Synthesis and Surface Modification of Nanoporous Poly(ε-caprolactone) Membrane for Biomedical Applications

Dissertation

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ABSTRACT

A constant and well-controlled drug release rate is of paramount importance to implantable drug delivery systems, which are promising therapies against cancers and chronic diseases. Nanoporous membranes can be used to obtain the controlled drug release rate that is required. This dissertation presents a novel approach to preparing nanoporous poly(ε-caprolactone) (PCL) membranes. Moreover, the application of the nanoporous PCL membrane for controlled release is shown in this study.

The nanoporous PCL membranes were prepared via the combination of thermally- and nonsolvent-induced phase separations. For the phase separation process, nonsolvent has significant effect on pore formation and drug release rate. In nonsolvent-induced phase separation, a large amount of nonsolvent was added to casting solutions in order to improve pore connectivity within the membrane. The use of a Teflon plate for membrane casting can result in uniform nanoporous membranes and consistent lysozyme diffusion. Pore connectivity was improved significantly when coagulation bath temperature was lowered. By using a 5°C water coagulation bath in the wet-process precipitation, the average pore size reduced from 90 nm to 55 nm while increasing the casting solution
concentration from 15 wt% to 25 wt% PCL. Thus, by varying the polymer concentration of the casting solution, the lysozyme release rate can be manipulated with precise control. The potential application of nanoporous PCL membranes to achieve the preferable zero-order release rate is demonstrated in this dissertation.

Along with achieving the zero-order release rate, the nanoporous PCL membranes also provide immunoprotection for cell-based therapies/devices. Immunoisolation can be achieved by preventing Immunoglobulin G (IgG) from diffusing through the nanoporous PCL membranes. With appropriate pore size, the nanoporous PCL membranes can allow the diffusion of therapeutic agents (lysozyme) and block the diffusion of immune molecules (IgG). The application of the nanoporous PCL membranes to cell-based therapies/devices is also demonstrated in this dissertation.

Extensive fibrosis induced by the healing process can be detrimental to the long-term performance of implantable applications. The prevention of fibroblast adhesion to the nanoporous PCL membrane surface is crucial for constant and well controlled drug release. This study shows a novel method to modify the nanoporous PCL membrane surface with poly(ethylene glycol) (PEG). To achieve this goal, oxygen plasma and PEG(400) monoacrylate were used to graft the PEG onto the membrane surface through covalent bonding. Initially, various plasma treatment conditions were investigated to optimize the PEG-grafting quality and to achieve minimum fibroblast adhesion. After the treatment, water contact angle measurements and attenuated total reflectance-Fourier
transform infrared spectroscopy (ATR-FTIR) spectra confirmed that PEG was successfully grafted onto the PCL membrane. X-ray photoelectron spectroscopy (XPS) revealed that different plasma powers and treatment times can change the surface composition of membranes. Cell adhesion and morphology studies indicate that either lower plasma power or shorter treatment time is able to significantly improve resistance to cell adhesion. With the use of appropriate plasma treatment conditions, the effects of grafting density and PEG chain length on reducing fibroblast adhesion were also investigated in this study. PEG-diacrylates were also investigated for their influence on fibroblast adhesion. For PEG-diacrylates, increasing molecular weight can lead to a higher resistance against cell adhesion. However, PEG-diacrylates were not as effective as PEG(400)-monoacrylate for providing the resistance to cell adhesion.
DEDICATION

Dedicated to my family
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NOMENCLATURE

d  Density
D  Droplet size at time t
D_0  Droplet size at time t_0
K  Growth rate constant
t  Time
t_0  Time at zero
V  Volume
W  Weight

Greek letters
Φ  Porosity

Subscripts
p  Polymer
CHAPTER 1

INTRODUCTION

1.1. Implantable drug delivery devices

Implantable drug delivery devices are promising systems for treatment of chronic diseases. Chronic patients always suffer from severe health problems which come from permanent illness and/or organ failure. Hence, medical methodology which can be effective for a long period of time has attracted a lot of attention. To optimize the pharmacotherapy, long-term drug release has to be well controlled in accordance with the remedy during a treatment course. Ideal drug delivery devices are able to release the desirable amount of drug to the targeted site for a required period of time [1]. Because the drug is delivered locally, other than systematically, the uncomfortable side effect can be alleviated. Brem et al. used implantable wafers to locally deliver chemotherapeutic drugs for the treatment of brain cancer [2]. It was reported that 31% of the patients receiving this therapy were alive; however, only 6% of patients having conventional brain tumor treatment survived [3]. One of the current challenges is the compromise between the small scale of the devices and the amount of drugs clinically necessary [4]. From a logistic viewpoint, the devices must not be too small to deliver the required
quantity of drugs during a medical treatment course. Therefore, meso-scale devices, having a large enough reservoir, are more preferable than nano- and micro-devices when treating chronic diseases.

A unique feature of this implantable drug delivery device composed of a reservoir and a semipermeable membrane is to separate the formulation of the drug from its delivery mechanism [5]. This feature maximizes drug stability and uses the membrane to control drug release and deliver the drug to desired sites. In this research work, a schematic of the device is shown in Figure 1.1.

Many polymeric drug delivery systems typically have the form of drug dispersing in the specific polymers. Changing the drug loading in such systems can result in different drug release patterns. Besides, a large amount of effort in the pharmaceutical industry has been on studying the optimized drug formulation that prolongs drug activity. Matrix systems, such as microcapsules, and microspheres, have some disadvantages. For example, the efficiency of encapsulating the drug molecules into the matrix system is restricted significantly. The poor efficiency might result from the low drug solubility in the specific solvents where polymers are dissolved. Also, during the physical mixture process, some drug could be denatured by the organic solvents, high shear stress, and local high-temperature heating. However, the proposed drug delivery device can decrease the risk of deactivating drugs during the manufacturing process.
This newly-developed implantable drug delivery device is also called a diffusion chamber, which has two kinds, i.e., (1) drug-releasing chambers and (2) cell chambers. For drug-releasing chambers, drugs can be loaded in the chamber and sealed with a semipermeable membrane. Nanoporous membranes can be used to achieve the desirable drug release profile during the medication course. For cell chambers, living cells can be stored in a chamber and sealed with a membrane that is impermeable to host immune response components, but allows free exchange of nutrients and bioactive substances to cross the membrane. Nanoporous membrane can offer immunoisolation; therefore not only immunosuppressive drugs can be eliminated but also usage of xenogeneic cells is tolerant to solve the shortage of donated organs. This drug delivery strategy has many advantages over systemic drug therapy. These advantages are (1) lower doses, (2) long-term drug activity, (3) fewer number of administrations, (4) less possibility to induce antibiotic resistance, (5) potential ability to manipulate several drugs release, and (6) superior control of drug concentration within the therapeutic window. Therefore, implantable drug delivery devices can significantly reduce the side-effect and improve patient compliance.

1.2. Zero-order drug release rate

To constantly-sustained release drugs has been an emerging therapy for cancers and chronic diseases. For chronic patients, it is more preferable to deliver the required amount of a drug for a desirable period of time other than a short-term repeated
administration. Repeated intravenous injection can not only decrease patients’ comfort but also carry significant risks. In the case of endostatin, it was reported that constant release for a long time, other than bolus injection, enhances therapeutic efficacy for pancreatic cancer [1, 6]. For an ideal therapy, to maintain a drug concentration within the specific therapeutic window is required. Therefore, many adverse effects can be suppressed or eliminated, such as dizziness, headache, nausea and high fever. Furthermore, keeping drug concentration above the effective level would be a more efficient way to consume valuable drugs. The constant and sustained release of drug implies that the designed drug delivery system can achieve a zero-order release rate over the entire treatment course.

1.3. Application of nanoporous membranes for zero-order release rate

Cohen and Rubner schematically revealed a model to rationalize that a zero-order release rate can be obtained with the nanoporous films, as compared to porous films [7]. In their discussion, the drug release rate might follow the Fickian diffusion that does not give a zero-order release rate for the microporous films. Because of the unique geometry within the nanoporous films, the nanopores can bring on a different diffusion mechanism. Cohen et al. and Rubner et al. demonstrated that Ketoprofen and Cytochalasin D can be released with a zero-order release rate by using the poly(allylamine hydrochloride)/poly(acrylic acid) nanoporous film [7]. LaVan and his coworkers reported that a drug delivery device
comprising of a nanoporous membrane can control the release rate from a drug-releasing chamber.

This kind of device can be designed to release different drugs for a variety of diseases, such as diabetes and cancers [4]. Orosz et al. and Gupta et al. used an aluminum oxide filter with pores of 20 nm to release antiangiogenic and antioxidant drugs for the treatment of proliferative diabetic retinopathy and age-related macular degeneration [8]. Desai’s group used microfabrication to create silicon nanoporous membranes. The implantable device with the nanoporous membrane is a potential remedy against diabetes up to 14 days [9—12].

1.4. Application of nanoporous membranes for cell-based therapies/devices

Cell chambers, also called artificial organs, offer a useful method to treat chronic diseases, including diabetes [13, 14], hemophilia B [15], dwarfism [16], anemia [17], parathyroid [18], Parkinson’s disease [19], Alzheimer’s disease [20], and Huntington’s disease [21]. Allografted or xenografted cells can be stored in the chamber and sealed with semipermeable membranes. Porous membranes not only act as a barrier to isolate these cells and prevent attacking by immune system, but also allow the required amount of oxygen and nutrients to diffuse into the chamber, as shown in Figure 1.2 [22]. Cell chambers can manufacture bioactive substances such as erythropoietin [23], insulin [24],
factor XI [15], human growth factor [16], interferon-α [25], and ciliary neurotrophic factor (B). Moreover, certain cancer-fighting mechanisms might be stimulated by the chamber containing cancer cells [26].

Nanoporous membranes play an important role in the cell chambers, and they provide immunoisolation to maintain the viability and normal function of transplanted cells. For successful transplantation of immunoisolated cells, the nanoporous membranes have to be biocompatible. Hence, immune rejection would not be very acute when the device is implanted. There has been a concern about the mechanical stability of membrane. Membrane breakage might result in a serious problem. It was reported that the tubular chambers had broken in dogs after six months post-implantation and, the membrane material was fragile and susceptible to breakage [27]. It is suggested that an increase in the membrane thickness could be helpful. However, it might raise another problem as normal kinetics of glucose sensing and timely release of bioactive substances by the cell would be affected by the membrane thickness. For example, a thicker membrane wall could decrease the islet cell dynamic responses due to a longer pathway that glucose has to diffuse.

It was suggested that polymer alloy membranes would offer an approach to increase membrane mechanical stability. Polymeric additives can impart the mechanical strength to the matrix material while retaining its intrinsic properties, such as biocompatibility [28]. For example, Uchiyama et al. added segmented polyurethane (SPU) into the blood
compatible and biocompatible copolymer consisting of 2-methacryloyloxyethyl phosphorylcholine (MPC) [28]. Segmented polyurethane can increase the toughness of MPC matrix and does not decrease biocompatibility significantly.

For successful transplantation, ideal membranes are required to prevent components of cellular and humoral immune responses from passing through the membranes. Thus, the immune system could produce antibodies or lymphocytes against foreign bodies. For example, once immunoglobulin G (IgG) enters into the chamber, it would lead to the destruction of the transplanted cells. However, in case of diabetes treatment, the membranes must be permeable to glucose and insulin. Also, the porous membranes have to allow sufficient passage of oxygen and nutrients to maintain cell viability. For above reasons, pore size and uniformity are key factors in the immunoisolation.

Micromachined silicon membranes offer small enough pores and uniform pore size distribution [10], and they are almost able to block the passage of IgG. However, the vascular and dense fibrous tissue layer would encapsulate the silicon device. This dense layer could increase the diffusion path [12]. Therefore, there is not enough oxygen and nutrients to promote the long-term performance of the cell chambers. Moreover, the fibrous encapsulation could inhibit the diffusion of bioactive molecules, such as insulin. Eventually, the device will fail and lose its function.
Some materials could be helpful to avoid host immune attack due to intrinsic properties. They provide immunoisolation which does not rely on pore size control. Polyanions, such as poly(styrene sulfonic acid) (PSSA), can have such effect on the classic pathway of the serum complement. PSSA can suppress the cascade activation reaction of the classic pathway and the cytolytic phase via disrupting C1q(r2s2) complex formation [29]. When the drug delivery device is implanted in the human body, the immune complements could not be activated. This mechanism gives another promising approach to achieving immunoisolation.

Successful cell chambers also rely on the sufficient oxygen and nutrient supply [4]. These devices have a strong demand on the adequate mass transfer to and from the bloodstream. Foreign body reaction might result in the formation of fibrous capsules around the chambers. It is reported that, in the porous material, there are two extreme cases to cause fibrous encapsulation. One is that the pores are too small (~0.22 μm) to allow cells to immigrate. Inflammatory macrophages and foreign giant cells stay at the interface. After a while, they are overlaid by a thick layer of tissue matrix. The other case is that the pores are so large that host cells can enter into the pore structure; then adhere and flatten on the internal structure [30]. This fibrous layer consists of macrophage, body giant cells, vascular region and fibroblast embedded in a collagen matrix. It has been studied that cells could be broken and fragmented after a certain time [31]. Even preserved cells could be affected by necrosis. Shortages of oxygen and nutrient supplies are contributable to these deleterious phenomena. Typically, nutrients
include glucose, albumin, and transferring and growth factors [32]. To overcome this kind of problem, neovascularization is highly desirable.

Brauker et al. [33] and Colton et al. [30] found that the specific microporous membrane structure can induce the neovascularization at the interface between host tissue and immunoisolation membranes. They reported that the membranes which induce neovascularization have a suitable pore size (~8 μm). Such pores allow host inflammatory cells to invade the interstices. When these inflammatory cells enter into the pores, most of these cells remain a rounded morphology and do not adhere to the pores. Moreover, Colton et al. [34] and Knighton et al. [35] proposed that some host inflammatory cells which enter into the tortuous pores could encounter locally low oxygen partial pressure. This hypoxia environment might make some cells to secrete angiogenic factors to promote neovascularization.

Colton et al. also suggested that the asymmetric pore structure, prepared by phase separation, could be better for cell chambers than other structures. The inner membrane, having smaller pore size and facing the transplanted cells, might prevent immune response substances from diffusing into the device. The outer membrane has larger pore interstices which are able to induce neovascularization. Surface modification also gives another approach to accelerating neovascularization. Williams et al. [36] discovered the binding of extracellular matrix protein onto the membrane surface could trigger the
neovascularization at the interface. It is a potential solution to the shortage of oxygen and nutrients as well.

In Desai’s study, the membrane can provide not only a well-controlled release rate but also immunoisolation for cell-based biomedical applications [9—12]. The nanopores can allow the diffusion of insulin and glucose and retard the permeation of immunoglobulin G. Nanoporous membranes have played an important role in the development of implant drug delivery devices [4, 8]. The membranes have potential for cell-based drug delivery devices to offer immunoisolation for the devices. For immunoisolation, the membranes have to be able to allow for free diffusion of nutrients, metabolic wastes and cell-secreted bioactive molecules while impeding the passage of immune molecules such as Immunoglobulin G (IgG) [9]. In this study, IgG diffusion through the membrane was also investigated. Nanoscale pores offer not only the control of release rate, but also the suitability for immunoisolation [10]. However, most of available membranes are made of non-degradable materials. It might be necessary to perform surgery in order to retrieve the device. This medical operation would result in discomfort in addition to the cost and inconvenience. Hence, biodegradable polymer is a favored material in the most of drug delivery systems.
1.5. Preparation of nanoporous PCL membranes

Recently, poly(ε-caprolactone) (PCL) has drawn a lot of attention in biomedical applications. PCL has several advantages including low cost, biocompatibility, and biodegradability. Moreover, PCL is a U.S. Food and Drug Administration approved implantable material, such as suture. Thus, PCL is a superior material to fabricate an affordable and implantable drug delivery device.

The porous membranes play an important role in a variety of drug delivery/release systems [9, 10, 37—41]. Several factors, including porosity, turtuosity, and pore size, have a crucial effect on controlling the rate of drug diffusion through the membrane. Currently, porous PCL membranes have been prepared by solvent-cast-leaching method [42, 43], bi-axial stretching [44, 45], thermally-induced phase separation [46, 47], nonsolvent-induced phase separation [48—50], and vapor-induced phase separation [51, 52]. However, state-of-the-art porous PCL membranes which are prepared via above methods have the structure of micro-scale pores. Due to the large pores, the mechanism governing diffusion phenomena could be Fickian diffusion and a constant release rate would not be achieved. However, a nanopore could provide a different release mechanism allowing for a constant release rate. When a protein drug of interest is released from a nanopore, the nanopore will be refilled by another protein at a similar rate [7]. In other words, only one molecule can pass through the nanopore at a time. The observed release behavior is dominated by the zero-order release mechanism. Thus, it is
desirable to prepare a nanoporous PCL membrane, as a barrier, to achieve a zero-order release rate.

