vs. electroplating time plot with positive and alternating electrospun nanofibers.
Summary

In summary, a unique nanocomposited metallic interconnect with conductor loss reduction is investigated in theory and in experiment for high frequency applications. The modified electrospinning techniques are reported for minimizing charge repulsion and nanofiber alignment. Electrospun nanofibers and photolithographical patterning processes are used for the template and the electroplating process is utilized for metallization. This process allows the realization of high precision porous conductors with easy control of porosity, low cost, and manufacturability.
CHAPTER 7
CONCLUSIONS AND FUTURE WORK

Conclusion

In this dissertation, four advanced nanofiber fabrication techniques are proposed for improving manufacturability, degrees of freedom in design parameters, patterning aspect ratio and resolution, and conformal packaging for future nanofiber based devices and systems. Additionally, two different categories of electrospun nanofibers, biodegradable and electrically conductive, are investigated and implemented for multiple applications such as drug delivery system, active tissue scaffolding, smart wearable strain gauge sensors, \textit{in-vitro} neural signal recording, and radio frequency (RF) transparent neural probe with functional magnetic resonance imaging (fMRI) compatibility. The main motivation of this work is to propose feasible solutions for developing nanofiber technology so it can be utilized in many different disciplines.

First, to adapt the advantages of electrospun nanofiber, some fabrication challenges need to be solved. Tube nozzle electrospinning is developed to enhance manufacturability. A 12.5 times improvement in mass throughput has been achieved with a 2 tube with 8 nozzle architecture. Dual syringe electrospinning is proposed to offer more degrees of freedom in design parameters such as two polymers with different degradation times and concentrations, dual drug release, and sandwiched multilayer architectures with differing morphologies. Nanofiber patterning with micron-level resolution precision can be achieved by using index matching oil-immersion lithography. The major limiting factor, UV light scattering effects, has been suppressed with replacing the air medium by an index matching medium. As a result, three times higher aspect ratio microstructures are obtained with this technique, as well as a great
reduction in dispersion-assisted pattern expansion. Since nanofibers offer microporous morphology, packaging becomes a major challenge for microfluidic applications. Conformal sealing is demonstrated by utilizing thermal reflow properties of the uncross-linked negative photoresist polymer nanofiber.

Second, electrospun nanofiber has been widely utilized in tissue engineering and bio-applications. An electrospun biodegradable polymer, Polycaprolatone (PCL), has been identified for long term drug delivery applications due to its longer term degradation profile, around one year. Additionally, the electrospun PCL dimensions, its crystallinity with different electrospinning conditions, the architecture of electrospun membrane and the effect on releasing profile have been studied. Approximately, a release profile over one month long is obtained with the hybrid structure which consists of two components with very different dimension, microbeads and nanofibers. A triple layer membrane consisting of a drug loaded microbead layer sandwiched by two nanofiber layers without drug loading is fabricated by dual syringe electrospinning, and shows an extended linear drug release for over 80 days. Moreover, we also explored the potential of incorporating magnetic nanoparticles in PCL nanofiber for an active cell culture. Magnetic nanofibrous scaffolds are fabricated and stem cell proliferation enhancement is obtained.

Third, electrospun polymer derived conductive carbon nanofiber (CNF) with both high surface to volume ratio and good electrical conductivity has been studied. The internet of things and smart wearable devices have gained a lot of attention recently. A strain gauge sensor based on carbon nanofiber embedded by polydimethysiloxane (PDMS) is proposed and implemented, specifically designed for these systems.
Approximately, 12-30 times higher sensitivity (gauge factor) than other advanced strain
gauge sensors such as graphene, carbon nanotube (CNT) nanocomposite, and silver
nanowire is obtained, which opens a new discipline for electrospun nanofiber
technology. Microelectrode arrays (MEAs) have been widely utilized for neural studies.
The carbon nanofiber MEA design has been optimized to improve the yield rate to over
90%. In fact, 1.5 times higher neurite length per area on CNF than advanced CNT is
observed, which proves that CNF based MEAs have a higher sensitivity than CNT
based MEAs. Moreover, neural signal and action potential waveforms recorded by CNF
MEAs is demonstrated for the first time. In addition to CNF MEAs, in-vivo neural probes
based on CNF are proposed and developed. The highly flexible polymer coated CNF
neural probe is fabricated and successfully surgically implanted. More importantly, the
unique conductivity and dimension of CNF suppresses Eddy current generation at radio
frequencies, which allows the CNF neural probe to be fMRI transparent. CNF neural
probes with high precision patterning and fMRI compatibility up to 4.7T
are demonstrated for the first time.