1.6. Surface modification for the prevention of biofouling

Biomedical devices implanted into the human body would initiate a sequence of host reactions, listed in Table 1 [53]. After implantation of drug delivery devices, monocytes and macrophages would promote healing response. Following the action, fibroblasts and vascular endothelial cells can proliferate at the implant site and, in turn, lead to the formation of granulation tissue. During the development of granulation tissue, fibroblast still can proliferate and synthesize collagen and proteoglycans. In the late stage of granulation tissue development, collagen, especially type I collagen, is responsible for the formation of fibrous capsules. Also, fibrous encapsulation is involved in the end-stage healing response. Within the wound-healing process, the injured tissues can be replaced by connective tissues which are mainly composed of fibrous capsules. In general, the fibrosis can separate an implanted device from the local tissue environment. Particularly, fibrous encapsulation around implants can bring about serious complications, for example, capsular contracture of breast implants and scarring of heart valve sewing rings [53, 54].

Poly(ethylene glycol) (PEG)/poly(ethylene oxide) (PEO) can prevent fibrosis and biofouling. There are three possible mechanisms involved in the biomolecules-repelling
property of PEG/PEO chains on various substrates in aqueous solution [55]. They are interfacial energy, steric stabilization effect, and mobility. One hypothesis about the interfacial free energy is that the driving force for biomolecules adsorption/adhesion decreases while the interfacial free energy gets closer to zero. Biomolecules, such as proteins and cells, at or near the lower interfacial energy surface would have more attractive interaction with the bulk solution than with the surface. Thus, non-specific protein adsorption and cell adhesion would not take place when zero interfacial energy surfaces are provided. Interfacial free energy of many materials can be calculated according to contact angle measurements. It is shown that there is a relatively low interfacial free energy at the interface between water and PEG. As a result, a very low driving force can be available for biomolecule attachment.

Figure 1.3 demonstrates the effect of the steric stabilization on preventing protein from adsorption [55]. The steric stabilization effect can be classified into two main categories, i.e., volume restriction effect and excluded-volume effect. For the volume restriction effect, it is assumed that two approaching layers are impenetrable. While one layer approaches the other, the adsorbed layer would be compressed, and the polymer segments within the interaction region may lose their configuration entropy. Therefore, these polymer segments may occupy fewer possible configurations in the compressed condition than in the incompressible condition. The reduction in entropy leads to an increase in Gibbs free energy and the formation of the repulsive force between the surfaces. In the case of the excluded-volume effect, it is assumed that the adsorbed layers of two surfaces
are able to overlap. This contact between the segments, from each surface, can cause an increase in the enthalpy of mixing ($\Delta H_M$). Due to higher segment concentration in the overlapped region, the configuration entropy ($\Delta S_M$) of the adsorbed polymer would be reduced. Consequently, the total Gibbs free energy in this system would increase, and the repulsive interaction between two surfaces can be developed. While protein is approaching the surface, the volume accessible to the grafted PEG/PEO chains reduces. The PEG/PEO molecules intend to maintain the original chain conformations, resulting in the repulsive force for preventing the protein from adsorption [55].

Because PEG has a unique structure, it can fit in the tetrahedral structure of water without distortion of the water lattices. Hence, PEG has high solubility in water, and tendency for the hydrophobic interaction between PEG and water molecules can be inhibited. The hydrophilicity and solubility of PEG result in the surfaces with a liquid-like film, allowing the polymer chains with tremendous flexibility and mobility. Moreover, PEG chains would not be hindered sterically in aqueous solutions because there are no bulky side groups in the structure. Figure 1.4 displays the effect of PEG mobility on the prevention of protein adsorption. In order to attach on the surfaces firmly, biomolecules have to contact with a surface for a certain period of time. Fast movement of hydrated PEG chains can significantly prevent proteins and cells from staying on surfaces because of shorter contact time. It is reported that long PEG chains, rather than short chains, can prevent biofouling more effectively [55].
Wisniewski and Reichert have shown that membrane biofouling has a detrimental effect on implantable needle-type glucose biosensors [56]. As shown in Figure 1.5, fibrous encapsulation around the implanted site can retard analyte diffusion to the sensor, such as glucose. Hence, the protein adsorption and cell adhesion can deleteriously influence the sensor response. It is necessary to eliminate biofouling on the sensor membrane surface for the development of long-term implantable biosensors. Desai and coworkers have demonstrated that the drug release rate decreases significantly due to fibrosis, especially for a large protein [57]. Fibrous encapsulation, a result of host response to implantation, can limit the long-term performance of drug delivery devices. Thus, it is required to obtain a nonfouling membrane surface. It is reported that surface modification with PEG can be the most efficient approach for biofouling prevention [58].

1.7. Scope of current work

The objective of this study is to develop nanoporous PCL membranes for biocompatible and biodegradable drug delivery devices with a constant, controllable and long-term release rate. A review of current techniques to prepare polymeric porous membranes is shown in Chapter 2. Chapter 3 displays a novel process to prepare the nanoporous PCL membranes. In Chapter 3, the nanoporous poly(ε-caprolactone) (PCL) membranes have been successfully prepared via the combination of thermally- and nonsolvent-induced phase separation. The results show that zero-order release rate can be achieved with
nano-scale pores. Moreover, the nanoporous PCL membrane has the potential for the immunoisolation of the cell-based drug delivery devices.

In Chapter 4, the importance of surface modification of the membranes is illustrated. Chapter 4 also introduces a variety of methods to accomplish the surface modification. Chapter 5 presents a simple and efficient approach to modifying the nanoporous PCL membrane surface with EPG. In an attempt to prevent cell adhesion, poly(ethylene glycol) (PEG) was grafted onto the membrane surface by using PEG-monoacrylate and oxygen plasma. The results show that fewer cells can adhere onto the PEG-modified nanoporous PCL membrane surface.
**Table 1.1.** Sequence of host responses to implantation of biomedical devices [53].

<table>
<thead>
<tr>
<th>Response</th>
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<tbody>
<tr>
<td>Injury</td>
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<tr>
<td>Blood-material interactions</td>
</tr>
<tr>
<td>Provisional matrix formation</td>
</tr>
<tr>
<td>Acute inflammation</td>
</tr>
<tr>
<td>Chronic inflammation</td>
</tr>
<tr>
<td>Granulation tissue</td>
</tr>
<tr>
<td>Foreign body reaction</td>
</tr>
<tr>
<td>Fibrosis/fibrous capsule development</td>
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</tbody>
</table>
Figure 1.1. Schematic illustration of the implantable drug delivery device which were developed in this study.
Figure 1.2. Illustration of transport requirements for different species and the role of porous membrane [22].
Figure 1.3. The effect of steric stabilization on preventing protein from adsorption [55].
Figure 1.4. The effect of PEG mobility on the prevention of protein adsorption [55].
Figure 1.5. Illustration of causes for the deleterious response of a subcutaneously implanted needle-type glucose biosensor. For the case of fibrous encapsulation, glucose diffusion is significantly limited from the tissue to the sensor [56].
CHAPTER 2

OVERVIEW OF POROUS MEMBRANE PREPARATION METHODS

Abstract

This chapter provides a general overview of porous membrane preparation methods. Several techniques can be used to prepare porous polymeric membranes. They are sintering, stretching, track etching, phase separation, selective etching of self-assembly of block copolymers and phase inversion with self-assembly of block copolymers. Moreover, this chapter presents a literature review about how to prepare porous PCL membranes.

2.1. Sintering

In this process, polymeric particles are compressed at a given pressure and sintered at a specific temperature. During the sintering, the interfaces between the contacting particles disappear and separate particles can be merged into a unity. A schematic illustration of this method is shown in Figure 2.1. The porous structure of the obtained membrane is
determined by the particle size and particle size distribution. Smaller particle size can give smaller pore size in the resultant membrane. In addition, the narrower particle size distribution can lead to the more uniform pore size within the membrane matrix. The pore sizes are allowed to be 0.1—10 µm. [59]

2.2. Stretching

During the preparation, an extruded film made of semi-crystalline polymer is stretched perpendicular to the direction of the extrusion, as shown in Figure 2.2 [60]. With application of a mechanical stress, small fractures form, and a porous membrane can be obtained. Huang et al. studied the effects of lubricant content, extrusion speed, stretching ratio, asymmetry heating temperature and heating time on the porous structure of polytetrafluoroethylene (PTFE) membrane [61]. Figure 2.3 displays the SEM picture of the PTFE porous membrane which was prepared with a stretching ratio of 50% and 320°C stretching temperature. The pore size of the stretched membrane is usually larger than 100 nm.

2.3. Track-etching

The simplest pore geometry, i.e., parallel cylindrical pore with a uniform size, in a membrane can be produced via this method. During the track-etching process (Figure 2.4), a polymeric film is exposed to high-energy particle radiation such as Ar\(^{9+}\). The
accelerated heavy ion particles can bombard the membrane surface and create tracks through the membrane. Then, the membrane is immersed in an etching solution usually a strong acid and alkaline. The damaged region is etched away along the track to form a cylindrical or conical pore [59]. A wide range of beam sizes, ion energies, fluxes and angle of incidence can be used to obtain a nanoporous membrane with different pore sizes and porosities. A membrane with pore size of 15—100 nm can be prepared via this track-etching method, as shown in Figure 2.5. Moreover, the composition of the etching bath plays an important role in the pore geometry and the pore structure as well. With addition of a surfactant, the pore geometry can be manipulated according to the etching speed, i.e., the concentration of the etchant. Fast etching can result in a conical pore, and slow etching can lead to a cylindrical pore, as illustrated in Figure 2.6 [63]. During the etching process, the diffusion rate of the surfactant molecules in the small volume of the etched pore is significantly different with that of etchant molecules. When the etchant diffusion rate is higher than the surfactant, a conical pore can be formed. In contrast, surfactant molecules can have enough time to rearrange on the wall of the etched pore when the diffusion rate of the etchant is much lower than that of the surfactant molecules. Therefore, a cylindrical pore can be formed.

2.4. Selective etching of self-assembly of block copolymers

Nanostructure can be obtained inherently with self-assembly of a block copolymer. An AB diblock copolymer with two-third A monomers and one-third B monomers can
organize to form a long cylinder of B with a nanodiamter (5—50 nm) entrapped in a continuous matrix of A. There are two basic requirements for this process. One is that the block copolymer has to compromise two chemically distinct polymer chains. The other is that the major component of the block copolymer has to be etch-resistant, and the minor component has to be chemically, thermally, or photo-chemically etchable. The concept for this method is schematically shown in Figure 2.7 [64]. A spin-coated poly(styrene-b-methyl methacrylate) (PS-b-PMMA) film on a substrate was displayed in Figure 2.8 (a). On heating, a cylindrical domain of PMMA can be formed within the film. Figure 2.8 (b) shows the same film after exposure to ultraviolet (UV) radiation and rising with alcohol [65].

2.5. Phase separation

Phase separation is the most popular method to prepare polymeric porous membranes. A variety of porous structures can be produced by using this method. During a phase separation process, the state of a polymer is transformed from liquid to solid. A phase separation process is initiated by decreasing the solubility of a polymer solution. There are several techniques to change the solubility and induce a phase separation process, such as precipitation by solvent evaporation, precipitation from the vapor phase, thermal precipitation, and immersion precipitation.
For the precipitation by solvent evaporation, a polymer is dissolved in a solvent and cast on a substrate. The solvent is usually volatile and allowed to evaporate for a certain time. When the amount of solvent decreases, the solubility would decrease and the porous membrane would precipitate out eventually. During the precipitation from the vapor phase, a polymer solution is cast on a substrate; and further the cast film is placed in a vapor phase. The vapor phase usually contains nonsolvent, and the nonsolvent is miscible with the solvent. Once the nonsolvent is introduced into the cast film, phase separation would begin to take place. While the solubility decreases to a certain point, a porous structure would be obtained.

For the thermal precipitation, a temperature change is used to make phase separation happen. Initially, a polymer is dissolved in a solvent at elevated temperature. After a homogeneous solution is obtained, the solution can be cooled to enable the phase separation process, and a porous membrane can be obtained as well.

Immersion precipitation is a well-studied method to prepare porous membranes. Nanopores can be easily achieved via this method. During the membrane preparation process, a polymer solution is cast on a substrate and, in turn, the cast film is immersed in a coagulation bath. The major component of the coagulation bath is nonsolvent; the nonsolvent is miscible with the solvent. The phase separation can occur because the nonsolvent can exchange with the solvent and lower the solubility [59].
2.6. Phase inversion with self-assembly of a block copolymer

Peinemann et al. used phase inversion with self-assembly of a block copolymer to prepare highly uniform nanoporous membranes [66]. Initially, the block copolymer solution was cast on a glass plate. Next, the case film was allowed for 10-second evaporation, and the self-assembly can occur. Then, the cast film was immersed into water to make the phase inversion happen. This process has several advantages, such as ease of up-scaling, high mechanical strength, and long-range order of the uniform pore size. Figure 2.9 shows the SEM pictures of this kind of the membrane prepared by Peinemann et al.

2.7. Preparation of porous PCL membranes

Recently, PCL has become a desirable material in the fields of tissue engineering and drug delivery because of its biocompatibility, biodegradability, low cost and commercial availability. Lin’s group [42, 43] used a solvent-cast-leaching method to prepare PCL microporous membranes. In their studies, various ratios of PCL and PEG were dissolved in acetone or dichloromethane. Then, the solutions were cast onto glass molds. After complete drying, the membranes were soaked in de-ionized water for 14 days. Because PEG has high solubility in water, it can be easily leached out from the membrane matrix in an aqueous solution. Herein, PEG was used as a pore former in this process. However, the pore size was in microns, and the application for a constant release rate would not be likely.
Khor et al. fabricated PCL microporous membranes with 100 μm pores on the surface via the biaxial stretching method [45]. PCL pellets were dissolved in methylene chloride to form a 6 w/w% solution and cast on a glass plate. After the solvent was completely removed by evaporation, the cast film was heated to improve the uniformity and reduce the defects. Moreover, the film was pressed at a specific pressure. Finally, the film was biaxially drawn to obtain the porous structure. Such large pore size would be appropriate for tissue engineering rather than for controlled drug release.

Tanaka and Yamamoto used vapor induced phase separation to produce porous PCL membranes [51, 52]. PCL and a copolymer of dodecylacrylamide and ω-carboxyhexylacrylamide, as a surfactant, were dissolved in various solvents, including chloroform, benzene, toluene, and xylene. The porous PCL membranes were prepared under blowing highly humid air. Due to existence of the surfactant, the fabricated porous membranes had a uniform pore size, but larger than 1 μm.

Coombes and his coworkers prepared porous PCL matrices by using nonsolvent induced phase separation [45, 49, 50]. Initially, PCL was dissolved in acetone at a certain temperature to produce 12.5-17 w/v% solution. After a period of time, the solution was poured into a mold, i.e., polypropylene syringe body. Methanol, a nonsolvent, was slowly added into the mold. Because acetone and methanol are miscible, methanol can replace acetone to influence the thermodynamic stability. Hence, addition of methanol
can cause precipitation and produce microporous PCL matrices. The resulting large pore size might be suitable for tissue engineering instead of drug release.

Takashi and Lloyd et al. used thermally-induced phase separation to produce PCL microfiltration membranes [46]. They prepared PCL solution in a mixed diluent of 1,4-dioxoane and water (87:13) at 80°C. In the experiment, they used 1,4-dioxane as a solvent and water as a nonsolvent. With an appropriate composition, the solution would become thermodynamically stable at high temperature and undergo phase separation at low temperature. Then, the solution was heated at 80°C to obtain a clear and homogeneous solution. This solution was well sealed in a metal pan. In the first step, the solution was cooled to 56°C which was 4°C above the cloud point of this solution. Next, the solution was quenched to 0°C in a water bath. After 2 hours, the formed membrane was washed with chilled water to remove the organic residuals. In this process, PCL is soluble in the mixed diluent at 80°C. However, the solubility decreases as the temperature is lowered. Because of solubility change, PCL would be precipitated from the solution at the lower temperature. Further research showed that the pore size can be smaller when the solution was quenched in liquid nitrogen (-196°C) [47]. However, the pore size could be too large to achieve a zero-order release rate. Therefore, a novel method to prepare nanoporous PCL membranes is developed and demonstrated in this dissertation.
Figure 2.1. Illustration of sintering process.
Figure 2.2. Schematic of the stretching process [60].
Figure 2.3. SEM picture of the PTFE porous membrane made by Huang et al. [61].
Figure 2.4. The drawing of the track-etching method to prepare a nanoporous membrane.
Figure 2.5. SEM of nanoporous track-etched membrane [62].
Figure 2.6. (A) Slow etching with 5M NaOH (B) Fast etching with 6M NaOH and 0.025 % sodium dodecyl diphenyloxide disulfonate [63].
Figure 2.7. Illustration of the nanoporous membrane formation by selective etching from self-assembly of block copolymers [64].
Figure 2.8. (A) A PS-b-PMMA film (~40 nm thick) with ~20 nm cylindrical microdomains of PMMA on a substrate. (B) The same film after UV-radiation exposure and rinsing with alcohol. The inset is field-emission SEM of the film, demonstrating the uniform pore size. The image size is 2 µm × µm [65].
Figure 2.9. SEM images of polystyrene-block-poly(4-vinylpyridine) nanoporous membrane. (Left) A view of the edge (Right) A view of the top [66]
CHAPTER 3

PREPARATION OF NANOPOROUS PCL MEMBRANES FOR ZERO-ORDER DRUG RELEASE RATE

Abstract

A constant and well-controlled drug release rate is of paramount importance to implantable drug delivery systems in finding the remedy against chronic diseases. This chapter describes the synthesis and potential application of nanoporous poly(caprolactone) (PCL) membranes to achieve the zero-order release rate. Nanoporous PCL membranes were prepared via the combination of thermally- and nonsolvent-induced phase separations. In the membrane preparation, 1,4-dioxane and 2-methoxyethanol were used as solvent and nonsolvent, respectively, resulting in uniform nanoporous membranes and consistent lysozyme diffusion using a Teflon plate for membrane casting. Pore connectivity was improved significantly when coagulation bath temperature was lowered from 35 to 5°C. Also, nonsolvent has paramount effect on porosity and pore connectivity. With sufficient addition of nonsolvent, it is more likely to obtain open porous structure within the membrane matrix. By using a 5°C water coagulation bath in the wet-process
precipitation, the average pore size reduced from about 90 nm to 55 nm while increasing the casting solution concentration from 15 wt% to 25 wt% PCL. Thus, by varying the polymer concentration of the casting solution, the lysozyme release rate was well controlled. The potential of nanoporous PCL membranes for an implantable drug delivery device to achieve the zero-order release rate was demonstrated in this study. In addition, this dissertation presents the potential application of the nanoporous PCL membranes for the cell-based therapies/devices. With appropriate pore size, the membranes can allow the diffusion of drug and block the passage of immune species simultaneously.

3.1. Introduction

Sustained and well-controlled release of protein- and DNA-based drugs has been an emerging therapy for the treatment of chronic diseases. For patients suffering from a chronic illness, it is preferable to deliver a drug of interest for a desirable period of time. In the case of endostatin, the constant release for a long period of time, other than bolus injection, enhanced therapeutic efficacy for pancreatic cancer [1, 6]. An ideal controlled release system is able to maintain a drug concentration within the specific therapeutic window [67]. Several side effects, such as nausea and high fever, resulting from a drug concentration above the toxic level, can be prevented. Moreover, keeping drug concentration above the effective level can improve the efficient usage of a drug. To maintain constant drug concentration in the body implies that the designed drug delivery
system can offer a zero-order release rate over the entire treatment course. However, delivering a drug of small molecular size to the targeted tissues and cells in a safe, well-controlled, and patient-friendly manner remains a significant challenge [68]. Therefore, a novel method to engineer the release rate of such small molecules has to be developed.

For a membrane-based drug delivery system, the porous membrane structure, such as pore size, porosity and tortuosity, has a crucial effect on controlling the drug release rate [37—41, 69, 70]. The nanoporous structure is a determinant factor in the achievement of the zero-order release rate. Based on their experimental results and schematic model, Berg et al. showed and explained that a zero-order release rate can be achieved by using nanoporous polyelectrolyte films, instead of microporous films [7]. Paulose et al. displayed that a zero-order diffusion system can be obtained with utilization of the nanoporous TiO₂ membrane [71]. Ferrari et al. used nanoporous silicon membranes to deliver interferon-α with a zero-order release rate [11, 69]. Moreover, a mathematical model was proposed to fundamentally understand the zero-order release behavior with the nanoporous membranes [72, 73]. Therefore, a nanoporous membrane with tunable structure can offer a means to perform a versatile constant drug release rate during the entire medical treatment.

In medical and pharmaceutical fields, the drug or cell-loaded chambers sealed with a semipermeable membrane are capable of releasing the molecule of interest for a long period of time [4]. The nanoporous membrane can form a barrier between the
surrounding and the inside of the chamber. When the pore size is small enough, a well-controlled drug release rate can be obtained. In our previous study, an implantable membrane-based drug delivery device was successfully developed, and its schematic design is shown in Figure 3.1 [75]. Such a system can provide several potential advantages over microparticles and bulk materials for protein delivery, including high loading efficiency without use of any solvents with limited solubility of biomolecules, less denature of cells and/or protein drugs without exposure to organic solvents during assembling the device, availability of long-term release with the reservoir of a large volume, capability of loading optimized formulation [5], and a pre-designed release profile with an appropriate nanoporous structure [1]. Thus, the nanoporous membrane will be the key component to achieve the desirable constant release rate.

Furthermore, nanoporous membranes have potential for cell-based drug delivery devices. The nanoporous membranes have to offer immunoisolation for the devices. The membranes capable of immunoisolation have to be able to allow for free diffusion of nutrients, metabolic wastes and cell-secreted bioactive molecules while impeding the passage of immune molecules such as Immunoglobulin G (IgG) [9]. In this study, IgG diffusion through the membrane was also investigated. Nanoscale pores offer not only the control of release rate, but also the suitability for immunoisolation [10].

Silicon, alumina and titanium oxide-based nanoporous membranes are chemically inert and mechanically stable. They have highly uniform and well-defined pore structures [11,
69, 71, 76]. However, if implanted, they must be surgically removed at the end of the medical treatment. Inexpensive polymers such as polycaprolactone (PCL), on the other hand, can be fully biodegradable, so a surgical retrieve is not necessary. Moreover, PCL is a U.S. Food and Drug Administration approved implantable material. Therefore, PCL is an excellent candidate to make implantable drug delivery devices.

Currently, porous PCL membranes have been prepared by a solvent-cast-leaching method [42, 43], bi-axial stretching [44, 45], thermally-induced phase separation [46, 47], nonsolvent-induced phase separation [48—50], and vapor-induced phase separation [51, 52]. However, state-of-the-art porous PCL membranes which are prepared via above methods have a micro-scale pore structure. Due to the large pores, the mechanism governing diffusion phenomena may be Fickian diffusion, and the zero-order release rate will not be achieved [7]. Thus, a reliable and promising method is sought to synthesize the desirable nanoporous PCL membrane.

None of studies so far have reported the synthesis of nanoporous PCL membranes for controlled drug release. In this study, nanoporous PCL membranes were successfully prepared via the combination of thermally- and nonsolvent-induced phase separations. A sufficient amount of nonsolvent was used to bring thermally-induced phase separation into effect. Lysozyme was used as a model drug. The consistent lysozyme diffusion rate, indicative of uniform membrane structure, was obtained when a Teflon plate was used to prepare the free-standing nanoporous PCL membranes. The effect of varying coagulation
bath temperature on the pore structure and lysozyme diffusion rate was investigated. Different polymer concentrations for the casting solution were used to control the lysozyme diffusion rate. The potential of nanoporous PCL membranes for the implantable drug delivery device (Fig. 3.1) to achieve the zero-order release rate was demonstrated in this study. Moreover, this dissertation shows that the nanoporous PCL membranes can block the diffusion of IgG to provide potential immunoprotection for cell-based therapies and devices.

3.2. Experiment

3.2.1. Materials

Polycaprolactone (Mₙ ~80,000) was purchased from Aldrich Chemicals (Milwaukee, WI). 1,4-Dioxane was obtained from Mallinckrodt Chemicals (Philipsburg, NJ). 2-Methoxyethanol (ACS reagent, ≥ 93%), lysozyme (from chicken egg white, lyophilized powder, ~95% protein, and ~50,000 units/mg) and Immunoglobulin G (from bovine serum, reagent grade, ≥ 95%) were purchased from Sigma-Aldrich (St. Louis, MO). Phosphate buffered saline solution (PBS) was purchased from Fisher Scientific Inc. All chemicals were used as received without further purification.

3.2.2. Preparation of nanoporous membranes by phase separation

Nanoporous polycaprolactone membranes were prepared using a combination of thermally- and nonsolvent-induced phase separations. In the preparation of the casting
solution, PCL at a suitable concentration (e.g., 20 wt%) was dissolved in a diluent which consisted of 15 wt% 1,4-dioxane as solvent and 65 wt% 2-methoxyethanol as nonsolvent. The solution was well stirred and heated at 50°C for approximately 2 hours to ensure that it was clear and homogeneous. A general membrane preparation procedure is described below. The PCL solution at 50°C was cast on a Teflon plate, and the cast film along with the Teflon plate was then immediately immersed into a coagulation bath. After 4 – 6 h, the newly-formed membrane on the Teflon plate was removed from the bath. To remove the membrane from the Teflon plate, isopropanol was poured onto the membrane. After 20 – 30 minutes, the membrane was peeled off from the Teflon plate. The resultant PCL membrane was around 50 μm thick. In this study, nanoporous PCL membranes were prepared in four different ways.

1. Different casting substrates: The casting solution composed of 20 wt% polycaprolactone, 65 wt% 2-methoxyethanol and 15 wt% 1,4-dioxane was cast on a glass plate and a Teflon plate. Immediately, the cast film was immersed in a 5°C water bath. Then, the membranes were peeled off and dried in air.

2. Different coagulation bath temperatures: The same casting solution described above was cast on the Teflon plate. Then, the cast film was immersed into 5, 15, 25 or 35°C water.

3. Different PCL concentrations in the casting solution: Three casting solutions of
15, 20 and 25 wt% PCL were prepared in the diluents mixture of 2-methoxyethanol and 1,4-dioxane at the 65/15 weight ratio. Each of the solutions was cast on the Teflon plate and then immersed into 5°C water.

4. Thermally-induced phase separation: The 20 wt% PCL solution described earlier was cast on the Teflon plate, and the cast film was placed in a 5°C close chamber to decrease the evaporation of solvent and nonsolvent. After 6 hours, the formed membrane was soaked in 5°C water for the removal of organic residual.

3.2.3. Diffusion of lysozyme through nanoporous PCL membranes

The ability of membranes to control lysozyme diffusion was examined in vitro by using a permeation cell made of polycarbonate. The cell consisted of two parts, permeate and retentate compartments, with equal volume of 3.7 mL. The membrane was fixed between these two compartments, sealed with an O-ring and screwed together. The permeation cell was rotated on a shaker at 37°C with a constant speed of 130 rpm to ensure homogeneous solutions and no boundary layer effects on the mass transfer behavior. In addition, different rotating speeds (100 – 260 rpm) including the aforementioned constant speed were tested to show that they had no effect on the mass transfer behavior. At time zero, the permeate compartment was filled with fresh PBS (phosphate buffered saline, pH = 7.4) solution, and the feed compartment was loaded with 4 mg/ml lysozyme or 1 mg/ml IgG. Every 24 hours, 0.5 ml solution was collected from the permeate compartment. The volume withdrawn was replaced with fresh PBS. The concentration of lysozyme
was determined at 280 nm using a UV-VIS spectrophotometer (UV-1700, Shimadzu Inc., Columbia, MD). The IgG concentration was measured by the plate-reader (Tecan, Durham, NC) at 595 nm.

### 3.2.4. Observation of membrane morphology by scanning electron microscopy

For cross-section observation, the PCL membranes were soaked in isopropanol. Then, the membrane was fractured in liquid nitrogen and mounted on the sample stage vertically. The surface of the sample was coated with Au/Pd. Scanning electron microscopes (SEMs) (S-3000H, Pleasanton, CA and Zeiss Ultra 55 Plus FE-SEM, Peabody, MA) with an accelerating voltage of 5 and 1kV were used to study the membrane morphology.

### 3.2.5. Determination of cloud point

A cloud point was measured by visual observation of appearance of turbidity. A solution consisted of 20 wt% PCL, 65 wt% 2-methoxyethanol and 15 wt% 1,4-dioxane was heated at 80°C and mixed thoroughly in a glass vial for 2 hours. Then, the vial was quenched in 5°C water to estimate a possible range of the cloud point. Within the possible range of the cloud point, a vial was placed in an oven at a specific temperature for one hour to observe the appearance of turbidity in order to decide the exact cloud point.
3.3. Results and discussions

3.3.1. The effect of casting substrates

Ma et al. [77] and Zeng et al. [78] reported that casting substrates had significant effects on the structure of polymeric films. It was shown that the crystallinity of poly(vinylidene fluoride) / poly(methyl methacrylate) film was affected by various casting substrates [77]. Zeng et al. illustrated that the miscibility and mechanical properties of cross-linked waterborne polyurethane and carboxymethylchitin films could be improved when a hydrophobic substrate, Teflon plate, was used [78].

In our study, a hydrophilic substrate, glass plate, and a hydrophobic substrate, Teflon plate, were used to investigate their effects on the PCL membrane formation. The uniformity of the nanoporous PCL membranes prepared was examined by measuring the diffusion rate of lysozyme. From the same piece of a membrane which was cast on a glass plate or a Teflon plate, several samples were cut to study the lysozyme diffusion profile. Figures 3.2(A) and 3.2(B) display that the cumulative lysozyme diffusion profile, in terms of the percentage of the original amount of lysozyme in the feed solution diffused across the membrane, would be more consistent when the membrane was cast on the Teflon plate instead of the glass plate. Aerts et al. also showed that more uniform porous structure could be obtained while the dope solution was cast on a Teflon plate [79]. As explained by Zeng et al., the use of the Teflon plate might be able to improve the miscibility of the casting solution, resulting in a uniform porous structure [77].
3.3.2. The effect of coagulation bath temperature

The rates of lysozyme diffusion through nanoporous PCL membranes which were prepared by using 5, 15, 25, and 35°C water coagulation baths were compared. The casting solution consisted of 20 wt% PCL, 65 wt% 2-methoxyethanol, and 15 wt% 1,4-dixoane. Figure 3.3 illustrates the effect of various coagulation bath temperatures in membrane preparation on the lysozyme diffusion rate. These results indicate that the rate of lysozyme diffusion through the PCL membrane could be higher when a lower water bath temperature was used.

The SEMs in Figure 3.4 exhibit the important effect of using coagulation bath temperatures at 5, 15, 25 and 35°C on the pore morphology of the top surface for each of the membranes. For the coagulation bath temperatures of 5, 15 and 25°C, Figure 3.5 displays the corresponding pore size distribution for each of these three membranes. For each membrane, the pore size distribution was obtained by analyzing the sizes of pores shown in the SEM. As shown in Figures 3.4 and 3.5, the average pore size for the top surface of the membrane increased when the coagulation bath temperature was increased. In Figures 3.4 (A) and 3.5, the average pore size was around 70 nm (20 – 200 nm) for the water bath temperature of 5°C. When water temperatures were increased to 15°C and 25°C, the average pore sizes became about 150 nm (30 – 300 nm) and 350 nm (80 – 900 nm), respectively. Pore coarsening and the use of the nonpolar plate, i.e., Teflon, were presumably responsible for the formation of noncircular and irregular pore structure.
shown in Figure 3.4 [80]. For the water bath temperature of 35°C, there were very few
pores (>1 µm) appeared on the surface of the membrane.

The pore morphology underneath the top surface can be significantly affected by the pore
formation mechanism, a result of phase separation with different coagulation bath
temperatures. Figure 3.6 displays the distinct influence of the coagulation bath
temperature on the pore structure in the cross-sections of the membranes. This figure
shows that the cellular morphology which is ascribed to liquid-liquid demixing during the
membrane formation. The cellular pore is a typical characteristic of nucleation and
growth of a polymer-lean phase. As observed in this figure, higher water bath
temperature could increase the pore size in the cross-section of the membrane. The result
and conclusion from Figure 3.6 are similar to those from Figure 3.4. Moreover, it
appears that the pores are well-connected, i.e., open cells, as seen in Fig. 3.6A, within the
membrane matrix which was prepared by using a 5°C water bath. On the other hand, as
seen in Figure 3.6(D), the pore structure appears as several single isolated droplets, i.e.,
close cells. These cells look unconnected to each other within the membrane matrix,
which was prepared using a 35°C coagulation bath.