Fourth, for nano/microfluidic applications, a self-packaged fabrication method for a
nanofibrous microchannel has been demonstrated. The effective nanochannel
characteristics have been studied using a flow test and the equation derived from the
Navier-Stokes theorem. With the overall channel dimensions in microscale, the
microchannel can be easily integrated with other microfluidic systems while maintaining
nanofluidic characteristics and functionalities. The results indicate that the hybrid
micro/nano structure is suitable for nanoscale transport applications.
Finally, a unique nanocomposited metallic interconnect with conductor loss reduction by suppressing eddy current generation is investigated in theory and in experiment for high frequency applications. The modified alternating and dynamic rotating electrospinning techniques are reported for minimizing charge repulsion and nanofiber alignment. Electrospun nanofibers and photolithographical patterning processes are used for the template and the electroplating process is utilized for metallization. This process allows to realize high precision porous conductors with easy control of porosity, low cost, and manufacturability.

**Recommendations for Future Work**

There are several areas that would enhance the impact of CNF neural probe greatly. Additional experiment with collaborative assistance from medical experts and integration of CNF neural probe with optogenetics functionality are discussed in the following sections.

**Wireless *in-vivo* Neural Stimulation and Recording**

High neural interactions on electrically conductive CNF MEAs have been demonstrated for an *in-vitro* study. Additionally, CNF based neural probes have been successfully fabricated and shown to be fMRI compatible up to 4.7T. Higher magnetic field fMRI compatibility can be studied. The impedance of the CNF neural probe is around 10-30 kΩ, which is suitable for stimulation and recording. To prove the functionally of CNF neural probe, *in-vivo* stimulation and recording are needed. Behavior response and disease diagnosis using CNF neural probe can be studied with assistance from collaborators in the medical discipline.

Moreover, read-out circuitry with Bluetooth communication capability is recommended to achieve wireless neural stimulation or recording functionality for
behavior studies. The analog front-end circuitry consists of two stages which are a low-noise amplifier (LNA) (INA116, Texas Instruments), and a band-pass filter. Figure 7-1 shows the recommended schematic of analog front end circuitry. The first stage LNA and second stage filter provide a total gain of 1,500 V/V. The band-pass filter is designed to have a low and a high cut-off frequencies of 100Hz and 10 kHz. The

Figure 7-1. Schematic of analog front-end circuitry.

Figure 7-2. Simulated of (a) frequency responses and (b) output voltage with an input voltage of 100 mV. VM1 = Input, VM2 = Output of LNA, and VM3 = Output of band-pass filter.
simulated frequency response and outputs after INA and band-pass filter with an input voltage of 100 mV are shown in Figure 7-2(a-b), respectively. The amplified and filtered neural signal is then processed through Bluetooth integrated microcontroller (CC2650, Texas Instruments). The suggested microcontroller has an on-chip analog to digital convertor (ADC) with a sampling frequency of 200 kHz and a 12-bit resolution which is sufficient to digitize the analog neural signal. Finally, processed neural signal can be transmit wirelessly through the Bluetooth communication protocol. Such a readout circuit would alleviate many problems associated with in-vivo studies such as limited cord length and flexibility of animal motion.

**CNF Neural Probe Integrated with Optogenetics**

Optogenetics has been a popular research area for neural engineers due to its unique activation method. Optogenetics is a recently discovered technique which involves the use of light to either activate or deactivate the specific cells corresponding to different light wavelengths. It provides far more precision compared to conventional electrical stimulation. An integration of an optical waveguide and CNF neural probe will have great impacts in neural studies, disease diagnosis, and even fundamental study of the brain network.

Polymer optical waveguide can be utilized for optogenetics activation. Typical optical waveguides utilize the total internal reflection phenomenon. Optical waves reach a boundary with different refractive indices where the wave will, in general, be partially refracted at the boundary surface and partially reflected. If the angle of incidence is greater than the critical angle, then the wave will not cross the boundary, but will instead be totally reflected internally. The critical angle is defined as the incident angle at which light is refracted such that it travels along the boundary. This can only
occur when the wave in a medium with a higher refractive index reaches a boundary with a medium of lower refractive index. The core material of the polymer waveguide can be SU-8, which has good chemical stability and mechanical strength. The refractive index of SU-8 is around 1.57-1.59. Therefore, the coating material must have a lower refractive index and a compatible deposition method. Some potential candidates are Indium Tin Oxide (ITO) and Parylene.
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BIOGRAPHICAL SKETCH

Sheng-Po Fang was born in 1989 in Taipei City, Taiwan ROC. In 2005, he came to the United States of America and attended South Dade Senior High School in Miami, FL. After he graduated from South Dade Senior High School in 2008, he attended Miami Dade College and received his associate's degree in 2010. He then transferred and began his time at the University of Florida (UF) in Gainesville, Florida. Sheng-Po joined MnM and started exploring in microfabrication area in Summer 2011 under Dr. Yong-Kyu Yoon's guidance. He received his Bachelor of Science in Electrical and Computer Engineering (ECE) at UF in May 2012. Sheng-Po started his doctoral program in ECE Department under Dr. Yoon's supervision in Fall 2012. He completed his doctoral degree in Spring 2017 and relocated to Portland, Oregon to begin a career at the Intel Corporation as an integration engineer.