In addition, asymmetric pore structure is a major characteristic of the membrane prepared
via phase inversion [81]. For the asymmetric membranes prepared in this study, both
skin layer and sublayer could affect the lysozyme diffusion rate. When the high-
temperature coagulation bath was used, larger pores were observed on the top side of the
Due to the pore coalescence, these large pores might be close pores, i.e., unconnected pores. Thus, lysozyme would not diffuse through these large pores. However, a few small pores could still appear on the top side. It was likely that these small pores were connected and allowed lysozyme to diffuse through.

### 3.3.3. Membrane formation mechanism

Table 3.1 lists the measured bulk porosities of the membranes which were prepared from the casting solution of 20 wt% PCL, 65 wt% 2-methoxyethanol and 15 wt% 1,4-dioxane with different coagulation bath temperatures. The density and porosity of the membrane were calculated by measuring the volume and weight of the membrane. The density, $d$, of the membrane was determined by the equation shown below:

$$d = \frac{W}{V}$$

where $W$ is the measured weight, and $V$ is the measured volume. The membrane porosity, $\Phi$, is calculated by the following equation:

$$\Phi = 1 - \frac{d}{d_p}$$

where $d_p$ is the PCL density ($d_p = 1.103$ g/cm$^3$).

As illustrated in Table 3.1, the colder the coagulation bath, the higher the membrane porosity. The trend in the measured bulk porosities is in accordance with the observation shown in Figure 3.6. From Figure 3.6 and Table 3.1, it is concluded that pore connectivity can be improved as coagulation bath temperature reduces. In other words,
the measured bulk porosity can be indicative of pore connectivity within the resultant membranes. Moreover, the measured cloud point of this casting solution (20 wt% PCL, 65 wt% 2-methoxyethanol and 15 wt% 1,4-dioxane) was around 27°C. When a coagulation bath temperature, e.g., 35°C, was higher than the cloud point, lysozyme could not diffuse through the membrane, as shown in Figure 3.3, in which the pore structure was of the close cell type. On the contrary, the membrane, which was immersed into the water bath at a temperature (5, 15 or 25°C) below the cloud point, was able to allow the lysozyme to diffuse through. Thus, the measured bulk porosity and pore connectivity would be further enhanced when thermally-induced phase separation is involved in the membrane formation more substantially.

How each of thermally- and nonsolvent-induced phase separations can affect the lysozyme diffusion and membrane structure was also investigated in this study. The casting solution consisting of 20 wt% PCL, 65 wt% 2-methoxyethanol and 15 wt% 1,4-dioxane was used again. The solution was clear and homogeneous at 50°C by visual observation, and it was maintained at this temperature for casting. Since the cloud point of this solution was around 27°C, the cast membrane was placed into a 5°C close system for the membrane preparation involving only thermally-induced phase separation. On the other hand, for the membrane preparation involving nonsolvent-induced phase separation mainly, the cast membrane was immersed into a 35°C water coagulation bath. However, for the membrane formation involving both thermally- and nonsolvent-induced phase separations, the cast membrane was immersed into a 5°C water coagulation bath.
Figure 3.7 shows the role of the thermally- or nonsolvent-induced phase separation or the combined phase separation on the lysozyme diffusion. As seen in this figure, the general shape of the lysozyme diffusion profile is significantly affected by the membrane preparation method. For example, no lysozyme could diffuse through the membrane which was made by using a 35°C water coagulation bath, i.e., via nonsolvent-induced phase separation. In the case of the membrane prepared via thermally-induced phase separation, diffusion profile was characterized by burst-type diffusion, followed by reaching nearly a plateau. However, combining both thermally- and nonsolvent-induced phase separations contributed to the suitable membrane formation, and lysozyme was able to diffuse through the membrane with the zero-order rate. Thus, the membrane formation mechanism has a critical impact on the lysozyme release profile. When only nonsolvent-induced phase separation was involved in the membrane preparation, a close pore structure was obtained. On the other hand, for the membrane prepared by only thermally-induced phase separation, the free diffusion was a result of large pores (Figure 3.8). However, the nanoporous PCL membrane with the zero-order release rate could be achieved by combining thermally- and nonsolvent-induced phase separations to prepare such a desirable membrane.

The morphology of the membrane which was formed via thermally-induced phase separation without wet-process precipitation, i.e., a 5°C close system, is illustrated in Figure 3.8. As illustrated in this figure, the interstitial domains might be around 5 µm at the top surface and the cross section. Such a large pore size would lead to the free
diffusion and the burst effect, as observed in Figure 3.7. Within the membrane matrix, the leafy, crystalline and lamellar structure could be attributed to the crystallization process [82]. Moreover, the calculated porosity was 73%, which was identical to the porosity of the membrane, which was prepared through immersion into 5°C water. The low temperature environment, involving thermally-induced phase separation, had a significant effect on the improvement of the PCL membrane porosity.

In combining thermally- and nonsolvent-induced phase separations, the possible composition pathway is proposed and illustrated in Figure 3.9. As shown in the 3D phase diagram, the composition pathway can cross the crystallization area more easily and deeply because of chilly nonsolvent. However, when the coagulation bath temperature increases, the crystallization area will become smaller. Therefore, it is less possible for the composition pathway to move into the crystallization region in a higher temperature coagulation bath.

McHugh and his coworker [83] have demonstrated that the pore structure which results from the liquid-liquid phase separation would coarsen due to pore coalescence [84] and Ostwald ripening [85]. The droplet coarsening can be ceased when the growth is frozen by the crystallization of the polymer-rich phase.

Moreover, the nucleation growth rate at the late stage of spinodal decomposition [86] and other models, such as droplet coalescence and Ostwald ripening, can be expressed as:
\[ D^3 = D_0^3 + K(t - t_0) \] where \( D \) is droplet size at the time \( t \), the \( D_0 \) is the droplet size at the end of the early stage, \( t_0 \) is the time when the late stage begins, and \( K \) is the domain growth rate. As shown by McHugh and his coworker [83], \( K \) would drop with decreasing quenching temperature below the crystallization temperature. \( K \) would be smaller in a 5°C than in a 35°C coagulation bath, i.e., the pore size would be smaller in a 5°C than in a 35°C coagulation bath as evident in Figure 3.6. The pore growth rate would be slower at 5°C. Furthermore, it took a shorter time to harden the casting solution in a 5°C coagulation bath from the experimental observation. This phenomenon might originate from the fact that the composition pathway crosses the crystallization line more quickly and deeply. By exploring the parameters in the 3D phase diagram, a limited range of the desired pore structure might be obtained via the combination of thermally- and nonsolvent-induced phase separations [87].

### 3.3.4. The effect of nonsolvent (2-Methoxyethanol) concentration

Nonsolvent plays a crucial role in the formation of porous membranes via phase separation methods. With the addition of nonsolvent into the casting solutions, it is more likely to obtain open-pore structure within the membrane matrix. Figure 3.10 shows the effect of adding nonsolvent (2-methoxyethanol) on lysozyme diffusion rate. When only 25 wt% 2-methoxyethanol existing in the casting solution, lysozyme could not diffuse through the PCL membrane. However, lysozyme is able to diffuse through the PCL membrane when the nonsolvent (2-methoxyethanol) concentration was increased to 65 wt%. Table 3.2 provided the porosity for the membranes which were prepared with two
distinct compositions (20 wt% PCL, 25 wt% 2-methoxyethanol and 55 wt% 1,4-dioxane; 20 wt% PCL, 65 wt% 2-methoxyethanol and 15 wt% 1,4-dioxane). As nonsolvent concentration was increased from 25 to 65 wt%, the porosity would increase from 36 to 73%. Table 3.2 clearly indicates that the adding nonsolvent can improve pore connection with the membrane matrix significantly. Figure 3.11 shows the pore morphology of PCL membranes which were prepared from the solutions containing 25 and 65 wt% 2-methoxyethanol. As shown in Figure 3.11, a close-pore structure might be observed in the PCL membrane which was prepared from the solution which contains 25 wt% 2-methoxyethanol. Therefore, lysozyme is not able to diffuse through the PCL membrane. When 65 wt% 2-methoxyethanol was added into the casting solution, an open-pore structure would be obtained after complete phase inversion. Hence, this membrane could be used for drug delivery/release purpose.

3.3.5. The effect of casting concentration

Various amounts of PCL (15, 20, and 25 wt%) were dissolved in a diluent mixture which contained 2-methoxyethanol and 1,4-dioxane at the ratio of 65/15 by weight. All the cast films were immersed into a 5°C water bath to prepare the membranes via the combined thermally- and nonsolvent-induced phase separation. Figure 3.12 illustrates pore morphologies at the top surfaces of the membranes which were made from 15%, 20%, and 25% PCL in the casting solutions. By analyzing the sizes of the pores shown in the SEMs for each membrane, Figure 3.13 presents the corresponding pore size distributions of the membranes. As shown in this figure, increasing polymer concentration gave rise to
a smaller pore size. When the membrane was prepared from 15% PCL solution, the average pore size was approximately 90 nm (30 – 250 nm). For the membranes which were produced by 20% and 25% PCL solutions, the average pore sizes were approximately 70 nm (20 – 200 nm) and 55 nm (20 – 150 nm), respectively.

The rates of lysozyme diffusion through the PCL nanoporous membranes made from different polymer concentrations were measured. Figure 3.14 shows the lysozyme cumulative diffusion profiles for these membranes. As shown in this figure, the lysozyme release profile displays an initial burst behavior for the membrane prepared from the 15 wt% PCL casting solution. This membrane had pores (~ 90 nm) too large to achieve a zero-order release rate. However, for the membranes made from the increased PCL concentrations, the profiles changed from the bursting type to the zero-order release rate. For these membranes, the average pore sizes were small enough (55 – 70 nm) to obtain the zero-order release rate.

Cohen and coworkers prepared micro/nano porous polyelectrolyte multilayer films for controlled drug release [7]. A Fickian diffusion of the drug was observed with microporous films (300 nm to 2 μm pore size). However, nanoporous films displayed the zero-order release kinetics. A schematic model was proposed to rationalize the zero-order release behavior with the nanoporous structure, instead of microporous films. The micropores are visualized as open containers where the drug can be free to diffuse through the pores into the environment. For the nanoporous films, the number of
nanopores is relatively smaller than that of drug molecules. When the drug molecule is released from the nanopore, it will be replaced by another in a fast manner. Therefore, the nanopore-controlled zero-order release behavior can be obtained.

Martin et al. showed experimentally that a zero-order release profile can be achieved when molecular hydrodynamic diameter is close to the membrane pore size [69]. The unexpected effect, deviated from Fickian diffusion, can be explained by single file diffusion. In the case of single file diffusion, the molecules cannot pass each other as they diffuse through the nanopores. Thus, a concentration gradient can have little effect on the diffusion behavior.

Cosentino et al. proposed a mathematical model to predict the zero-order release rate [72]. In their model, the nanopores were considered as a bottleneck. In addition, it was hypothesized that the mass transfer rate would remain the same over a certain level of concentration gradient. With this model, it is more likely to release a drug of interest in a controlled manner with application of nanoporous membranes.

In this study, increasing PCL concentration resulted in a higher viscosity for the casting solution. During the phase separation in membrane formation, the droplets, which were formed by nucleation and growth of a polymer lean phase, would grow slowly in a highly viscous solution [47]. Hence, a smaller pore size could be obtained with utilization of concentrated casting solution. For the asymmetric membranes which are prepared
involving wet-process phase inversion, the pore size at the skin layer is much smaller than that at the sublayer. Therefore, the skin layer has a substantial effect on controlling the diffusion behavior of a drug. As seen in Figure 3.14, increasing polymer concentration could bring about a linear and lower lysozyme release rate due to a smaller pore size in nanoscale. Thus, the drug release rate, according to the medical need, can be tailored by varying the polymer concentration of the casting solution for the membrane formation.

3.4. Nanoporous PCL membrane to achieve immunoprotection for cell-based therapies/devices

Immunoprotection for cell-based therapies/devices is another important application for the nanoporous PCL membrane. Immunoprotection can be achieved by decreasing the pore size to prevent Immunoglobulin G (IgG) from diffusing through the membranes. In this study, IgG (Sigma-Aldrich, St. Louis, MO) diffusion through the membranes of different pore sizes was also investigated.

Figure 3.15 shows the diffusion profiles for the different membranes prepared from 15, 20, and 25 wt% PCL casting solutions. This figure indicates that Immunoglobulin G can diffuse through the membrane, which was produced via a 15 wt% PCL casting solution. However, for the IgG diffusion through the other two membranes (20 and 25 wt% PCL), no signal above the minimum detectable level could be observed in the retentate side.
after 10 days. The mass transfer resistance to IgG is from not only pore size (55—70 nm), but also tortuous pore structure, i.e., not straight pores. The pore geometry can explain why IgG cannot diffuse through the PCL membranes, even when pore size is somehow larger than IgG molecule. This study suggests that the PCL nanoporous membranes are able to block IgG diffusion more significantly while PCL concentration increases.

In addition, asymmetric pore structure is a major characteristic of the membrane prepared by phase inversion [81]. It has two advantages for cell-based drug delivery devices. One is that the layer where nanopores exist is less than around 1 μm thick. Therefore, it is expected that the rapid dynamic response of cells can be achieved because of the thin layer. Moreover, the membrane can allow fast diffusion of oxygen and nutrients for the living cells. The other advantage is that neovascularization can be obtained at the interface between tissue and membrane surface with larger pore structure (0.8 – 8 μm) [88]. The neovascularization can allow a bioactive, which is secreted by the cells, to be delivered into a blood stream via the newly-grown blood vessel. As shown in this study, it is possible to retard IgG diffusion and simultaneously to maintain the fast diffusion of other small molecules, such as glucose. Hence, the PCL nanoporous membranes presented in this study have great potential for the cell-based drug delivery devices.
3.5. Release of Interferon-α from membrane-based drug delivery devices

In this study, the potential application of the nanoporous PCL membranes for the achievement of zero-order release rate is displayed. The schematic illustration of the device is shown in Figure 3.16. To demonstrate the real application, a nanoporous PCL membrane was prepared via phase separation methods and a drug reservoir was fabricated by using hot-embossing technique. For the membrane preparation, 25 wt% PCL was dissolved in a diluent. The diluent was a mixture of 1,4-dioxane as solvent and 2-methoxyethanol as nonsolvent with a ratio of 15 to 65 by weight. After complete dissolution, the solution was cast on a Teflon plate, and immediately immersed in a 5°C water bath. After 6 hours, the membrane was peeled off from the Teflon plate and dried in vacuum overnight. Then, the membrane was modified with poly(ethylene glycol), as shown in Chapter 5. PCL drug reservoirs (4.5 mm in diameter and 1.8 mm height) were fabricated by using hot embossing technique. Finally, the reservoirs were filled with Interferon-α (Schering, Kenilworth, NJ) and sealed with the nanoporous PCL membrane.

To conduct the release study, the device were placed in 6 mL PBS medium and rotated on a shaker at 37°C with a constant speed of 130 rpm. Every day, the medium was withdrawn and replaced with fresh PBS solution. Interferon-α concentration was characterized by using human interferon alpha (Hu-INF-α) serum sample ELISA kit (PBL Biomedical Laboratories, Piscataway, NJ).
Figure 3.16 displays the release profile of interferon-α from two identical devices. During the first 5 days, it would take time for the PBS solution to penetrate the porous structure, diffuse through the membrane, and dissolve interferon-α powders. After the period of time, interferon-α can be released out. As shown in Figure 3.16, the released amount would be in the desirable range of 0.02 to 0.1 mg from the 6th to 29th day. This study presents a great potential of the nanoporous PCL membrane for the sustained and constant drug release.

3.6. Concluding remarks

The nanoporous PCL membrane to control the drug release rate for an implantable drug delivery device in promising therapeutic application has been described. The drug release rate can be well tailored by varying the temperature of the coagulation bath and the polymer concentration of the casting solution. Nanoporous PCL membranes with well-connected pore structure can be prepared via the combination of thermally- and nonsolvent-induced phase separations. A uniform nanoporous PCL membrane would be obtained when the Teflon plate, instead of the glass plate, was used for the membrane preparation. The porous PCL membrane which was exclusively formed via thermally-induced phase separation had high porosity but large pore size (~ 2 μm) at the top surface. Wet-process precipitation, i.e., using water as the coagulation bath, also played an important role in the formation of nanoporous PCL membranes. Lowering the water coagulation bath temperature from 35 to 5°C improved the bulk porosity and pore
connectivity significantly. In addition, the addition of nonsolvent can enhance the pore connectivity. As long as a 5°C water bath and sufficient amount of nonsolvent were used, a well-connected nanoporous membrane with high porosity could be obtained, in which the average pore size could be reduced to around 55 nm.

The drug release rate can be well controlled by varying the polymer concentration of the casting solution. The average pore sizes of membranes which were prepared via 15, 20 and 25 wt% PCL solutions were around 90, 70 and 55 nm, respectively. By using a 15 wt% PCL solution for the membrane formation, the diffusion of lysozyme through the membrane behaved as the first-order rate. However, the higher polymer concentrations resulted in smaller pore sizes, leading to a lower drug release rate with the zero-order rate behavior. Moreover, immunoprotection can be provided by using the nanoporous PCL membranes to block the passage of IgG.
**Table 3.1.** Porosities of the PCL membranes prepared with different water bath temperatures.

<table>
<thead>
<tr>
<th>Coagulation bath temperature (°C)</th>
<th>Porosity (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>5</td>
<td>73</td>
</tr>
<tr>
<td>15</td>
<td>68</td>
</tr>
<tr>
<td>25</td>
<td>63</td>
</tr>
<tr>
<td>35</td>
<td>56</td>
</tr>
</tbody>
</table>

**Table 3.2.** Porosities of the PCL membranes prepared with two distinct compositions.

<table>
<thead>
<tr>
<th>Composition of casting solution</th>
<th>Porosity (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>20 wt% PCL, 25 wt% 2-Methoxyethanol and 55 wt% 1, 4-Dioxane</td>
<td>73</td>
</tr>
<tr>
<td>20 wt% PCL, 65 wt% 2-Methoxyethanol and 15 wt% 1, 4-Dioxane</td>
<td>36</td>
</tr>
</tbody>
</table>
Figure 3.1. Schematic design of the membrane-based reservoir-type drug delivery device.
Figure 3.2. Cumulative lysozyme diffusion through each of three samples which were cut from the same piece of the PCL membrane cast on (A) a glass plate and (B) a Teflon plate.
Figure 3.3. Various diffusion profiles of lysozyme through the PCL membranes prepared with different coagulation bath temperatures (5, 15, 25, and 35°C). The number of samples tested, n, was 5 for the error bars shown.
Figure 3.4. SEM images of the PCL membranes (top surface) which were prepared at different coagulation bath temperatures: (A) 5°C, (B) 15°C, (C) 25°C, and (D) 35°C.
Figure 3.5. Pore size distribution of the membranes which were prepared with 5, 15 and 25°C coagulation baths. n is the number of pores measured.
Figure 3.6. SEM images of the PCL membranes (cross-section) prepared with a casting solution of PCL/2-methoxyethanol/1,4-dixoane at the weight ratio of 20/65/15 at different coagulation bath temperatures: (a) 5°C, (b) 15°C, (c) 25°C, and (d) 35°C. / (A) enlarged (a) 5°C, (B) enlarged (b) 15°C, (C) enlarged (c) 25°C and (D) enlarged (d) 35°C.
Figure 3.7. Effect of membrane formation mechanism on lysozyme diffusion profile.

The number of samples tested, n, was 5 for the error bars shown.
Figure 3.8. SEM images of the membranes prepared from the PCL/2-methoxyethanol/1,4-dioxane casting solution at the weight ratio of 20/65/15 and cooled from 50°C to 5°C via thermally-induced phase separation, i.e., without using a coagulation bath: (A) Cross section, (B) Top surface, (C) Enlarged view of the cross section, and (D) Enlarged view of the top surface.
Figure 3.9. Proposed composition pathway in the combined thermally- and nonsolvent-induced phase separation.
Figure 3.10. Effect of adding nonsolvent on lysozyme diffusion profile. The number of samples tested, n, was 5 for the error bars shown.
Figure 3.11. SEM images of the membranes (cross section) prepared with two distinct compositions (a) 20 wt% PCL, 25 wt% 2-Methoxyethanol and 55 wt% 1, 4-Dioxane (b) 20 wt% PCL, 65 wt% 2-Methoxyethanol and 15 wt% 1, 4-Dioxane. / (A) the enlargement of (a) and (B) the enlargement of (b).
Figure 3.12. SEM images of the membranes (top surface) prepared via the combined thermally- and nonsolvent-induced phase separation from different PCL concentrations: (A) 15%, (B) 20%, and (C) 25%.
Figure 3.13. Pore size distributions of the membranes which were prepared via the combined thermally- and nonsolvent-induced phase separation from 15, 20 and 25% PCL solutions. n is the number of pores measured.
Figure 3.14. Various diffusion profiles of lysozyme through the membranes prepared via the combined thermally- and nonsolvent-induced phase separation from different PCL concentrations of 15%, 20%, and 25%. The number of samples tested, n, was 5 for the error bars shown.
Figure 3.15. Diffusion of IgG through different PCL nanoporous membranes.
Figure 3.16. Release profile of interferon-α from the membrane-based drug delivery device.
CHAPTER 4

SURFACE MODIFICATION METHODS

4.1. Introduction

Chapter 3 shows that the nanoporous PCL membranes can be utilized to achieve zero-order drug release rate for implantable drug delivery devices. Once the implantation of the membrane-based drug delivery devices, fibrosis might easily form on the membrane surface and interfere the diffusive transport of bioactive agents. After surgery, a series of host reactions would be induced. These reactions include injury, blood-material interactions, provisional matrix formation, inflammation, granulation tissue, foreign-body reaction, and fibrous capsule development. At the late stage of a healing process, fibroblasts can proliferate to a certain extent. In turn, the fibroblasts can secrete collagen around the implant site. Collagen, a major component of connective tissue fibrils, can form a dense fibrous capsule surrounding the implanted devices in the late stage [53]. Intensive fibroblast activity will result in more fibrous capsulation and scar formation. Consequently, fibrous matrix would become firm and dense around the implant. It has been reported that the fibrosis around porous membrane-based drug delivery devices could lead to pore clogging and, in turn, limit the long-term performance of the devices.
Moreover, cells encapsulated in a diffusion chamber will not survive after a certain time because of the shortage in the oxygen and nutrient supply, for cell-based devices [31].

To prevent fibrous encapsulation, it is of importance to prohibit fibroblast cultivation on the surface. Because fibroblast is one of adherent cells, the most appropriate strategy is to prevent fibroblast from attaching to the surface. Therefore, the surface property is critical to the success of implantable biomedical devices. In order to maintain the properties and structures of bulk materials, the surface properties and/or structures have to be altered. Many literatures suggest the use of a smooth and clean device surface, biocompatible materials, and anti-inflammatory agents or the collagen synthesis inhibitor to inhibit fibroblast adhesion [90—92]. In addition, coating a device surface with biocompatible material may mediate the acute inflammatory response, minimize the fibrosis formation, and maintain the release performance of the implanted devices. Preferred coating materials include poly(ethylene glycol) (PEG), albumin, collagen, etc. Among these materials, PEG has been the center of interest in the surface modification of implantable biomedical applications because it is a nontoxic, non-antigenic, non-immunogenic and FDA approved material for internal consumption [58]. Moreover, PEG molecules are effective in reducing protein adsorption and cell adhesion because of the rapid mobility, the steric stabilization effect, and the minimal interfacial energy with water [55, 93—95]. The strategies for the immobilization of PEG molecules on surface include physical adsorption [55, 96, 97], silane coupling [98—99], wet chemical reaction [100, 101], UV
irradiation [102—104], and plasma discharge [105, 106]. This chapter presents a general review related to methods for the immobilization of PEG on a surface.

4.2. Physical adsorption

Physical adsorption of amphiphilic PEG copolymers onto hydrophobic substrates is the simplest method to immobilize PEG on the surface for the anti-biofouling purpose [55]. Amiji et al. treated hydrophobic dimethyldichlorosilane-treated glass and low-density polyethylene surfaces with poly(ethylene oxide)/poly(propylene oxide)/poly(ethylene oxide) (PEO/PPO/PEO) triblock copolymers to prevent fibrinogen adsorption and platelet adhesion [97]. Bridgett et al. also modified polystyrene surfaces with PEO-PPO-PEO to control staphylococcus epidermidis adhesion [96]. However, a disadvantage of this approach is that the PEG-containing copolymers could not stay permanently on the surfaces due to relatively weak non-covalent bonding. Hence, it might not be a desirable way to modify various surfaces of the biomedical devices for long-term applications.

4.3. Silane coupling

Desai et al. modified nanoporous silicon membranes with PEG through the silane coupling method [76, 98, 99]. Initially, a functional group, i.e., trichlorosilane SiHCl₃, was added to one end of the PEG chain because trichlorosilane has the fastest reaction rate with silicon surfaces among other chlorosilane derivates. PEG-silane (PEG-OSiCl₃)
was synthesized by reacting PEG (MW 1000) with silicon tetrachloride. The reaction has
to be performed in the presence of triethylamine as a catalyst and under anhydrous
conditions to avoid hydrolysis and side reactions. Then, PEG-silane can react with
hydroxyl groups on the silicon surface to form Si-O-Si network bonding. Therefore, the
PEG chain is immobilized on the nanoporous silicon membranes with covalent bonding.
Further research in Desai’s group used the same technique to graft PEG on nanoporous
alumina membranes. Although the silane coupling is widely used to immobilize PEG on
inorganic substrates, it would not be a proper protocol to modify a polymeric membrane
surface, such as PCL.

4.4. Wet chemical processes

Kiss et al. grafted PEO onto polyethylene (PE) surfaces through covalent bonding. At
first, PE was sulfochlorinated by \( \text{SO}_2\text{Cl}_2 \) in carbon tetrachloride in the presence of
azobisisobutyronitrile (AIBN) as a catalyst \cite{100}. The reaction was performed at 55-
60°C for 14 minutes. Then, the sample was washed thoroughly with a series of
acetonitrile and water. After complete drying in a vacuum, the chlorinated substrate was
treated with a halogen exchange reaction. Brominated and iodinated PE surfaces were
synthesized by trans-halogenation of chlorinated PE samples with \( \text{AlBr}_3 \) and \( \text{NaI} \),
respectively. In the case of bromination, \( \text{AlBr}_3 \) was added in an anhydrous solvent, \( \text{CS}_2 \).
For the iodination, the chlorinated PE sample was reacted with \( \text{NaI} \) in acetone.
According to Williamson’s ether synthesis, PEG can react with the brominated and
iodinated PE surfaces. In this process, a hydroxyl group of PEG can react with potassium tert-butoxide in anhydrous benzene or acetonitrile at 25°C for 40 hours to obtain PEG-grafted PE surface. Wet chemical processes usually require high temperature and prolonged reaction time to successfully modify an inert material, such as PE, with PEG. Because PCL has a low melting point (~60°C), high reaction temperatures can damage the pore structure of the nanoporous PCL membranes. Consequently, the wet chemical process is not suitable in our study.

4.5. Ultraviolet irradiation

Wang et al. used UV irradiation to attach PEG onto poly-L-lactide (PLLA) films [102]. During the process, PLLA films were soaked in a hydrogen peroxide solution under UV irradiation. Then, the treated films were washed with de-ionized water to remove the excess hydrogen peroxide. These films were immersed into 10 v/v % PEG (MW 2000) solution in a quartz container purged with nitrogen to perform UV irradiation for 60 minutes at 30°C. Finally, the films were rinsed with water at 70°C to remove the residual PEG. Cheo et al. modified natural rubber (NR) latex films with PEG by UV-induced grafting [103]. In their study, the films were subjected to argon plasma to produce reactive functional groups, such as peroxide and hydroperoxide, on the surface. The films were immersed into methoxy-PEG-monomethacrylate solution and exposed to UV irradiation. Then, the films were washed with ethanol or water to remove the excess reactants. Altankov et al. immobilized PEG on polysulfone (PSf) through a relatively
complex procedure with the aid of UV-irradiation [104]. Firstly, they prepared 4-azido benzoyl chloride from 4-aminobenzoic. They synthesized \( \alpha \)-4-azidobenzoyl-\( \omega \)-methoxy (polyethylene glycol) (ABMPEG) from 4-azido benzoyl chloride and monomethoxy poly(ethylene glycol) (MW 10,000). After precipitation of ABMPEG from \( \text{CH}_2\text{Cl}_2 / \text{diethyl ether} \) and diafiltration, ABMPEG aqueous solution was placed onto the PSf substrate and kept in the dark for several hours. The substrate was gently rinsed with water and immediately treated with UV light. Although high reaction temperature and prolonged reaction time might not be necessary for the UV-irradiation method, the tedious procedures, including pure nitrogen purging and synthesis of reactive PEG-derivatives, could still be a barrier for practical purposes.

4.6. Plasma

Song et al. modified PSf porous membranes with PEG via a low-temperature plasma technique [105]. Firstly, PSf membranes were placed in a 0.5% PEG (MW 4000) solution containing 25 wt % isopropanol and 75 wt % water for more than 24 hours. After drying in air, the pre-soaked membranes were exposed to nitrogen plasma. Then, the membranes were washed with water to remove the excess PEG. Wang et al. dipped poly(vinylidene fluoride) (PVDF) microporous membranes into a chloroform solution with different PEG concentrations for 5 minutes [106]. After drying, the pretreated PVDF membranes were subjected to argon plasma at the pressure of 100 Pa and under the gas flow rate of 20 standard cubic centimeters per minute (sccm). The power was
controlled from 0 to 90 W, and treatment time was varied from 0 to 120 seconds. 

Afterward, the membranes were washed with chloroform and water to remove the ungrafted PEG. Though plasma treatment is the most efficient approach for immobilization of PEG on the polymeric substrate, the treatment conditions including power and time must be tailored and optimized. With the ideal conditions, the desirable properties, such as the prevention of protein adsorption and cell adhesion, can be achieved by changing grafting density and PEG molecular weight.

Physical bonding is usually achieved through van der Waals force, electrostatic force or hydrogen bonding. The interaction between PEG and the substrate is not stable, and PEG could easily detach from the surface. Hence, a chemical bonding is highly desirable, especially for the long-term stability. Wet chemical processes require elevated temperature and prolonged reaction time to accomplish the surface modification. However, the melting point for PCL is just around 60°C, so wet chemical processes are not preferable to meet our requirements. Although silane coupling is used to modify the surface of inorganic materials, it is not an appropriate method to modify the nanoporous PCL membrane surface. As a tedious and complex process is needed to accomplish the surface modification by using UV irradiation, so UV irradiation is not desirable. Therefore, a novel, simple and efficient approach to modifying the nanoporous PCL membrane surface is needed. Chapter 5 presents such approach. For this approach, oxygen plasma is utilized to create reactive functional groups on the PCL membrane surface because PCL is an inert polymer. In addition, oxygen plasma can be operated at
room temperature. Thus, the membrane structure would not be altered due to temperature change. To facilitate the grafting of PEG onto the PCL membrane surface, PEG-acrylates are used to provide the reactive double bonds of the acrylates. As a result, a chemical bonding between PEG and the surface can be easily obtained.
CHAPTER 5

SURFACE MODIFICATION TO PREVENT FIBROBLAST ADHESION

Abstract

The extensive fibrosis induced by a healing process will be detrimental to the long-term performance of implantable applications. The objective of this study is to modify the polycaprolactone (PCL) membrane surface with poly(ethylene glycol) (PEG) to prevent fibroblast adhesion. To achieve this goal, oxygen plasma and PEG(400) monoacrylate were used to graft the PEG onto the membrane surface through covalent bonding. Various plasma treatment conditions were investigated to optimize the PEG-grafting quality and to achieve minimum fibroblast adhesion. After the treatment, the water contact angle decreased significantly. Attenuated total reflectance-Fourier transform infrared spectroscopy (ATR-FTIR) spectra indicated that PEG was successfully grafted onto the PCL membrane with the appearance of the PEG characteristic peaks. X-ray photoelectron spectroscopy (XPS) revealed that different plasma powers and treatment times can change the surface composition of membranes. To evaluate the applicability of
this new strategy for prevention of fibrosis, NIH 3T3 fibroblast was used as a model cell line. Cell adhesion and morphology studies indicate that either lower plasma power or shorter treatment time is able to improve resistance to cell adhesion. This chapter demonstrates that this simple and efficient method can be applied to inhibit biofouling on the surface of implantable biomedical devices. With the application of optimized plasma conditions, the effects of grafting density and chain length are investigated in this study.

5.1. Introduction

In our previous study, the nanoporous PCL membranes can be utilized to achieve zero-order drug release rate for implantable drug delivery devices. However, for implantable biomedical devices, one of the most desirable abilities is prevention of biofouling, a result of protein adsorption and/or cell adhesion on their surfaces [5, 56, 75 107—111]. Implantation of biomedical devices can induce host reactions, including injury, blood-material interactions, provisional matrix formation, inflammation, granulation tissue, foreign-body reaction, and fibrous capsule development. During the development of granulation tissue, fibroblasts can proliferate and synthesize collagen around the implant site. Collagen can form a dense fibrous capsule surrounding the implanted devices in the late stage [53]. Extensive granulation tissue will lead to more fibrous capsulation and scar formation. Consequently, fibrous encapsulation around the implants can cause complications and poor healing [54]. It has been reported that the fibrosis around porous membrane-based drug delivery devices could result in pore clogging and, in turn, limit
the long-term performance of the devices [89]. In addition, cells encapsulated in a diffusion chamber will not survive after a certain time because of the shortage in the oxygen and nutrient supply [31].

In order to prevent or delay the occurrence of the inflammatory response and the growth of the fibrous capsule, several strategies have been proposed. These strategies suggest the use of a smooth and clean device surface, biocompatible materials, and anti-inflammatory agents or the collagen synthesis inhibitor [90—92]. Moreover, coating a device surface with biocompatible material may mediate the acute inflammatory response, minimize the fibrosis formation, and maintain the release performance of the implanted devices. Preferred coating materials include poly(ethylene glycol) (PEG), albumin, collagen, etc. Among these materials, PEG has been the center of interest in the surface modification of implantable biomedical applications because it is a nontoxic, non-antigenic, non-immunogenic and FDA approved material for internal consumption [58]. Moreover, PEG molecules are effective in reducing protein adsorption and cell adhesion because of the rapid mobility, the steric stabilization effect, and the minimal interfacial energy with water [55, 93—95].

Surface modification with PEG molecules can be accomplished through physical adsorption [55, 96, 97] or covalent bonding [76, 98—104]. The physical adsorption of PEG on a surface provides the simplest approach to obtaining the anti-biofouling property on the substrate. This interactive force between PEG and the surface could
result from hydrogen bonding, hydrophilic/hydrophobic interaction, van der Waals force, etc. However, this kind of interaction is quite weak compared to a covalent bond. Therefore, the physically-adsorbed PEG will not permanently remain on the substrate, and the physical adsorption is not an ideal method to create cell and protein-resistant surfaces for long-term biomedical applications [55]. On the other hand, covalent bonding of PEG onto the substrate is the most effective way to prepare a stable biocompatible surface. The strategies for covalent bonding of PEG molecules include silane coupling [76, 98, 99], wet chemical reaction [100, 101], UV irradiation [102—104], and plasma discharge [105, 106]. Silane coupling is usually used to graft PEG onto inorganic materials, such as glass, silicon, and silicon dioxide. Accordingly, silane coupling cannot be utilized to attach PEG on the polymeric PCL membrane surface. To successfully immobilize PEG on the surface, a wet chemical method usually requires high temperature and prolonged reaction time. This approach is applicable only if there are some reactive functional groups on the surface. Because PCL is an inert polymer and has a low melting point (~60°C), this type of approach is not suitable for our study. UV irradiation involves complex and tedious procedures, which are needed to permanently attach PEG on the surface. Therefore, UV irradiation is not preferred.

In this study, a simple and efficient approach has been developed to immobilize PEG on the nanoporous PCL membrane surface by using PEG(400) monoacrylate and oxygen plasma. The schematic illustration of this method is shown in Figure 5.1. Initially, PEG(400) monoacrylate was physically adsorbed onto the surface. Subsequently, the
oxygen plasma was applied to produce radicals on the surface. PEG chains were then
grafted onto the nanoporous PCL membrane surface. In this protocol, plasma power and
treatment time are critical. Therefore, operating parameters were explored to achieve a
high quality of PEG grafting and obtain an anti-biofouling surface. The PEG-grafted
nanoporous PCL membranes were characterized by using ATR-FTIR and the contact
angle measurement. Furthermore, X-ray photoelectron spectroscopy was used to analyze
the chemical composition of the surfaces. Fibroblast adhesion on unmodified and PEG-
grafted surfaces was also evaluated in this work. The cell morphology on the PEG-
modified surface was observed by using a fluorescence microscope. By using the
appropriate plasma treatment conditions, the effects of grafting density and chain length
are also explored. Besides, this chapter compares the effects of mono-acrylate and di-
acrylate of PEG. It should be noted that this newly-developed approach can be
applicable in the development of implantable biomedical devices, such as biosensors and
drug delivery devices, to prevent biofouling.

5.2. Experiment

5.2.1. Materials

Polycaprolactone (Mn ~80,000) was purchased from Aldrich Chemicals (Milwaukee, WI).
1,4-Dioxane was obtained from Mallinckrodt Chemicals (Philipsburg, NJ). 2-
Methoxyethanol (ACS reagent, ≥93%) was acquired from Sigma-Aldrich (St. Louis,
MO). Polyethylene glycol-200 monoacrylate (PEG(200)MA), polyethylene glycol-400 monoacrylate (PEG(400)MA) and polyethylene glycol-1000 monoacrylate (PEG(1000)MA) were purchased from Monomer-Polymer & Dajac Labs (Feasterville-Trevose, PA). Polyethylene glycol diacrylate (Mn = 258), polyethylene glycol diacrylate (Mn = 500) and polyethylene glycol diacrylate (Mn = 700) were obtained from Sigma-Aldrich (St. Louis, MO). NIH 3T3 (mouse embryonic fibroblast cell line, CRL-1658) was purchased from American Type Culture Collection (Manassas, VA). Dulbecco’s modified Eagle medium (DMEM/F-12), Dulbecco's phosphate buffered saline (D-PBS), L-glutamine, sodium pyruvate, newborn calf serum (heat-inactivated), trypsin-EDTA (0.25% trypsin and 1 mM EDTA-4 Na), and calcein-AM were obtained from Invitrogen Inc. (Carlsbad, CA). Isopropanol was purchased from Fisher Scientific Inc. (Fair Lawn, NJ). All chemicals were used as received without further purification.

5.2.2. Preparation of nanoporous PCL membranes

The nanoporous polycaprolactone membrane was prepared using a combination of thermally- and nonsolvent-induced phase separations, as described in Chapter 3. To prepare a 20 wt% PCL casting solution, the PCL pellets were dissolved in a diluent which consisted of 15 wt% 1,4-dioxane as solvent and 65 wt% 2-methoxyethanol as nonsolvent. The solution was well stirred and heated at 50°C for approximately 2 h. A general procedure for membrane preparation is described below. The PCL solution was cast on a Teflon plate. Subsequently, the cast film was immediately immersed in a 5°C coagulation bath, i.e., water. After 4–6 h, the membrane on the Teflon plate was
removed from the bath, and isopropanol was poured onto the top of the solidified membrane. After 20–30 minutes, the membrane was separated from the Teflon plate. Then, the membrane was dried in air and further in vacuum.

5.2.3. PEG grafting via oxygen plasma technique

Although PCL is an inert polymer without any reactive functional groups, radicals can be easily generated on the membrane surface with the aid of oxygen plasma to produce free radicals. Because of the high reactivity of PEG(400) monoacrylate, a covalent bond can be formed from the reaction between the acrylate and PCL surface through the radicals. The following procedure was used to treat PCL membranes for PEG grafting. The nanoporous PCL membrane was firstly dipped into isopropanol and then de-ionized (DI) water to clean the surface. After the membrane was dried completely in air for around 2 hours, it was soaked for 2 h in a 0.1M PEG(400) monoacrylate solution using a solvent mixture, which consisted of 80% ethanol and 20% water. After the PEG(400)MA-adsorbed membrane was dried in air overnight, the bottom of the membrane was exposed to oxygen plasma. The plasma-induced grafting process was conducted using the Technics/Micro-RIE 800-II reactive ion etcher at a radio frequency of 30 kHz. During the plasma treatment, the oxygen flow rate was 20 standard cubic centimeters per minute (sccm) and the pressure inside the chamber was around 150 mTorr. To study the effect of the plasma treatment conditions on the biofouling resistance, the plasma power was adjusted from 25 to 100 W and the treatment time was varied from 5 to 20 seconds. To investigate the influence of grafting densities, the nanoporous PCL membrane were
dipped into PEG(400)MA solutions of different concentrations (0.05—0.2M). Then, the membranes were treated with an optimized oxygen plasma condition. Moreover, the effect of PEG chain length on anti-biofouling performance is demonstrated in this dissertation. The nanoporous PCL membrane were soaked into 0.1M PEG(200)MA, PEG(400)MA and PEG(1000)MA solutions; and further, the membranes were treated with an appropriate plasma condition. PEG-diacylate (PEGDA) was also used for the surface modification of nanoporous PCL membranes. For the comparison of the effect of PEGDA molecular weight, the nanoporous PCL membranes were immersed into 0.1M PEGDA(200), PEGDA(575) and PEG(700) solutions. And the membranes were exposed to oxygen plasma with a desirable condition. Following the completion of the reaction, the treated membrane was immersed into a copious amount of isopropanol and then DI water to remove any organic residuals for further analysis.

5.2.4. Fourier transform infrared spectroscopy (FTIR) analysis

A Nexus 470® FTIR spectrometer with a zinc selenide crystal prism (Thermo Nicolet, Madison, WI) in the attenuated total reflection (ATR) mode was used for evaluation of chemical compounds on the modified and unmodified nanoporous PCL membranes. A small piece of the sample after surface treatment was mounted in the sample holder. Thirty-two scans were collected for each spectrum. Each scan was conducted from 400 to 4000 cm⁻¹. The background spectrum and the sample spectrum were obtained for analysis.
5.2.5. Contact angle measurement

To investigate the surface hydrophilicity, droplets of 10 µL DI water were placed on the surface of a membrane sample at room temperature. The water drop profile on the surface was captured with a high-performance charge-coupled device camera acquired from COHU (San Diego, CA). The contact angle was obtained by measuring the sessile drop profile analyzed with a MATLAB code [112].

5.2.6. X-ray photoelectron spectroscopy

Surface compositions of the membranes were analyzed using the Kratos Axis Ultra X-ray photoelectron spectroscopy instrument with a monochromated Al K\(_{\alpha}\) X-ray at a power of 130 W and a photon energy of 1486.7 eV. Each of the membrane samples was attached on a carbon tape, mounted on a sample holder and evacuated overnight in a chamber holder. Then, the sample was moved into the ultra high vacuum (~10\(^{-9}\) torr) analysis chamber. During the measurement, the charge neutralization was used with 2.1 A filament current, 2.3 V filament charge, and 1.3 V filament bias. A binding energy of 285.0 eV for C-H was used to compensate for the charging effects. High-resolution C1s core level (the 1s orbital of carbon) spectra were taken with a power of 80 W passing through the detector and further resolved into individual peaks, i.e., C-H, C-O, C=O, and O-C=O, by using the software supplied by the manufacturer [113].
5.2.7. Fibroblast adhesion study

NIH 3T3 fibroblast cells were cultured in DMEM/F12, which was supplemented with 2 mM L-glutamine, 1 mM sodium pyruvate, and 10% v/v newborn calf serum. Prior to the cell adhesion study, the membranes were fixed at the bottom of 48-well cell culture plates and sterilized with exposure to an ultraviolet light at a power of 30 W (Sylvania, Danvers, MA) overnight. The cells were seeded onto the sterilized membrane surfaces at a density of approximately $1 \times 10^5$ cells/cm$^2$ and incubated at 37°C with 5% CO$_2$. After 6 h incubation, the cell-seeded membranes were removed from the cell culture plates and gently rinsed in fresh D-PBS to remove non-adherent fibroblasts. Subsequently, the membranes with attached cells were trypsinized with trypsin-EDTA for 10 minutes at 37°C, and the number of fibroblasts was counted using a hemocytometer. Cell counts were performed at least three times. Non-treated PCL membranes were used for control.

5.2.8. Fibroblast morphology study

NIH 3T3 fibroblast morphologies on the PEG-modified PCL membranes were observed using fluorescent calcein-AM stain and a fluorescent microscope. The PCL membranes were treated with two distinct plasma conditions, i.e., at a power of 25 W for 5 seconds and a power of 100 W for 20 seconds. Initially, the cells at a density of 100,000 cells/cm$^2$ were seeded on the membranes which were already sterilized and fixed at the bottom of 48-well cell culture plates. After 6 h incubation at 37°C with 5% CO$_2$, the membranes were rinsed with D-PBS to remove the unattached cells. Then, a 200 μL of 10 μM calcein-AM in D-PBS was prepared to label the adherent fibroblasts on each of
the modified membranes in the 48-well cell culture plate at 37°C. After 20 minutes, the membranes were rinsed with D-PBS and placed on the glass slide with a cover slip on the top. The cell morphology was visualized with a Nikon Eclipse TS100 fluorescent microscope.

5.3. Results and discussion

5.3.1. Optimization of oxygen plasma operating conditions (Power and time)

5.3.1.1. Attenuated total reflection-Fourier transform infrared analysis

The plasma-based surface modification is generally used in order to improve surface properties like surface free energy for better adhesion properties and printability of frequently chemically quite inert and hardly wettable polymers. ATR-FTIR analysis was applied to the treated and untreated nanoporous PCL membranes to identify the chemical functional groups on the surface. Figure 5.2 shows the existence of the peak at 810 cm\(^{-1}\), which can be attributed to the C=C stretching on the PEG(400)MA-adsorbed membrane surface without the plasma treatment. For the membrane which was treated with the plasma at a power of 25 W for 5 seconds, the spectrum displays the disappearance of the acrylate’s double bond peak at 810 cm\(^{-1}\), implying the complete conversion of the acrylate double bonds [114]. Therefore, a covalent bond was formed from the reaction between the acrylates of the PEG(400)MA and the PCL surface through the radicals generated by using oxygen plasma.
Figures 5.3 (A)–(C) compare the functional groups between the untreated and the PEG-modified membranes under different plasma treatment conditions. For the PEG-modified membranes, the spectra show the presence of the strong PEG characteristic peak at 1106 cm\(^{-1}\), corresponding to C-O-C stretching vibration of the PEG. In the spectra of the PEG-modified membrane, the intensities of the peaks at 2866 cm\(^{-1}\) and 3440 cm\(^{-1}\), ascribed to CH\(_2\)-stretching and OH-stretching of the PEGs, are relatively high compared to the unmodified membrane [115]. Therefore, ATR-FTIR analysis provides solid evidences that PEG was successfully grafted onto the nanoporous PCL membrane from the reaction between the double bond of PEG(400) monoacrylate and the PCL surface through the radicals generated by oxygen plasma. Moreover, there exists no significant difference among the spectra of the PEG-modified membranes, which were treated with a variety of plasma conditions (25–100 W and 5–20 seconds).

### 5.3.1.2. Contact angle measurement

Figure 5.4 shows the static water contact angles of the modified and unmodified PCL nanoporous membranes. For the pristine membrane, the contact angle was around 107°, suggesting that the surface was quite hydrophobic. Upon the plasma treatment, the water contact angle dropped to the average of 43°. This significant decrease in the contact angle might indicate the presence of hydrophilic PEG on the surface. However, the contact angles still remained around 43°, when the plasma power and the treatment time were varied. Thus, further increasing the plasma treatment intensity had little influence on the wettability of the modified surfaces.
5.3.1.3. Surface elemental composition analysis

Figure 5.5 illustrates the chemical compositions of the plasma-treated membranes as a function of plasma power (25, 50 and 100 W) and treatment time (5, 10 and 20 seconds). High-resolution C1s core-level spectra for all treated membranes could be resolved into three to four component peaks. The peaks were located at the binding energies of 285.0 eV for C-H, 286.4 eV for C-O, 288.0 eV for C=O, and 289.1 eV for O-C=O. For the unmodified PCL membrane, Figure 5.5 (J) displays that the signal of C-H is much higher than the signals of C-O and O-C=O. This result is in agreement with PCL molecular structure. For the membranes which were treated with the oxygen plasma at a power of 25 W for 5, 10 and 20 seconds, there were only three component peaks involved in the spectra, which were C-H, C-O, and O-C=O. The signals of C-H and O-C=O could come from the backbone of PEG(400)MA and/or the nanoporous PCL membrane. The strong intensity of the C-O peak could provide the evidence that PEG was successfully grafted onto the nanoporous PCL membrane surface.

When the oxygen plasma power was increased from 25 to 50 and 100 W, a new species, C=O, started to appear on the surface due to the application of overdosed oxygen plasma. Additional radicals generated by the overdosed oxygen plasma could lead to the formation of C=O after exposure to air [116, 117]. When the plasma power was fixed at 100 W and the treatment time was increased to 20 seconds, significant increase in the intensity of the C-H peak and decrease in the intensity of the C-O peak were observed in the spectrum. These results can be attributed to the overexposure of the oxygen plasma.
The prolonged plasma treatment can result in a substantial etching process [118]. Due to the strong plasma power, some ether and/or hydroxyl (C-O) groups might be etched away from the PEG chains. As a result, a relatively low intensity of the C-O peak and a high intensity of the C-H peak can be seen in Figure 5.5 (I).

Based on the XPS results, Figure 5.6 shows the calculated ratio of carbonyl groups (C=O and O-C=O) to the ether and/or hydroxyl groups (C-O) as a function of plasma power and treatment time. For the unmodified PCL membrane, the ratio would be equal to 0.81. However, the ratios would be in the range of 0.1 to 0.2 for the PEG-modified membranes; these results indicate that the ether and/or hydroxyl groups (C-O) could be a major component on the membrane surface. Therefore, it may be concluded that PEG was successfully grafted on the membrane surface.

Carbonyl groups, for example C=O and O-C=O, might be a result of the over-dosage and overexposure to the oxygen plasma. The ether and/or hydroxyl group, C-O, could directly come from the PEG chains immobilized on the nanoporous PCL membrane surface. An increase in the ratio can suggest that a larger number of oxygen-containing functionalities is generated by the oxygen plasma and less intact PEG chains are grafted onto the surface. Therefore, this ratio can be indicative of the quality of PEG grafting under the oxygen plasma treatment.

As shown in Figure 5.6, the ratios became higher when both the plasma power and treatment time were increased. This indicates that higher plasma power and longer
treatment time might result in the breakage of the PEG chains and consequently a smaller number of ethylene glycol groups that could be attached onto the membrane surface. On the other hand, higher plasma power and longer treatment time might produce a lot of radicals on the membrane surface. When the membrane surface was exposed to air, the reaction between the radicals and air will lead to the formation of carbonyl groups, i.e., C=O and O-C=O. It is also likely that the breakage of PEG chains can react with air to form carbonyl groups under severe oxygen plasma treatment.

In this study, however, the lowest ratio was obtained with application of the lowest plasma power and the shortest treatment time. This implies that intact PEG could be grafted onto the membrane surface and few carbonyl groups would appear on the surface with the mildest plasma treatment condition. With the high quality of PEG grafting, the PEG-grafted nanoporous PCL membrane surface is supposed to better prevent fibroblast adhesion.

**5.3.1.4. Fibroblast adhesion study**

The effects of the oxygen plasma treatment conditions on biofouling prevention were examined by studying fibroblast adhesion to the PEG-modified PCL membrane surfaces. Figure 5.7 shows the ratio of fibroblast adhesion between a PEG-modified PCL membrane surface and the untreated PCL membrane surface for various oxygen plasma treatment conditions. As shown in this figure, either lower plasma power or shorter treatment time led to a reduction in fibroblast adhesion. For the PEG-modified
membrane which was treated with oxygen plasma at the power of 25 W for 5 seconds, there was a significant decrease in cell adhesion by around 72%, as compared to the untreated membrane. However, a more than 5-fold increase (greater than 6 times of the cell number) in fibroblast adhesion was observed when the PEG-modified membrane was exposed to the oxygen plasma at a power of 100 W for 20 seconds. Therefore, the results indicate that the milder plasma treatment conditions, i.e., lower power and shorter treatment time, can result in less fibroblast adhesion.

The observed fibroblast adhesion is in agreement with the XPS results as shown in Figures 5.5 and 5.6. During the plasma process, higher power and longer treatment time might not only break PEG chains but also generate additional oxygen-containing functionalities. Figures 5.5(I) and 6 indicate that severe oxygen plasma treatment could etch away ethylene glycol groups and therefore make PEG chains shorter. It is reported that short PEG chains cannot prevent cell adhesion well enough in comparison with long PEG chains [119—121]. Because shorter PEG chains may have lower mobility, they cannot move so fast to decrease the contact time between proteins/cells and the surfaces. Thus, it is more likely that fibroblasts can adhere onto the membrane surfaces as in the case that the surfaces were treated with severe plasma conditions [55]. Also, low-molecular-weight PEGs can provide fewer polymer segments. When proteins and/or cells approach the surfaces, there is a minimal change in a conformation number and a configuration entropy. Therefore, the developed repulsive force would not be strong enough to prevent fibroblast adhesion.
Furthermore, based on the XPS results, a large number of carbonyl groups, such as aldehyde, ketone and carboxylic acid, were produced due to the severe plasma treatment. The carbonyl groups might promote significant protein adsorption with a specific confirmation, in turn, leading to a substantial increase in cell adhesion [122—124]. Thus, a drastic increase in fibroblast adhesion to the PEG-grafted surfaces was observed due to the severe oxygen plasma treatment. According to the result displayed in Figure 5.7, the 5-second oxygen plasma treatment at a power of 25 W might be sufficient enough to graft PEG onto the surfaces without too much formation of carbonyl compounds to prevent fibroblast adhesion.

5.3.1.5. Fibroblast morphology investigation

To further investigate the effects of plasma conditions on the interaction between fibroblasts and the treated membrane surfaces, the cell morphology on the membrane surfaces was observed by using fluorescent microscopy and calcein-AM stain. The PEG-modified nanoporous PCL membranes were treated with two distinct plasma conditions, namely 25 W for 5 seconds and 100 W for 20 seconds, and NIH 3T3 fibroblast morphologies on both cases were studied. As shown in Figure 5.8 (A), few cells adhered to the PEG-modified membrane surface, which was treated with oxygen plasma at a power of 25 W for 5 seconds. Most of the adhered cells were still round without stretch as normal 3T3 cell growth pattern. This observation indicates that low affinity existed between fibroblasts and the membrane surface. However, Figure 5.8 (B) displays that a large number of fibroblasts adhered to the PEG-modified nanoporous PCL membrane
which was treated with oxygen plasma at a power of 100 W for 20 seconds. Also, the extensive cell spreading suggests high affinity between the fibroblasts and the membrane surface. The observation of fibroblast morphology is in agreement with the results shown in Figures 5.5, 5.6 and 5.7.

Cell attachment and morphology on a specific surface is considerably affected by adsorption of cell-adhesive glycoproteins, such as fibronectin and vitronectin. With the same contact angle, it is reported that other properties instead of the surface hydrophilicity is mainly responsible for protein adsorption and conformation on the surface [123]. Several studies showed that surface chemistry plays an important role in the adsorption and conformation of the glycoproteins and, in turn, modulates cell adhesion and morphology [123-128]. Groth et al. showed that the carboxyl group can improve the fibroblast adhesion and lead to the spread-out morphology on the surface [93]. Figure 5.6 indicates that a considerable formation of carbonyl groups, i.e., \( \text{C}=\text{O} \) and \( \text{O-} \text{C}=\text{O} \), can be induced by using the severe oxygen plasma treatment. These two functional groups may promote the adsorption of cell-binding proteins with the proper conformation. Fibroblasts can consequently adhere to the membrane surfaces with the spread-out morphology. On the other hand, the PEG-grafted membrane surface which was treated with the appropriate plasma condition, i.e., lower power and shorter treatment time, with less amounts of these two groups, had a positive effect on suppressing cell adhesion and spreading. With obtaining optimized oxygen plasma operating conditions,
other parameters can be explored their effects on fibroblast adhesion, such as grafting density and PEG chain length.

5.3.2. Effect of grafting density

Grafting density would be crucial to the success of preventing cell adhesion. Herein, the nanoporous PCL membrane was soaked in 0.05, 0.1 and 0.2 M PEG(400)MA solutions for 2 hours. Then, the membranes were dried in air and further treated with an optimized plasma condition, i.e. 25W for 5 seconds. Figure 5.9 shows the effect of grafting density/PEG(400)MA concentration on static water contact angle. It can be observed that the contact angle would not change significantly as a function of grafting density/PEG(400)MA concentration. Figure 5.10 shows the functional groups of the membrane surface. Among the plasma-treated samples, the membrane, which was modified with the 0.05M PEG(400)MA solution, could give the lowest strength of PEG’s characteristic peaks at 1100, 2870 and 3340 cm\(^{-1}\). However, the characteristic peaks of PEG (1100, 2870 and 3340 cm\(^{-1}\)) would not vary substantially when 0.1 and 0.2M PEG(400)MA solutions were used for the surface modification. Thus, the membrane surface might be already well covered with PEG(400)MA when the immersion of the membrane in the 0.1M PEG(400)MA solution. Figure 5.11 illustrates how the grafting density/PEG(400)MA concentration affect fibroblast adhesion. When the 0.05M PEG(400)MA solution was used for the surface modification, a significant increase in fibroblast adhesion can be observed, relative to the control sample. However, fibroblast adhesion can be reduced as the PEG(400)MA concentration was increased to 0.1 and
0.2M. As shown in Figure 5.11, there is no obvious change in fibroblast adhesion while 0.1 and 0.2M PEG(400)MA solutions were used for the surface modification. With the immersion of the nanoporous PCL membranes into a 0.1M PEG(400)MA solution, the membrane surface might be fully saturated with PEG(400)MA molecules. Increasing PEG(400)MA concentration (> 0.1M) might have no effect on reducing fibroblast adhesion. Figure 5.12 displays the elemental composition of the PEG-modified membrane surface. As shown in Figure 5.12, the ratio of $\text{C}=\text{O}$ plus $\text{O-C}=\text{O}$ to $\text{C-O}$ would decrease significantly when the PEG(400)MA concentration increases from 0.05 to 0.1M. In addition, the ratio would not change substantially between the membranes which were modified with 0.1 and 0.2M PEG(400)MA solutions. While the concentration was equal to 0.05M, the membrane surface might not be fully covered with PEG(400)MA molecules. There was space between each PEG(400)MA molecule; and the uncovered membrane surface would be exposed to oxygen plasma. Hence, the carbonyl groups, i.e., $\text{C}=\text{O}$ and $\text{O-C}=\text{O}$, could be produced due to exposure to oxygen plasma. On the contrary, a limited amount of the carbonyl groups would be produced as the membrane surface was fully packed with PEG(400)MA molecules, i.e., when 0.1 and 0.2M PEG(400)MA solutions were used.

**5.3.3. Effect of chain length**

PEG chain length also plays an important role in the prevention of fibroblast adhesion. In this study, the nanoporous PCL membranes were immersed into 0.1M PEG(200)MA, PEG(400)MA and PEG(1000)MA solutions. The pre-treated membranes were exposed
to oxygen plasma at a power of 25W for 5 seconds. Figure 5.13 displays the variation of contact angle as a function of PEG chain length. For the membrane modified with PEG(400)MA, the lowest contact angle can be achieved, compared to other two samples. As shown in Figure 5.13, the membrane modified with PEG(1000)MA would provide the highest contact angle (~64°) among these three samples. Figure 5.14 illustrates the ATR-FTIR analysis of the PEG-modified membranes. For the membrane modified with PEG(400)MA, it can give higher PEG signals (1100, 2870 and 3340 cm⁻¹) than the membranes modified with PEG(200)MA and PEG(1000)MA. Moreover, the membrane modified with PEG(1000)MA provides the lowest strength of the PEG characteristic peaks.

Figure 5.15 shows the relation between PEG chain length and fibroblast adhesion. Among these three samples, only the membrane modified with PEG(400)MA can prevent fibroblast adhesion. For the membranes modified with PEG(200)MA and PEG(1000)MA, they might improve fibroblast adhesion instead of reducing fibroblast adhesion. For the PEG(200)MA, the chain length could be too short to provide enough mobility and steric stabilization effect. Therefore, increasing hydrophilicity might be attractive to fibroblast and promote fibroblast adhesion. However, when PEG molecular weight increases, the reactivity between PEG-monoacrylate and the membrane surface would decrease.
Figure 5.16 presents a brief explanation why it is more difficult to modify the membrane surface with higher PEG molecular weigh in this study. Once PEG molecular weight increases, the reactive group (acrylate CH=CH$_2$) would be hidden within the polymer matrix. Hence, it is more unlikely for the acrylate groups to react with the radicals produced by oxygen plasma. For the membrane pre-treated with PEG(1000)MA, the oxygen plasma might generate a large amount of carbonyl groups other than covalent bonding between PEG and the membrane surface. That is a reason why the membrane modified with PEG(1000)MA would provide the lowest contact angle, the weakest PEG signals; and further, it can increase fibroblast adhesion.

**5.3.4. Comparison of effect of mono-acrylate and di-acrylate of PEG**

In this study, PEG-diacylates were also investigated for their effect on the prevention of fibroblast adhesion. The 0.1M PEGDA(258), PEGDA(575) and PEGDA(700) solutions were prepared and used to modify the nanoporous PCL membranes with the suitable plasma conditions. Table 5.1 shows the numbers of ethylene glycol groups for PEGDA(258), PEGDA(575), PEGDA(700) and PEG(400)MA. Figure 5.17 displays the variation of contact angle as a function of PEGDAs and PEG(400)MA. When the molecular weight of PEGDA or the number of ethylene glycol becomes higher, the contact angle would decrease. Moreover, there is no significant difference in contact angle between the membranes modified with PEGDA(700) and PEG(400)MA.
Figure 5.18 presents the surface function groups of the membranes modified with PEGDA(258), PEGDA(575), PEGDA(700) and PEG(400)MA. The spectra shows that the membrane modified with PEGDA(258) provides the lowest intensity for the characteristic peaks of PEG among these four samples. When the number of ethylene glycol groups increases to 9 (PEG(400)MA), 10 (PEGDA(575)) and 13 (PEGDA(700)), two major PEG peaks (1100 and 2870 cm$^{-1}$) would increase considerably. Furthermore, the peak for the hydroxyl group at 3340 cm$^{-1}$ can be observable when the 0.1M PEG(400)MA was used for the surface modification. For the PEG(400)MA, two ending functional groups are the acrylate group and the hydroxyl group. Once the acrylate group forms a covalent bonding with the membrane surface, the hydroxyl group would be stretched out and be detected by ATR-FTIR analysis.

Figure 5.19 shows the variation of fibroblast adhesion as a function of PEG(400)MA and PEGDAs. For the membranes modified with PEGDAs, the fibroblast adhesion would be reduced as the number of ethylene glycol groups increases. When more ethylene glycol groups were immobilized on the membrane surface, the chain mobility would become higher and the steric stabilization effect would be more significant. Nevertheless, the membrane modified with PEG(400)MA could reduce the fibroblast adhesion more than the membranes modified with PEGDAs. Although PEGDA(700) has a larger number of ethylene glycol groups than PEG(400)MA, PEG(400)MA still can reduce more fibroblast adhesion than PEGDA(700). As shown in the insert of Figure 5.19, the chain mobility of PEGDAs would be limited significantly because two ending functional groups are
immobilized on the membrane surface. For PEG(400)MA, only one ending group was fixed on the membrane surface, i.e., acrylate group. Hence, the other ending group, i.e., hydroxyl group, can be stretched out and move freely in the surroundings. With the high mobility, PEG(400)MA can provide the best anti-biofouling ability in this study.

5.4. Concluding remarks

In this study, a new and simple approach to grafting PEG from PEG monoacrylate onto the nanoporous PCL membrane to prevent fibroblast adhesion has been developed. To accomplish the optimized PEG-grafting on the membrane surface, a variety of oxygen plasma treatment conditions were investigated. As a result, the water contact angle dropped from 107° for the unmodified membrane to an average of 43° for all PEG-modified membranes, revealing the appearance of the hydrophilic PEG on the surface. ATR-FTIR spectra provided evidence that the PEG was successfully grafted onto the membrane surface from the existence of PEG characteristic peaks at 1100, 2870, and 3440 cm⁻¹. Disappearance of the peak at 810 cm⁻¹ suggested that complete conversion of acrylate group was achieved. XPS results showed that the surface composition was changed according to the different plasma treatment conditions. More gentle plasma treatment led to the lower ratio of C=O and O-C=O to C-O, suggesting that a higher quality of the PEG-grafting was obtained. The severe plasma treatment not only generated carbonyl groups on the surface but also broke the PEG chains. It is concluded
that the plasma treatment condition can affect the surface chemistry and play an important role in the fibroblast adhesion to the surface.

It is found that either lower plasma power or shorter treatment time can cause a decrease in fibroblast adhesion. When the membrane was treated with the oxygen plasma at a power of 25 W for 5 seconds, fibroblast adhesion was reduced by 72%, as compared to the untreated membrane. From the cell morphology study, few round fibroblasts were observed on the membrane which was treated with the mildest conditions (25 W for 5 seconds), indicating a low affinity between the cells and the substrate. The approach of this investigation holds great potential to improve the performance of current implantable drug delivery devices. It can also be applied to other implantable biomedical applications.
Table 5.1. The number of ethylene glycol groups for PEGDA(258), PEGDA(575), PEGDA(700) and PEG(400)MA.

<table>
<thead>
<tr>
<th>Species</th>
<th>Number of ethylene glycol</th>
</tr>
</thead>
<tbody>
<tr>
<td>PEGDA(258)</td>
<td>3</td>
</tr>
<tr>
<td>PEGDA(575)</td>
<td>10</td>
</tr>
<tr>
<td>PEGDA(700)</td>
<td>13</td>
</tr>
<tr>
<td>PEG(400)MA</td>
<td>9</td>
</tr>
</tbody>
</table>
Step 1. Physical adsorption

Step 2. Generation of radicals

Step 3. Formation of covalent bonds

Figure 5.1. The scheme for the surface immobilization of PEG onto the nanoporous PCL membrane.
**Figure 5.2.** ATR-FTIR spectra of the untreated, PEG(400)MA-adsorbed and plasma-treated nanoporous PCL membranes.
Figure 5.3. ATR-FTIR spectra of the pristine and PEG-modified nanoporous PCL membranes which were treated with oxygen plasma at power of (A) 25 W, (B) 50 W and (C) 100 W for 5, 10 and 20 seconds.
Figure 5.3. continued

(B) 50 W

Untreated

5 seconds

10 seconds

20 seconds

Absorbance

Wavenumber (cm\(^{-1}\))

3340 cm\(^{-1}\)

2870 cm\(^{-1}\)

1100 cm\(^{-1}\)
Figure 5.3. continued

Absorbance

Wavenumber (cm\(^{-1}\))

Untreated

5 seconds

10 seconds

20 seconds

(C) 100 W

3440 cm\(^{-1}\)

2870 cm\(^{-1}\)

1100 cm\(^{-1}\)
Figure 5.4. Static water contact angles of unmodified and PEG-modified nanoporous PCL membranes which were treated with oxygen plasma at the power of 25–100 W for 5–20 seconds.
Figure 5.5. High-resolution C1s XPS spectra for the membranes which were treated with a variety of plasma conditions. (A) 25 W for 5 seconds, (B) 25 W for 10 seconds, (C) 25 W for 20 seconds, (D) 50 W for 5 seconds, (E) 50 W for 10 seconds, (F) 50 W for 20 seconds, (G) 100 W for 5 seconds, (H) 100 W for 10 seconds, and (I) 100 W for 20 seconds (J) PCL membrane without any treatment.
Figure 5.6. Effects of plasma power and treatment time on the ratio of $\text{C} = \text{O}$ plus $\text{O-C} = \text{O}$ to $\text{C-O}$.
Figure 5.7. Ratio of NIH 3T3 fibroblast adhesion between a treated PCL membrane surface and the untreated PCL membrane surface for various oxygen plasma treatment conditions.
Figure 5.8. Fluorescence microscopy images of NIH 3T3 fibroblast adhered onto PEG-grafted nanoporous PCL membranes which were treated with two extremely distinct plasma conditions: (A) 25 W for 5 seconds and (B) 100 W for 20 seconds.
Figure 5.9. Variation of water contact angle as a function of grafting density/PEG(400)MA concentration.
Figure 5.10. ATR-FTIR spectra of the untreated and PEG-modified nanoporous PCL membranes which were modified with various grafting densities/PEG(400)MA concentration (0.05—0.2M).
Figure 5.11. Ratio of NIH 3T3 fibroblast adhesion between the treated PCL membrane surface and the untreated PCL membrane surface for three PEG(400)MA concentrations, 0.05, 0.1 and 0.2 M.
Figure 5.12. Surface elemental composition as a function of PEG(400)MA concentration.
Figure 5.13. Variation of water contact angle as a function of the molecular weight of PEG-monoacrylate.
Figure 5.14. ATR-FTIR spectra of the untreated and PEG-modified nanoporous PCL membranes which were modified with various PEG-monoacrylate molecular weights.
Figure 5.15. Ratio of NIH 3T3 fibroblast adhesion between the treated PCL membrane surface and the untreated PCL membrane surface for PEG(200)MA, PEG(400)MA and PEG(1000)MA.
Figure 5.16. Schematic illustration of the relation between PEG molecular weight and surface reactivity
Figure 5.17. Variation of water contact angle as a function of PEG-diacrylate’s molecular weight and PEG(400)MA.
Figure 5.18. ATR-FTIR spectra of the untreated and PEG-modified nanoporous PCL membranes which were modified with PEG(400)MA and different PEGDA’s molecular weights.
Figure 5.19. Ratio of NIH 3T3 fibroblast adhesion between the treated PCL membrane surface and the untreated PCL membrane surface for PEGDA(258), PEGDA(575), PEGDA(700), and PEG(400)MA.
CHAPTER 6

CONCLUSIONS AND RECOMMENDATIONS

6.1. Conclusions

1. Preparation of nanoporous PCL membranes via the combination of thermally- and nonsolvent-induced phase separations

The nanoporous PCL membrane to control the drug release rate for an implantable drug delivery device in promising therapeutic application has been described. The drug release rate can be well tailored by varying the temperature of the coagulation bath and the polymer concentration of the casting solution. Nanoporous PCL membranes with well-connected pore structure can be prepared via the combination of thermally- and nonsolvent-induced phase separations. A uniform nanoporous PCL membrane would be obtained when the Teflon plate, instead of the glass plate, was used for the membrane preparation. The porous PCL membrane which was exclusively formed via thermally-induced phase separation had high porosity but large pore size (~2 μm) at the top surface. Wet-process precipitation, i.e., using water as the coagulation bath, also played an
important role in the formation of nanoporous PCL membranes. Lowering the water coagulation bath temperature from 35 to 5°C improved the bulk porosity and pore connectivity significantly. As long as a 5°C water bath was used, a well-connected nanoporous membrane with high porosity could be obtained, in which the average pore size could be reduced to around 55 nm.

The drug release rate can be well controlled by varying the polymer concentration of the casting solution. The average pore sizes of membranes which were prepared via 15, 20 and 25 wt% PCL solutions were around 90, 70 and 55 nm, respectively. By using a 15 wt% PCL solution for the membrane formation, the diffusion of lysozyme through the membrane behaved as the first-order rate. However, the higher polymer concentrations resulted in smaller pore sizes, leading to a lower drug release rate with the linear release profile.

2. Surface modification of nanoporous PCL membrane with PEG

In this study, a new and simple approach to grafting PEG from PEG monoacrylate onto the nanoporous PCL membrane to prevent fibroblast adhesion has been developed. To accomplish the optimized PEG-grafting on the membrane surface, a variety of oxygen plasma treatment conditions were investigated. As a result, the water contact angle dropped from 107° for the unmodified membrane to an average of 43° for all PEG-modified membranes, revealing the appearance of the hydrophilic PEG on the surface. ATR-FTIR spectra provided evidence that the PEG was successfully grafted onto the
membrane surface from the emergence of PEG characteristic peaks at 1100, 2870, and 3440 cm\(^{-1}\). Disappearance of the peak at 810 cm\(^{-1}\) suggested that complete conversion of acrylate group was achieved. XPS results showed that the surface composition was changed according to the different plasma treatment conditions. More gentle plasma treatment led to the lower ratio of C=O and O-C=O to C-O, suggesting that a higher quality of the PEG-grafting was obtained. The severe plasma treatment not only generated carbonyl groups on the surface but also broke the PEG chains. It is concluded that the plasma treatment condition can affect the surface chemistry and play an important role in the fibroblast adhesion to the surface.

It is found that either lower plasma power or shorter treatment time can cause a decrease in fibroblast adhesion. When the membrane was treated with the oxygen plasma at a power of 25 W for 5 seconds, fibroblast adhesion was reduced by 72%, as compared to the untreated membrane. From the cell morphology study, few round fibroblasts were observed on the membrane which was treated with the mildest conditions (25 W for 5 seconds), indicating a low affinity between the cells and the substrate. The approach of this investigation holds great potential to improve the performance of current implantable drug delivery devices. It can also be applied to other implantable biomedical applications.
6.2. Recommendation for future work

This dissertation demonstrates an important application of nanoporous PCL membranes to biomedical devices. However, this section introduces another application of membrane technology, artificial organs. Due to the shortage of organ and/or tissue donors, it has been increasingly important to find an efficient treatment against chronic diseases, such as diabetes, Parkinson’s disease, and Alzheimer’s disease. Cell-based therapy has been a new and exciting strategy to replace organ transplantation [3, 13, 22]. Primary cells or genetically-engineered cell lines with specific secretions can be used as “source” to produce therapeutic compounds [74, 75]. For example, pancreatic B cell can secrete insulin for diabetic therapy, and certain cancer cells can stimulate natural cancer-fighting mechanisms. Biohybrid artificial organs, i.e., devices containing living cells and semipermeable membranes, can be used as a substitute for unhealthy organs and tissues.

A proposed device configuration for the treatment of diabetes is shown in Figure 6.1. The most critical requirement for artificial organs is to maintain cell viability in the devices. It is required to prepare a nanoporous membrane to isolate living cells, such as islet of Langerhans cells, from being attacked by immune system. Simultaneously, this membrane has to allow enough oxygen and nutrients to reach the encapsulated living cells. To achieve rapid dynamic response, the membrane must give little mass transfer resistance to a stimulus (e.g., glucose) and a cell-produced drug (e.g., insulin). With a suitable membrane structure (e.g., asymmetric), all metabolic wastes can leave the devices with a sufficient rate to ensure a clean environment for the living cells (Theme I,
see Figure 6.2). In addition, the surface modification of the nanoporous membrane with certain polymers/oligomers, e.g., polyethyleneglycol, is able to achieve immunoprotection, to prevent fibrosis, and to induce neovascularization (Theme II, see Figure 6.3). Cell culture construct also plays a significant role in the long-term performance of the devices. Electrospun fibers with a nanoporous structure can offer an ideal environment for cell seeding, infiltration, proliferation, and differentiation (Theme III, see Figure 6.4).

6.2.1. Synthesis of asymmetric nanoporous membranes with uniform pore size (Theme I)

The immunoprotection of transplanted cells and tissues by size-based semipermeable membranes is crucial to the success of artificial organs [9]. This is shown schematically in Figure 6.2. As shown in this figure, the membranes have to provide different mass transfer resistance against various species. For example, the membranes have to block the passage of immune molecules, such as IgG. However, the membranes should allow the controlled release of bioactive drugs, such as insulin. Moreover, the membranes should allow the fast diffusion of metabolic wastes produced by living cells, in order to maintain cell viability. Currently, most bio-polymeric porous membranes have not been able to possess uniform pore structure in the range of tens of nanometers. Because of broad pore size distribution, these membranes cannot completely block the diffusion of immune molecules. Even if 1% of pores are large enough to allow the passage of antibodies, the immunoreactions can be deleterious to all of encapsulated cells and
significantly restrict the long-term function of the devices [129]. Microfabrication has 
been used to create inorganic membranes with well-controlled pore size down to tens of 
nanometers [11, 24, 70]. However, the inorganic membranes (silicon) are not 
biocompatible. Therefore, the application of the microfabricated silicon membranes to 
implantable devices is limited. Due to the limited mechanical strength, the membranes 
will be quite thick, and the porosity will be extremely low. As a result, the transport of 
stimulus (glucose), drug (insulin), oxygen, nutrients, and metabolic wastes will be too 
slow to maintain cell functionality and to ensure therapeutic effectiveness. Here, I 
propose to synthesize biocompatible block-copolymers. The self-assembly of block 
copolymer can result in a highly-ordered nanoporous structure [64]. This technique 
typically requires either an inorganic support or a minimum thickness to obtain sufficient 
mechanical strength. Hence, I suggest combining a self-assembly technique and a phase 
inversion method to prepare asymmetric membranes with uniformly nanoporous 
structures [66]. This membrane, prepared via phase inversion, can give high porosity, 
very thin top layer and excellent mechanical strength. This study can not only meet the 
requirements for artificial organs but also achieve extremely high selectivity for certain 
separation purposes.

Because poly(ε-caprolactone) is a biocompatible material and has relatively low 
degradation rate, it is a promising candidate for the constituent of artificial organs. The 
self-assembly of amphiphilic block copolymers can yield materials with tunable nano-
scale features. I suggest to synthesize poly(ε-caprolactone)-b-poly(ethylene oxide) and
poly(ε-caprolactone)-b-poly(hydroxyethyl methacrylate) block copolymers, which are biocompatible materials. The effects of molecular weights and the ratios of two monomers on self-assembled structures should be investigated thoroughly. The self-assembly of amphiphilic block copolymers has been incorporated with phase inversion to prepare asymmetric nanoporous membranes with extremely uniform pore size. Several parameters involved in the phase inversion process should also be investigated, for example, solvents, nonsolvents, additives, evaporation times, and various compositions of casting solutions.

6.2.2. Surface modification to manipulate the response of human body (Theme II)

The surface modification of the immunoisolation membranes with specific materials can lead to the pre-designed host response. This is illustrated schematically in Figure 6.3. As illustrated in this figure, a variety of molecules can be immobilized onto the membrane surface. Surface-modified molecules can prevent certain immune species from approaching to the surface. In addition, some surface-modified molecules can induce pre-programmed human response, such as cell adhesion and neovascularization. It has been reported that the size-based immunoisolation is not sufficient enough to completely block the passage of immune molecules [12]. Surface modification with ionic polymer can inhibit the serum complement activity [29]. To assure the cell viability, a large amount of oxygen and nutrients supplied from blood circulation is usually required. Therefore, neovascularization around the implant is preferable [36]. The newly-
developed blood vessels can be close to the membrane surface to provide enough oxygen and nutrients for the living cells. The metabolic wastes, produced by the transplanted cells, will not remain in the devices and can be removed by the new blood vessels rapidly. Fibrosis is a critical issue to the success of implantable devices. Surface modification with certain materials can prevent the membrane surface from fibroblast adhesion [93]. Therefore, I suggest modifying the membrane surface with a variety of materials, e.g., polyethyleneglycol or its derivatives. The interaction between the modified membrane surface and the host response can be optimized according to any specific requirements.

6.2.3. Synthesis of electrospun micro/nano fibers with nanoporous structures (Theme III)

The primary factor in determining the success of artificial organs is the ability to maintain cell viability and function. In addition to protection from immune rejection, providing an appropriate cell-culture environment is another promising strategy to achieve the long-term performance of cell-based therapy. Figure 6.4 depicts Theme III schematically. As depicted in this figure, living cells, which can function as the source of drug, can be seeded on and cultured within the electrospun fibers where nanopores appear.

Electrospun micro/nanofibrous scaffolds have emerged as a new and efficient methodology in the field of tissue engineering [130]. Figure 6.5 shows the schematic illustration of electrospinning apparatus and micro/nano fibers with nanopores. The scaffolds can improve and manipulate mammalian cell attachment, proliferation,
infiltration, differentiation and function by tailoring their physical and spatial architectural geometries. Nanopores can be produced on and/or within the fibers by using the combination of electrospinning and phase inversion approaches. With nanopores located throughout individual fibers, the mechanical strength of scaffolds can be increased [131]. Most of cells can exhibit a particular response to a nanostructure. A nanoporous surface is able to improve cell proliferation and the uniformity of cell spreading on the scaffolds [132]. Nanoporous structure, formed via the phase inversion technique, can generate additional space and surface area within as-spun fibrous scaffolds. This unique structure will allow for the oxygen and nutrient requirements for cell viability to be met [133]. This dual-porosity scaffold is also capable of increasing the transport rate of metabolic wastes through the nanoporous structure to provide better culture environment [134].
Figure 6.1. Overall schematic illustration of the three themes for membrane technology and artificial organs research.
Figure 6.2. Schematic illustration of Theme I (Immuinoisolation membrane).
Figure 6.3. Schematic illustration of Theme II (Surface modification to manipulate biological responses).
Figure 6.4. Schematic illustration of Theme III (Cell-culture construct).
Figure 6.5. Schematic illustration of electrospinning apparatus and micro/nanofibrous scaffold with nanoporous structure.
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